The Expanding Role of Biomarkers in the Assessment of Smoking-Related Parenchymal Lung Diseases

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Recent advances in the field of clinical biomarkers suggest that quantification of serum proteins could play an important role in the diagnosis, classification, prognosis, and treatment response of smoking-related parenchymal lung diseases. COPD and idiopathic pulmonary fibrosis (IPF), two common chronic progressive parenchymal lung diseases, share cigarette smoke exposure as a common dominant risk factor for their development. We have recently shown that COPD and interstitial lung disease may represent distinct outcomes of chronic tobacco use, whereas others have demonstrated that both diseases coexist in some individuals. In this perspective, we examine the potential role of peripheral blood biomarkers in predicting which individuals will develop COPD or IPF, as well as their usefulness in tracking disease progression and exacerbations. Additionally, given the current lack of sensitive and effective metrics to determine an individual's response to treatment, we evaluate the potential role of biomarkers as surrogate markers of clinical outcomes. Finally, we examine the possibility that changes in levels of select protein biomarkers can provide mechanistic insight into the common origins and unique individual susceptibilities that lead to the development of smoking-related parenchymal lung diseases. This discussion is framed by a consideration of the properties of ideal biomarkers for different clinical and research purposes and the best uses for those biomarkers that have already been proposed and investigated.

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Abbreviations: BODE = BMI, airflow obstruction, dyspnea, and exercise capacity; CCL-18 = CC-chemokine ligand-18; CRP = C-reactive protein; IPF = idiopathic pulmonary fibrosis; KL-6 = Krebs von den Lungen-6; MMP = matrix metalloproteinase; SP = surfactant protein; TNF- α = tumor necrosis factor- α

A biologic marker, or biomarker, has been defined by the National Institutes of Health Biomarkers Definitions Working Group¹ as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” Although there is controversy surrounding what is considered a biomarker as opposed to a physiologic test or an outcome, commonly used lung biomarkers in

clinical and research settings have included physiologic variables (lung function, exercise capacity), patient-reported outcomes and characteristics (dyspnea, health-related quality of life, BMI), exacerbation frequency and severity, and radiologic parameters (quantitative texture analysis,^{2,3} sequential visual assessment score⁴) (Table 1). Protein biomarkers have achieved prominence in the pulmonary literature because of their potential role in the classification, prognosis, and treatment response in several lung diseases. Protein biomarkers can be measured in a variety of compartments,

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Table 1—Different Types of Biomarkers

Type of Biomarker	Examples
Physiologic variable	Lung function Exercise capacity
Patient-reported outcome	Dyspnea Health-related quality of life
Patient characteristic	BMI
Exacerbation	Frequency and severity
Radiologic parameter	Quantitative texture analysis Sequential visual assessment score
Proteins	Exhaled breath condensate and volatile gases Sputum Bronchial biopsies BAL fluid Urine Peripheral blood

including exhaled breath condensates and volatile gases, sputum, bronchial biopsies, BAL fluid, urine, and peripheral blood (Table 1).^{5,6} In this perspective, we focus our discussion on peripheral blood protein biomarkers because they appear to be more sensitive, better standardized, and more reproducible. Additionally, they have the practical advantages of being minimally invasive, cost effective, readily available in clinical settings, and easily measured during exacerbations.⁵⁻⁸ Finally, peripheral blood biomarkers have been shown to correlate with mortality and other important clinical outcome measures (Table 2).⁹⁻¹¹ Of note, although there is emerging research exploring other innovative peripheral blood-based biomarkers, such as changes in cell populations (fibrocytes,¹² T-cell subtypes,¹³ monocytes), transcriptional changes (genes, microRNAs, noncoding RNA), autoantibodies, markers of oxidative stress, proteomics, and metabolomics, these areas are beyond the scope of this review. Throughout the rest of this perspective, we use the term “biomarker” to refer exclusively to a peripheral blood protein biomarker.

POTENTIAL BIOMARKER APPLICATIONS

Biomarkers have the potential to play an important role in the assessment of common smoking-related parenchymal lung diseases such as COPD and idiopathic pulmonary fibrosis (IPF). However, identifying the ideal biomarker for different clinical and research purposes requires a careful assessment of the best clinical setting in which to implement the assay and an understanding of the limitations of biomarkers in general (Table 3). To gain insight into the potential future uses and existing limitations of biomarkers in common chronic lung diseases, it is helpful to carefully examine the successful implementation of biomarkers in other fields of medicine. For instance, in cancer and

cardiovascular disease, biomarkers have been used to noninvasively establish diagnoses, guide management, stratify risk (often in a model that includes established risk factors), predict prognosis and mortality, direct treatment strategies, and provide insight into the cause and underlying disease pathobiology.¹⁴⁻¹⁶ Importantly, in multiple clinical research trials, biomarkers are now being used as substitutes for clinical end points, commonly called surrogate end points. According to the National Institutes of Health Biomarkers Definitions Working Group,¹ a surrogate end point “is expected to predict clinical benefit (lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.” Substituting biomarkers for clinical end points can potentially enable investigators to design trials of shorter duration with smaller numbers of subjects enrolled.^{1,17} We anticipate that in the near future, biomarkers may help us identify individuals at risk of developing common smoking-related parenchymal lung diseases such as COPD or IPF. Further, biomarkers could improve our ability to monitor disease progression and exacerbations, predict mortality when added to established risk factors, serve as clinical outcome measures in research trials, and, in some cases, provide insight into disease mechanisms.

BIOMARKER APPROACHES AND LIMITATIONS

When critically evaluating the rapidly expanding literature examining lung biomarkers and their potential future uses, it is important to keep in mind the properties of an ideal biomarker. A biomarker should be related to the pathobiology of the disease and be an indicator of disease progression with a longitudinal relationship to a health outcome of interest. It should be reproducible, free from bias and other threats to validity, and sensitive to effective interventions.¹⁷ Common approaches to biomarker discovery include (1) hypothesis-driven assessment of candidate

Table 2—Advantages of Peripheral Blood Protein Biomarkers Over Other Compartments

Advantage
Technical
More sensitive
Better standardized
More reproducible
Other
Minimally invasive
Cost effective
Readily available in clinical settings
Easily measured during an exacerbation
Correlated with mortality and other clinical parameters
Studied longitudinally

Table 3—Potential Future Uses and Existing Limitations of Pulmonary Biomarkers

Potential Future Uses	Existing Limitations
Identify individuals predisposed to COPD vs IPF	Threats to validity:
Establish diagnosis	Chance
Stratify risk	Generalizability
Predict prognosis and mortality	Bias
Correlate with disease progression and exacerbations	Reproducibility
Guide management	Overfitting
Direct treatment strategies	Confounding
Provide insight into the cause and underlying pathobiology of diseases	Analytical issues
Act as surrogate end points	Concerns regarding processing/storage
	False-positive findings due to multiple hypothesis testing
	Ability to establish incremental usefulness beyond existing markers and other clinical tools
	Use of cross-sectional vs longitudinal observations
	Reflective, not predictive, of disease process
	Paucity of replication cohorts
	Lack of a clearly established relationship with:
	Disease development
	Disease severity
	Disease progression
	Response to therapy
	Health outcomes of interest:
	Mortality
	Exacerbations
	Changes in lung function
	Health-care resource use

IPF = idiopathic pulmonary fibrosis.

genes or proteins one-by-one; (2) discovery-based studies that use high-throughput techniques to screen large portions of the genome or proteome, such as RNA expression microarrays or mass spectroscopy of an entire proteome of serum^{7,18}; and (3) pattern-based exploration using expression signatures or algorithm-interpreted proteomic patterns while not needing to understand precisely which genes or proteins account for the pattern.^{16,18}

Although significant progress has occurred in the field of biomarkers, important limitations must be considered. General limitations of biomarker research include threats to validity, including chance, generalizability, bias, reproducibility, overfitting, confounding,^{7,14} analytical issues,¹⁹ concerns regarding processing/storage, and false-positive findings due to multiple hypothesis testing, as well as failure to establish incremental utility beyond existing markers¹⁵ and other clinical tools.^{16,18} Common limitations in pulmonary biomarker literature include small studies, the lack of replication cohorts, and the paucity of

longitudinal studies assessing the change of candidate protein levels over time. This last shortcoming currently limits our ability to determine if a candidate biomarker is truly associated with clinical end points such as disease severity, progression, and response to therapy, or health outcomes of interest, including mortality, exacerbations, changes in lung function (ie, FEV₁), and health-care resource use (Table 3).^{1,5-7} Therefore, although a biomarker may correlate with the presence of a lung disease, it may not correlate with clinical outcomes, which becomes of critical importance when biomarkers are used as surrogate end points for the development of pharmacologic therapy and other interventions.^{1,7,17}

BIOMARKERS OF COPD AND IPF

COPD and IPF share cigarette smoking as a common dominant risk factor for their development. Although emphysema and pulmonary fibrosis can coexist in the same individual,²⁰ recent evidence suggests that these patterns of disease may be distinct consequences of smoking that reflect unique individual susceptibilities.²¹ We have shown that interstitial lung abnormalities are inversely associated with total lung capacity and the severity of radiographic emphysema in smokers, suggesting that smoking may lead to two distinct patterns of injury.²¹ Alternatively, there is evidence that some heavy smokers can develop a combined emphysema and pulmonary fibrosis overlap syndrome. These individuals are often men with severe dyspnea on exertion, relatively preserved lung volumes, and marked reductions in diffusing capacity.^{20,22} Patients with combined COPD and IPF have a high prevalence of pulmonary arterial hypertension and lung cancer and are at a greater risk of death than are those with IPF alone.²³

It is theorized that lung-based protein biomarkers can be investigated in peripheral blood because changes in markers in the blood may reflect lung biology. For example, lung inflammation and injury may cause the biomarkers to leak into the systemic circulation or may cause changes in extrapulmonary production and/or clearance from the circulation.⁸ In this section, we examine the possibility that biomarkers could have a role in predicting which individuals will develop COPD, IPF, or an overlap syndrome. It is also possible that changes in levels of select proteins in the peripheral blood compartment can provide mechanistic insight into the pathogenic mechanisms by which smokers develop emphysema and/or lung fibrosis. Finally, we summarize the current body of literature evaluating the potential ability of biomarkers to correlate with disease progression, predict mortality, and identify exacerbations.

It is well recognized that COPD is not only a respiratory inflammatory condition, but also one marked by low-grade, chronic, systemic inflammation with extrapulmonary manifestations, including reduced BMI, skeletal muscle dysfunction, cardiovascular disease, and osteoporosis.²⁴ As such, biomarker discovery has centered on both extrapulmonary biomarkers of systemic inflammation and pulmonary candidate molecules that are derived from respiratory tract inflammation and lung destruction/repair.²⁵ The concept that COPD is a systemic disease has led to the postulation that biomarkers of common inflammatory pathways may be useful in the assessment of COPD.

C-Reactive Protein: C-reactive protein (CRP), an acute-phase protein linked to the total systemic burden of inflammation, was initially promising because it was shown to be increased in COPD, to correlate with variables predictive of outcomes, and to be reduced in patients with COPD using inhaled corticosteroids. However, there is lack of evidence of a causal association or direct correlation with survival.²⁶⁻³¹ As such, it has been suggested that elevated levels of CRP may not reflect mortality from COPD, but rather vascular events,³² or it may be secondary to increases in other proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), IL-6, IL-8, or fibrinogen.^{10,17,26,33}

Surfactant Protein-D: Surfactant protein (SP)-D is thought to play an important role in the pathogenesis of COPD, including oxidant production, inflammatory responses in alveolar macrophages, and apoptotic cell clearance. As such, it has been shown that serum SP-D levels were higher in individuals with COPD and tracked well with changes in health status.^{10,34,35} However, SP-D has also been linked to changes in BMI and atherosclerosis, raising the question as to whether it is disease specific or related to systemic manifestations of COPD.³⁶ Foreman and colleagues³⁶ demonstrated that certain genetic variants of SP-D are associated with changes in serum concentrations of SP-D and lung function, suggesting that SP-D is involved in the pathogenesis of COPD. In addition, Sin and colleagues³⁷ showed that inhaled corticosteroids with long-acting β agonists reduced lung-specific SP-D levels, but not the more generalized biomarkers of systemic inflammation (CRP and IL-6).

Interleukins, Fibrinogen, TNF- α , and Clara Cell Protein-16: Vestbo and colleagues⁹ recently demonstrated that none of the biomarkers believed to reflect

systemic inflammation, including CRP, SP-D, fibrinogen, IL-6, IL-8, and TNF- α , was associated to changes in FEV₁ over time. However, this study did reveal that Clara cell protein-16 levels were significantly associated with the rate of change of FEV₁, suggesting a role as a biomarker of COPD. The authors caution that this association was weak and has yet to be validated as biologically meaningful.

CC-Chemokine Ligand-18 (or Pulmonary and Activation-Regulated Chemokine): Another promising biomarker of COPD is CC-chemokine ligand-18 (CCL-18), a lung-predominant chemokine that is associated with reduced FEV₁ and BMI, airflow obstruction, dyspnea, and exercise capacity (BODE) index, and it indicates poor prognosis.^{10,11,38} CCL-18 is also associated with clinical outcomes, including cardiovascular morbidity, overall mortality and response to treatment.^{38,39} However, it is unclear what role this chemokine may play in disease pathogenesis.³⁸

Biomarker Panels: In addition to the hypothesis-driven research detailed in the previous sections, the use of newer high-throughput techniques has led to the discovery of a number of candidate biomarkers that are under investigation. Bon and colleagues⁴⁰ used a multiplex protein array to identify significant associations between several serum inflammatory proteins and quantitative radiographic measurements of FEV₁ % (including matrix metalloproteinase [MMP]-1 and MMP-7), airway thickening (including IL-6 and CRP), and parenchymal emphysema (IL-6, TNF- α , and MMP-7). We showed that a panel of 24 markers of inflammation and tissue destruction/repair was significantly related to lung function, exercise capacity, the BODE index, and exacerbation frequency. It is important to note that because of the cross-sectional nature of this study, the proteomic profile was not shown to be related to mortality.¹¹ We also showed in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study that a combination of candidate biomarkers can improve the accuracy of predicting the risk of death in patients with COPD when added to clinical variables, such as age, FEV₁, BODE index, and hospitalizations, known to predict mortality in COPD.¹⁰ The addition of fibrinogen, CCL-18, SP-D, CRP, Clara cell protein-16, IL-6, IL-8, and TNF- α to clinical variables significantly improved the predictive model for mortality, with IL-6 having the greatest impact.¹⁰ Given the complex and heterogeneous nature of COPD, it is unlikely that a single biomarker will be sufficient to predict outcomes. We believe that future research should focus on continued development of a panel of biomarkers that improves the assessment of COPD when added to established clinical metrics.

Biomarkers of IPF

IPF is a chronic, progressive, fibrotic lung disease of unknown cause with a variable and unpredictable course and a poor prognosis. Clinical and physiologic parameters have had limited success as predictors of disease progression or mortality in individuals with IPF, leading some investigators to explore the potential uses of biomarkers. Although some biomarkers have been shown to correlate with disease progression and survival, the use of biomarkers in IPF remains experimental.^{41,42}

Krebs von den Lungen-6 (or Mucin 1): Levels of Krebs von den Lungen-6 (KL-6), a high-molecular-weight glycoprotein, are elevated in a variety of interstitial lung diseases and have potential as markers of disease activity in patients with pulmonary fibrosis.⁴³ Yokoyama and colleagues⁴⁴ showed that initial KL-6 levels can predict IPF prognosis, specifically survival over 3 years.

SP-A and SP-D: SP-A and SP-D, members of the collectin family, which are secreted by alveolar type 2 pneumocytes, appear to increase in the blood following disruption of the alveolar-capillary barrier. They have also been shown to have prognostic value for mortality in IPF; concentrations of SP-A may indicate a more destructive phenotype, and concentrations of SP-D appear to relate to the extent of deterioration in pulmonary function per year and the degree of radiographic abnormality.⁴⁵⁻⁴⁸ Kinder and colleagues⁴⁶ demonstrated that the addition of SP-A and SP-D to an IPF clinical prediction model improved performance in the prediction of 1-year mortality.

Matrix Metalloproteinases: MMPs are expressed in multiple lung cell types including the alveolar epithelium; they are important for extracellular remodeling and their expression is considered an essential component of lung injury and repair, which is why metalloproteases are thought to play an important role in the pathogenesis of pulmonary fibrosis.^{49,50} Levels of MMP-1, MMP-2, MMP-7, and MMP-9 are increased in IPF. In one of the first studies to look for a pattern-based biomarker signature in IPF, we found significantly higher concentrations of MMP-1 and MMP-7 in IPF. These metalloproteases were two main components of a five-protein signature that distinguished IPF from controls (MMP-1, MMP-7, MMP-8, insulin-like growth factor binding protein-1, and tumor necrosis factor receptor superfamily, member 1A).⁵¹ This study also demonstrated that MMP-1 and MMP-7 levels distinguished IPF from other interstitial lung diseases such as sarcoidosis and hypersensitivity pneumonitis, as well as from COPD. MMP-7 levels were elevated in subclinical interstitial lung disease⁵² and

negatively correlated with FVC % and carbon monoxide diffusing capacity %.⁵¹ In families affected by pulmonary fibrosis, MMP-7 concentrations were significantly increased and were correlated with disease severity.⁵¹ Importantly, a subsequent study by Richards and colleagues⁵³ showed that MMP-7 concentrations also predicted disease progression and mortality in patients with IPF, as did intracellular adhesion molecule-1, IL-8, vascular cell adhesion molecule-1, and S100A12 levels. This study also demonstrated that an MMP-7 clinical prediction rule (the combined clinical and molecular outcome index) predicts mortality better than do clinical rules alone. These data suggest that MMPs may have a role as diagnostic biomarkers and as markers of disease severity and functional impairment, in addition to their potential to predict disease progression and survival.

Other Biomarkers Related to IPF: An additional biomarker of interest that is elevated in IPF is serum CCL-18, which has been shown to have a predictive value in the change in total lung capacity and FVC at 6 months, as well as in IPF survival.⁵⁴ Connective tissue growth factor, which was correlated negatively with 6-month change of FVC,⁵⁵ and YKL-40, which was significantly correlated positively with serum KL-6 and negatively with carbon monoxide diffusing capacity and PaO₂,⁵⁶ have also been investigated. Although all this research is promising, additional investigation, particularly into the development of a panel of biomarkers that have additive value to established clinical parameters, will be necessary to establish which biomarkers will be useful in clinical and research settings.

Overlapping Biomarkers of IPF and COPD

To the best of our knowledge, a direct comparison between biomarkers of COPD and IPF has not been published before. But, through highlighting some of the recent advances in the COPD and IPF biomarker literature, it is evident that such a comparison emphasizes that some biomarkers are distinct, indicating pathobiologic mechanisms unique to COPD or IPF, whereas a number of biomarkers are common to both diseases (Fig 1). Overlapping biomarkers could highlight similarities in disease pathogenesis or, in some cases, could be a feature of combined COPD and IPF. Alternatively, the observed biomarker signature overlap could be driven in part by shared risk factors such as cigarette smoke exposure and, thus, could provide insight into common disease pathways induced by smoke-related lung injury. For instance, some of the biomarkers that overlap (IL-6, IL-8, and SP-D) are markers of inflammation, suggesting that injury induced by cigarette smoke may trigger an inflammatory process that contributes to the pathogenesis

FIGURE 1. Specific and overlapping biomarkers of IPF and COPD. CC-16 = Clara cell protein 16^{9,10}; CCL18 (PARC) = CC-chemokine ligand-18/pulmonary and activation-regulated chemokine⁵⁴; CCN2 = connective tissue growth factor, CTGF^{10,38,55}; CRP = C-reactive protein^{10,26-30}; CXCL10 = CXC chemokine ligand-10⁵⁷; ICAM-1 = intracellular adhesion molecule-1⁵³; IGFBP1 = insulin-like growth factor binding protein-1⁵¹; IL = interleukin^{10,26,33,53,58}; IPF = idiopathic pulmonary fibrosis; KL-6 (MUC1) = Krebs von den Lungen-6 (Mucin 1)^{43,44,58-60}; MMP = matrix metalloproteinase^{11,40,49-51,53}; MPIF-1 = myeloid progenitor inhibitory factor-1⁶¹; S100A12⁵³; SP = surfactant protein^{10,34-37,46,48,62,58-60}; TNF- α = tumor necrosis factor- α ^{10,26}; TNFRSF1A = tumor necrosis factor receptor superfamily, member 1A⁵¹; VCAM-1 = vascular cell adhesion molecule-1⁵³; YKL-40.⁵⁶

of COPD and IPF.⁶³⁻⁶⁵ Other biomarkers associated with lung destruction and remodeling (MMPs) highlight the importance of extracellular matrix remodeling in both diseases. Unfortunately, although the overlap between the biomarkers of COPD and IPF may provide mechanistic insight into the common origins that lead to the development of smoking-related parenchymal lung diseases, it can also be a significant limitation when attempting to identify a unique biomarker signature that correlates with a specific disease. Although this limitation may be overcome through the integration of biomarkers with other clinical parameters, the specificity of any biomarker signature developed for COPD or IPF should be assessed subsequently in other smoking-related lung diseases. As such, it is critical that studies directly compare COPD and IPF, using such publically available data sets as the Lung Genomics Research Consortium, a resource that contains detailed transcriptomic, genomic, and epigenetic data on well-phenotyped patients with COPD or IPF, as well as smoking and nonsmoking control subjects.⁶⁶

Biomarkers of COPD and IPF Exacerbations

Some of the biomarkers discussed in the previous sections may also play a role in the management of, clinical decision making for, and prediction of outcomes in COPD and IPF exacerbations. Assessment of COPD exacerbations relies mostly on clinical

presentation, which makes them variable and difficult to predict.⁶⁷ The most promising biomarker of COPD exacerbations is CRP, which increases at the onset of the exacerbation and subsides during its course.⁶⁷ CRP is also useful in confirming the diagnosis of COPD exacerbation when combined with the presence of at least one major exacerbation symptom.⁶¹ Other potential biomarkers include SP-D, whose levels are increased during exacerbations,^{35,62} and CXC chemokine ligand-10, which helps differentiate between bacterial-, viral-, and eosinophilic-associated exacerbations.⁵⁷ Similarly, MMP-9,¹¹ IL-8, TNF- α , IL-6, and myeloid progenitor inhibitory factor-1 may have a potential role in identifying COPD exacerbations.⁶¹ A limited number of biomarker studies have been conducted in patients with IPF exacerbations, in contrast to COPD exacerbations. Collard and colleagues⁵⁸ found significant elevations in KL-6, SP-D, and IL-6 in patients with acute IPF exacerbations when compared with patients with IPF and stable disease, suggesting that type 2 alveolar epithelial cell injury and/or proliferation is an important component of an acute exacerbation.⁵⁸ Additional research into biomarkers associated with COPD and IPF exacerbations will likely lead to further insight into the pathobiology of an exacerbation and improved treatments for these exacerbations.

FUTURE OF BIOMARKERS IN SMOKING-RELATED PARENCHYMAL LUNG DISEASES

Although there are currently no established clinical applications for biomarkers of the smoking-related parenchymal lung diseases, it is evident from the research detailed here that our research community can be expected to develop a panel of biomarkers that can be used clinically to correlate with disease progression, identify exacerbations, and predict mortality. In this context, biomarkers are unlikely to substitute for clinical parameters, but rather will improve diagnostic accuracy through their incorporation into integrated clinical, molecular, and genetic predictive models. We anticipate that a single biomarker or biomarker panel could serve as a surrogate end point for functional outcome measures in clinical research trials. Using a biomarker or biomarker panel that more effectively measures disease outcomes when compared with a conventional clinical end point such as lung function will allow for the design of clinical trials of shorter duration with smaller study cohorts. Biomarkers may eventually help us identify high-risk individuals (ie, smokers) likely to develop COPD or IPF or the combined COPD or IPF syndrome. Additionally, biomarkers are likely to play a prominent role in the diagnosis of COPD and IPF, enabling us

to identify clinical phenotypes such as rapid decliners and frequent exacerbators. Finally, we anticipate that biomarkers will be a part of a more stratified and personalized approach to the classification, prognostication, and treatment of the smoking-related parenchymal lung diseases.

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