CHEST

Biomarker Development in COPD

Moving From *P* Values to Products to Impact Patient Care

Zsuzsanna Hollander, PhD; Mari L. DeMarco, PhD; Mohsen Sadatsafavi, MD, PhD; Bruce M. McManus, MD, PhD; Raymond T. Ng, PhD; and Don D. Sin, MD

There is a great interest in developing biomarkers to enable precision medicine and improve health outcomes of patients with COPD. However, biomarker development is extremely challenging and expensive, and translation of research endeavors to date has been largely unsuccessful. In most cases, biomarkers fail because of poor replication of initial promising results in independent cohorts and/or inability to transfer the biomarker from a discovery platform to a clinical assay. Ultimately, new biomarker assays must address 5 questions for optimal clinical translation. They include the following: is the biomarker likely to be (1) superior (will the test outperform current standards?); (2) actionable (will the test change patient management?); (3) valuable (will the test improve patient outcomes?); (4) economical (will the implementation of the biomarker in the target population be cost-saving or cost-effective?); and (5) clinically deployable (is there a pathway for the biomarker and analytical technology to be implemented in a clinical laboratory?)? In this article we review some of the major barriers to biomarker development in COPD and provide possible solutions to overcome these limitations, enabling translation of promising biomarkers from discovery experiments to clinical implementation.

CHEST 2017; 151(2):455-467

KEY WORDS: biomarkers; COPD; personalized medicine

COPD is the third leading cause of death in the United States affecting > 25 million US adults ≥ 40 years of age and killing 150,000 every year.[1] Globally, there may be as many as 380 million people with the disease and accounting for 3 million deaths annually.[2,3] In 2010, COPD was responsible for $50 billion direct and indirect costs in the United States.[4] By 2020, the costs are expected to escalate to $90 billion per year, with direct costs consuming half of the total costs.[5] Smoking is the principal environmental

ABBREVIATIONS: ALK = anaplastic lymphoma kinase; CRP = C-reactive protein; EGFR = epidermal growth factor receptor; FDA = Food and Drug Administration; NSCLC = non-small cell lung cancer

AFFILIATIONS: From the Centre for Heart and Lung Innovation, James Hogg Research Centre (Drs Hollander, McManus, Ng, and Sin), St. Paul's Hospital, Vancouver, BC, Canada; the Institute for Heart + Lung Health (Drs Hollander, Sadatsafavi, McManus, Ng, and Sin), the Division of Respiratory Medicine, Department of Medicine (Dr Sin), the Department of Pathology and Laboratory Medicine (Drs DeMarco and McManus), and the Department of Computer Sciences (Dr Ng), University of British Columbia, Vancouver, BC, Canada; the PROOF Centre of Excellence (Drs Hollander, Ng, and McManus), Vancouver, BC, Canada; and the Centre for Clinical Epidemiology and Evaluation (Dr Sadatsafavi), Vancouver Coastal Health Research Institute, Vancouver, BC, Canada.

FUNDING/SUPPORT: This study was funded by Genome Canada: Genome British Columbia, Genome Quebec, the Canadian Institutes for Health Research (CIHR), the Networks of Centres of Excellence for Commercialization and Research, the St. Paul's Hospital Foundation, and the Canadian Respiratory Research Network, which is funded by the Institute of Circulatory and Respiratory Health Emerging Network of CIHR, the Canadian Lung Association, the Canadian Thoracic Society, and the Canadian Respiratory Health Professionals; the British Columbia Lung Association; with industry partner funding from AstraZeneca, Boehringer-Ingelheim (Canada) Ltd., GlaxoSmithKline Inc., and Novartis Pharmaceuticals Canada Inc.

CORRESPONDENCE TO: Don D. Sin, MD, St. Paul's Hospital, 1081 Burrard St, Vancouver, BC, Canada V6Z 1Y6; e-mail: Don.Sin@hli.ubc.ca

DOI: http://dx.doi.org/10.1016/j.chest.2016.09.012

driver of COPD; although there has been a substantial decline in the smoking rate in the United States and elsewhere, 44 million US adults still smoke on a daily basis (representing 19% of the adult population) and 50% are ex-smokers.[6] COPD mortality is currently increasing by 1.6% per year (3.7% per year in women and 0.4% per year in men),[1] driven largely by the aging population and more successful treatment of other major drivers of mortality, such as ischemic heart disease.[7,8]

Although there has been a substantial improvement in the understanding of COPD pathogenesis over the last 30 years, there are no disease modifiers (aside from smoking cessation and domiciliary oxygen therapy for those who are hypoxemic). Currently, COPD is largely treated symptomatically using bronchodilators to improve airflow, reduce dyspnea, and prevent exacerbations.[9] One major barrier to novel therapies is the heterogeneity of COPD pathogenesis. Although COPD is characterized by airflow limitation, the molecular processes that drive the airflow limitation are thought to be highly variable.[10] To capture this heterogeneity, there has been a focused effort on finding simple biomarkers of disease activity. Recognizing the need for new COPD therapeutics and the primal role of biomarkers in this process, the US Food and Drug Administration (FDA) has published a set of guidelines for biomarker development to accelerate drug discoveries.[11] This guidance has enabled some important progress to be made as evidenced by the recent success of the COPD Foundation Biomarker Qualification Consortium in qualifying plasma fibrinogen as a drug development tool for COPD.[12] Notwithstanding this success, biomarker discovery and implementation remain extremely challenging, with most biomarkers failing to make it beyond the discovery stage.[13] In this article we will focus primarily on blood biomarkers and outline the characteristics of an ideal biomarker in COPD that can be used clinically, summarize a number of common pitfalls in biomarker translation, and discuss ways of moving biomarker discoveries from great *P* values to tangible products that will impact patient care.

Characteristics of an Ideal Biomarker

There are numerous different definitions of biomarkers. In 2001, a working group, under the auspices of the US National Institutes of Health, recommended the use of a standardized definition of biomarkers. It defined a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[14] In its broadest context, physiologic parameters, such as FEV_1, and bioimaging features, such as low density area and emphysematous holes on CT scan, could qualify as biomarkers. In practice, however, most understand biomarkers as a test or series of tests that provide an objective and reproducible indication of a disease state.[13] More recently with the advent of precision or personalized medicine, a biomarker is perceived as an indicator to enable tailoring of treatment interventions for specific patients that will maximize therapeutic benefits and minimize the risk of treatment.[15] There are different types of biomarkers (Table 1),[16,17] and their use will be dictated by the clinical context. Notwithstanding, there are accepted ideal characteristics of biomarkers. For common diseases such as COPD, biomarkers must be safe, accurate, inexpensive, and easy to measure. Ideally, biomarkers should also be modifiable with effective therapy and whose results are reproducible across sex and age, and across different racial and ethnic backgrounds. Most importantly, ideal biomarkers should enable clinicians to better manage their patients with COPD. Typically, this means that the result of the biomarker test will guide clinicians to intervene with more effective therapies for those who need them and eliminate the use of ineffective (or even harmful) therapies for whom the interventions are not indicated. Therefore, the basic assumption (which needs to be proven for each clinically implemented biomarker) is that a biomarker-guided approach will lead to improved health outcomes (and experience) for patients compared with standard non-biomarker-guided approaches either at reduced costs (ideally) or slightly increased costs (in which the gained benefits of the biomarker results outweigh their costs). Biomarkers that do not modify disease management are generally not very useful clinically because they have no or little impact on patient-related health outcomes and are unlikely to be cost-effective. Figure 1 depicts a typical pipeline for biomarker development, and Table 2 summarizes some of the common pitfalls in clinical translation of biomarkers.

Clinical Traits for Which Biomarkers Are Desirable

Biomarkers for FEV_1 Decline

Recognizing the increasing burden of COPD in the United States and elsewhere, and the paucity of effective therapeutics to reduce its burden, in 2007, the FDA

TABLE 1] Definitions of Terms Commonly Used in Biomarker Development

Term	Definition
Area under the receiver operating characteristic curve	A measure of the ability of a test to accurately discriminate a result, indicating a particular disease state from a result not indicating that disease state.
Biomarker	A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to an intervention.
Diagnostic biomarker	Biomarker used to identify or determine the presence of absence of a disease or condition.
Exacerbation	An acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads in extreme cases to a state of breathless paralysis.
Expression quantitative trait loci	Genomic loci that contribute to variation in expression levels of messenger RNAs.
Overfitting	Occurs when the model fitting process unintentionally exploits characteristics of the data that are caused by noise, experimental artifacts, or other chance effects that are not shared between datasets, rather than to the underlying biology that is shared between datasets.
Phase I trial	A trial in a small number of patients in which the toxicity and dosing of an intervention are tested.
Phase II trial	A trial in which the safety and preliminary efficacy of an intervention are tested in patients.
Phase III trial	A large-scale trial in which the safety and efficacy of an intervention are tested in a large number of patients. The FDA requires these trials before a drug can be put on the market.
Precision medicine	Model for tailoring of medical treatment to the individual characteristics of each patient, taking into account variability in genes, environment, lifestyle, and so forth, specific to the individual.
Prognostic biomarker	A biomarker used to assess the likelihood of a clinical event, disease recurrence, or progression.
Predictive biomarker	A biomarker used to identify the individuals who will likely experience a positive or negative effect from a specific drug or exposure.
P value	Probability of observing these results given the null hypothesis of no difference.
Targeted discovery	Biomarker discovery from a list of preselected targets (eg, preselection because of availability of assays or a priori knowledge).
Untargeted approach	Analytical approach that is global in scope and outputs comprehensive data of biologic molecules (transcripts, proteins or metabolites).
Untargeted (unbiased) discovery	Biomarker discovery without an a priori hypothesis where molecules are measured using an untargeted approach.

Detailed explanations of definitions are available elsewhere.[16,17] FDA = Food and Drug Administration.

created the white pages entitled "Guidance for Industry: Chronic Obstructive Pulmonary Disease: Developing Drugs for Treatment,"[11] with the goal of accelerating drug development and implementation in COPD. The document states that "with the exception of lung function tests, there are no well-validated biomarkers or surrogate end points that can be used to establish efficacy of a drug for COPD."[11] To date, bronchodilators, which impact lung function, have been the most successful COPD medications. However, none of the bronchodilators has been shown to attenuate disease activity or modify the rate of decline in lung function[18] except in small studies, post hoc analyses of large datasets, or as secondary end points,[19-21] There are no biomarkers to guide development of disease modifiers in COPD. Data on the rate of change of FEV_1, the most commonly used measure of lung function for disease modification, are very noisy, often associated with a coefficient of variation > 1.50.[22,23] Without biomarkers for lung function decline, no small studies can be conducted to determine the therapeutic promise of potential disease-modifying compounds. Therefore, drugmakers are prematurely forced either to abandon development or commit millions of dollars for a phase III trial without clear and compelling phase II data demonstrating efficacy of the compound.[24] Given the conservative nature and constraints on resources of big pharmaceutical companies, most choose the former

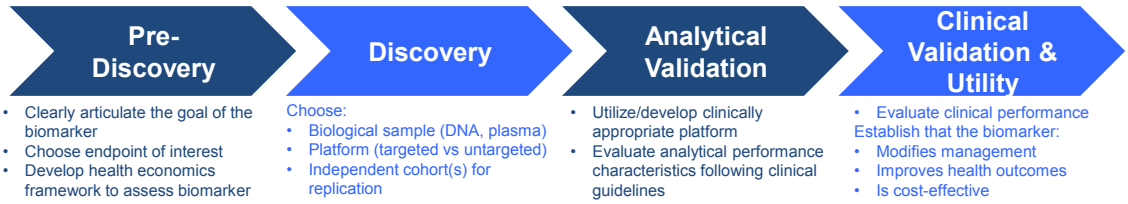

Figure 1 – *Example workflow for biomarker development. The first critical steps in biomarker development are to clearly articulate the clinical goals of the biomarker, to choose a clinical end point of interest (eg, FEV_1 decline, exacerbation, mortality), and to develop an economic framework for assessing the value of the biomarker (see text for further details). The second step is to identify samples (eg, DNA, RNA, plasma) from well-phenotyped COPD cohort(s) and to subject these samples to –omics interrogation for discovery. Ideally, results from the initial discovery experiment should be replicated in multiple other cohorts to ensure stability and generalizability of data. To avoid poor replication, investigators should choose statistical methods that will minimize overfitting. The third step is to use a technology suitable for clinical use and determine the analytical performance characteristics. The fourth step is to determine the clinical validity and usefulness of the new assay by evaluating the diagnostic performance of the biomarker test and determining whether it can modify patient management, improve health outcomes, and/or is cost-effective.*

option. There is a pressing need to develop a robust biomarker for prediction of rate of decline in lung function.

Biomarkers for Exacerbations

Exacerbations are the principal drivers of hospitalizations and mortality in patients with COPD and are a source of significant costs for society. Furthermore, severe exacerbations, but not mild to moderate exacerbations, are associated with rapid progression of disease.[22,25] In general, for drug approval, FDA mandates two independent 52-week trials, both demonstrating unequivocally that the investigational product significantly reduces the rate of exacerbations as compared with an appropriate comparator product or placebo. To ensure that there will be a sufficient number of end points in these trials, investigators typically enrich the study population for exacerbators by selecting patients with moderate to severe airflow limitation and those with at least one exacerbation in the year prior to enrollment. Although on average the rate of exacerbations increases with increasing severity of airflow limitation, there is tremendous variation in this relationship.[26] Similarly, although a previous history is the single best predictor of future exacerbations, only approximately 50% of the patients with one exacerbation in the previous year will experience another exacerbation in the ensuing year[27] (meaning that only one-half of the patients enrolled based on these criteria will contribute meaningful data to the primary end point for exacerbation trials). As well, there is no standardized definition of exacerbations, and as a result, in the real world there is tremendous variation in the way in which COPD exacerbations are diagnosed,[28] further reducing the statistical power of studies to detect a difference in the exacerbation rate between investigational drugs and their comparators. These and other factors increase the statistical noise of clinical trials and increase the risks and costs for drug developers. Not surprisingly given these constraints, drugmakers have focused on low-hanging fruits by evaluating drug classes with proven effectiveness for exacerbations, such as bronchodilators (singly or in multiple combinations)[29] or anti-inflammatories.[30] However, we may have reached the therapeutic limits of this approach because newer combination therapies (consisting of beta 2 agonists and antimuscarinic agents) appear to be no better than single agents for COPD exacerbation prevention despite significant improvements in FEV_1.[31]

There are 2 types of generic COPD biomarkers that could accelerate drug development (especially for novel classes of compounds) for prevention or mitigation of exacerbations. The first is a robust biomarker that could accurately predict patients who are likely to exacerbate within a relatively short window period (eg, 3-6 months) independent of a prior history of exacerbation. This class of a biomarker can be referred to as prognostic. The second is a biomarker that could accurately diagnose a serious exacerbation, which can be labeled as a diagnostic biomarker (see Table 1 for definitions).

The major challenge in the development of a prognostic biomarker is the unpredictable nature of exacerbations. With some events, there is a clear prodrome, whereas others are stochastic.[32] The principal drivers of exacerbations are an environmental trigger (usually a viral infection), the host responses to that trigger, and the underlying disease activity within that individual. Because all of these factors can vary within and across individuals, there may be a tremendous amount of noise inherent to prognostic biomarkers, making clinical translation difficult, if not impossible. To mitigate this risk, biomarker discovery should (1) limit the time window of prediction to a reasonable period (eg, within

TABLE 2] Common Issues That Prevent Translation of Promising *P* Values From Biomarker Discovery Experiments to Clinically Meaningful Tests and Possible Solutions to These Problems

Problems	Phase of Biomarker Development	Possible Solutions
Biomarker(s) may not be clinically relevant or useful	Prediscovery	• Clearly specify the phenotype of interest and determine the clinical need for a biomarker for this phenotype • Develop a lucid economic framework to assess the clinical utility of biomarkers
Overfitting of data (overoptimism)	Discovery	• Use large sample sizes ($>$ 100, preferably $>$ 500) • Use stringent and conservative statistical tests (eg, cross-validation methods) • Avoid data contortion or overanalysis to maximize *P* values or area under the curve values
Poor replication of data	Discovery	• Avoid overfitting of data in the discovery experiment • Consider replication of results using samples from independent cohort(s) as part of the initial discovery experiments
Unable to successfully transfer biomarkers from a discovery to clinically appropriate platform	Analytical development	• Consider using an in-use clinical platform right from the initial discovery stage (eg, mass spectrometry) or at the replication stage • Involve laboratory medicine professionals early in the discovery and replication stages to ensure that clinical translation will be possible
Clinical application of the biomarker test may be uncertain	Clinical validation	• Carefully perform discrete choice or similar preference elicitation experiments with patients, physicians, and payers to understand how the biomarker test may impact COPD management
Biomarker test may not be clinically useful	Clinical validation/utility	• Perform a clinical utility study demonstrating that the biomarker test modifies management strategy and/or improves health outcomes • Provide clear affirmative answers to the 5 SAVED questions[a]
Despite potential clinical utility, the value of real-world implementation of biomarker and subsequent disease management (biomarker-treatment bundle) is not demonstrated	Clinical utility	• Perform an evidence-informed, realistic decision-analytical modeling study to examine the cost-effectiveness of implementing the biomarker combined with disease management strategies over a sufficient time horizon to capture all relevant outcomes and translate the results into costs and health effects

[a]SAVED questions include the following: Is the biomarker likely to be (1) superior (will the test outperform current standards?), (2) actionable (will the test change patient management?), (3) valuable (will the test improve patient outcomes?), (4) economical (is the cost of the test nominal or will there be downstream health care savings?), and (5) clinically deployable (is there a pathway for the biomarker and analytical technology to be implemented in a clinical laboratory?

3 months after blood draw rather than after 12 months); (2) focus on biomarkers that reflect disease activity (eg, proinflammatory proteins) or increase the host's susceptibility to respiratory infections (eg, reduced antibody titers); (3) relate the biomarkers to hard outcomes (eg, hospitalizations) for which there is greater consensus and a great clinical need rather than softer outcomes (eg, mild exacerbations), which may have only modest clinical relevance; and (4) prioritize biomarkers based on their performance characteristics and robustness across different cohorts (eg, patients with multiple comorbidities vs patients with no or very few comorbidities) and different settings (eg, hospitalized vs outpatient).

In contrast with the issues faced by prognostic biomarkers, the major challenge in the development of a diagnostic biomarker is a lack of a gold standard. Currently, COPD exacerbations are diagnosed based on clinical gestalt and/or health service utilization. Given the imprecise nature of clinical assessment, there are numerous different definitions of COPD exacerbation,[33]

some of which are entirely based on symptoms (eg, Anthonisen criteria[34]), others which are event based (eg, change in regular COPD medications[35]), and still others which are a combination of both.[36] The validity of the case definition of exacerbations becomes even more problematic in older patients who have multiple comorbidities (eg, congestive heart failure, pulmonary embolism) which can provoke similar symptoms (eg, shortness of breath, chest discomfort or cough) as those related to COPD exacerbations.[37-39] To address this gap, standardized patient-reported outcomes questionnaires, such as the EXAcerbations of Chronic pulmonary disease Tool (http://www.exactproinitiative.com/), have been developed to reduce the variation in the diagnosis of COPD exacerbations.[40] These tools are increasingly being used by pharmaceutical companies in phase II trials to make important decisions about phase III trials (eg, either to proceed with or terminate development).[41] With further development and refinement of patient-reported outcomes and other questionnaires, it is possible in the future that they may become benchmarks against which novel biomarkers can be assessed. For now, biomarkers are being evaluated against clinical judgment. Not surprisingly, given the imprecise and highly variable nature of clinical assessment, performance characteristics of biomarkers have been less than ideal[42,43] and poorly reproducible. Performance characteristics of diagnostic biomarkers may be improved (perhaps to the point of clinical translation and application) by the addition of acute cardiac biomarkers, such as troponin T and amino-terminal pro-brain natriuretic peptide, to rule out common mimickers or confounders of COPD exacerbations related to left ventricular cardiac failure, which is remarkably common in patients with COPD.[44] Large-scale replication studies are required to validate this notion.

Biomarkers as Companion Diagnostics

Biomarkers could be developed as companion diagnostics for new or existing drugs to determine its value to a specific patient. We will call these predictive biomarkers. The FDA defines companion diagnostic as a "medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product."[45] These biomarkers can guide therapeutic decisions by accurately identifying individuals who are most likely to benefit and/or those who are likely to experience harm from treatment. These biomarkers can also inform regarding the optimal dosing information for a specific patient. The discovery of a companion diagnostic biomarker for a new drug should ideally begin early in the drug developmental process. If the purpose of the predictive biomarker is to provide information on the ideal dosing regimen for patients, then discovery should start in phase I. If biomarkers are developed to target novel therapeutic compounds for patients who are most likely to benefit and least likely to cause harm (or vice versa), their development should start at the latest in phase II of drug development. Biomarker development is difficult and may take years to clinically implement. In most cases, after discovery and replication, successful biomarkers need to be redeveloped and validated in a clinically relevant platform, which may take years to successfully complete. If FDA approval is sought for the biomarker, the application for the novel drug and the companion diagnostic should ideally be submitted contemporaneously, such that the biomarker is available when the drug is approved.

There are several companion diagnostic tests for respiratory disease patients that have obtained FDA approval. All of these tests have been developed for use in patients with non-small cell lung cancer (NSCLC). The most successful biomarkers have been those that evaluated mutations of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK). One example of a successful companion diagnostic is the cobas EGFR test (Roche Molecular Systems).[46] It is used to determine whether or not patients with NSCLC with the EGFR mutation are eligible for treatment with erlotinib. Another EGFR mutation-related test is called the therascreen EGFR RGQ PCR Kit (QIAGEN). It is used to guide therapeutic decisions for gefitinib or afatinib. The VENTANA ALK (D5F3) CDx Assay (Ventana Medical Systems, Inc.) has been developed for the detection of ALK protein to guide therapeutic decisions on crizotinib treatment for patients with NSCLC. For the same drug, the Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Inc.) has been developed to detect rearrangements in the ALK gene.

Biomarkers for Precision Medicine

Another application of biomarkers is in effecting precision medicine.[15] Although there is no consensus on the definition of precision medicine, for operational purposes, precision medicine can be defined as "a treatment targeted to the needs of patients based on…characteristics that distinguish a given patient from other patients with similar presentations (or diseases)."[15] The ultimate goal of precision medicine is to maximize

therapeutic benefits for patients at the lowest possible cost while exposing them to the lowest possible risk of harm. Precision medicine can be implemented using a variety of different methods and tools (eg, genetic and genomic markers, psychosocial traits, lung function measures, clinical profiling), the most promising of which are biomarker-based. There are already several well-known examples in which biomarker-based precision medicine has been successfully operationalized in the treatment of airway disease. For instance, gene expression of the tumor for *EML4* ALK or EGFR mutations guides therapeutic decisions for patients with NSCLC as noted previously.[47,48] In asthma, serum IgE concentrations are used in concert with clinical assessment to determine the potential utility of anti-IgE therapy with omalizumab.[49] In cystic fibrosis, genetic biomarkers are increasingly being used to determine therapeutic choices for patients by targeting treatments specific to the individuals' cystic fibrosis transmembrane conductance regulator mutation[50] and thereby achieving large clinical benefits with minimal adverse effects (although very costly). In COPD, clinicians may use serum concentrations of alpha-1 antitrypsin, along with clinical assessment, to guide implementation of replacement therapy with an alpha-1 proteinase inhibitor.[51] In patients with advanced COPD, CT scans demonstrating predominance of upper lobe emphysema have been used to guide patient selection for lung volume reduction surgery.[52]

There are emerging (but not quite ready for prime time) areas in which biomarkers may guide therapeutic choices for patients with COPD. For example, although vitamin D supplementation therapy does not generally reduce exacerbations in COPD, in patients with serum 25-hydroxyvitamin D $<$ 10 to 20 ng/mL, supplementation therapy may decrease the rates of moderate or severe exacerbations.[53,54] Another emerging example is the use of peripheral blood eosinophils to guide therapeutic choices regarding inhaled corticosteroids. Although in general inhaled corticosteroids are not indicated in patients with COPD, in those with significant elevations in peripheral eosinophil counts, they appear to have a therapeutic role by significantly reducing exacerbation rates.[55,56] Before widespread clinical translation can be advocated, however, prospective clinical trials are required to establish the optimal cutoff values of peripheral eosinophil counts at which benefits clearly outweigh the risks of inhaled corticosteroid therapy. Peripheral eosinophils are also being used as a biomarker in the development of IL-5 receptor antagonists (benralizumab and mepolizumab),[57,58] whereas serum periostin is being developed as an accompanying biomarker for IL-13 antagonist, lebrikizumab.[59] For treatment of acute exacerbations, peripheral eosinophil counts may enable separation of patients who are more likely to respond to corticosteroids vs those who would benefit from non-steroid-based therapies.[60] It is clear that COPD is not a single disease entity; it consists of many different endotypes, each driven by a unique set of molecular pathways.[61] Accordingly, biomarkers are urgently required for each of these unique pathways to enable development of therapeutics that would maximize clinical benefits and reduce the risk of adverse effects for patients in each of these endotypes. Given the heterogeneity of pathogenesis, a generic drug developmental approach, wherein patients with airflow limitation regardless of their endotype are evaluated in clinical trials, is likely doomed for failure.

Biomarker Discovery to Clinical Implementation

Similar to the drug developmental pipeline, biomarker development is a long and arduous process, fraught with many pitfalls and perils (Table 2). We have previously discussed this issue in detail[13] and summarized this process in Figure 1. Briefly, the biomarker developmental process begins with a strong clinical motivation and a focused question (eg, discovering biomarkers to diagnose severe COPD exacerbations vs discovering COPD biomarkers of exacerbation). A biomarker discovery phase can be approached using targeted or nontargeted approaches. The major advantage of a targeted approach is that the biology of the targeted biomarker is usually well known and is plausible with respect to the pathogenesis of COPD. Moreover, with well-known targets, discovery assays may be already available and tested in multiple settings. The disadvantage, however, is that given the complexity of COPD, a targeted approach is limited by our current understanding of disease pathogenesis. The multiomics (nontargeted) approach, on the other hand, increases the statistical chances of finding a unique signature for COPD endotypes; however, given the huge number of features (eg, probe sets) inherent in these datasets, many findings may represent false discoveries.[62] Although the use of a very high statistical threshold can mitigate this risk, the flip side is that these penalization methods can be overly stringent, yielding false negatives.[63] The methods to surmount these limitations include the

following: (1) increasing the sample size (to hundreds of thousands in some cases); (2) replication of results across multiple (independent) cohorts; (3) determining the lung specificity of the signal (preferably using COPD lung tissues) as expression or protein quantitative trait loci or something similar; and (4) understanding the biologic relevance of the signals using existing knowledge and bioinformatic tools, complemented by in vivo and ex vivo experiments (eg, cell culture, immunohistochemistry of relevant lung tissues and cells).[63] The importance of large datasets (N > 1,000, preferably) and replication using external cohorts cannot be overstated. Small sample sizes and lack of replication are two (of several) major drivers for "why most published research findings are false."[64] Collaborative research and data sharing are pivotal in biomarker discovery, and as such investigators should make every effort to make their data open and publicly available.[65]

With large datasets generated by multiomics experiments, big analytical solutions are required for data evaluation and interrogation. The first step in this process is to create an a priori analytical plan that carefully outlines the statistical approach for data analysis (eg, data cleanup, normalization, univariate feature ranking, multivariable feature ranking, classifier generation, module creation). Investigators must focus beyond the *P* value in this process and consider the performance characteristics of the discovered biomarker(s) in the clinical context in which the biomarkers may be used. Further, investigators must avoid the trap of overfitting datasets by contorting the analysis to maximize *P* values and receiver operating characteristics curves without considering the uniqueness of their dataset or the possible errors inherent to the various features contained in their dataset.[66] Overfitting of datasets is a major cause of nonreproducibility of biomarker results.[65]

The next phase after successful biomarker discovery is biomarker qualification/verification by an alternate analytical process.[67] To enable observation of hundreds to thousands of biomarkers in the discovery phase, analytical workflows used are typically less rigorous than those used in confirmatory experiments and for patient care purposes. For discovery platforms, the priority is to comprehensively and efficiently (both in time and cost) explore many potential targets; therefore, sacrifices are made on other fronts. For instance, a discovery proteomics experiment using mass spectrometry surveying thousands of targets relies on relative, not absolute, quantitation of biomarker targets. Identified biomarkers can then be confirmed by quantitative mass spectrometric analysis that uses an internal standard and a calibration curve for each peptide sequence being measured—an approach currently used to accurately quantify proteins in clinical laboratories.[68] Similarly, expression of specific sequences from a discovery gene array experiment should be confirmed by a more robust method, such as quantitative polymerase chain reaction, before proceeding. Similar to validation, this verification step should be performed on samples that closely resemble the target clinical population on which the test would be used.

With verification that the signal observed on discovery platforms can be reproduced using a more analytically robust technology, investigators can consider moving biomarker targets forward. However, before investigators take this step, the following 5 questions should be asked of the biomarker. Is the biomarker likely to be (1) superior (will the test outperform current standards?), (2) actionable (will the test change patient management?), (3) valuable (will the test improve patient outcomes?), (4) economical (will the implementation of the biomarker in the target population be cost-saving or cost-effective?), and (5) clinically deployable (is there a pathway for the biomarker and analytical technology to be implemented in a clinical laboratory?). A no response to any of these 5 questions should be a warning sign to investigators to stop and reconsider their biomarker before proceeding to the next phase because the biomarker in its current state is unlikely to be of clinical use.

Once these hurdles are surmounted, the investigators can begin the process of clinical assay development (as necessary) and assessment of analytical and clinical performance. The complexity of this stage should not be underestimated because the resources in terms of regulatory knowledge, personnel, specimens, time, and funds are significant.[69] In the United States, the FDA regulates medical devices intended for use "in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man…"[70] The type of medical device to be used (eg, immunoassay, mass spectrometry, next-generation sequencing), the intended use, and the indications for use will determine the path forward from a regulatory perspective. Investigators must also consider the existing clinical laboratory landscape, whether they want to fast-track clinical deployment, and how they might build an

assay that could be ported onto instrumentation common to clinical laboratories. Therefore, during this stage, the leadership of a laboratory medicine professional, such as a clinical chemist, or partnership with an in vitro diagnostics company, is recommended.

Although there are many important considerations when pursuing regulatory approval for a new medical device to be implemented in more than one laboratory (a test designed, manufactured, and used within a single laboratory is considered a laboratory-developed test and is subject to different regulations), herein we will consider three key topics: device class, intended use, and method validation. To be compliant with FDA's quality system regulation, the device (which includes both the instrument and software) must be manufactured following good manufacturing practices, and FDA clearance must be obtained. The FDA broadly categorizes medical devices into three tiers, denoted classes 1 through 3. The categorization of a device depends on the potential risk to patients.[71] The risk is assessed through evaluation of the test's intended use and indications for use,[72] including evaluation of not only the invasiveness of the test but also the potential harm to the patient because of a false-positive or false-negative result. Tests with the greatest risk are considered class 3 devices, whereas a test with minimal risk to the patient is classified as a class 1 device. Take for example two existing in vitro diagnostic devices registered with the FDA: C-reactive protein (CRP) measured by immunoassay and *Mycobacterium tuberculosis* detected by nucleic acid analysis. FDA documentation states, "A CRP immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the CRP in serum and other body fluids. Measurement of CRP aids in evaluation of the amount of injury to body tissues…"[73] It also states the following:

> …nucleic acid-based in vitro diagnostic devices for the detection of *Mycobacterium tuberculosis* complex in respiratory specimens are qualitative nucleic acid-based in vitro diagnostic devices intended to detect *Mycobacterium tuberculosis* complex nucleic acids extracted from human respiratory specimens. These devices are non-multiplexed and intended to be used as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings. These devices do not include those intended to detect the presence of organism mutations associated with drug resistance. Respiratory specimens may include sputum (induced or expectorated), bronchial specimens (eg, bronchoalveolar lavage or bronchial aspirate), or tracheal aspirates.[74]

The CRP and *M tuberculosis* tests previously described are considered class 1 and class 3 tests, respectively. CRP is one of many possible tests a physician could use to assess the amount of injury to body tissues; therefore, the potential harm to the patient is low if the test result is incorrect. Conversely, the *M tuberculosis* test specifically guides the selection of therapy; therefore, the potential risk associated with an incorrect test result (misdiagnosis) is high. Beyond classes 1 through 3, devices are further classified to determine the type of submission required. This includes—in order of decreasing submission burden—premarket applications, premarket notifications (commonly referred to as 510(k) clearances), and investigational device exemptions. Notably, most (74%) class 1 devices are exempt from the premarket application process.[72]

Method validation requirements are also dependent on the classification of the medical device.[75] A list of validation experiments that form the foundation of any method validation for a quantitative method can be found in Table 3. Included in Table 3 are the associated Clinical & Laboratory Standards Institute guidelines recognized by the FDA, which set out the details for how method validation experiments should be performed, analyzed, and reported. Additional requirements, such as interlaboratory reproducibility studies required for 510(k) submissions, can be accessed from the FDA Recognized Consensus Standards Database (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm). Circumvention of these well-established clinical laboratory practices—enacted to ensure patient safety—will impede deployment of a biomarker assay in clinical care. For a better appreciation of the regulatory approval process required for widespread biomarker deployment in health care,

TABLE 3] Core Experiments for a Clinical Method Validation

Evaluation	CLSI Guideline[a]
Analytical sensitivity	EP17-A2
Accuracy (method comparison, bias)	EP09-A3, EP15-A3
Precision	EP05-A3, EP15-A3
Reportable range (linearity)	EP6-A
Interferences and commutability	EP07-A2, EP14-A3
Reference intervals	EP28-A3c
Quality control	C24-A3

CLSI = Clinical & Laboratory Standards Institute.
[a]Because guidelines are frequently updated, please refer to the Food and Drug Administration Recognized Consensus Standards Database for the most current versions.

investigators are directed to published mock 510(k) FDA submissions for two protein-based multiplex clinical assays.[76] Although regulatory requirements vary by country, these two cases provide an example of the rigorous analytical and clinical assessments necessary for submission and feedback such filings receive from regulatory agencies.

Economic Framework for Biomarker Discovery

If the target of biomarker development is to facilitate drug development, the economic return will be in terms of the reduced drug development time and risks. One of the major potential benefits of companion diagnostic biomarkers in drug development is to reduce the overall cost and duration of drug development, which is now estimated to be $2.6 billion per pill[77] by harnessing a stratified patient population identified through the companion diagnostics. Furthermore, the reduction in development risk afforded by the companion diagnostic combined with a faster path to market provides additional motivation for drugmakers to develop novel drugs for COPD. This in turn would positively affect the economic outcome of future financings and partnerships for the company. Finally, a biomarker-based companion diagnostic could increase the pharmaceutical company's ability to compete successfully in the COPD therapeutic space once the drug reaches market. However, the pathway for the qualification of biomarkers for drug development is lengthy, complex, and fraught with risks. The resources that are required are often prohibitive for individual pharmaceutical companies and public funding agencies alike. Strategic and coordinated planning is required, requiring investment and leadership by governments and international agencies. An example is the Biomarker Qualification Process, which is part of the agency's Drug Development Tools Qualification Program.[78] Given the strategic aspects of such an investment, the focus of the economics aspects of biomarker development in this review is on biomarker development for other clinical uses.

Ultimately, the merits of such a biomarker depends on whether it provides good health value for the additional resources that society will have to spend when the biomarker is implemented. The key issue to consider is the notion of opportunity costs; that is, if the resources that are spent on the implementation of the biomarker would have been spent on the next best alternative option (eg, improving adherence to available medications, investment in new therapies), would society be at a better position in terms of the overall stock of health? Attempts at addressing this question invokes complex and multidisciplinary research and knowledge synthesis activities that involve (1) reconciling evidence from diverse sources (eg, biomarker performance studies, comparative effectiveness of available treatments), (2) extrapolating beyond the available data to predict long-term outcomes, (3) translating intermediary performance metrics (eg, biomarker sensitivity and specificity) to policy-relevant messages on cost and effectiveness of implementation decisions, and (4) systematically quantifying uncertainties in the underlying evidence and translating them to the uncertainty in cost and effectiveness outcomes. For the most part, these objectives can be achieved through formal decision-analytical modeling and computer simulation of outcomes of biomarker implementation.[79] The main outcome of this practice will be the incremental cost-effectiveness ratio of implementation of the biomarker compared with usual care. In addition to this main outcome, this framework can also produce informative predictions about the projected uptake of the biomarker, budget impact, and change in clinical outcomes (eg, number of hospitalizations attributable to the disease). Key parameters to inform such a practice will come from the clinical validation studies of the biomarker under the development which is combined with the evidence on the natural history of the disease and the impact of treatments. Complementary studies might be required to elicit patient preferences for different aspects of the biomarker (eg, to what degree patients would be willing to trade false-positive and false-negative test results).

Economic considerations in biomarker development should be contemplated early, even before the investigator team launches the study (Fig 1). Evidence on the burden of the disease of interest in the target population can be combined with plausible characteristics of a hypothetical biomarker to estimate the potential population-level impact and return on investment. Predictions of this type can back up requests for funding from the granting agencies and other sponsors. Midcycle biomarker development can benefit from formal decision-analytical approaches for example to determine the optimal cutoff on an assay value which results in the lowest cost-effectiveness ratio. Late-cycle economic evaluation is often necessary to prove the cost-effectiveness of a commercial product for approval processes in many jurisdictions, including the United States.

There are established guidelines and best practice standards for development and validation of decision-analytical models.[80] A challenge that such models for biomarkers face is the paucity of evidence on real-world aspects of biomarker implementation (eg, market price, update in the target population, use outside of the recommended indication). Estimates from previous similar technologies, combined with expert opinion, can provide initial estimates. Sensitivity analyses exploring different assumptions and what if scenarios can add to the value. Another challenge is the requirement for considering between-individual variability (heterogeneity) of the disease process. Biomarkers, by their core definition, characterize individual-specific attributes that can impact care. Decision-analytical models should concordantly consider the full spectrum of heterogeneity in the disease process. This often requires departure from the traditional cohort-based modeling (eg, Markov models) to more advanced microsimulation methods, such as discrete event simulation.[81]

Conclusions

There is a pressing need to develop biomarkers as diagnostic, prognostic, and predictive biomarkers to enable precision medicine and to markedly improve health outcomes in patients with COPD. However, there are significant barriers which prevent clinical translation. Although most investigators focus on strong *P* values for discovery purposes, *P* values alone provide no meaningful insight on the potential translatability of these biomarkers to the clinic. It is essential that investigators right from the onset carefully consider the various stages of the biomarker developmental pipeline (Fig 1) and thoughtfully craft a plausible plan to take their most promising biomarkers from discovery to the clinic. Ultimately, the plan should address the following 5 questions to ensure clinical relevance: Is the biomarker likely to be (1) superior (will the test outperform current standards?), (2) actionable (will the test change patient management?), (3) valuable (will the test improve patient outcomes?), (4) economical (is the cost of the test nominal or will there be downstream health care savings?), and (5) clinically deployable (is there a pathway for the biomarker and analytical technology to be implemented in a clinical laboratory?)? An affirmative answer to all of these questions should motivate significant allocation of resources to rapidly implementing these biomarkers as novel assays to address the growing epidemic of COPD throughout the world.

Acknowledgments

Author contributions: D. D. S. takes responsibility for the integrity of the data. All of the authors contributed equally to the conception and write-up of the manuscript.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following: D. D. S. is a Tier 1 Canada Research Chair in COPD. None declared (Z. H., M. L. D., M. S., B. M. M., R. T. G.).

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

References

1. Ma J, Ward EM, Siegel RL, et al. Temporal trends in mortality in the United States, 1969-2013. *JAMA*. 2015;314(16):1731-1739.
2. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2095-2128.
3. Adeloye D, Chua S, Lee C, et al. Global and regional estimates of COPD prevalence: systematic review and meta-analysis. *J Glob Health*. 2015;5(2):020415.
4. COPD Foundation. Impact of COPD on health care costs. https://www.copdfoundation.org/pdfs/Impact%20on%20Costs.pdf. Accessed June 21, 2016.
5. Centers for Disease Control and Prevention. Increase expected in medical care costs for COPD. http://www.cdc.gov/features/ds-copd-costs/. Accessed June 21, 2016.
6. Center for Disease Control and Prevention. Current cigarette smoking among adults –United States, 2011. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6144a2.htm?s_cid=%20mm6144a2.htm_w. Accessed June 21, 2016.
7. Ford ES. Trends in mortality from COPD among adults in the United States. *Chest*. 2015;148(4):962-970.
8. Ford ES. Hospital discharges, readmissions, and ED visits for COPD or bronchiectasis among US adults: findings from the nationwide inpatient sample 2001-2012 and Nationwide Emergency Department Sample 2006-2011. *Chest*. 2015;147(4):989-998.
9. Niewoehner DE. Clinical practice. Outpatient management of severe COPD. *N Engl J Med*. 2010;362(15):1407-1416.
10. Lange P, Celli B, Agusti A, et al. Lung-function trajectories leading to chronic obstructive pulmonary disease. *N Engl J Med*. 2015;373(2):111-122.
11. United States Food and Drug Administration. Guidance for industry: chronic obstructive pulmonary disease: developing drugs for treatment. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm071575.pdf. Accessed June 21, 2016.
12. Miller BE, Tal-Singer R, Rennard SI, et al. Plasma fibrinogen qualification as a drug development tool in chronic obstructive pulmonary disease. Perspective of the Chronic Obstructive Pulmonary Disease Biomarker Qualification Consortium. *Am J Respir Crit Care Med*. 2016;193(6):607-613.
13. Sin DD, Hollander Z, DeMarco ML, et al. Biomarker development for chronic obstructive pulmonary disease. From discovery to clinical implementation. *Am J Respir Crit Care Med*. 2015;192(10):1162-1170.
14. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95.
15. Jameson JL, Longo DL. Precision medicine–personalized, problematic, and promising. *N Engl J Med*. 2015;372(23):2229-2234.
16. FDA-NIH Biomarker Working Group. *BEST (Biomarkers, EndpointS, and other Tools) Resource*. Silver Spring, MD: Food and Drug Administration/National Institutes of Health; 2016.
17. Committee on the Review of Omics-based Tests for Predicting Patient Outcomes in Clinical Trials, Board on Health Care Services, Board on Health Sciences Policy, Institute of Medicine. *Evolution of*

Translational Omics: Lessons Learned and the Path Forward. Washington, DC: The National Academies Press; 2012.

18. Tashkin DP, Celli B, Senn S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med*. 2008;359(15):1543-1554.
19. Vestbo J, Anderson JA, Brook RD, et al. Fluticasone furoate and vilanterol and survival in chronic obstructive pulmonary disease with heightened cardiovascular risk (SUMMIT): a double-blind randomised controlled trial. *Lancet*. 2016;387(10030):1817-1826.
20. Celli BR, Thomas NE, Anderson JA, et al. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med*. 2008;178(4):332-338.
21. Lapperre TS, Snoeck-Stroband JB, Gosman MM, et al. Effect of fluticasone with and without salmeterol on pulmonary outcomes in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med*. 2009;151(8):517-527.
22. Vestbo J, Edwards LD, Scanlon PD, et al. Changes in forced expiratory volume in 1 second over time in COPD. *N Engl J Med*. 2011;365(13):1184-1192.
23. Zafari Z, Sin DD, Postma DS, et al. Individualized prediction of lung-function decline in chronic obstructive pulmonary disease. *CMAJ*. 2016;188(14):1004-1011.
24. Rennard SI, Dale DC, Donohue JF, et al. CXCR2 Antagonist MK-7123. A phase 2 proof-of-concept trial for chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2015;191(9):1001-1011.
25. Mannino DM, Davis KJ. Lung function decline and outcomes in an elderly population. *Thorax*. 2006;61(6):472-477.
26. Agusti A, Calverley PM, Celli B, et al. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res*. 2010;11:122.
27. Hurst JR, Vestbo J, Anzueto A, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med*. 2010;363(12):1128-1138.
28. Roede BM, Bindels PJ, Brouwer HJ, et al. Antibiotics and steroids for exacerbations of COPD in primary care: compliance with Dutch guidelines. *Br J Gen Pract*. 2006;56(530):662-665.
29. Wedzicha JA, Decramer M, Ficker JH, et al. Analysis of chronic obstructive pulmonary disease exacerbations with the dual bronchodilator QVA149 compared with glycopyrronium and tiotropium (SPARK): a randomised, double-blind, parallel-group study. *Lancet Respir Med*. 2013;1(5):199-209.
30. Calverley PM, Rabe KF, Goehring UM, et al. Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised clinical trials. *Lancet*. 2009;374(9691):685-694.
31. Decramer M, Anzueto A, Kerwin E, et al. Efficacy and safety of umeclidinium plus vilanterol versus tiotropium, vilanterol, or umeclidinium monotherapies over 24 weeks in patients with chronic obstructive pulmonary disease: results from two multicentre, blinded, randomised controlled trials. *Lancet Respir Med*. 2014;2(6):472-486.
32. Aaron SD, Donaldson GC, Whitmore GA, et al. Time course and pattern of COPD exacerbation onset. *Thorax*. 2012;67(3):238-243.
33. Pauwels R, Calverley P, Buist AS, et al. COPD exacerbations: the importance of a standard definition. *Respir Med*. 2004;98(2):99-107.
34. Anthonisen NR, Manfreda J, Warren CP, et al. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med*. 1987;106(2):196-204.
35. Cazzola M, Di Perna F, D'Amato M, et al. Formoterol Turbuhaler for as-needed therapy in patients with mild acute exacerbations of COPD. *Respir Med*. 2001;95(11):917-921.
36. Thompson WH, Nielson CP, Carvalho P, et al. Controlled trial of oral prednisone in outpatients with acute COPD exacerbation. *Am J Respir Crit Care Med*. 1996;154(2 Pt 1):407-412.
37. Jaiswal A, Chichra A, Nguyen VQ, et al. Challenges in the management of patients with chronic obstructive pulmonary disease and heart failure with reduced ejection fraction. *Curr Heart Fail Rep*. 2016;13(1):30-36.
38. Wang WQ, Huang HL, Zhu S, et al. High-sensitivity cardiac troponin T in patients with acute myocardial infarction in acute exacerbation of chronic obstructive pulmonary disease. *Clin Lab*. 2015;61(8):1083-1093.
39. Rizkallah J, Man SF, Sin DD. Prevalence of pulmonary embolism in acute exacerbations of COPD: a systematic review and metaanalysis. *Chest*. 2009;135(3):786-793.
40. Leidy NK, Wilcox TK, Jones PW, et al. Standardizing measurement of chronic obstructive pulmonary disease exacerbations. Reliability and validity of a patient-reported diary. *Am J Respir Crit Care Med*. 2011;183(3):323-329.
41. Aquinox. Aquinox Pharmaceuticals announces results from FLAGSHIP Trial with AQX-1125 in chronic obstructive pulmonary disease patients with frequent exacerbations. http://investor.aqxpharma.com/releasedetail.cfm?releaseid=921316. Accessed June 21, 2016.
42. Hurst JR, Donaldson GC, Perera WR, et al. Use of plasma biomarkers at exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2006;174(8):867-874.
43. Asiimwe AC, Brims FJ, Andrews NP, et al. Routine laboratory tests can predict in-hospital mortality in acute exacerbations of COPD. *Lung*. 2011;189(3):225-232.
44. Abroug F, Ouanes-Besbes L, Nciri N, et al. Association of left-heart dysfunction with severe exacerbation of chronic obstructive pulmonary disease: diagnostic performance of cardiac biomarkers. *Am J Respir Crit Care Med*. 2006;174(9):990-996.
45. U.S. Food and Drug Administration. Medical devices: companion diagnostics. U.S. Department of Health and Human Services website. http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm407297.htm. Accessed June 21, 2016.
46. cobas. The cobas® EGFR Mutation Test v.2. http://www.cobasegfrtest.com/. Accessed June 21, 2016.
47. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med*. 2014;371(23):2167-2177.
48. Cataldo VD, Gibbons DL, Perez-Soler R, et al. Treatment of non-small-cell lung cancer with erlotinib or gefitinib. *N Engl J Med*. 2011;364(10):947-955.
49. Busse WW, Morgan WJ, Gergen PJ, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med*. 2011;364(11):1005-1015.
50. Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med*. 2011;365(18):1663-1672.
51. Chapman KR, Burdon JG, Piitulainen E, et al. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;386(9991):360-368.
52. Fishman A, Martinez F, Naunheim K, et al. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med*. 2003;348(21):2059-2073.
53. Martineau AR, James WY, Hooper RL, et al. Vitamin D3 supplementation in patients with chronic obstructive pulmonary disease (ViDiCO): a multicentre, double-blind, randomised controlled trial. *Lancet Respir Med*. 2015;3(2):120-130.
54. Lehouck A, Mathieu C, Carremans C, et al. High doses of vitamin D to reduce exacerbations in chronic obstructive pulmonary disease: a randomized trial. *Ann Intern Med*. 2012;156(2):105-114.
55. Pascoe S, Locantore N, Dransfield MT, et al. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. *Lancet Respir Med*. 2015;3(6):435-442.
56. Pavord ID, Lettis S, Locantore N, et al. Blood eosinophils and inhaled corticosteroid/long-acting beta-2 agonist efficacy in COPD. *Thorax*. 2016;71(2):118-125.
57. Brightling CE, Bleecker ER, Panettieri RA Jr, et al. Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a

randomised, double-blind, placebo-controlled, phase 2a study. *Lancet Respir Med*. 2014;2(11):891-901.

58. Pharmacy Times. COPD: evaluating the pipeline. http://www.pharmacytimes.com/publications/directions-in-pharmacy/2015/march2015/copd-evaluating-the-pipeline. Accessed June 21, 2016.
59. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med*. 2011;365(12):1088-1098.
60. Aaron SD, Vandemheen KL, Maltais F, et al. TNFalpha antagonists for acute exacerbations of COPD: a randomised double-blind controlled trial. *Thorax*. 2013;68(2):142-148.
61. Woodruff PG, Agusti A, Roche N, et al. Current concepts in targeting chronic obstructive pulmonary disease pharmacotherapy: making progress towards personalised management. *Lancet*. 2015;385(9979):1789-1798.
62. Ioannidis JP, Allison DB, Ball CA, et al. Repeatability of published microarray gene expression analyses. *Nat Genet*. 2009;41(2):149-155.
63. Obeidat M, Hao K, Bosse Y, et al. Molecular mechanisms underlying variations in lung function: a systems genetics analysis. *Lancet Respir Med*. 2015;3(10):782-795.
64. Ioannidis JP. Why most published research findings are false. *PLoS Med*. 2005;2(8):e124.
65. Ioannidis JP. How to make more published research true. *PLoS Med*. 2014;11(10):e1001747.
66. Subramanian J, Simon R. Overfitting in prediction models - is it a problem only in high dimensions? *Contemp Clin Trials*. 2013;36(2):636-641.
67. Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol*. 2006;24(8):971-983.
68. Annesley TM, Cooks RG, Herold DA, et al. Clinical mass spectrometry-achieving prominence in laboratory medicine. *Clin Chem*. 2016;62(1):1-3.
69. Fung ET. A recipe for proteomics diagnostic test development: the OVA1 test, from biomarker discovery to FDA clearance. *Clin Chem*. 2010;56(2):327-329.
70. U.S. Food and Drug Administration. Regulatory information: Federal Food, Drug and Cosmetic Act (FD&C Act). U.S. Department of Health and Human Services website. http://www.fda.gov/regulatoryinformation/legislation/federalfooddrugandcosmeticactfdcact/. Accessed June 21, 2016.
71. U.S. Government Publishing Office. 21 U. S. C. 360C - classification of devices intended for human use. https://www.gpo.gov/fdsys/granule/USCODE-2010-title21/USCODE-2010-title21-chap9-subchapV-partA-sec360c/content-detail.html. Accessed June 21, 2016.
72. U.S. Food and Drug Administration. Medical devices: classify your medical device. U.S. Department of Health and Human Services website. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/ClassifyYourDevice/. Accessed June 19, 2016.
73. U.S. Food and Drug Administration. CFR - code of federal regulations title 21. U.S. Department of Health and Human Services website. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=866.5270. Accessed June 21, 2016.
74. U.S. Food and Drug Administration. CFR - code of federal regulations title 21. U.S. Department of Health and Human Services website. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=866.3372. Accessed June 21, 2016.
75. Zhao Z, Sacks D. *Assay Development in Pathobiology of Human Disease. A Dynamic Encyclopedia of Disease Mechanisms*. Cambridge, MA: Academic Press; 2014:3194-3206.
76. Regnier FE, Skates SJ, Mesri M, et al. Protein-based multiplex assays: mock presubmissions to the US Food and Drug Administration. *Clin Chem*. 2010;56(2):165-171.
77. Avorn J. The $2.6 billion pill–methodologic and policy considerations. *N Engl J Med*. 2015;372(20):1877-1879.
78. U.S. Food and Drug Administration. Biomarker qualification program. Department of Health and Human Services website. http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm284076.htm. Accessed June 21, 2016.
79. Buxton MJ, Drummond MF, Van Hout BA, et al. Modelling in economic evaluation: an unavoidable fact of life. *Health Econ*. 1997;6(3):217-227.
80. Caro JJ, Briggs AH, Siebert U, et al. Modeling good research practices–overview: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force–1. *Value Health*. 2012;15(6):796-803.
81. Karnon J, Stahl J, Brennan A, et al. Modeling using discrete event simulation: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force–4. *Value Health*. 2012;15(6):821-827.