Novel Outcomes and End Points

Biomarkers in Chronic Obstructive Pulmonary Disease Clinical Trials

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Biomarker development in chronic obstructive pulmonary disease (COPD) is a nascent field, in part because of the complexity underlying COPD pathogenesis. The objective of this review is to provide examples of how biomarkers may be effectively applied in clinical trials of COPD by limiting their use to specific contexts and using them to answer well delineated questions. Types of novel outcomes or "biomarkers" that may be useful in clinical trials in COPD include analyses performed on bronchoscopically obtained samples, sputum, exhaled gases, blood, and urine and "ex vivo" assays performed using biological samples obtained from trial participants. These novel biological outcomes are rarely useful as primary end points in phase III clinical trials in COPD, because they are not typically recognized by the U.S. Food and Drug Administration or other regulatory agencies. More commonly, the applications of these outcomes include "proof-of-concept" decisions, demonstration that the intervention had the intended pharmacologic or biological effect, identification of patient subgroups that benefit most, and safety monitoring. Examples given in this review include outcomes used in a phase IIA study of an inhaled small molecule inhibitor of epidermal growth factor receptor. Large observational studies of COPD, including the ECLIPSE, COPDGene, and SPIROMICS studies will further inform our use of biomarkers in COPD clinical trials. To encourage the application of novel biomarkers in clinical trials, the Food and Drug Administration has developed a new process for biomarker "qualification." This process has been designed to be more efficient and to promote consensus building and sharing of preclinical data.

Keywords: biomarker; chronic obstructive pulmonary disease; clinical trials

The National Institutes of Health defines a biomarker 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" (1). More recently, Sin and Vestbo proposed a refined definition of biomarkers specifically for chronic obstructive pulmonary disease (COPD) (2), which was intended to incorporate two rigorous criteria for biomarker validation that were promulgated by Bucher and colleagues (3): (1) is there a strong, independent, consistent association between the surrogate end point and the clinical end point? and (2) is there evidence from randomized controlled trials that improvement in the surrogate end point with a drug consistently leads to improvement in the target (clinical) outcome? These proposed criteria represent a very formal and rigorous approach to the development of biomarker criteria, which is laudable from the perspective of understand-

ing molecular and cellular mechanisms of disease. However, from the perspective of clinical trials and drug development, biomarkers that fall short of these formal criteria may still be valuable in specific settings. Indeed, the requirement that changes in a biomarker *result in* changes in important clinical outcomes may not be possible in human studies unless the biomarker is also the specific target of the intervention. One goal of this review is to present examples of novel biomarkers that have been valuable in the clinical trial setting. In most instances, these biomarkers do not fulfill these rigorous criteria. However, the information that they provide can still be quite valuable.

TYPES OF COPD BIOMARKERS

A nonexhaustive list of biomarkers that may be useful in COPD clinical trials would include analytes that directly reflect cellular and molecular events occurring in the lung (e.g., from exhaled gases, bronchoscopic samples, and sputum) and those which reflect either the peripheral signature of lung pathology or secondary effects of lung disease (e.g., measured in the blood and urine) (Table 1). In addition, this review considers assays performed *ex vivo* using patient samples that were collected during the clinical trial. Clearly, samples obtained from the lung will have the greatest sensitivity for events occurring within the lung, but their sensitivity will be inversely proportional to the difficulty and expense required for their measurement. Apart from exhaled gases, these measures will not ultimately be valuable in the clinic. However, their use in the research setting may provide critically important information that is not otherwise obtainable. Furthermore, they may be used in the research setting to establish "gold standards" for the subsequent development of less invasive biomarkers. Samples that are obtained bronchoscopically include bronchial biopsies (which sample the mucosal and submucosal compartments of the airway [4]), epithelial brushings (which yield a mixture of epithelial cells as well as inflammatory cells that have migrated to the airway [5]), and bronchoalveolar lavage, which typically captures soluble mediators that reflect the distal lung. Alternatively, bronchial wash can be performed, using a smaller volume in the first aliquot of a lavage and processing it separately, to preferentially assess the more proximal airways (6).

An alternative scheme for the classification of biomarkers useful in COPD would be to classify them based on the aspect of COPD physiology that they reflect. The molecular and cellular mechanisms of COPD are thought to begin with an inflammatory response to an inhaled exposure. This inflammatory response involves the innate immune system (neutrophils and/or macrophages) during the initial exposure, as well as adaptive responses later in the disease (7,8). Other processes, such as oxidative stress (9), tissue injury with abnormal repair (10), apoptosis (11), and extracellular matrix destruction, may also contribute. These processes are modified by genetic factors and epigenetic factors, such as airway infection (12). The subsequent chronic inflammatory responses lead to mucus hypersecretion (13), airway remodeling (14), and alveolar destruction. Based on this complex

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TABLE 1. TYPES OF BIOMARKERS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Sample types for biomarker measurement

Blood
 Urine
 Sputum
 Exhaled gases
 "Ex vivo" assays
 Bronchoscopic samples
 Biopsies
 Epithelial brushings
 Bronchoalveolar lavage and wash

Examples of biomarkers by pathophysiological process

Inflammation
 CRP, IL-6, fibrinogen
 Oxidative stress
 8-isoprostane
 Tissue injury
 Surfactant protein D
 Matrix destruction
 Desmosine
 Infection
 Procalcitonin

Definition of abbreviation: CRP = C-reactive protein.

pathophysiology underlying COPD, a broad range of biomarkers might be useful in COPD clinical trials, depending on which aspect of COPD pathophysiology one wished to investigate (Table 1). For example, biomarkers of inflammation in COPD include serum C-reactive protein (15–17), IL-6 (18), and fibrinogen (19, 20). Biomarkers of matrix destruction include urinary desmosine (21), and biomarkers of infection include procalcitonin (22). However, this review is not intended to be a comprehensive review of these cellular and molecular pathways that contribute to COPD, or of the biomarkers of those pathways. Rather, this review argues that the complexity of the pathophysiology underlying COPD renders the development of universally useful biomarkers difficult in COPD. There is no biomarker in COPD that serves the same role as the troponin assay in the assessment of acute coronary syndromes. Nor is it likely such a universal biomarker will be developed, because so many different factors contribute to COPD. The main objective of this review is to demonstrate that we can nonetheless make very practical use of biomarkers to ask very specific and context-dependent questions that support the interpretation of clinical trials in COPD.

POTENTIAL APPLICATIONS OF BIOMARKERS IN CLINICAL TRIALS

Biomarkers have not historically been useful as primary end points in phase III clinical trials in COPD, because they are not recognized for that purpose by the U.S. Food and Drug Administration (FDA) or other regulatory agencies. Whether a biologically relevant and validated blood biomarker could be developed in COPD is very uncertain. COPD (when defined in terms of airway obstruction) ultimately results from complex physiological interactions in which tissue destruction and remodeling have the greatest impact on patient symptoms, which are less likely to yield a blood biomarker than inflammatory, oxidative, or metabolic processes. More commonly, the applications of biomarkers in COPD include: (1) "proof of concept" for early stage trials; (2) demonstration that the intervention had the intended pharmacologic or biological effect; (3) identification of patient subgroups that benefit most; and (4) safety monitoring.

BIOMARKERS FOR PROOF OF CONCEPT FOR EARLY STAGE TRIALS

Biomarkers may be particularly helpful in phase II studies, especially those that are evaluating drugs with new mechanisms of action. One example is a clinical trial we recently undertook of an epidermal growth factor receptor (EGFR) inhibitor, BIBW 2,948 BS (23). The rationale for this study is that the molecular and cellular pathways that underlie increased mucin stores in the airway epithelium in COPD are thought to include activation of the EGFR pathway (24, 25). BIBW 2,948 BS is inhaled via a dry powder inhaler, and is rapidly hydrolyzed by plasma esterases to an inactive zwitterion form to minimize systemic effects. To prove the concept that EGFR pathway blockade could inhibit mucin production in COPD, we studied epithelial mucin stores in current smokers with COPD and symptoms of chronic bronchitis using bronchoscopic techniques in a 4-week randomized placebo-controlled trial. Subjects received either BIBW 2,948 BS at one of two doses (15 or 30 mg twice daily) or corresponding placebo. The changes in mucin stores that we observed were not significantly different in the pooled BIBW 2,948 BS group versus placebo (Figure 1). However, the reduction in epithelial mucin stores was numerically greater in the group that received 30 mg twice daily. These data suggest that the higher dose preparation might indeed achieve reduction in mucin stores if it can be delivered effectively and safely. But the data did not prove this conclusively. Therefore, the next important question to ask was whether BIBW 2,948 BS had the intended biological effect (blocked activation of EGFR in airway epithelial cells) at the doses administered.

BIOMARKERS TO DEMONSTRATE THE INTENDED BIOLOGICAL EFFECT

To document effective EGFR blockade by BIBW 2,948 BS, we measured the effect of BIBW 2,948 BS on EGFR phosphorylation and internalization in epithelial brushings in a subset of patients using an *ex vivo* EGFR internalization assay. Because EGF binding leads to EGFR dimerization, transphosphorylation, and rapid receptor internalization (26–29), this assay allowed us to monitor BIBW 2,948 BS-mediated EGFR inhibition through the measurement of EGF-induced receptor internalization. Inhaled BIBW 2,948 BS induced a dose-related

Figure 1. The effect of BIBW 2,948 BS on airway mucin stores. No significant difference was observed in the change from baseline in the volume of mucin per surface area of basal lamina ($V_s \mu_b \text{ ala}$) in the pooled placebo and pooled treatment groups (all $P > 0.05$).

Figure 2. BIBW 2,948 BS induced epidermal growth factor receptor (EGFR) inhibition after 4 weeks of treatment in airway epithelial cells obtained from study subjects and studied *ex vivo*. Samples from 24 patients treated with 30 mg/15 mg twice daily BIBW 2,948 BS or matching placebos were analyzed. "Perinuclear EGF spots per cell" at baseline was set to 100% for each patient. The % control (ctl) value, as indicated on the y axis, is calculated as the "perinuclear EGF spots per cell" at end of treatment, normalized to this baseline value. % ctl values shown are means ($\pm$ SEM) ($n = 4$; 30 microscopic images per experiment). The inset provides the means ($\pm$ SEM) of the four patient subgroups (i.e., treated with 30 mg/15 mg twice daily BIBW 2,948 BS or with matching placebos).

inhibition of EGFR internalization in epithelial cells from treated subjects (Figure 2). In addition, the degree of EGFR inhibition correlated with decreases in mucin stores in the group receiving 30 mg twice daily (Figures 3A–3C), suggesting that effective EGFR inhibition with higher doses does, indeed, lead to decreases in mucin stores. However, BIBW 2,948 BS was also associated with a dose-related increase in reversible adverse effects, including liver enzyme elevation ($n = 2$), and reduction in FEV₁.

In summary, the use of an *ex vivo* biomarker (EGFR internalization assay) suggested that the higher dose of BIBW 2,948 BS achieved the intended biological effect (i.e., EGFR blockade), but that the lower dose did not. In addition, we found that the doses at which therapeutic effects on mucin stores may occur overlap with the doses at which reversible adverse effects occur. Whether the adverse effects of BIBW 2,948 BS are a class effect associated with EGFR inhibition in the airway or are specific to BIBW 2,948 BS is not clear, but the application of this *ex vivo* biomarker allowed useful inferences in a study that was otherwise negative, and informed the decision as to whether to move forward with the development of this specific compound.

BIOMARKERS FOR PATIENT SELECTION

With respect to patient selection, there are excellent examples from other fields of biomarkers that identify drug responders. For example, CCR5 serves as such a biomarker in the context of maraviroc (30), which is approved for the treatment of acquired immune deficiency syndrome on the basis of its ability to block viral entry by CCR5-tropic, but not CXCR4-tropic, viruses. This example, however, reflects a very specific drug action coupled with a very specific pathogenic mechanism of disease, and such elegant simplicity is less common in complex diseases. Nonetheless, progress is being made in phenotyping and biomarker development in complex lung diseases as well. In asthma, for example, sputum analyses have led to an understanding of distinct cellular phenotypes of disease (31–33), and genomic analyses of

lung tissues have identified corresponding molecular phenotypes (34). Both these cellular and molecular phenotypes have therapeutic relevance, identifying responders and nonresponders to inhaled corticosteroid therapy, for example (35). On the basis of these studies, sputum cytology has been used in clinical studies to guide therapy (34, 36, 37) and to select patients likely to respond to novel biological therapies, such as mepolizumab (38, 39). Blood biomarkers that may reflect molecular phenotypes are also under investigation, guided by the genomic studies that identified those phenotypes (5, 34).

By analogy, there is ample evidence that patients with COPD are also heterogeneous (40, 41). However, our understanding of the molecular and cellular mechanisms underlying that heterogeneity is in its infancy (42). At least three large observational studies in COPD are designed to better understand heterogeneity in COPD and develop radiological, biological, and genetic markers of these phenotypes (the ECLIPSE, COPDgene, and SPIROMICS studies). The ECLIPSE study has been completed, and the initial publications are now forthcoming (43–45), yielding information on the value of sputum neutrophils (46) and serum levels of CC-16 (47), surfactant protein D (48), and PARC/CC-16 (48) as biomarkers in COPD. The COPDgene study has nearly completed enrollment, and preliminary data on biomarkers should be presented soon (49). The SPIROMICS study has just begun enrollment, and has the advantage that it will obtain bronchoscopic samples (in a subset of 300 subjects) for comparison with blood or other non-invasively obtained samples in the overall study population ($n = 3,200$). The rationale for bronchoscopic sampling is that sampling of the lung will provide a better "gold standard" for molecular and cellular analyses relevant to the disease phenotyping, and will facilitate the development of blood biomarkers.

BIOMARKERS FOR SAFETY MONITORING

Another emerging role for biomarkers in clinical trials is in preclinical and clinical development. Although safety biomarkers have yet to become prominent in COPD-related clinical de-

Figure 3. Correlation between reduction in EGFR internalization and reduction in mucin stores (A) in pooled BIBW 2,948-treated groups, (B) in the group treated with BIBW 2,948 at 15 mg twice daily, and (C) in the group treated with BIBW 2,948 at 30 mg twice daily ($r = 0.98$; 95% confidence interval, 0.71–0.99). Inhibition of EGFR internalization was associated with decreases in airway mucin stores in the 30-mg subgroup. b.i.d. = twice daily.

velopment, industry, academia, and regulatory agencies are collaborating to develop and qualify safety biomarkers for general use. One example is the Nephrotoxicity Working Group (50, 51) of the Predictive Safety Testing Consortium, which was created as part of the FDA's Critical Path Initiative. Other Predictive Safety Testing Consortium groups are currently involved in qualifying biomarkers to detect hepatotoxicity, vascular injury, nongenotoxic carcinogenicity, and myopathy (52). Safety biomarkers might be valuable in lung diseases in at least two ways: (1) to identify toxicity to other organs, such as the kidney early in drug development; and (2) to identify lung toxicity either during drug development or in postmarketing studies. However, the specific relevance of safety biomarkers to clinical trials in COPD at this time is unclear.

BIOMARKER QUALIFICATION

Concerned that the development and approval of new drugs has lagged behind advances in molecular biology and genetics, the FDA has sought strategies to speed drug development. One strategy has been to develop a "biomarker qualification" process that is intended to speed development of exploratory biomarkers and their acceptance in drug development and

regulatory review (53, 54). The term "qualified" indicates that the presence of the biomarker has been statistically shown to reflect a biological change and accepted by the appropriate regulatory agency for use in a specific context (55). The initial target of this program has been the qualification of biomarkers that will be generally useful in drug development, especially safety biomarkers that reflect various aspects of nephrotoxicity (51). The hope is that wide acceptance of these biomarkers will speed preclinical development of candidate drugs. Qualification for a specific context of use requires two stages (55): (1) consultation that begins with a letter summarizing the biomarker, its context of use, and the data available to support this context of use; and (2) submission of the full data package for review by the Biomarker Qualification Review Team, which is recruited from throughout the Center for Drug Evaluation and Research (CDER) and other FDA centers, as needed. Completion of this process enables the use of such biomarkers for regulatory review of drug submissions. This process should indeed speed use of safety biomarkers in preclinical development; however, whether it will materially influence the other applications of biomarkers described here or facilitate the use of biomarkers as primary outcomes in clinical trials is uncertain.

CONCLUSIONS

One important factor that will ultimately limit the use of a given biomarker as a primary endpoint in clinical trials is that regulatory approval and labeling of that drug will likely be limited to its ability to influence the biomarker rather than the disease itself. Ultimately, the value of such an indication depends on both the biological evidence that the biomarker is tightly linked to disease (using rigorous criteria, such as those outlined by Sin and Vestbo [2]) and public awareness that such a link is important. Such public awareness has been achieved in some instances (e.g., lipid profiles in cardiovascular disease), but, in the realm of COPD, and lung diseases generally, we are far from making such convincing links between biomarkers and disease. Clearly, much more needs to be done to establish biomarkers that track with disease severity and progression in COPD, and large observational studies are contributing to this effort. In the meantime, biomarkers can still play specific roles in clinical trials in COPD, particularly with respect to well circumscribed applications, such as proof-of-concept studies, demonstration that the intervention had the intended pharmacologic or biological effect, and identification of patient subgroups that benefit most. For these applications, biomarkers need not fulfill all the rigorous criteria proposed for biomarkers generally. Rather, the biomarkers must be applied rationally, based on evidence, within a specific context of use.

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