PROTOCOL

Version 6.0R

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1. Introduction

The Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) is an observational study of COPD patients designed to inform future development of therapies for COPD by 1) providing robust criteria for subclassifying COPD participants into groups most likely to benefit from a given therapy during a slinical trial, thereby improving the chances of successful outcome, and by 2) identifying biomarkers/phenotypes that can be used as intermediate outcomes to establish clinical benefit during therapeutic trials, thus reducing costs associated with clinical trials.

The purpose of the study protocol is to delineate the rationale, methods, and procedures for: (1) selecting and enrolling participants; (2) conducting study related visits; (3) collecting, processing, and storing biological samples; (4) collecting, processing, and storing data; (5) conducting planned statistical analyses; and (6) providing overall study administration.

2. Background

2.1. Literature and Studies

COPD is a chronic, progressive disease characterized by airflow obstruction that reflects defects in airway function and/or abnormalities in the alveolar parenchyma. COPD is the fourth leading cause of death in the US, currently affecting between 12,000,000 and 24,000,000 people. Most (~90%) COPD is associated with cigarette smoking, although only ~20% of the smoking population exhibits this phenotype (Gerald and Bailey, 2002). COPD typically manifests in the mid-thirties to mid-forties with changes in spirometry (lung function), but clinically apparent symptoms are often not apparent until patients are in their mid-fifties (Pauwels, et al., 2001). There are few, if any, specific therapies for this disease. For all patients, smoking cessation is important. For patients with mild disease (GOLD Stage I) inhaled short-acting bronchodilators (e.g., β_2-agonists and/or anticholinergics) are used on an as needed basis. Maintenance long-acting bronchodilator treatment is recommended in patients with moderate disease (GOLD Stage II). Addition of inhaled corticosteroids is added to GOLD Stage II and IV treatment to improve health status, primarily by reducing the frequency of exacerbations. Long-term oxygen therapy may be needed in GOLD State IV patients. Importantly, none of the presently used pharmacological treatments for COPD are documented to prospectively modify the long-term decline in lung function; rather they are used primarily for symptom relief and to reduce the frequency of serious exacerbations (Gross, 2008; Cazzola, 2009).

Complicating the therapeutic scenario is the fact that the disease is highly heterogeneous, with cigarette smoke-induced chronic bronchitis (CB) and emphysema subsumed within the COPD definition, even though CB and emphysema likely reflect histologically and clinically distinct entities. Recent literature suggests that dividing patients into these two phenotypes is insufficient to characterize patients suffering from lung disease attributable to cigarette smoking, and other classifications have been proposed. The recognition of COPD as a systemic disease, affecting extra-pulmonary systems, including cardiovascular and muscle functions, further complicates the disease phenotype. Friedlander and Lynch (2007) suggest as many as seven clinical phenotypes

and nine physiologic phenotypes are needed to fully characterize the disease spectrum. This complex phenotypic picture suggests the need for phenotype-specific treatments, which would require careful evaluation and classification of individual patients in order to be applied appropriately. Indeed, it has recently been proposed that COPD be classified as an "orphan disease," despite the large numbers of individuals afflicted with the disorder, partially because the disease itself is so heterogeneous and each individual subtype of the disease would likely benefit from its own unique therapeutic regimen (Rennard and Vestbo, 2008).

2.1.1. Pulmonary Disease pathophysiology

Chronic Bronchitis:

The chronic bronchitis (CB) phenotype predominates in approximately 80% of patients with COPD (ALA, 2005, Pauwels et al, 2001). Patients with CB have persistent cough, sputum production, and evidence of airflow obstruction in pulmonary function tests, including a decreased FEV_1. These changes are the result of narrowing of the airway lumen (its overall cross-sectional area), due to mucus production and airway wall thickening from the inflammatory response. The pathology is located within the bronchi and bronchioles of the lungs. It includes swelling of the airway wall due to an acute and chronic inflammatory process and the copious production of mucus by the epithelial cells that line these airways. A striking feature is the proliferation of goblet cells that produce mucus, as well as metaplasia of epithelial cells to a squamous cell phenotype. The inflammatory process results in thickening due to both edema and to proliferation of the connective tissue underlying the epithelium. Hyperplasia of smooth muscle may also play a role. The inflammatory response includes neutrophils, macrophages and lymphocytes, which infiltrate both the airway wall and the lumen. This response may result from 1) smoke-induced injury of the epithelial cells or airway macrophages that subsequently induce cytokines, chemokines, growth factors, and other inflammatory mediators that recruit leukocytes; 2) the effect of defects in mucus and the clearance of mucus, which is an excellent culture media for proliferation of many potential pathogens.
The CB phenotype of COPD may thus reflect two interrelated pathophysiologies: (1) a derangement of the primary innate immune defense of the airway surface, i.e., mucus clearance; and (2) an inflammatory/scarring disease of the airway walls. Recent pathophysiologic studies suggest that the defect in innate defense in CB reflects the relative dehydration of mucus (Randell et al, 2006). 'Mucus dehydration' in CB can be produced by either, or both, too little salt and water on airway surfaces (due to dys-regulated ion transport) or excessive secretion of mucins, which are secreted 'dry' by goblet cells so they must 'hydrate' using the ambient liquid on airway surfaces (Verdugo, 1984). The cigarette smoke-induced CB responses that produce mucus dehydration likely reflect both an increase in the mucin content and a reduction in the water content of airway surface liquid (ASL).

Hogg et al., (2007) present data suggesting that there may also be important sub-types of CB, (1) patients with large and small airways symptoms of CB, e.g., chronic cough, sputum production, and mucus adhesion/plugging; and (2) patients with predominantly small airway wall abnormalities and mucus plugging without symptoms of CB. The possibility of these two subtypes has implications for patient selection and specification of outcomes in future clinical trials, and one can envision drug development programs focused on mucus adhesion, infection, and inflammation as a result. One of the major goals of SPIROMICS will be to further

characterize these patient subgroups in an effort to provide useful biomarkers for phenotyping and for clinical endpoints that pertain to each individual subtype.

Emphysema:

The emphysema phenotype accounts for approximately 30% of patients with COPD (ALA, 2005). The pathophysiology of emphysema is complex. Many mechanistic processes are thought to contribute to the alveolar enlargement and destruction, including oxidative and proteolytic processes, particularly those that damage the elastin in the alveolar walls. Leukocyte and inflammatory responses to inhaled cigarette smoke, and to the mediators produced by the lungs in response to smoke, are critical in modulating the tissue damage. At some point in disease development, stimulated neutrophils and macrophages are recruited through the pulmonary circulation into the lung parenchyma. At the same time, because cigarette smoke-related disease produces systemic responses, the bone marrow is stimulated to produce more leukocytes, which often show evidence of stimulation and activation prior to reaching the lungs. Epithelial cells are also critical players in the development of alveolar enlargement as a source of mediators and as regulators of the oxidative and proteolytic environment of the lungs. Additionally, the mechanical properties of the lungs are another major consideration in the development of enlarged alveoli. The interplay of centrifugal and centripetal forces during inspiration and expiration and the effect of leukocyte trafficking and tissue damage on the balance of forces come together to result in the observed structural changes, including the loss of tethering of alveolar walls to the connective tissue of the airways. The loss of alveolar walls results in diffusion defects of oxygen and carbon dioxide between the alveolar spaces and the blood, resulting in poor gas exchange and defects in oxygenation as measured by the diffusion capacity of the lungs. This defect, which can result in hypoxia and hypercapnia, accounts for some of the systemic manifestations of COPD.

These pathophysiologic mechanisms described above interact to produce the often heterogeneous pathologic phenotypes of emphysema in COPD. Emphysema itself can be categorized as centrilobular (centriacinar) or panacinar, based on the anatomical location of the disease, which is most clearly documented by multidetector-row computed tomography (MDCT) scans. Centrilobular emphysema results when the alveoli in the central, most proximal, region of the lobule are enlarged and these changes occur most commonly in the upper regions of the lungs. Centrilobular emphysema is more often associated with CB. It can contribute to the clinical phenotype by decreasing the surface area of the alveolo-capillary bed and causing defective exchange of oxygen and carbon dioxide, which results in gas diffusion defects as well as the airway obstruction caused by CB. In panacinar emphysema, the alveoli are diffusely enlarged throughout the lobule. The pathogenesis and relationship between centrilobular and panacinar emphysema have been contested for many years. Centrilobular emphysema may result from obstructive changes in the small airways of the lungs due to inflammation within these small conducting airways that occurs early in the course of COPD (Hogg, 2004; Hogg et al., 2004). As centrilobular emphysema progresses, a panacinar distribution may develop. However, panacinar emphysema need not be preceded by centrilobular changes. It is usually not accompanied by CB, and it tends to occur in the lower regions of the lungs. Whether these are distinct diseases that result from different processes initiated by the same cigarette smoke is an important question that will impact on therapy and clinical course.

2.1.2. COPD as a systemic disease

It is now appreciated and accepted that COPD is associated with important systemic, extra-pulmonary symptoms, especially in patients with more advanced disease (Couillard et al 2010, Papaioannou et al, 2009). Although the underlying mechanisms of these systemic effects are still under investigation, they are thought to be due to persistent inflammatory disease, imbalanced oxidative stress, or abnormal immunological function. Many patients with COPD have decreased fat-free mass, impaired systemic muscle function, anemia, osteoporosis, depression, pulmonary hypertension, and cor pulmonale, all of which can contribute to outcome. Indeed, dyspnea, body mass index, and timed six minute walk distance have been reported to be better predictors for mortality than lung function tests, such as FEV_1. Understanding these systemic aspects of COPD is clearly important for a complete phenotypic classification of COPD in the individual patient.

2.1.3. Exacerbations in COPD

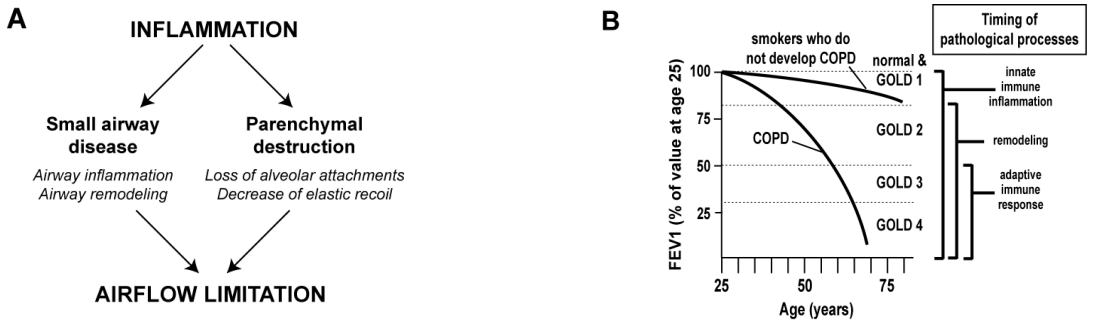
Exacerbations, which are defined by the GOLD document as "an event in the natural course of the disease characterized by a change in the patient's baseline dyspnea, cough, or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication," are of extreme importance in clinical management of COPD (GOLD Executive Committee, 2008). Exacerbations have a profound effect on the patient, and they contribute significantly to the overall morbidity and mortality associated with this disease (Anzueto 2010; Niewowhner 2006). Exacerbations are often associated with worsening airflow obstruction and can result in more rapid decline in lung function, increased peripheral muscle weakness, and decreased quality of life. Patients with frequent exacerbations have a reduced quality of life and increased mortality. It is now accepted that during an exacerbation, increased inflammation both in the lungs and the systemic circulation can affect many organs and systems. Numerous abnormalities can be found outside the lungs during and after an exacerbation that affect nutritional status, skeletal muscle function, and bone mass as well as the cardiovascular and endocrine systems. Thus, prevention and treatment of exacerbations are major objectives in the clinical management of COPD.

2.1.4. COPD as an inflammatory disease

A seminal discovery of the last decade is that as COPD worsens the lungs are progressively infiltrated by cells of the innate and adaptive immune system (Hogg et al, 2004). It is highly plausible, but formally unproven, that these immune cells contribute centrally to the airways remodeling, mucus hyperplasia, and alveolar destruction that combine variably to determine the ultimate pathophysiological phenotype of individual COPD patients (Curtis et al, 2007) (Figure 1A). Inflammation in COPD begins as an accentuation of the normal innate defenses of the lower respiratory tract (mucociliary clearance, epithelial integrity) and by subtle recruitment of neutrophils and macrophages, which are found even in smokers with normal lung function. This innate inflammation is amplified in the minority of smokers who develop COPD, with appearance of adaptive immune response in the lungs in advanced COPD (Figure 1B). Inflammation also clearly drives the symptoms of acute exacerbations (Papi and Luppi et al, 2006), but it remains uncertain how to modulate the pulmonary immune response therapeutically without increasing the frequency and severity of these fundamentally infectious events (Papi and

Bellettato et al, 2006). Finally, inflammation almost certainly underlies many of the systemic manifestations of COPD (Barnes et al, 2009).

Figure 1. Contemporary understanding of the role of inflammation & adaptive immunity in COPD pathogenesis.



A. Primary role of inflammation in COPD. Adapted from GOLD Report 2008 (GOLD, 2008). B. Proposed correlation between the onset of pathological process in COPD and GOLD disease severity stage. The natural history of the decline in FEV_1 in males followed by Fletcher et al.(1976, 1977) is shown with the GOLD stages (GOLD, 2008) superimposed as dotted horizontal lines. Although infiltration of the lungs by inflammatory cells is seen even in smokers with normal lung function (Niewoehner et al, 1974; Hogg et al, 2004), evidence of an adaptive immune response increased sharply in severe and very severe COPD (Hogg et al, 2004). Modified from Curtis et al (2007).

Understanding the inflammatory response will address the goals of SPIROMICS to provide robust criteria for subclassifying COPD participants into groups most likely to benefit from a given therapy during a clinical trial and to identify biomarkers and phenotypes that can be used as intermediate outcomes to establish clinical benefit during therapeutic trials. The key findings cited above came mostly from cross-sectional analysis using surgical specimens. SPIROMICS proposes to accelerate progress towards controlling COPD via longitudinal analysis of samples more appropriate for integration into clinical monitoring schemes. Because inflammation is so central to COPD pathogenesis and outcomes, state-of-the-art analysis of innate and adaptive immune cells, and of their inflammatory mediators, will be integrated into multiple intermediate outcome measures (blood and induced sputum). These data will be available for correlation with all other data to define unique COPD phenotypes and to identify useful biomarkers.

2.2. Rationale for this Study

The abilities to identify homogeneous subpopulations with the potential to benefit from new treatments and to identify clearly defined and measurable endpoints, for which treatment effects can be accurately and efficiently assessed in a shorter time frame than is typically required for accepted measures of morbidity and mortality, are both important aspects of efficient drug development programs targeted at COPD. SPIROMICS offers the opportunity to address these critical issues by ascertaining potentially important biomarkers and other surrogate endpoints while simultaneously identifying homogeneous subtypes of COPD. If successfully executed, this research initiative will significantly inform the development of new therapies for COPD.

A recent draft guidance document from the US Food and Drug Administration Center for Drug Evaluation and Research (FDA-CDER, 2007) cites the need for new COPD therapies due to a rising prevalence worldwide and the significant morbidity and mortality associated with the disease. Available therapies provide only symptomatic relief and have not been proven to significantly alter the underlying disease process. The stated purpose of this guidance document is to focus research efforts on what is now a better understanding of the pathophysiology of COPD, including the role of inflammation, by clearly describing the regulatory requirements of a drug development program in COPD.

Possible targets for COPD therapies include improving airflow obstruction, providing symptom relief, modifying or preventing exacerbations, altering disease progression, and modifying lung structure. Drug development programs must be designed to show benefit of treatment with respect to clinical endpoints appropriate for the therapy's intended target. Possible endpoints include objective physiological assessments (pulmonary function testing and exercise capacity), subjective patient or rater assessments (symptom scores, activity scales, and health-related quality of life scales), and biomarkers or surrogate endpoints. The draft guidance notes that, to date, no validated biomarkers or surrogate endpoints have been established for use in COPD clinical trials beyond pulmonary function testing.

With the increased understanding in recent years of the pathogenesis of COPD, it is now clear that the critical factor in conducting successful therapeutic development programs is the ability to identify appropriate patient populations for a particular intervention and to have an accurate assessment of the safety and efficacy of that intervention as early as possible in phase 2 and 3 clinical trials. Hence the need for a longitudinal clinical study designed to accomplish both of these objectives.

2.3. Primary Hypotheses

The primary hypotheses of SPIROMICS are that 1) by investigating a large number and variety of phenotypic/clinical parameters and biological markers, some will be found that enable COPD patients to be divided into homogeneous subgroups for targeted enrollment in future therapeutic clinical trials; and that 2) the same, or a different subgroup, of phenotypic/clinical and biological markers can be used as intermediate outcomes for use as clinical trial endpoints.

3. Study Objectives

3.1. Primary Aims

3.1.1. Primary Aim 1: Phenotype Identification

The first primary aim is to identify homogeneous subgroups of COPD patients for targeted enrollment in future therapeutic clinical trials. The current system of subclassifying COPD into either chronic bronchitis or emphysema provides little guidance when developing novel treatments or designing clinical trials. By better defining COPD phenotypes, SPIROMICS aims to classify this complex disease into several consistent patient populations for targeting new therapies.

This aim theorizes that COPD patients can be successfully subclassified into functionally useful categories by evaluation of a wide variety of clinical/biological parameters accompanied by appropriate statistical tests to group patients according to a few commonly shared phenotypes. Hence, the goal is to conduct as complete an evaluation as possible, taking into account what is presently known about the pathophysiology of the disease, the heterogeneity of the disease phenotype, the extent and severity of disease symptoms, and previous research/findings in smaller studies.

In order to remain current, as the study reaches maturity, newer hypotheses and research findings, particularly in the areas of mucus and airway surface liquid biology as well as inflammatory responses, will be incorporated as feasible. The extensive collection of multiple biological samples as outlined for the study visits (see Section 6.2) reflect this philosophy.

The general approach to subgroup finding assumes that homogenous subgroups can be defined in terms of multiple measurement domains (including clinical, radiological, proteomic, and genomic) through the application of statistical methods appropriate for high-dimensional data structures. These methods include hierarchical cluster analysis based on standard parametric methods for Gaussian clusters as well as Bayesian statistical approaches, and principal components analysis. Discovery of novel clustering or classification schemes, such that the within-cluster variation is significantly smaller than the overall variation in the study population, is the goal of this aim.

A more targeted approach to subgroup finding will also be undertaken as part of this aim based on the hypothesis that therapies can be individualized to a patient's genotype. Statistical analyses that incorporate the results of genetic tests will allow an evaluation of this hypothesis in SPIROMICS.

Another targeted approach to subgroup finding corresponds to prospectively identifying components of COPD syndrome that are associated with a worse prognosis, as measured by morbidity over a 3-year study period, when compared to participants without those components. The longitudinal study design allows an evaluation of subgroups defined at baseline or during the first year of follow-up with respect to long-term outcomes such as number and severity of exacerbations and decline in FEV_1.

The statistical analysis plan for evaluation of this aim is described in greater detail in Section 7,

3.1.2. Primary Aim 2: Surrogate Endpoint Discovery and Validation

The 2nd primary aim is to identify and validate intermediate biological or clinical outcomes for use as clinical trial endpoints. More specifically, SPIROMICS will identify intermediate outcome measures that predict long-term clinical endpoints of morbidity. Such outcomes may be markers of short-term change (1 year) that predict long-term change (3 years), or they may be markers that change with exacerbations and are predictive of more severe long-term morbidity/mortality.

A surrogate endpoint is any indicator of a normal biological process, pathogenic process, or pharmacologic response that operates as a substitute for a clinical endpoint (Biomarkers Definitions Working Group, 2001). Clinical endpoints considered as valid measures of COPD progression include mortality, morbidity (hospitalizations; use of IV antibiotics), quality of life (QOL), and FEV_1. Follow-up periods of several years are typically needed to show treatment effects with respect to these endpoints. Our goal is to identify intermediate outcomes that may show short-term changes in response to treatment that are predictive of longer-term clinical benefit. Such intermediate markers are candidates for validation as surrogate endpoints in COPD clinical trials.

Validation of a surrogate endpoint with respect to a clinical endpoint in the context of a therapeutic clinical trial typically involves establishing that there is a statistically significant and clinically meaningful treatment effect evident with respect to both the surrogate endpoint and clinical endpoint based on the clinical trial data. In addition, the two endpoints should be strongly correlated with each other. Further, a regression model fit to the clinical endpoint as the outcome variable and including effects for both the surrogate endpoint and treatment would ideally show no significant relationship between either independent variable (surrogate endpoint or treatment) after controlling for the other variable in the model (Chakravarty, 2003; DeGruttola, 2001). While the SPIROMICS design does not currently include therapeutic interventions, statistical methods available to provide a preliminary evaluation of intermediate outcomes are described in more detail in Section 7.4. Further, induced phenotypes may be incorporated into the study design going forward to aid in the evaluation of potential surrogates.

Within the context of this protocol, it is believed that a subset of the same clinical and biological parameters used to define subclassifications of COPD patients can be used to define surrogate endpoints, if these parameters are followed over time and if they are shown to be associated or correlated with disease progression. Hence, the protocol includes a measure of these clinical/biological features not only at a baseline visit, but again at one year and at the end of the study (three years).

3.2. Secondary Aims

3.2.1. Secondary Aim 1: Cohort Building

Recruit, enroll, and maintain the SPIROMICS cohort to support longitudinal evaluation, ancillary studies, and possible enrollment in concurrent clinical trials.

Thirty-two hundred (3,200) participants will be recruited and enrolled at six clinical centers into four strata (non-smokers, smokers, mild/moderate COPD, severe COPD). Participants will be equally distributed between males and females and will be representative of the national racial/ethnic populations. A variety of data will be collected for each participant including physiological, radiographic, biological, and quality of life indicators. In addition to meeting the primary objectives outlined above, the maintenance of this extremely well-characterized cohort will provide valuable information about the progression of COPD and the various factors underlying this progression as well as identification of subpopulations that may be interested in participating in future therapeutic clinical trials. Contacting potential participants will be governed by the informed consent process, described more fully in Section 5.3.

3.2.2. Secondary Aim 2: COPD Controlled Vocabulary

Develop and curate a COPD controlled vocabulary using both automatic and manual methods.

A carefully selected, comprehensive, and up-to-date vocabulary set is essential for supporting a variety of critical informatics functions. A COPD Vocabulary set, called SPIRO-V, will be created to accurately describe all the SPIROMICS cohorts, phenotypes, and outcome measures. SPIRO-V will follow an ontological structure, to capture hierarchical semantic associations, and it will be stored in a database. The procedure followed in creating SPIRO-V will have three phases: 1) method for concept identification, 2) concept aggregation and generation using an ontological structure, and 3) evaluation.

For the first phase, a comprehensive survey of existing initiatives on development of COPD vocabularies will be conducted and a process for selecting vocabularies manually and for generating new vocabularies automatically will be established. A conceptual database model for the SPIRO-V ontology will be created and will be implemented as a database. For the second phase, systems and interfaces will be created for importing manually selected and automatically generated vocabularies into the SPIRO-V database in an efficient and effective manner. Finally, for the last phase, usability experiments will be conducted to evaluate the quality of the SPIRO-V vocabularies in terms of accuracy and utility.

3.2.3. Secondary Aim 3: Support Ancillary Studies

Facilitate the development of ancillary studies that will address additional research hypotheses.

An ancillary study is one that contributes new or additional data to the SPIROMICS study but whose aims do not necessarily address the primary aims of the parent study. Also, ancillary studies are not funded as part of the parent study.

Ancillary study proposals are subject to review and approval by the Ancillary Studies Committee, Steering Committee, Project Office, and OSMB to ensure consistency with the parent study objectives, determine appropriateness of additional participant contacts, and monitor overall participant burden. Genomics and Informatics Center (GIC) statisticians provide study design and management support to investigators submitting ancillary study proposals. The GIC also offers review and advice on data management methods to ensure that data collected can be merged with the SPIROMICS parent study data.

4. Rationales for Outcome Measures

4.1. Rationale for the Selection of Long-Term Outcome Measures

4.1.1. Morbidity

Morbidity in SPIROMICS will primarily be measured by assessing acute exacerbations in the SPRIOMICS cohort. As indicated in the background section, the chronic, progressive course of COPD is often aggravated by acute periods of worsening symptoms (increased cough, dyspnea, and sputum production). Most exacerbations are caused by viral or bacterial infections, and they have a negative impact on health status (Schmier et al, 2005; Doll, 2005). They are also the most

frequent cause of medical visits, hospital admissions, and death. At present, there are no validated diagnostic tests of biomarkers of exacerbations despite the fact that one of the main objectives of COPD treatments is to reduce the frequency and severity of such events.

4.1.1.1. Hospitalization Resulting from Pulmonary Exacerbation

Morbidity will be assessed via hospitalizations, which will be self-reported at clinic visits and through quarterly follow-up phone calls over the three-year study period. Medical records associated with all hospitalizations will be obtained locally by clinic personnel, and blinded hard-copies or scanned copies sent to the GIC for central abstraction. Hospitalizations will be classified as being related to a pulmonary exacerbation, not related to a pulmonary exacerbation, or undetermined. The Morbidity and Mortality Committee will review summaries of the abstracted data and outcomes of the classification process, and adjudicate undetermined cases and a random sample of those able to be classified. The abstracted data as well as the outcome of the adjudication process will become part of the SPIROMICS central database.

4.1.1.2. Clinically Identified Pulmonary Exacerbation without Hospitalization

During routine follow-up calls and clinic visits, participants will also be queried about their COPD-related antibiotic use, visits to the emergency department, and contacts with their physician. Because of the volume and nature of the records relating to these visits, these records will not be reviewed by the site investigators. These patient reports will provide a count of the number of exacerbations that did not require hospitalization.

4.1.1.3. Patient reported outcomes

<u>Health Related Quality of Life (HRQoL)</u>

Because the consequences of COPD will generally worsen, patients' health related quality of life typically declines over time. HRQoL instruments have been designed to provide a standardized method of assessing the impact of the disease on the patients' daily lives, activity, and well-being. They generally take the form of questionnaires, of which many have been developed (Cazzola, et al., 2008). The specific questionnaires chosen for SPIROMICS reflect the need to monitor several specific aspects of the patients' health status, including respiratory symptoms, anxiety/depression, and sleep. Please see Section 6.4.7 for a list of questionnaires administered in SPIROMICS.

<u>Dyspnea</u>

Dyspnea, or breathlessness, is the primary reason that COPD patients seek medical care and measurements of dyspnea provide insight into patient health. One way to measure dyspnea is to measure a clinical rating based on activities of daily living through questionnaires, which consider activities such as walking or climbing stairs. Please see Section 6.4.7 for a list of questionnaires administered in SPIROMICS.

4.1.2. Lung function

SPIROMICS includes a number of measures of pulmonary function taken at each visit. As mentioned above, COPD is characterized by physiological problems, such as airflow limitations and abnormalities of gas exchange and lung hyperinflation. These features of lung function are accessed objectively in the laboratory setting using spirometry/plethysmography, which can measure such parameters as FEV_1 (forced expiratory volume in one second), FVC (forced vital capacity or total volume of air exhaled after full inspiration), FRC (functional residual capacity or volume of gas remaining in the lung at the end of tidal expiration), and IC (inspiratory capacity or maximum volume of gas that can be inspired from end-tidal expiration). The FDA preferred primary endpoint for assessment of alteration in disease progression in COPD is serial measurements of FEV_1 over three years (FDA-CDER, 2007). Other objective physiologic assessments considered by the FDA, in the draft guidelines, are RV/TLC, and exercise results such as the six-minute walk test. The annual rate of decline in the post-bronchodilator FEV_1 has been the primary outcome for most trials attempting to demonstrate disease modification for COPD. FEV_1 is also the usual outcome for studies of bronchodilation. The GOLD guidelines stage the severity of COPD using the post-bronchodilator FEV_1 (GOLD, 2007).

Methods for conducting spirometry have been standardized, and reference equations based upon distributions in the normal population are available. In COPD, post-bronchodilator FEV_1 has been used to classify patients by severity and describe disease progression (Anthonisen et al, 1994). FEV_1 measurements are generally reproducible in most patients. Changes 5-10% from the patient's baseline values are considered clinically important, and poor lung function is predictive of mortality. As a result, FEV_1 is considered a valid clinical endpoint for use in phase 3 or confirmatory clinical trials in COPD by most regulatory authorities.

4.1.3. Mortality

Deaths of SPIROMICS participants will be identified during follow-up calls and attempts to schedule clinic exams during the three-year study period, and deaths will be recorded in the clinical database. The cause of death will be determined via chart review and adjudication, and deaths attributable to COPD worsening or exacerbation will be recorded as confirmed clinical endpoints, in addition to contributing to the endpoint of all-cause mortality.

4.1.3.1. BODE Index

Several variables, such as BMI (body mass index), dyspnea, and FEV_1, are known to be predictors of mortality in COPD patients, but the relative strength of each individual variable remains difficult to determine. Recent data suggest that the BODE index, can be useful and predict changes in prognosis (Cote, 2005; Imfeld et al, 2006). This is an index that takes into account body mass (B), obstruction (O), dyspnea (D) and exercise endurance (E). Use of this index in the context of SPIROMICS is in line with the overall philosophy to utilize multiple parameters to define patient disease status. This index will be calculated for the Year 3 visit for use as a long-term clinical endpoint.

4.2. Rationale for the Selection of Assessments to Define Phenotypes and Ascertain Intermediate Outcome Measures

4.2.1. Computed Tomography

FEV_1 and other lung function evaluations are nonspecific in that they do not represent the relative contribution of airflow obstruction arising from emphysema, chronic bronchitis, and bronchiectasis. SPIROMICS investigators believe that progress toward specific treatments of COPD will be accelerated by including a precise diagnosis of the specific airway lesions responsible for measured declines in lung function. Thus, multidetector-row computed tomography (MDCT) scans are included at the baseline and Year 1 clinic exam. Studies within the radiology center (Hoffman lab) laboratory have served to validate CT as a tool for assessing lung volume (Hoffman et al, 1983), regional air content (Hoffman et al, 1985; Chon et al, 2006), regional lung expansion (Chon et al, 2006; Hoffman et al, 1986; Reinhardt et al, 1998), airway segmentation (Reinhardt et al, 1998; Tschirren, Hoffman, et al, 2005; Tschirren, McLennan, et al, 2005; Liu et al, 1986), and vessel segmentation (Liu, 1986, Shikata et al, 2004). The segmentation of the lung, lobes, airway, and pulmonary vascular bed are described along with methods for assessing lung texture (parenchymal pathologies).

4.2.2. Exercise

As mentioned above, dyspnea is an important feature in COPD. Aside from questionnaires, dyspnea can be rated by monitoring its extent while the patient performs an exercise task. In general, these ratings provide different and distinct information than that obtained by clinical ratings of dyspnea. Additionally, the ability to exercise is an important clinical outcome and a marker of other outcomes, such as HRQoL and mortality. Exercise testing is useful to assess the degree of impairment, prognosis, and the effects of interventions (Cazzola et al, 2008). Hence, SPIROMICS proposes to utilize standardized exercise tests as a parameter to define the patient phenotype.

4.2.3. Induced Sputum

For biomarker studies in clinical trials, it will be important to have relatively easy access to the samples containing the biomarker. As with blood and urine, induced sputum offers this opportunity (Cazzola et al., 2008) as well as allowing direct query of the pulmonary phenotype. Many COPD patients produce sputum spontaneously, but because spontaneous sputum often contains a large proportion of dead cells, which complicate measurements, induced sputum is the present procedure of choice. In addition, sputum induction will be required to obtain samples from healthy control participants.

A variety of biomarkers can be evaluated in induced sputum, including those that will query multiple disease mechanisms, such as mucociliary clearance (mucins; regulators of airway surface liquid), inflammation (cell counts, cytokines, and chemokines), infection (microbiome), oxidative stress, and protease/anti-protease activity. Recent results reporting potential biomarkers in induced sputum reflect the concept that additional markers with even greater value may be found through proteomics analyses. Thus, SPIROMICS places high value on the

evaluation of induced sputum to address primary study objectives, patient sub-classification, and biomarker identification.

4.2.4. Microbiome

The microbiome, i.e., what organism/s is/are present in the lungs of COPD patients, is very likely to play a critical role in disease progression. It is hypothesized that the presence of a subset of microbes in patient samples might not only suggest a patient subpopulation, but it might also predict exacerbation rate and/or rate of lung function decline. Thus, the SPIROMICS investigators believe it is prudent to include assays on the biospecimens that will identify the organisms present in the participants and to quantify these organisms as much as technologically feasible. Molecular methods, such as T-RFLPs and arrays, rather than culture plate methods, are proposed to increase the scope and accuracy of the microbiome determinations, which will include assays for both bacterial and viral infections.

4.2.5. Blood/Plasma/Serum

The collection of plasma/serum for analysis is based upon the hypothesis that low-grade systemic effects, including inflammation and oxidative stress, play an important role in the pathogenesis of COPD. A number of markers of systemic inflammation have been shown to be associated with smoking and reduced FEV_1 (Barnes et al, 2006). Several markers, including C-reactive protein, fibrinogen, leukocytes, platelets, TNF-α, IL-6, and IL-8 have been researched. In general, it is believed that more longitudinal studies with larger sample sizes are needed to confirm the specificity and sensitivity of plasma biomarkers (Cazzola et al, 2008), and SPIROMICS offers this unique opportunity. SPIROMICS investigators believe that combining data obtained on various plasma/blood markers with other parameters measured will produce a clearer picture of COPD as well as provide useful markers for subclassification and intermediate outcomes. The SPIROMICS protocol includes collection of both plasma (standard and protease protected) and serum, to accommodate the measurement of a wide variety of analytes and to provide an opportunity for proteomics-type ancillary studies. Further details related to the proposed analysis of these samples are found elsewhere in this protocol.

4.2.6. Urine

Collection of urine is non-invasive and can query certain aspects of systemic disease. For example, previous studies have identified elastin degradation products (desmosine) in COPD patients, particularly those with emphysema (Boschetto et al., 2006). 8-isoprostane, which is believed to be a byproduct of lipid peroxidation, thus a marker of oxidative processes, is another potential biomarker that can be measured in urine, as demonstrated for other systemic diseases. Present concepts support the idea that additional markers of systemic COPD could be discovered in the urine, thus, the potential use of proteomics within the context of SPIROMICS and urine collection. On a practical level, SPIROMICS investigators will be able to utilize urine samples for measurement of cotinine levels to verify smoking status of participants.

4.2.7. Biomarker Identification from Biospecimens

One of the primary aims of SPIROMICS is to identify measurable biomarkers for classification of disease phenotype and to monitor disease progression. Within this overall scope, multiple sample types, including blood (serum/plasma), urine, and induced sputum are taken from individual participants at two or more clinic visits (baseline, year 1, and year 3). The multiple sample types, derived to query as many aspects of COPD as possible, have the potential to be a powerful source of biomarker information, ultimately being related back to phenotypic information obtained from PFTs, questionnaires, microbiome data, and MDCT images. The development of the biobank of samples is one of the most critical aspects of SPIROMICS.

However, samples are only interesting if they can be studied, and the abundance of samples becomes a disadvantage if a solid plan to analyze them is not in place. The choice of biomarker assays represents two overarching goals. The first revolves around hypothesis-driven biomarker analysis, which is based upon the idea that there is enough known about COPD and its pathogenesis in the present literature to propose a series of biomarkers with a high likelihood of producing useful information for disease phenotyping and monitoring of disease progression. In this case, various aspects of the disease pathogenesis would be queried by measuring specific analytes, such as cytokines/chemokines to monitor inflammation and elastin by-products for alveolar wall destruction. This feature of the analysis also allows previously studied biomarkers, which have shown promise in COPD populations (such as surfactant protein D and Clara Cell Secretory Protein 16 as reported from the ECLIPSE study (Lomas et al, 2008; Lomas et al. 2009), to be further validated. The second takes into account the fact that not all potentially useful biomarkers have been identified or can be predicted based upon what is known. In this case, discovery proteomics and metabolomics come into play. SPIROMICS samples will be collected in such a manner as to accommodate both types of analyses.

4.2.7.1. Hypothesis-based biomarker detection

Blood plasma and induced sputum samples will provide a rich resource for biomarker detection methodologies and/or multiplex arrays for protein detection. Utilization of the same arrays for samples derived from the lung and the blood will be useful for determining how the analytes relate to the underlying biology and to determine the most appropriate sample type for querying specific disease phenotypes.

The identification of the following classes of analytes will provide the ability to query different aspects of COPD pathogenesis (the list following is representative of the analyte classes of interest, but is not totally inclusive):

- *Inflammation:* This class involves the analyses of cytokines/chemokines, including well-studied proteins, such as interleukin-8 and 10, and TNF-alpha as well as less well-studied proteins, such as interleukin 7, 16, and 17E, and including proteins believed to be important for the biology of multiple immune cell types, including macrophages, lymphocytes, neutrophils, eosinophils, and dendritic cells. Acute phase reactants, such as C reactive protein, ceruloplasmin, and haptoglobin are also included in this category, as are cell-type specific products, such as eosinophil cationic protein, neutrophil elastase, and interferon-gamma.

- *Proteolytic/antiproteolytic processes:* Included in this class are proteases/antiproteases, such as MMPs (proteases released during airway wall destruction) and TIMPs (inhibitors of MMPs).

- *Epithelial function:* Clara cell secretory protein and surfactant protein D are of interest in order to query airway epithelial cell integrity.

- *Cardiovascular disease:* Analytes such as von Willebrand Factor, brain natriuretic peptide, and fibrinogen are included in this class.

- *Airway wall destruction:* Elastin breakdown products, such as desmosine or PGP are included to query emphysema development.

- *Other processes:* Repair processes can be queried with proteins such as fibronectin, a wound repair protein that has been associated with all-cause mortality in COPD (Man et al, 2008). Overall metabolic processes can be queried with analytes such as insulin-like growth factor 1 and leptin.

4.2.7.2. Discovery-based biomarker detection

SPIROMICS will follow a general approach to discovery-based biomarker detection, which involves sample collection, analysis of samples using unbiased methods appropriate for the sample (e.g., mass spectrometry), statistical approaches to identify candidate biomarkers associated with disease phenotype and/or disease progression as defined from separate SPIROMICS data, and final confirmation of identified biomarkers by targeted quantitative methods. Additionally, because of the nature of the biobanking as part of the SPIROMICS protocol, it will be feasible to evaluate these same biomarkers in different samples, (e.g., urine versus blood) to determine the most robust sample type for individual biomarker detection.

1. Proteomics

Proteomic technologies based on mass spectrometry (MS) have emerged as a valid strategy for discovery of diagnostic and prognostic biomarkers, and are of interest for use in SPIROMICS for both blood plasma and induced sputum. Blood plasma will be collected and stored in special P100 tubes to prevent protein degradation. Induced sputum, likewise, will be stored with a mind toward protecting proteome integrity. Appropriately prepared samples (as defined by individual protocols) be able to be analyzed by systems such as nanoflow high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Software, such as Mascot (Matrixscience) is available for screening the database NCBInr for proteins corresponding to the peptide maps that are obtained.

Established statistical methods will be used to identify candidate biomarkers. For proteomics analysis, based upon previous searches for biomarkers in other studies, we anticipate a high false-discovery rate during the initial discovery phase. Furthermore, we anticipate that it will not always be possible to develop a targeted assay, which will often depend upon high quality antibody reagents that are often not available. One approach that is being considered is to utilize

multiple reaction monitoring (MRM) coupled with stable isotope dilution (SID)-MS for direct quantification of candidate proteins to confirm the candidates in the samples of interest. This method has been successfully employed in a multi-laboratory study to reproducibly detect low μg/ml proteins in plasma (Addona et al., 2009).

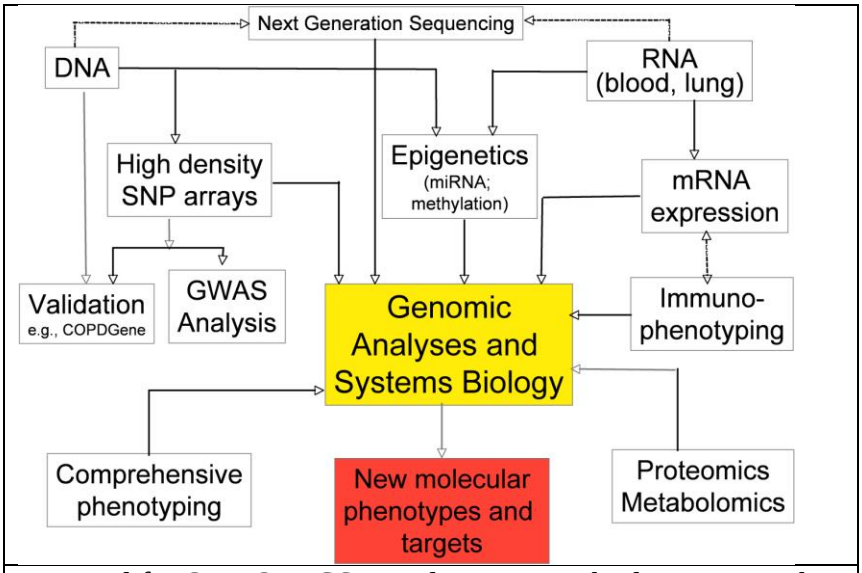
4.2.8. Integrated Genomics

By virtue of collection of both DNA and blood cell RNA from a large number of well-characterized participants with COPD (GOLD II-IV), smokers without COPD, and healthy non-smokers, the SPIROMICS study will provide a new opportunity to explore a "systems biology" approach to COPD (see Figure 1). Additional insights provided by proteomic and metabolomic analyses will add further power.

It is widely believed that genetic modifiers that predispose individuals to, or protect from, development of COPD and predict degree of disease severity will provide insight into specific molecular pathways that may contribute to specific subsets of patients with the disease. Recent work has implicated α-nicotinic acetylcholine receptor (CHRNA3/5), hedgehog interacting protein (HHIP), and SERPINE2 (Pillai et al, 2009; Zhu et al.2007) as well as other genetic variants identified by candidate gene studies that either interact with tobacco smoke (IL13, Sadeghnejad et al.; 2007) or affect pulmonary function in participants with COPD such as ADAM33 (Sadeghnejad et al.; 2009). Additionally, there is a high likelihood that additional modifiers will be identified based upon larger, more comprehensive studies, such as the COPD Foundation funded initiative known as COPDGene, which will data from over 10,000 COPD participants.

The great advantage of the SPIROMICS study will be the ability to relate these genetic polymorphisms to gene expression, protein expression, and aspects of cellular inflammation in the lungs of affected patients. In addition, the use of lung tissue specimens will allow assessment of epigenetic changes, which are likely to be important in this disease which has a prominent environmental component. The integrated analysis of these genomic data will comprise a cutting-edge approach to the identification of disease-relevant molecular pathways (see Figure 2). The use of blood cells for gene expression and miRNA analyses has some ancillary value in: 1) phenotyping participants based on the systemic, inflammatory component of the COPD phenotype, and 2) the potential to develop non-invasive tests for specific molecular phenotypes.

Figure 2. Genomic Analyses and Systems Biology



Potential for SPIROMICS to utilize systems biology approaches to generate new molecular phenotypes and targets for COPD. A combination of DNA and RNA analyses (with potential for next generation assays), extensive phenotyping, including proteomics and metabolomics from each SPIROMICS patient (at multiple time points from multiple sample sources) will provide the necessary data for comprehensive "systems biology" analyses. See text for further details.

5. Study Design

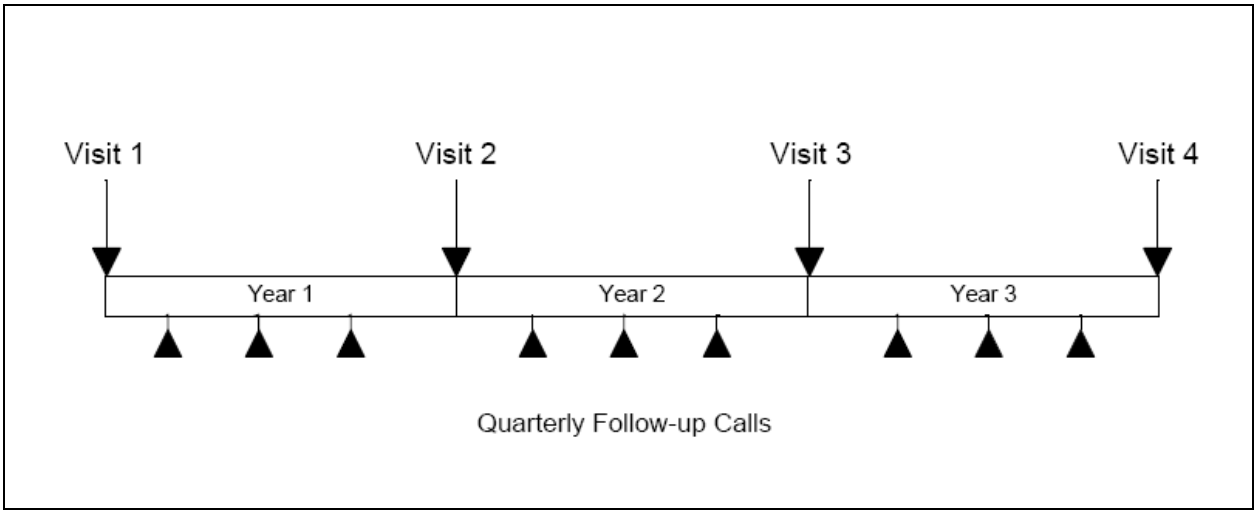
5.1. Overview or Design Summary

SPIROMICS is a prospective cohort study that will enroll approximately 3,200 participants at six clinical centers with a total of 11 recruitment locations over three years. All participants will have up to four main study-related visits (Baseline and Follow-up visits at years 1, 2, and 3 post-baseline). During the study visits, clinic staff conduct physical examinations and tests, collect biological specimens, and administer a series of questionnaires to study participants. Participants also receive quarterly follow-up calls to assess health status and determine if an exacerbation has occurred.

5.2. Schedule of Study Contacts

Figure 3 depicts the schedule of clinical visits and follow-up calls in SPIROMICS.

Figure 3. Schedule of Study Contacts



5.3. Participant Selection and Withdrawal

5.3.1. Inclusion/Exclusion Criteria

The 3,200 participants will be distributed across four enrollment strata (i.e., Non-smokers, Smokers without COPD, Mild/Moderate COPD, and Severe COPD) as shown in Table 1. Participants must fall into one of the four categories described in Table 1 in addition to meeting the inclusion/exclusion criteria described Section 5.3.1, 5.3.1.1,and 5.3.1.2 in order to be included in the study. With regards to race/ethnicity, sites' recruitment will reflect national racial distributions. All centers will recruit equal numbers of males and females.

Table 1. SPIROMICS Enrollment Strata

	Non-Smokers (Stratum 1)	**Smokers (Stratum 2)**	**Mild/Moderate COPD (Stratum 3)**	**Severe COPD (Stratum 4)**
Smoking Status	< 1 pack-year	> 20 pack-years	> 20 pack-years	> 20 pack-years
Bronchodilator Status for Assessing Lung Function	Pre-bronchodilator	Post-bronchodilator	Post-bronchodilator	Post-bronchodilator
FEV1/FVC ratio criteria	$FEV_1/FVC > .7$	$FEV_1/FVC > .7$	$FEV_1/FVC < .7$	$FEV_1/FVC < .7$
Other Lung Function Criteria	FVC>LLN	FVC>LLN	$FEV_1 > 50\%$ pred.	$FEV_1 < 50\%$ pred.
Sample Size	N = 200 (6.25%)	N = 900 (28.13%)	N = 1500 (46.88%)	N = 600 (18.72%)

Regarding participation in other studies:

1. Participants may participant in concurrent observational studies, excluding the COPDGene Study (COPDGene Investigators, 2010)
2. Participants in therapeutic clinical trials can be included after the treatment is unmasked.
3. Participants in ongoing, open-label clinical trials can be enrolled if the participant is in the control group of such a study.
4. Participants recruited into SPIROMICS can be recruited into other interventional studies after SPIROMICS baseline values are obtained. Clinic staff will maintain records documenting activity in interventional studies.

5.3.1.1. Inclusion/exclusion criteria

Table 2. Inclusion/Exclusion Criteria

	Non-smokers	Smokers	Mild/ Mod COPD	Severe COPD
Inclusion Criteria				
Between age 40 and 80 (inclusive) at Baseline Visit	X	X	X	X
Able to tolerate and willing to undergo study procedures	X	X	X	X
<1 pack-year history of smoking	X			
>20 pack-year history of smoking		X	X	X
Pre-bronchodilator: FEV_1/FVC >=.7 and FVC>LLN	X			
Post-bronchodilator: FEV_1/FVC >=.7 and FVC>LLN		X		
Post bronchodilator: FEV_1/FVC < .7 and FEV_1 > 50% pred			X	
Post bronchodilator: FEV_1/FVC < .7 and FEV_1 < 50% pred				X
Able to understand English and/or Spanish	X	X	X	X
Exclusion Criteria				
Women only: Cannot be pregnant at baseline or plan to become pregnant during the course of the study	X	X	X	X
Dementia or other cognitive dysfunction which in the opinion of the investigator would prevent the participant from consenting to the study or completing study procedures	X	X	X	X
Has plans to leave the area in the next 3 years	X	X	X	X
Smoking history of > 1 pack-year but <21 pack-years	X	X	X	X
Has a BMI > 40 kg/m^2 at baseline exam	X	X	X	X
Prior significant difficulties with pulmonary function testing	X	X	X	X
Hypersensitivity to or intolerance of albuterol sulfate or ipratropium bromide or propellants or excipients of the inhalers	X	X	X	X
Non-COPD obstructive lung disease (various bronchiolitides, sarcoid, LAM, histiocytosis X) or parenchymal lung disease, pulmonary vascular disease, pleural disease, severe kyphoscoliosis, neuromuscular weakness, or other conditions, including clinically significant cardiovascular and pulmonary disease, that, in the opinion of the investigator, limit the interpretability of the pulmonary function measures.	X	X	X	X
History of Interstitial lung disease	X	X	X	X
Current diagnosis of asthma	X			
History of Lung volume reduction surgery or lung resection	X	X	X	X
History of lung or other organ transplant	X	X	X	X
History of endobronchial valve therapy	X	X	X	X
History of large thoracic metal implants (e.g., AICD and/or pacemaker) that in the opinion of the investigator limit the interpretability of CT scans	X	X	X	X
Currently taking >=10mg a day/20mg every other day of prednisone or equivalent systemic corticosteroid	X	X	X	X
Currently taking any immunosuppressive agent	X	X	X	X
Current illicit substance abuse, excluding marijuana	X	X	X	X
History of or current use of IV Ritalin	X	X	X	X
History of or current use of heroin	X	X	X	X
History of illegal IV drug use within the last 10 years or more than 5 instances of illegal IV drug use ever	X	X	X	X
Known HIV/AIDS infection	X	X	X	X
History of lung cancer or any cancer that spread to multiple locations in the body	X	X	X	X
History of or current exposure to chemotherapy or radiation treatments that, in the opinion of the investigator, limits the interpretability of the pulmonary function measures.	X	X	X	X
Diagnosis of unstable cardiovascular disease including myocardial infarction in	X	X	X	X

the past 6 weeks, uncontrolled congestive heart failure, or uncontrolled arrhythmia				
Any illness expected to cause mortality in the next 3 years	X	X	X	X
Active pulmonary infection, including tuberculosis	X	X	X	X
History of pulmonary embolism in the past 2 years	X	X	X	X
Known diagnosis of primary bronchiectasis	X	X	X	X
Currently institutionalized (e.g., prisons, long-term care facilities)	X	X	X	X
Known to be a first degree relative of another, already enrolled participant (i.e., biological parent, biological sibling)	X	X	X	X

5.3.1.2. Temporary exclusion criteria

Temporary exclusion criteria are events, diseases, or treatments that require a waiting period to elapse before a participant can be screened for SPIROMICS. In addition, these criteria should be applied when scheduling the Years 1, 2, and 3 visits.

- Participants who present with a pulmonary exacerbation, either solely participant-identified or that has been clinically treated, in the last six weeks can be rescreened for the study once the six-week window has passed.

- Participants who present with an upper respiratory infection, either solely participant-identified or that has been clinically treated, in the last six weeks can be rescreened for the study once the six-week window has passed.

- Participants who present with current use of acute antibiotics or steroids can be rescreened for the study ≥30 days after discontinuing acute antibiotics/steroids. This does not apply to participants who are on chronic prednisone therapy of <10 mg per day or <20 mg every other day or participants who are currently on chronic, prophylactic, or suppressive antibiotic therapy.

- Participants who are currently on chronic, prophylactic, or suppressive antibiotic therapy must have started this therapy at least 6 weeks prior to the baseline visit.

- Participants who present with a myocardial infarction or eye, chest or abdominal surgery within six weeks can be rescreened after the six week window has passed. Study coordinators should consult with the site principal investigator prior to rescreening these participants.

- Female participants who present <3 months after giving birth will be asked to reschedule their visit until three months have passed since the birth.

5.3.2. Ethical Considerations

Prior to initiating enrollment at each clinical site, the informed consent document will be reviewed by the Steering Committee, Project Office, OSMB, and the Institutional Review Board (IRB) at each clinical site to insure that the document is consistent with study protocol, federal regulations, and individual institutions' policies. Enrollment will not commence until this

document has been approved by the Steering Committee, OSMB, Project Office, and each clinical site's IRB.

Participants in this study will receive an informed consent document thoroughly describing the study procedures, including the risks associated with these procedures, the amount of time required to complete study-related procedures, and the expected length of follow-up for the study. An investigator-designated research professional will review the informed consent document with each participant to insure he or she understands the study procedures and is aware of his or her rights.

5.3.2.1. Special Ethical Issues

Smoking Cessation
All enrolled participants who smoke will be provided with information about smoking cessation and, where available, referred to cessation programs.

Hold on Bronchodilator and Anticholinergic Therapy
In order to obtain accurate measurements of lung function, participants will need to abstain from using inhaled bronchodilators (e.g., albuterol) for six hours prior to pulmonary function testing and inhaled anticholinergics (e.g., ipratropium) for eight hours prior to pulmonary function testing. It will be at the discretion of the investigator as to whether it is medically advisable for a participant to abstain from bronchodilator therapy. Practicality may require some long-acting bronchodilators to simply be noted rather than withheld.

Genetics
SPIROMICS will be collecting specimens for and conducting analyses of genetic information on all participants. Strict confidentially standards are in place and will be maintained to protect the privacy of SPIROMICS participants. Biospecimen samples will be labeled with a unique identifier that does not contain any protected health information or otherwise identifies a participant.

In the event a new genetic marker is found during the course of SPIROMICS, participants with this hypothetical marker will not be notified. The analyses conducted as part of SPIROMICS are not intended to be diagnostic, and may not take place in a Clinical Laboratory Improvement Amendments (CLIA) certified lab. Some ancillary studies, however, may conduct genetic or other analyses in CLIA labs and may potentially include participant notification as part of the study protocol.

Specimen Storage for Future Studies
Some of the biospecimens collected as part of this study will be stored for future analyses. These specimens will not be individually identifiable by the laboratory or GIC personnel. The GIC will develop and maintain a tracking system whereby study participants can modify their level of consent. Participants can ask that any specimens still in storage be destroyed and not included in future analyses. The request to remove stored specimens will be made at the clinical centers to preserve participant confidentiality.

5.3.3. Participant Recruitment Plans and Consent Process

5.3.3.1. Internal sources

Patient referrals

Participants will be recruited from the patient population at each clinical center. Patients may be referred by their physician to study personnel or study personnel may, after obtaining permission from a patient's physician, approach patients directly regarding interest in the study. Study personnel may use the SPIROMICS Introductory Letter and/or SPIROMICS study pamphlet (both available on study website: www.cscc.unc.edu/spir) when contacting potential participants.

5.3.3.2. External sources

Clinical centers may solicit participants using posters or /flyers, email lists, and print media that comply with the official study template (available on study website: www.cscc.unc.edu/spir). It is important that any communication to potential participants be approved by the clinical center's IRB and comply with any institutional requirements. Flyers can only be placed in locations where they will be seen by individuals seeking medical care (e.g., doctor's office, pulmonary rehabilitation clinic). Flyers should not be placed in public locations, such as elevators or cafeterias.

Potential participants may self-refer via the SPIROMICS study website (www.spiromics.com). In the event that the GIC receives questions regarding participation in the study, the individual will be referred to the appropriate clinical center.

5.3.3.3. Consent Process

In order to participate in SPIROMICS individuals must be willing to participate in a research study and meet the eligibility criteria outlined in Table 2. Clinical center staff will provide a copy of the informed consent form to individuals who meet these criteria and allow them an opportunity to read the document. Staff members will then review the document with the potential participants, verbally explaining each section of the informed consent and answering any questions. If at the end of this review an individual feels comfortable proceeding, the clinic staff member will obtain the participant's signature. Clinic staff will also sign the consent form. A copy of the signed form will be given to the participant and the original will be stored by the clinical center.

With respect to the Health Insurance Portability and Accountability Act of 1996, IRBs generally require that informed consent documents describe the following information for participants:

1. Procedures that are followed to keep participant information, specimens, and tissues confidential.
2. A description of what information could be seen by researchers or other people (such as members of the DSMB, FDA)
3. A description of why researchers or other people would need to see this information.
4. A description of what happens to information after the study is over or if the participant leaves the study before it is finished

5. A description of when a participant's permission expires

In addition, participants will be asked if they would like to be contacted for future ancillary studies related to SPIROMICS. Please see the study website (www.cscc.unc.edu/spir) for the most current version of the Ancillary Studies Policy.

5.3.4. Risks to Participants

5.3.4.1. Clinical sites

There is a risk that someone not authorized to view participant information, including identifiable information, will gain access to this information. Clinical sites will take measures to prevent this from happening and will comply with local regulations. Risks related to individual procedures performed as part of SPIROMICS are included for each assessment starting in Section 6.4.

5.3.4.2. GIC

The only direct risk to participants originating from the GIC is the risk of breach of confidentiality. The GIC has many safeguards against this, including staff training, controlled access to computer and paper files, back up systems for electronic data, and protection against malicious code. More detail regarding the GIC's security plans and measures is available in Section 8 of this protocol: Data Handling and Record Keeping.

5.3.4.3. Change in or loss of insurance coverage

There is a risk that a participant may experience change in coverage, change in cost, or loss of insurance as a result of incidental findings during the course of the study. Tests performed as part of the SPIROMICS study are for research purposes only, and are not for clinical or diagnostic use. Therefore, the results of these tests will not be reported to insurance companies.

During the course of a study-related procedure, it is possible that a previously unknown disease or illness will be discovered (e.g., cancerous growth found during CT scan) or that a change in disease severity will be found (e.g., decreased lung function). If one of these two events occurs, the participant will be referred to care outside of the SPIROMICS study. The resultant diagnosis and care may result in changes to a participant's insurance.

5.3.5. Benefits to Participants

No direct benefits to the study participant are expected to result from participation in the proposed study, as will be stated in the informed consent. Potential benefits to the study participant may result if the clinical center examination and the measurements associated with it identify a health problem previously unknown to the affected individual.

Benefits to the COPD patients and to society are expected to accrue from the proposed research. The knowledge gained in this study will be used to develop methods of classifying

pathogenically homogeneous subpopulations of COPD patients for the purpose of targeting specific pathogenic processes. A second goal is to identify markers of disease progression and treatment response that will be useful for monitoring a patient's progress during a period of less than one year. These aims, if successful, would allow a targeted approach to disease treatment on an individual basis.

5.3.6. When and How to Withdraw Participants

5.3.6.1. Principal Investigator (PI) Discretion

A study participant can be withdrawn from the study if the clinical center PI determines that it is unsafe or unethical for the participant to continue in the study. Situations that might result in this kind of withdrawal include:

- bodily impairment such that the participant cannot complete the study protocol
- institutionalization (e.g., long-term care facility, prison)
- aggressive or antagonistic behavior towards clinical center staff

5.3.6.2. Participant Request

Participants may withdraw at any time for any reason. At the time of withdrawal participants can either 1) decline to provide any more data or specimens to the study but allow use of previously collected data and/or specimens, 2) withdraw all their data from study databases and request that any stored samples be destroyed, or 3) withdraw some portion of the data collected (i.e., participants may withdraw specimens but not exam data or vice versa).

5.3.6.3. Participant exit interview

If a participant chooses to withdraw from the SPIROMICS study, the study coordinator will conduct an exit interview to determine the disposition of the participants' study data (see Section 5.3.6). The coordinator will also provide the participant with any clinically relevant study results or establish how the participant would like to be contacted if relevant results become available in the future. The participant may decline to participate in the exit interview, in which case the consent in place at the time of study withdrawal will be used to determine the status of the participant's data.

6. Study Procedures

6.1. Screening for Eligibility

Potential participants will either contact the research staff directly or will be referred by their physician. Upon initial contact the research staff member will ascertain interest in participating in the study by providing a study description that references the procedures involved in the study and expected timeline. If the potential participant would like to continue, the research staff member will review the inclusion/exclusion criteria to determine eligibility. This initial screening can be conducted in person or over the phone, using the phone script available on the

study website. If the potential participant is eligible, the research staff member either will provide a copy of the informed consent for the potential participant to review or will proceed to schedule a time for the participant to come to the clinic to review the informed consent. Please see Section 5.3.3 for details on the consent process.

6.2. Schedule of Measurements

Table 3. Schedule of Study Measurements

	V1	V2	V3	V4
Physiological Measures				
Pulmonary Function Testing				
Exhaled CO (eCO)	X	X		X
Forced Vital Capacity	X	X	X	X
Slow vital capacity	X	X	X	X
Bronchodilation	X	X	X	X
Forced Vital Capacity	X	X	X	X
Slow Vital Capacity	X	X	X	X
Six minute walk test with continuous pulse oximetry	X	X	X	X
Anthropometric Measurements				
Height	X	X	X	X
Weight	X	X	X	X
Arm Span	X	X	X	X
Waist	X	X	X	X
Hip	X	X	X	X
Neck	X	X	X	X
Seated Blood Pressure	X	X	X	X
MDCT	X	X		
Biospecimen collection				
Blood				
Serum (Two - RT)	X	X		X
Plasma (One - P100)	X	X		X
Plasma (Three - Plasma/DNA w/EDTA and One CBC w/EDTA)	X	X		X
Plasma (One –with citrate)	X	X		X
RNA (One – PAX - Gene)	X	X		
Urine	X	X		X
Induced Sputum	X	X*	X*	X*
Questionnaires				
Medical History	X	X	X	X
St. George's Respiratory Questionnaire	X	X	X	X
MOT Short Form – 12	X	X	X	X
COPD Assessment Test	X	X	X	X
Modified Medical Research Council (MRC) Dyspnea Scale	X	X	X	X
Sleep questionnaires				
Berlin Questionnaire	X	X	X	X
Pittsburgh Sleep Quality Index	X	X	X	X
Veterans Specific Activity Questionnaire	X	X	X	X
Hospital Anxiety and Depression Scale (HADS)	X	X	X	X
FACIT	X	X	X	X
Questionnaire for Ease of Cough and Sputum Clearance	X	X	X	X

*Sample collection attempted only if initial collection at baseline failed.

6.3. Study Visit Scheduling

The procedures and assessments for primary study visits, i.e., Baseline, Year 1, Year 2, and Year 3, may take place over multiple days as long as all procedures are completed within 42 days of the start of a particular visit.

6.4. Description of Study Exams, Specimen Collection, and Questionnaires

6.4.1. Pulmonary Function Tests

6.4.1.1. Background and Rationale

The 2005 ATS/ERS guidelines for pulmonary function testing and interpretation will serve as the primary guidance for the conduct and interpretation of the spirometry measurements (Macintyre et al, 2005; Miller et al, Jul 2005; Miller et al, Aug 2005; Pellegrino et al, 2005; Wanger et al, 2005). The 2002 ATS statement will guide the six-minute walk test (ATS Statement, 2002).

The between-maneuver repeatability, which 90% of consecutive patients can meet, is 120 ml (6.1%) for FEV_1 and 150 ml (5.3%) for FVC (Enright et al, 2004). The established target is 150 ml for both measures (or 100 ml if the FVC is <1 L) (Miller et al, Aug 2005). The short-term (24.9±17.1 days) reproducibility in mild COPD participants is 113 ml (CV 4.1%) for FEV_1 and 150 ml (CV 3.5%) for FVC (10). The minimal clinically significant difference for FEV_1 is about 100 ml (Donohue et al, 2004).

6.4.1.2. Inclusion/exclusion specific to procedure

6.4.1.2.1. Inclusion criteria

There are no additional inclusion criteria for the PFT procedures.

6.4.1.2.2. Exclusion criteria

For six-minute walk, clinically significant cardiac, orthopedic or balance difficulties or resting hypoxemia (S_pO_2 <88% on room air, may be modified for altitude) are reasons for exclusion.

6.4.1.2.3. Notes related to inclusion/exclusion criteria specific to pulmonary function testing

Reversibility is neither an inclusion or exclusion criteria.

6.4.1.3. Methods

A central training session will be conducted prior to the start of enrollment. Pulmonary function technicians at each clinical center will have to be trained in the skills necessary to use the study specific equipment and methods. All technicians will be certified prior to data collection, with recertification annually.

Spirometry (SVC and FVC) will be performed on standardized equipment. Centralized quality assurance will be used.

Following is the brief testing sequence and methods for each visit (baseline, year 1, year 2, and year 3):

Prior to PFTs, participants will be asked to withhold/refrain from vigorous exercise (0.5 hours), smoking (1 hour), eating a large meal (2 hours), alcohol (4 hours), caffeine (6 hours), inhaled albuterol (6 hours), inhaled ipratropium (8 hours), and any other bronchodilators for 24 hours. Practicality may require some long-acting bronchodilators to simply be noted rather than withheld. Instructions for withholding bronchodilator medications prior to testing will stress the continued use of rescue medication if needed. The use of albuterol or ipratropium will generally relieve any symptoms related to the trough effect of long-acting bronchodilators. Failing to withhold/refrain from the above activities will not exclude a participant from continuing with PFTs.

Data will be collected in the following order:

1) Slow vital capacity (expiratory vital capacity method), including measurement of inspiratory capacity after a stable (at least 3 breaths) end-expired volume
2) Forced vital capacity
3) Bronchodilation with albuterol sulfate HFA and ipratropium bromide HFA four puffs each (30 minute waiting period before post-bronchodilator SVC or FVC)
4) Slow vital capacity, 30-120 minutes post bronchodilator (expiratory vital capacity method)
5) Forced vital capacity, 30-120 minute post bronchodilator
6) Six-minute walk test with continuous oximetry recording. S_pO_2 will be recorded every 2 seconds on a pulse oximeter. The test will be stopped if the S_pO_2 declines to <80%.

6.4.1.4. Bronchodilator dosing and re-dosing

The study dose of bronchodilators for post-bronchodilator spirometry is 4 puffs of ipratropium and 4 puffs of albuterol. If bronchodilators are used at home prior to the study visit, the dose is recorded on the Pulmonary Function Test Form (PFT Form, see study website). The use of bronchodilators at home prior to the visit does not change the study dose during spirometry. Participants may take their usual medications approximately 165 minutes after completing the post-bronchodilator spirometry.

Re-dosing of one or both bronchodilators is required prior to the six minute walk, CT, or sputum induction, if too much time has elapsed since the last puff of albuterol taken for post-bronchodilator spirometry. Table 4 provides the requirements for re-dosing for CT scan and six minute walk.

Table 4. Bronchodilator Dosing and Re-dosing Schedule for CT Scan and Six Minute Walk Conducted on the Same Day as Study Spirometry

Time after initial dose	Administer	Test by
< 165 minutes	No additional puffs	30-180 minutes after original doses
Participant takes usual medications approximately 165 minutes after post-bronchodilator spirometry		
>-165 and <300	2 puffs albuterol	15-180 minutes after new albuterol
>=300 minutes	2 puffs of ipratropium and 2 puffs albuterol	30-180 minutes after new albuterol

If the CT scan or six minute walk occurs on a separate day from the spirometry, then the participant should be instructed to take their medications as usual (i.e., no withholding), and should be dosed with 2 puffs of albuterol and 2 puffs of ipratropium.

For induced sputum, if COPD subjects have not had albuterol in the prior 165 minutes, regardless of whether the induction occurs on the same day as the study spirometry, then they should be dosed with 2 puffs of albuterol. Participants who are non-smokers or smokers without obstruction do not need albuterol for the sputum induction. The investigator should be contacted if a participant reports having asthma and would otherwise not receive albuterol.

6.4.1.5. Justification for the use of both albuterol and ipratropium to measure the response to a bronchodilator and determine the post-bronchodilator FEV_1, FVC and SVC (including inspiratory capacity)

The standard of practice in the community for assessing the response to a bronchodilator involves the performance of spirometry before and 15-30 minutes following administration of 2-3 inhalations of albuterol sulfate HFA (90 mcg/inhalation). However, according to the current GOLD guidelines (2008), "possible dosage protocols are 200 mcg β_2-agonist, up to 160 mcg anticholinergic or the two combined with repeat measurement of FEV_1 10-15 minutes after the β_2-agonist or 30-45 minutes after the combination" (GOLD Executive Committee, 2008).

According to the SPIROMICS protocol, spirometry will be performed before and 30-120 minutes following administration of 4 inhalations of albuterol sulfate HFA plus 4 inhalations of ipratropium bromide HFA (17 mcg/inhalation). The rationale for using the two different classes of short-acting inhaled bronchodilators (beta-adrenergic agonist plus anticholinergic) in twice the usual recommended therapeutic dose for assessing the bronchodilator response is based on well-established evidence of the additive bronchodilator effect of the two different types of inhaled bronchodilators (Dorinsky et al, 1999). Similarly, the reason for administering 4, rather than the conventional 2, inhalations of each bronchodilator is based on evidence that, in general, maximal bronchodilation is not achieved with only 2 inhalations of either agent (Harrison et al, 1983; Gross et al, 1989).

In SPIROMICS, pre- and post-bronchodilator spirometry will be performed both for assessing the degree of bronchodilator responsiveness and for determining the post-bronchodilator FEV_1, FVC and SVC (including IC). The degree of bronchodilator responsiveness may be considered a surrogate for airway responsiveness to methacholine, is a potential determinant of disease progression (as measured by the annual rate of change in FEV_1) (Anthonisen, Wright, et al, 1986; Tashkin et al, 1996) and may also help define a COPD phenotype, namely one characterized by pronounced versus minimal reversibility of airflow obstruction. The post-bronchodilator FEV_1 is the metric that has traditionally been used to define disease progression in COPD (Anthonisen et al, 1994) since it tends to minimize the day-to-day variability in pre-bronchodilator FEV_1 that is largely related to day-to-day variability in bronchomotor tone. SPIROMICS will rely on administering 4 inhalations of both a beta-agonist and an anticholinergic bronchodilator in an effort to optimize our ability 1) to determine maximal bronchodilator responsiveness and 2) to obtain reproducible values of FEV_1 (unconfounded by varying bronchomotor tone), thus affording greater precision in assessing the annual rate of decline in FEV_1.

The bronchodilator reversibility procedure employed in SPIROMICS, although different from what is currently the standard of practice, has the potential for setting a new standard for determining reversibility of airflow obstruction and hyperinflation, in addition to providing a more reliable measure of disease progression, as defined by the annual decline in lung function.

6.4.1.6. Measurements

1) FVC (liters and percent predicated for FVC) (Hankinson, 1999)
2) FEV_1 (liters per second and percent of predicted for FEV_1) (Hankinson, 1999)
3) Exhaled carbon monoxide as an indicator of recent smoking
4) IC
5) Bronchodilator reversibility of FEV_1 and FVC
6) FEV_1/FVC
7) Six-minute walk distance
8) Area above the oxygen saturation-time curve during the six-minute walk

6.4.1.7. Human Subjects' Protection and Risks

PFTs are a common medical procedure of generally low risk. Some participants may experience breathlessness, cough, fatigue, dizziness/lightheadedness (hyperventilation), all of which are brief, and very rarely headache, syncope, musculoskeletal chest pain, rib fractures, or ear injury. An episode of stress incontinence (urine leakage) may be caused by the PFT maneuvers in susceptible individuals. A seated position has been specified to reduce risk related to dizziness or syncope. Transmission of airborne disease is rare and minimized or eliminated with single-use filters.

Instructions for withholding bronchodilator medications prior to testing will stress the continued use of rescue medication if needed. The use of albuterol or ipratropium will generally relieve any symptoms related to the trough effect of long-acting bronchodilators.

Albuterol has been reported to cause urticaria, angioedema, paradoxical bronchospasm, angina, arrhythmias, QT prolongation, hypertension, hypokalemia, seizures, tremor, nervousness, headache, tachycardia, muscle cramps, palpitations, insomnia, and dizziness.
Ipratropium has been reported to cause cough, nausea, dry mouth, dizziness, headache, dyspnea, atrial fibrillation, tachycardia, paradoxical bronchospasm, laryngospasm, angioedema, anaphylaxis, hypersensitivity, and exacerbate narrow-angle closure glaucoma.

The dose used in testing is twice the usual dose of albuterol (one dose every four hours) or ipratropium (one dose every six hours) used chronically. However, home management of exacerbations includes increasing the dose and/or frequency of bronchodilator therapy (GOLD, 2007). Doses in patients hospitalized or visiting the Emergency Department for exacerbations may be ten times the usual dose. Repeat dosing after at least three hours, is unlikely to result in any additional side effects, if necessary for the scheduling of the plethysmography.

The six-minute walk test is self-paced, but participants are encouraged to cover as much distance as they can in six-minutes. As with any walk, the participant may stumble or fall. It is expected that more severe participants will become short of breath and may need to stop to recover before six minutes have elapsed. The walk test will be stopped if the participant's S_pO_2 falls below 80%.

Data transmission will be encrypted with a 128-bit VPN approach. The PFT software is designed to be HIPPA compliant for clinical use.

The participant may benefit from treatment or secondary prevention after study identification of unrecognized pulmonary disease. All study personnel are certified in the ethical conduct of human biomedical and genetics research and HIPPA information security.

6.4.2. Anthropometric Measurements

6.4.2.1. Background and rationale

Anthropometric measurements are an easy, cost-efficient method for calculating body composition. Numerous studies have shown that these measurements are predictive of health outcomes, including risk of cardiovascular disease and obstructive sleep apnea.
In patients with COPD, low body mass index (BMI) is independently predictive of all cause mortality (Landbo, et al, 1999). Conversely, high BMI negatively impacts exercise performance in the setting of COPD (Schols, et al, 1991). In addition, in a study by Chen, et al (2007), waist circumference was negatively associated with pulmonary function after adjusting for sex, age, height, weight, and pack-years of smoking.

6.4.2.2. Inclusion/exclusion specific to procedure

6.4.2.2.1. Inclusion criteria

There are no additional inclusion criteria.

6.4.2.2.2. Exclusion criteria

Participants must be able to stand and straighten spine.

6.4.2.3. Methods

6.4.2.3.1. Standing Height

Standing height will be assessed using a mounted stadiometer. The study coordinator will record the measurement to the nearest tenth of a centimeter.

6.4.2.3.2. Arm span

Arm span will be measured using a study-provided measuring tape. The participant will stand with his or her back against a wall. With his or her arms held level with his or her shoulders as widely as possible, the study coordinator will measure the distance from finger tip to finger tip.

6.4.2.3.3. Waist

To define the level at which the waist or abdominal circumference is measured, you must first locate and mark a bony landmark, the lateral border of the ilium (i.e., top of the hip). The recorder (if available) makes sure that the tape is parallel to the floor and that the tape is snug, without compressing the skin. Measurements are made at the end of a normal expiration and reported to the recorder to the nearest centimeter.

6.4.2.3.4. Hip

The hip girth is measured at the level of maximal protrusion of the gluteal muscles (hips).

6.4.2.3.5. Neck

Neck measurements should be taken between the midcervical spine and midanterior neck using a study-provided plastic measuring tape.

6.4.2.3.6. Weight

Participants will be asked to self-report weight prior to being weighed. Participants' weight will be measured using a digital scale. Study coordinators will record the digital weight readout (tenth of a kg).

6.4.2.4. Measurements:

1. Height
2. Arm Span
3. Waist Circumference

4. Hip Circumference
5. Neck Circumference
6. Weight
7. Body Mass Index (BMI)
8. BODE (calculated in part using BMI)

6.4.2.5. Human Subjects' Protection and Risks

There is a low probability of participants' experiencing an adverse or unanticipated event during anthropometric measurements. Clinical risks include syncope and vasovagal episodes. Participants may also experience feelings of embarrassment or discomfort with being measured. Efforts to minimize these risks include conducting the measurements in a quiet, private location. Study coordinators should approach participants in an open, tolerant manner, reassuring them during the procedures that they can stop at any time. Further, coordinators should carefully describe the measuring process before initiating it so that the participant is not surprised at any step.

6.4.3. Blood Pressure

6.4.3.1. Background and rationale

High blood pressure is a known risk factor for many diseases, including cardiovascular disease, stroke, and kidney failure. SPIROMICS will use the following cut points for determining the presence of hypertension (NHLBI, 2004):

Table 5. Blood Pressure Cutoffs

Category	**Systolic**		**Diastolic**
Normal	<120 mmHg	And	<80mmHg
Prehypertension	120-139mmHg	Or	80-89mmHg
HBP Stage 1	140-159mmHg	Or	90-99mmHg
HBP Stage 2	>160mmHg	Or	>100mmHg

6.4.3.2. Inclusion/exclusion specific to procedure

6.4.3.2.1. Inclusion criteria

There are no procedure specific inclusion criteria.

6.4.3.2.2. Exclusion criteria

Any condition that prevents the study coordinator from placing the blood pressure cuff on either of the participants' arms (e.g., amputation, rashes, small gauze/adhesive dressings, casts, arm withered, puffy, have tubes, open sores, hematomas, wounds, arteriovenous (AV) shunt, or any other intravenous access device) are exclusion criteria..

6.4.3.3. Methods

1. Blood pressure (BP) will be measured using an automated device. Coordinators should measure the participant's arm to determine appropriate cuff size.
2. BP will be taken from the right arm unless some condition (such as noted in the exclusion criteria) prevents use of that arm. Coordinators may use the left arm in this situation.
3. Participants will be asked to sit quietly with their feet on the floor and with their arm resting in their lap. Cuff will be placed on the arm and the automated BP device activated. Coordinators will take three consecutive measurements with 30 second intervals between each measurement.

6.4.3.4. Measurements:

1. Systolic BP
2. Diastolic BP
3. Pulse

6.4.3.5. Human Subjects' Protection and Risks

There are no significant risks associated with measuring blood pressure. Participants may experience discomfort in the form of mild pressure, tingling, or numbness while the BP cuff is active.

6.4.4. Multidetector-Row Computed Tomography (MDCT)

6.4.4.1. Background and Rationale

The Radiology Center for SPIROMICS will oversee the acquisition of a state-of-the-art computed tomography (CT) image data set acquired for the purpose of providing lung phenotypes associated with COPD. Data will be acquired appropriately, transmitted securely, assessed for quality and protocol adherence, archived safely with quantitative image analysis results, and results will be transmitted appropriately.

Multidetector-row computed tomography (MDCT) provides the ability to image the lung with a theoretical in vivo resolution of approximately 0.5mm. The whole lung can be imaged at this resolution in approximately 10 seconds, well within a single breath hold. Scanner rotation speeds are on the order of 300 ms per revolution. With advances in image processing methods, the lung, lobes, bronchial tree, and vascular trees can be extracted and quantitatively assessed. Density and texture measures of the lung parenchyma via MDCT imaging are now providing tools for establishing regional presence and distribution of lung pathology including emphysema-like lung tissue found at full inspiration and air trapping found at full expiration.

6.4.4.2. Inclusion/exclusion specific to procedure

All participants meeting primary inclusion and exclusion criteria for entrance into the SPIROMICS study will have a CT scan.

6.4.4.3. Methods

Prior to participant enrollment CT scanners at each site will be calibrated using the COPDGene Lung Phantom (CTP674, The Phantom Lab). Please see Section 8.5.3 for details on the CT quality assurance plan.

Although there are some variations between CT scanners, a standardization of the parameters has been developed to allow multiple scanners to output reliable quantitative measures across a given patient population. Please see the Imaging MOP for details. Upon arriving at the CT site the study coordinator will check with the radiology technician to insure that appropriate study protocol is followed. Study coordinators are provided with a cheat sheet (see Imaging MOP) to assist in confirming the appropriate CT scanner settings. BMI will be used to determine large (BMI>30), medium (BMI 20 to 30), or small (BMI <20) mAs setting. The BMI values will be provided to the radiology technician by the study coordinator.

All participants will be imaged in the supine body posture. Scout scans will be obtained to minimize the subject's exposure to radiation and must be performed at the lung volume for which anatomical boundaries are being evaluated. Each study site should perform scouts as deemed locally appropriate to specify a TLC and RV scan such that when spirally scanned, the full extent of the lung is acquired at the respective lung volumes and over-scanning is kept to a minimum (no more than 2 cm cephalad to the apical or 5 cm caudal to the basal lung borders). Inadequate scout scans may be repeated as per site-specific determination. (Spiral scans, however, may not be repeated.) The exposure factors (kV and mA) for the topogram should be set to the lowest available on the CT scanner that provides an acceptable image. For GE scanners 80 kv and 20 mA is sufficient to achieve satisfactory scout image quality. Two CT scans will be performed each at different lung volume. The first volume will be at Total Lung Capacity (TLC) followed by another CT scan at Residual Volume (RV). Study coordinators will be provided with a script to coach participants in the appropriate breathing technique (See Imaging MOP).

CT scans should be conducted after the participant has received the bronchodilator dose administered for post-bronchodilator spirometry. For CT scans occurring on the same day as spirometry, if the scan occurs more than 165 minutes after the last dose of bronchodilation, then the participant will need to be re-dosed as described in Section 6.4.1.4 and Table 4.

If the CT scan occurs on a separate day from spirometry, subjects should not withhold bronchodilators prior to the study visit for the CT scan. Subjects will receive 2 puffs of albuterol and 2 puffs of ipratropium before the CT scan as described in Section 6.4.1.4.

At all visits subsequent to the baseline visit, participants will be scanned at the same dose as was used during the baseline CT visit unless either 1) the original scan was at a higher than appropriate dose (e.g., small subject scanned as a medium) or 2) the participant’s BMI has changed more than three units on either side of the BMI thresholds for dose categories.

6.4.4.4. Measurements

1) Primary candidate outcomes / phenotype measures:

- Volume measures (assessed for whole lung, left and right lung and lung lobes):
- Total volume at TLC and RV
- Total air volume at TLC and RV
- Total tissue (non-air) volume at TLC and RV

Primary parenchymal measures to be assessed from the TLC scans on a per lung, right and left lung, and lobar basis in this study are:

- Lower 15^{th} percentile point (in Hounsfield Units: HU) of the lung density histogram assessed from TLC scans at baseline and the rate of leftward shift over time.
- Percent of voxels below -950, -910, 850, and their change over time.
- "Alpha" assessed for holes defined as connected voxels whose values fall below -950 HU.

Primary parenchymal measure to be assessed from the RV scans on a per lung, right and left lung and lobar basis in this study are:

- Percent of lung voxels below -850. (index of air trapping) and its change over time.

Primary airway measures to be assessed at each airway segment out to 6 generations (as detectable) along 6 paths (RB1, RB4, RB10, LB1, LB4, and LB10)

- Luminal area at baseline and over time
- Luminal perimeter at baseline and over time
- Average wall thickness at baseline and over time
- Wall area percent at baseline and over time

2) Secondary outcomes / phenotype measures:

These measures are less well tested in the literature and will be performed in an attempt to evaluate their utility in the identification of COPD phenotypes and disease progression.

Secondary parenchymal measures to be assessed from the TLC scans:

- Parenchymal measures assessed for sub-lobar (areas of influence of primary lobar airways) segments including change over time
- Raw texture measures on a lobar and sub-lobar basis including change over time

Secondary parenchymal measures to be assessed from the RV scans:

- Percent of voxels falling below -850 evaluated for sub-lobar segments and the change over time
- Pulmonary artery vascular volume assessed for the arterial volume within the borders of the lung out to the 5^{th} generation pulmonary arteries and the change over time

6.4.4.5. Human Subjects' Protection and Risks

There is a low probability of serious risks in these radiological studies. There is a theoretical risk from radiation exposure from the CT scans, but the doses of radiation are within acceptable exposure limits. This is not a therapeutic intervention study, and will not compromise standard, usual care.

The x-ray exposure during these studies will be limited to roughly the area covered by the rib cage. Although there are no proven harmful effects from the radiation received during this study, long-term effects of this radiation on participants' health cannot be excluded with certainty. The total radiation received over the course of the study will be equivalent to less than the annual radiation limit for a medical radiation worker.

These scanning images are not intended as a replacement for a physical examination or a substitute for a visit to the participant's doctor; nevertheless, they will be reviewed by a local radiologist. If there are any abnormalities observed by the clinical center radiologists these will be reported to the clinical site Principal Investigator. Furthermore, the Radiology Reading Center personnel will report abnormal results found during the central read to the GIC. The GIC will in turn direct those results to the appropriate clinical center.

The risks of the abnormal findings are:

- False positive scan, which is an abnormality that initially is of concern for cancer that is later found not to be cancer. Anxiety may result from false positive results.
- Detection of abnormalities unrelated to lung cancer that could lead to unnecessary testing or treatment
- Failure to detect a lung cancer that is present, and possibly miss an opportunity for care

6.4.5. Biospecimen Collection

6.4.5.1. Background and rationale

For large pharmaceutical trials, biomarkers for patient subgrouping or as measures of intermediate outcomes will optimally be obtained in samples that are patient-friendly and non-invasive, such as blood and urine.

6.4.5.2. Inclusion/exclusion specific to procedure

All participants meeting primary inclusion and exclusion criteria for entrance into the SPIROMICS study will have samples drawn for blood and urine at baseline, Year 1, and Year 3.

6.4.5.3. Methods

6.4.5.3.1. Collection of Blood:

In an effort to reduce experimental variability, venous blood samples should be drawn from participants in the morning after a fast initiated at midnight. The following blood samples should be collected, limited to no more that eight tubes of blood drawn per day/visit. The recommended order of the blood draw is as follows:

- 2 tubes of blood allowed to clot for serum, which will be processed within 2 hours of collection as described in the Biospecimen Collection and Processing Manual of Procedures, MOP 4, for future batch shipment on dry ice to the GIC.

- 1 tube of plasma collected in a P100 tube for preservation of plasma proteins in anticipation of proteomics analyses. Plasma should be processed within 2 hours of collection as described in MOP 4 for future batch shipments on dry ice to the GIC.

- 1 tube of plasma collected in EDTA for submission directly to the local clinical laboratories for complete blood count with white blood cell differential and platelet count.

- Three tubes of blood collected in EDTA for plasma and DNA which will be processed within 2 hours of collection as described in MOP 4 for future batch shipment to the GIC. After the baseline visit, only the plasma will be collected from these tubes unless a participants DNA sample is lost or damaged, in which case processing for DNA will occur during the Year 1 clinic visit.

- 1 tube of blood in PAX-Gene™ RNA tube. This tube will be shipped to the GIC laboratories for RNA extraction (including preservation of micro RNA species). RNA will be suitable for all down-stream applications (transcriptomics, RT-PCR).

- 1 tube of plasma collected in citrate to be processed for plasma and buffy coat cells within two hours for future batch shipments on dry ice to the GIC.

The above collections total approximately 70 ml per visit

- 1 additional tube may be collected if the following analysis is needed.
    - Some sites may require blood pregnancy test before MDCT, which is conducted on a sample of serum (red-top or serum gel tubes). This would only be required when urine pregnancy test is not acceptable at the site and for women of child-bearing age.
- Sites may redraw blood tubes up to two of the blood tubes if needed because of problems in specimen processing.

6.4.5.3.2. Blood sample processing:

The goal is to obtain an adequate number of very high quality aliquots to support all analyses within SPIROMICS and future ancillary studies. Quality assurance guidelines are outlined in the MOP 4. Briefly, plasma and serum tubes should be processed within 2 hours after collection according to the methods outlined in MOP 4. Aliquots range in volume from 100-500 µl. All

samples should be stored and batch shipped according to procedures outlined in MOP 4. As a general rule, buffy coat cells collected from the plasma tubes should be frozen for shipment to the GIC to serve as an alternative source of DNA if such a sample is needed.

6.4.5.3.3. Collection of Urine

In an effort to reduce experimental variability, a urine sample will be collected as soon as possible after the participant enters the clinic after a fast since midnight. Urine will be measured into aliquots with and without preservative (antioxidants). Samples will be frozen immediately after aliquoting at -80° C and batch shipped to the GIC.

6.4.5.4. Measurements

6.4.5.4.1. Blood

- Blood samples collected for Complete Blood Counts (CBC) with differentials will give:
    - White blood cell count per volume of blood
    - White blood cell differentials (neutrophils, lymphocytes, monocytes, eosinophils, and basophils
    - Red blood cell count per volume of blood
    - Hemoglobin
    - Hematocrit (percent red blood cells per volume of blood)
    - Platelet count (number of platelets per volume of blood)
    - Mean corpuscular volume (average size of red blood cells)
    - Mean corpuscular hemoglobin (average amount of oxygen-carrying hemoglobin inside a red blood cell)
    - Mean corpuscular hemoglobin concentration (average concentration of hemoglobin inside a red cell)
    - Red cell size distribution
- P100 plasma will be prioritized toward proteomics and/or metabolomics analyses that require high quality plasma where the analytes of interest are not predefined or known.
- EDTA plasma will be prioritized toward analysis of known biomarkers or hypothesis driven biomarker analyses where it is previously determined, or reasonably speculated, that EDTA plasma will serve as an excellent source to measure the analyte of interest.
- Serum will be prioritized toward analysis of known biomarkers or hypothesis driven biomarker analyses where it has been previously determined, or reasonably speculated, that serum will serve as the best source for analyte measurement.
- DNA from whole blood will be used for fine-mapping genotyping analyses, evaluating SNPs or sequencing of regions/genes as defined by other large studies, such as COPD-Gene that focus on identification of genetic markers associated with COPD.
- RNA will be used for transcriptomics of mRNA/microRNA or for analyses focused on single-gene expression. High quality is paramount to the success of these efforts.

6.4.5.4.2. Urine

- For women of child-bearing age, at those sites where urine tests are acceptable, urine will be used for pregnancy determination as measured using the analyte, human chorionic gonadotropin (hCG).
- Urine will be prioritized toward proteomics and/or metabolomics studies and/or hypothesis-based studies to define pathogenic features of COPD.
- Urine will be analyzed for cotinine to assess participants' smoking status.

6.4.5.5. Human Subjects' Protection and Risks

6.4.5.5.1. Blood draws

- Veins vary in size from one participant to another and from one side of the body to the other. Obtaining a blood sample from some people may be more difficult than from others.
- Other risks associated with having blood drawn are slight but may include:
    - Excessive bleeding
    - Fainting or feeling light-headed
    - Hematoma (blood accumulating under the skin)
    - Infection (a slight risk any time the skin is broken)
    - Discomfort related to needle stick
    - Rarely, injury to adjacent structures

6.4.5.5.2. Urine

- Urine collection is a benign procedure not associated with risk to the participant.
- Pregnancy testing may reveal unknown pregnancy, which will need to be appropriately communicated to the participant following strict confidentiality protocols.

6.4.6. Sputum Induction

6.4.6.1. Background and rationale

Sputum induction provides an opportunity to directly measure biomarker activity within the lung. These biomarkers are suggestive of disease mechanisms and include mucociliary clearance (e.g., mucins, regulators of airway surface liquid), inflammation (e.g., cell counts, cytokines, and chemokines), infection (e.g., microbiome), oxidative stress, and protease/anti-protease activity.

SPIROMICS will analyze and process induced sputum using the "whole sputum" method as opposed to the "select sputum" method, which involves manually removing sputum plugs from the sample. "Whole sputum" is defined as the raw, unaltered, total expectorated secretions collected from the participant at the conclusion of the induction. It contains whatever secretions were expectorated by the participant.

Sputum induction is first attempted at the baseline study visit. If for some reason the sample is not collected at baseline, additional attempts to collect the sample will be made a subsequent

visits. Participant must produce enough sputum to complete a cytospin slide in order for the collection to be considered "complete."

6.4.6.2. Inclusion/exclusion specific to procedure

6.4.6.2.1. Inclusion criteria

There are no additional inclusion criteria.

6.4.6.2.2. Exclusion criteria

- Participants with a known intolerance to albuterol or salmeterol
- Participants with a known history of poor outcomes with sputum induction
- Participants with a post-bronchodilator FEV_1 of <35% during screening

6.4.6.3. Methods

6.4.6.3.1. Instructions prior to commencing procedure

To conduct a sputum induction participants must not eat or drink anything (except water) for at least two hours prior to beginning the induction. Participants must also have been able to perform three reproducible flow-volume curves during the pulmonary function testing portion of the study visit. For participants at risk of bronchospasm, the post-bronchodilator values are acceptable.

Participants should be coached in the proper technique for saline inhalation, coughing, and nasal/oral/pharyngeal cleansing (see MOP 5 – Sputum Induction and Processing for specific instruction).

For induced sputum, if COPD subjects have not had albuterol in the prior 165 minutes then they should be re-dosed with 2 puffs of albuterol. Participants who are non-smokers or smokers without obstruction do not need albuterol for the sputum induction. The investigator should be contacted if a participant reports having asthma and would otherwise not receive albuterol. Please see Section 6.4.1.4 for bronchodilator dosing and re-dosing instructions.

6.4.6.3.2. Instructions for induction:

Induction requires approximately 20 minutes to complete once the participant has begun saline inhalation. Saline concentrations should be adjusted based on whether the participant's initial FEV_1 post albuterol is less than or greater than 50% predicted. Participants with a post albuterol FEV_1 of > 50% predicted will begin with 3% saline and participants with a post albuterol FEV_1 of < 50% and > 35% predicted will begin with 0.9% saline. Spirometry is repeated during the induction process at one, two, five, and seven minutes (FEV_1 <50% predicted) or two and seven minutes (FEV_1>50% predicted). Adjustments to the saline concentration can be made if a participant's FEV_1 falls more than 10% during the course of the induction. Please see MOP 5 for additional details.

6.4.6.3.3. Procedure Termination:

The procedure is terminated 1) if the FEV_1 falls by > 20% at any time point, or 2) if the participant requests that the procedure be stopped, or 3) after three seven-minute inhalation periods have been completed.

6.4.6.4. Instructions for sample collection

6.4.6.4.1. Sputum

Samples will be processed dependent on the type of analyte of interest. For assessments that do not need to be completed immediately, the processed samples will be aliquoted for storage at -80° C. Please see MOP 5 for additional details.

6.4.6.5. Measurements

The current protocol for sample processing will allow a variety of different measurements, including:

1) Mucin/water content including for example total mucin concentration, muc5AC and muc5B contributions to total mucins, mucus rheologic parameters, mucin complex discovery proteomics-mass spectrometry, and viscosity
2) Regulators of mucus water/mucin content such as nucleotides and nucleosides
3) Microbiology including both bacteria and viruses
4) Cells, for example inflammatory/respiratory epithelial cell numbers
5) Soluble biomarkers.

Please see discussion in other sections of the protocol for determining the markers to test in biological fluids (see Section 4.2.7) including cytokines, PGP, and discovery proteomics.

6.4.6.6. Human Subjects' Protection and Risks

6.4.6.6.1. Risks with hypertonic saline

Inhalation of hypertonic saline for sputum induction may result in wheezing, coughing, or chest tightness, particularly in susceptible individuals such as those with asthma. Asthmatic participants will be pre-medicated with albuterol in order to minimize this risk. Spirometry will be evaluated for all participants before induction as well as at prescribed intervals during each of the three levels of hypertonic saline. Participants may also experience transient throat irritation during the hypertonic saline inhalation but this generally resolves post-induction when the participant is provided with a snack and juice or water.

6.4.6.6.2. Risks with albuterol

See section 6.4.1

6.4.7. Medical History and Questionnaires

6.4.7.1. Medical History

A detailed medical history will be collected on each participant, including history of lung disease, cardiovascular disease, and cancer. Participants will be asked about their respiratory health history, including smoking status, second hand smoke exposure, as well as possible occupational and environmental exposures. Participants will also be asked to provide a medication list. The study coordinator will administer this form.

6.4.7.2. Questionnaires

6.4.7.2.1. St. George's Respiratory Questionnaire for COPD Patients (SGRQ-C)

The St. George's Respiratory Questionnaire for COPD Patients (SGRQ-C) is the recently revised and shortened form of the well-established The St. George's Respiratory Questionnaire. The SGRQ-C is targeted for patients with COPD and reduces the SGRQ from 50 questions to 40. It retains its score domains (symptoms, activity, and impacts) as well as a total score, and remains highly correlated with the original assessment (Merugo et al, 2007). Questions are either dichotomous (true/false) or Likert scale. Scores are weighted with a final score between 0 (no impairment) and 100 (worst possible health) (Merugo et al, 2006).

The SGRQ should be completed solely by the participant but a study coordinator should be available to answer questions (Jones, 2008). The original SGRQ takes approximately 10 minutes to complete, and it is expected the revised version will take no longer than this (Wilson et al, 1997).

6.4.7.2.2. Modified Medical Research Council (mMRC) Dyspnea Scale

The Modified Medical Research Council Dyspnea Scale (mMRC) is a five-item instrument to assess a patient's degree of breathlessness in relation to physical activity. Participants are asked to read each brief description of an activity and then select the statement that best describes their experience with dyspnea. This scale is used in calculating the BODE index, and takes approximately one minute to complete (Mahler et al, 1988).

6.4.7.2.3. Berlin Questionnaire

The Berlin Questionnaire is a 14-item instrument used to predict a patient's risk of sleep apnea. The questionnaire is grouped into three categories, and a participant is classified as "Higher Risk" if he or she scores positive in two of the three categories (Netzer et al, 1999). The assessment takes approximately five minutes to complete.

6.4.7.2.4. Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a 19-item questionnaire. The instrument is comprised of seven component areas: subjective sleep quality, sleep latency, sleep duration,

habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction. Each of the component areas are weighted on a 0-3 scale, are totaled for a final score of 0-21 with higher scores indicating poorer sleep. The questionnaire takes approximately 5-10 minutes to complete (Buysse et al, 1988).

6.4.7.2.5. Veterans Specific Activity Questionnaire

The Veterans Specific Activity Questionnaire (VSAQ) is a 13-item questionnaire. The instrument contains a list of daily activities, ranked from lowest metabolic equivalent (MET) value to highest MET value (1-13). The VSAQ is scored using a nomogram. Predicted exercise capacity is calculated based on the answer from the VSAQ and the participant's age (Myers et al, 2001). This questionnaire takes approximately 5 minutes to complete.

6.4.7.2.6. Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale (HADS) is a 14-item questionnaire. It has two subscales with seven questions each. One measures anxiety and the other measures depression (Funk et al, 2009). Each item is rated 0-3 resulting in scores between 0-21 for each subscale (Snaith, 2003). Scores >=8 in either subscale indicate possible depression or anxiety (Funk et al, 2009). The questionnaire takes approximately 2 to 5 minutes to complete (Snaith, 2003).

6.4.7.2.7. COPD Assessment Test

The COPD Assessment Test (CAT) is an eight-item questionnaire. These eight questions cover cough, phlegm, chest tightness, breathlessness going up hills/stairs, activity limitation at home, confidence leaving home, sleep, and energy. Each question is rated on a likert scale ranging from zero to five, with high scores indicating poorer COPD-related health status (Jones et al, 2009). The questionnaire take two to five minutes to complete.

6.4.7.2.8. MOT Short Form – 12

The Medical Outcomes Study Short Form – 12 (SF-12) is a twelve-item questionnaire. The questions cover eight health concept areas (Physical Functioning, Role Physical, Role Emotional, Mental Health, Bodily Pain, General Health, Vitality, and Social Functioning). Questions are either dichotomous (yes/no) or likert scale. Questions are scored on a weighted scale. This questionnaire take approximately 10 minutes to complete (Ware et al, 1996).

6.4.7.2.9. Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F)

The Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) questionnaire is a 40-item scale used to assess fatigue in the setting of chronic illness (Cella et al, 2005). The questionnaire covers five domains, and responses are graded on a 0 to 4 likert scale. Higher scores indicate better quality of life (i.e., less fatigue).

6.4.7.2.10. Questionnaire for Ease of Cough and Sputum Clearance

The Questionnaire for Ease of Cough and Sputum Clearance is an 8-item scale to assess participant perception of the severity and frequency of cough symptoms. Items are graded on a 1 to 5 scale with higher scores indicating worse symptom perception (Rubin et al, 1996). This questionnaire will be administered as part of the sputum induction.

6.4.7.3. Inclusion/exclusion specific to procedure

There are no additional inclusion or exclusion criteria for completing the study questionnaires.

6.4.7.4. Methods

1) Medical History should be collected during the first block of study procedures as it determines, in part, participant eligibility. Study coordinators should administer the medical history form, recording participants' answers directly into the data management system (DMS). Please refer to the MOP 1 – Clinical Center Procedures and Question by Question instructions for more details on how to answer participant questions and probe for more information.

2) The study questionnaires can be administered in blocks as outlined in MOP 1.

All questionnaires will be interviewer administered, and these questionnaires should be completed by the participant, and the data directly entered by him or her into the DMS. It is important that only the participant is answering the questions. If a family member or friend is present he or she should not assist in completing the instruments.

6.4.7.5. Measurements or Assessments

1. Social, Medical, and Exposures History
2. Health-related Quality of Life
3. Dyspnea
4. Risk of sleep Apnea
5. Sleep Quality
6. Predicted Exercise Capacity
7. Anxiety and Depression

6.4.7.6. Human Subjects' Protection and Risks

The expected risks associated with completing study questionnaires are low. Potential risks include potential discomfort and embarrassment in answering questions. The study coordinator will make effects to provide a safe, reassuring atmosphere and will emphasize that the participant does not have to answer any question he or she feels uncomfortable with.

6.4.8. Bronchoscopy Substudy

6.4.8.1. Background and Overview

Bronchoscopy with bronchoalveolar lavage, epithelial brushings and bronchial biopsies, can be used to directly assess the biology of the lung. The goal of our bronchoscopy sub-study will be to bank samples for future analyses using the modalities described below. We propose to study a subgroup of the overall SPIROMICS subjects, comprising 50 subjects per site (total n=300). Our strata enrollment will deviate slightly from that of the parent study because we have two additional specific goals: 1) to exclude subjects with very low FEV1 for safety (<30% predicted) and 2) to oversample non-smoking healthy controls and smokers with normal lung function to ensure a large enough control groups for sample analysis.

6.4.8.2. Bronchoscopy Enrollment Strata

	Non-Smokers (Stratum 1)	Smokers (Stratum 2)	Mild/Moderate COPD (Stratum 3)	Severe COPD (Stratum 4)
Smoking Status	< 1 pack-year	> 20 pack-years	> 20 pack-years	> 20 pack-years
Bronchodilator Status for Assessing Lung Function	Pre-bronchodilator	Post-bronchodilator	Post-bronchodilator	Post-bronchodilator
FEV1/FVC ratio criteria	FEV1/FVC > .7	FEV1/FVC > .7	FEV1/FVC < .7	FEV1/FVC < .7
Other Lung Function Criteria	FVC>LLN	FVC>LLN	FEV1 > 50% pred.	FEV1 < 50% pred.
Sample Size	N = 60 (20%)	N = 100 (33%)	N = 120 (40%)	N = 20 (7%)

6.4.8.3. Inclusion/exclusion specific to procedure

6.4.8.3.1. Inclusion criteria

Subjects enrolled in the SPIROMICS who consent to a research bronchoscopy and who meet all local requirements for a bronchoscopy (e.g., any laboratory tests that are required by institutional policy to be administered prior to a bronchoscopy), are eligible for this substudy.

6.4.8.3.2. Exclusion criteria

1. Age>80
2. History of cardiac disease or other comorbid condition severe enough to significantly increase risks based on investigator discretion.
3. Pao2/Sao2 that qualifies them for supplementary oxygen at rest (PaO2< 60 or SaO2<88%)
4. Post bronchodilator FEV1<30% predicted

5. Use of anticoagulation (patients on warfarin or clopidogrel will be excluded, patients on aspirin alone can be studied even with concurrent use)

6.4.8.4. Methods

Substudy will be conducted during two separate visits. The first visit will be for sputum induction in order to obtain a sputum sample for immunophenotyping (which will be compared to immunophenotyping performed in blood and BAL during the bronchoscopy visit). The second visit for the bronchoscopy, and must be scheduled at least 2 weeks but not more than 12 weeks after the sputum induction visit.

6.4.8.4.1. Sputum induction

Sputum should be induced on a separate visit using the methods described in MOP 5 – Sputum Induction and Processing. Subsequent processing and shipping should be performed as described in Section 7.0 of the Bronchoscopy MOP.

6.4.8.4.2. Bronchoscopy

The preferred order and locations of bronchoscopic procedures are as follows:

1. Blood draw for blood immunophenotyping, EDTA plasma, and CBC with differential
2. Mouthwash and tongue scraping (for microbiome analyses)
3. Protected brush specimen(s) in a lower lobe bronchus (3 brushes)
4. BAL/wash (approach: 20cc x1 [repeat if no return]/then 2x40cc, 1x50cc into one segment), manual or "low wall" suction. Repeat both the BAL and wash in a second segment.
5. Cytological brushes x 3 in ipsilateral lower lobe bronchi
6. Optional small airways brushings
7. Endobronchial biopsies in the contralateral lung (up to 8 adequate biopsies)

<u>Detailed Methods:</u>

1. 2 10ml tubes of blood will be drawn. One Heparin tube for immunophenotyping and an EDTA plasma tube. (described in MOP4)

2. CBC with differential should be drawn as well for normalization of immmunophenotyping dataCollect 2.5mL of sterile saline and transfer to 15mL conical tube containing 10mL of RNAlater. Flush another 2.5mL of sterile saline through the bronchoscopy prior to the start of the bronchoscopy and transfer to a second 15mL conical tube containing 10mL of RNALater.

3. Protected brush specimen(s) in a lower lobe bronchus (3 brushes)

4. These protected brushes will be used for microbiome analyses (will be initially processed in RNAlater and later undergo extraction of both RNA and DNA)

5. BAL/wash (approach: 20cc x1[repeat if no return]/then 2x40cc, 1x50cc into one segment). The first 20cc will be processed as an "airway wash specimen". The remaining aliquots will be used as "BAL specimens":

    a. Microbiome analyses (airway wash specimen and BAL specimen)
    b. Cell count, cytospin preps for cell differential (BAL and wash) and additional cytospins for future studies (BAL specimen)
    c. Supernatant (multiple aliquots, BAL and wash specimen)
    d. Alveolar macrophage isolation for RNA (miRNeasy)
    e. Additional cell pellet for Immunophenotyping

6. Cytological brushings x 3 in ipsilateral lower lobe bronchi. These brushings will be used for:
    a. Cell count, cytospin preps for diff
    b. Cell pellets for RNA (miRNeasy)

7. Optional small airway cytological brushings x 12. These brushings will be used for:
    a. Cell count, cytospin preps for diff
    b. Cell pellets for RNA (miRNeasy)

8. Endobronchial biopsies in the contralateral lung (up to 8 adequate biopsies).
    a. Two of these biopsies will be snap frozen and homogenized for RNA (miRNeasy).
    b. Six will be prepared for histology, quantitative morphometry and IHC

6.4.8.5. Specimens Collected

1. Blood for immunophenotyping and CBC with differential cell count
2. Tongue Scraping
3. Oral Rinse
4. Saline controls
5. Bronchial Wash
6. BAL
7. Epithelial Brushes (large airway brushings required, distal airway brushing optional)
8. Endobronchial Biopsies

6.4.8.6. Primary outcome(s):

The primary goal of the Bronchoscopy sub-study is to collect lung specimens that are optimally prepared for the following biological analyses. The scientific goal will be to use these samples in the main SPIROMICS study or in ancillary studies to phenotype patients with COPD at a molecular level.

- Characterization of inflammation

a. BAL Cell type and differential, multicolor flow cyometry (Immunophenotyping substudy)
b. Alveolar macrophages gene expression (incl miRNA)
c. BAL supernatant

Approach to analysis: Analyses of cellular and soluble markers of inflammation should identify subgroups of subjects with specific patterns of inflammation that may be the target of specific therapies.

- Analyses of resident lung cells
    a. Epithelial cell gene expression (incl miRNA)
    b. Bronchial biopsy gene expression (incl miRNA)

Approach to analysis: Genomic analyses or resident lung cells have the potential to distinguish subgroups of patients based on unsupervised and supervised methods of clustering and to identify wholly novel mechanisms of disease.

- Microbiome analyses
    a. For oral, airway and distal lung microbiome

Approach to analysis: Microbiome analyses will permit unbiased phenotyping of subjects with respect to bacterial colonization generally as well as with respect to specific patterns of colonization.

- Analysis of bronchial biopsies
    a. Assessment of pathology and quantitative assessment of airway remodeling
    b. Immunohistochemistry and in situ hybridization

Approach to analysis: Quantitative assessment of airway mucin stores and fibrosis can serve as a pathological measure of chronic bronchitis or remodeling for comparison with radiographic imaging. Immunohistochemistry and in situ hybridization will allow protein validation of findings made in genomics studies.

6.4.8.7. Sample size considerations

The Bronchoscopy sub-study plans to recruit 100 participants from the healthy controls stratum, 100 from the smokers without COPD, 120 from the mild/moderate COPD stratum and 20 from the severe COPD stratum. We consider power for three potential sub-group comparisons:

(a) n=120 vs. n=20 (mild/moderate COPD vs. severe COPD)
(b) n=120 vs. n=100 (mild/moderate COPD vs. smokers without COPD)
(c) n=50 vs. n=50 (two potential sub-groups identified in SPIROMICS).

Estimation of the power for various analyses in Woodruff et al., A Distinctive Alveolar Macrophage Activation State Induced by Cigarette Smoking, would require substantial simulation modeling. However, the results in that manuscript suggest that the sample size in this

sub-study would be adequate to detect meaningful differences. For instance, in that study a sample size of 15 smokers and 15 nonsmokers had complete separation of the two groups when considering the pattern of expression of the 200 most variable genes in the human alveolar macrophages. In this small sample, when looking at individual genes, 110 were significantly differentially expressed between the two groups even after a Bonferroni correction for multiple testing. On the other hand, there were substantially smaller differences between the 15 nonsmokers and 15 asthmatics, with none of the genes differentially expressed between these two groups being among those that were differentially expressed in smokers and nonsmokers.

6.4.8.7.1. Power for difference in means for a continuous variable

Our original proposal for SPIROMCIS expressed power for a continuous variable (FEV1) in terms of standard deviation units. For instance, stating "With the proposed disease severity subgroup sample sizes, we would have approximately 98% power to detect a difference of 0.20 times the standard deviation in FEV1 change from baseline values for the comparison of mild versus moderate COPD patients (n=800 and 1,000, respectively), similar to that observed in TORCH."

For the three sub-group comparisons described above, the sizes of the difference detectable with 90% power are (a) 0.79, (b) 0.44, and (c) 0.65 standard deviation units, respectively.

6.4.8.7.2. Power for difference between two proportions (that is, of a dichotomous variable)

The power to detect a difference in proportions depends not just on the difference but also on the proportion in the reference group. The table below gives the size of the difference detectable with 90% power for various values of the proportion in the reference group.

(a) n=120 vs. n=20

Proportion in reference group	0.05	0.10	0.15	0.20	0.25	0.30
Difference detectable with 90% power	0.27	0.31	0.34	0.36	0.37	0.37

(b) n=140 vs. n=60

Proportion in reference group	0.05	0.10	0.15	0.20	0.25	0.30
Difference detectable with 90% power	0.14	0.17	0.19	0.20	0.21	0.22

(c) n=50 vs. n=50

Proportion in reference group	0.05	0.10	0.15	0.20	0.25	0.30
Difference detectable with 90% power	0.24	0.27	0.29	0.30	0.31	0.32

In summary, the sub-study will be able to detect moderately large differences between groups. A primary aim of SPIROMICS is to refine classification of COPD patients, that is, being able to group them into distinct phenotypes. If meaningful distinctions do exist, they should be reflected in substantive differences in the underlying biology. Thus the sub-study should have adequate power to detect effects that are of a magnitude relevant to the goals of SPIROMICS.

6.4.8.8. Human Subjects Protection/Risks

The presence of COPD has been shown to increase the complication rate of bronchoscopy (where FEV1/FVC <50% or FEV1 <1 liter and FEV1/FVC <69%) (1). A complication rate of 5% occurred in the patients with severe COPD compared with 0.6% in those with normal lung function. The use of sedation in patients with severe COPD has increased risk relating to potential carbon dioxide retention. With this in mind, the Spiromics investigators are committed to optimizing safety through both judicious application of exclusion criteria and the institution of safety monitoring throughout the procedure. With similar procedures, research bronchoscopy has indeed been performed in large multi-center studies such as FORTE (2).

6.4.8.8.1. Potential Risks and Protections against those Risks.

The following are risks associated with the sputum induction and associated procedures:

1) Spirometry – Spirometry is generally low risk. Risks include breathlessness, cough, and dizziness, all of which are brief. Very occasionally, leakage of urine (from straining) or fainting can occur.

2) Sputum Induction – Breathing in the hypertonic saline (salty solution) may result in wheezing, coughing, or chest tightness, particularly in some people, such as those with asthma. In order to minimize this risk, subjects will be medicated with albuterol before beginning this test. In addition, spirometry values will be evaluated before induction as well as at prescribed intervals during each of the three levels of hypertonic saline, in order to reduce these risks. One may also experience temporary throat irritation while breathing in the saline solution but this generally gets better once the induction is over.

3) Risks associated with albuterol and ipratropium – As part of this visit subjects may be asked to inhale albuterol (a bronchodilator) and ipratropium (an anticholinergic). The side effects of albuterol have been reported to be rash, swelling, hives, paradoxical bronchospasm, angina (chest pain), arrhythmias (irregular heartbeat), high blood pressure, low blood potassium, seizures, tremor, nervousness, headache, fast heartbeat, muscle cramps, palpitations, insomnia, and dizziness. Ipratropium has been reported to cause cough, nausea, dry mouth, dizziness, headache, shortness of breath, irregular heartbeat, fast heartbeat, paradoxical bronchospasm, throat spasm, swelling, severe allergic reaction (anaphylaxis), and exacerbate narrow-angle closure glaucoma.

The following are risks associated with the bronchoscopy and associated procedures:

<u>Risks of Bronchoscopy:</u>

Discomfort: subjects may experience minor discomfort associated with the urge to cough when the saline solution is instilled during BAL.
Bronchoalveolar lavage: Bronchoalveolar lavage can induce coughing in some patients but is otherwise well-tolerated.

Bronchial brushings: Bronchial brushings are less likely to induce coughing but can cause a small amount of bleeding from the airway wall that is self-limited.

Endobronchial biopsies: Bronchial biopsies are usually accompanied by a mild tugging sensation with each biopsy, and may also cause some bleeding from the airway wall that is usually minor but may be noticed as blood streaking of sputum by the subject. The incidence of moderate or severe bleeding following biopsy is less than 1% in patients with normal blood clotting function. Serious bleeding resulting in the need for transfusion, serious breathing problems, or death have been very rare and have usually been confined to patients with an underlying bleeding disorder or in the setting of hematologic malignancies (cancers of the blood, bone marrow, or lymph nodes), or with low platelet (cells responsible for helping clot the blood) counts. Subjects will be monitored for bleeding as a part of the bronchoscopic procedure. A pneumothorax can result from the rupture (breaking) of lung tissues allowing air to enter the space surrounding the lung (pleural space) causing partial collapse of the lung. This complication, though potentially serious, occurs rarely (about 1 in 200 patients) when experienced personnel perform the bronchoscopic procedure. Furthermore, the anatomic location of the biopsies we propose here (at carina) makes them far less likely to cause a pneumothorax than transbronchial biopsies.
Mucosal inflammation: the mucosa is the lining of the airways. Mucosal inflammation (swelling) can occur when the bronchoscope is wedged into the airway. Although this can be seen at bronchoscopy, no clinically important consequences have been observed (that is, there were no serious or long-term problems).

Fever: a transient self-limited fever can occur after BAL. Fever lasting more than 24 hours requires further evaluation for a pneumonia that is a rare complication of bronchoscopy.
Infection of your lungs: Bronchitis or pneumonia can occur in the first days or even week after bronchoscopy. Bronchitis or pneumonia would be treated with antibiotics.
Shortness of breath and wheezing: The bronchoscopy procedure may cause one's lung disease (if one has any) to be worse in the days immediately after the procedure. If this occurs subjects will communicate with the investigators about whether another visit and/or increase in medications is necessary.

Sore throat: This usually occurs right after the bronchoscopy and can last for up to a day. Throat lozenges often help to treat this discomfort.

Vocal cord spasm: This can occurs very rarely when the bronchoscope is passed through the vocal cords. If it happens the bronchoscope will be removed, and the subject will be treated with Albuterol and given oxygen. The spasm will resolve by itself.

Risk of abnormal findings: The most likely abnormal result will be the unexpected finding of a visible abnormality that might be cancer or another important medical condition. If an abnormal

finding is seen, we will inform the subjects and their regular doctor. Such a finding might lead to additional tests at the recommendation of that doctor. There is the risk that such an abnormality, after testing or treatment, is found not to be disease-causing. Such findings may result in unnecessary anxiety for the subjects could expose the subject to the risks associated with those additional tests.

Risks of Associated procedures:

Spirometry: Spirometry will be done before and after the bronchoscopy. The risks are described above.

Albuterol: Four puffs of albuterol will be given before the bronchoscopy and may be given after the bronchoscopy if the subject requires it. The risks of albuterol are described above.
Lidocaine: lidocaine is the medicine used to suppress one's gag response and induce local anesthesia for the bronchoscopy procedure. To guard against lidocaine toxicity, we will limit the total lidocaine dose to less than 600 mg or 9mg/Kg, whichever is less.

Benzodiazepines: Intravenous midazolam is administered to decrease anxiety, this drug may cause hypotension and excessive drowsiness. If midazolam is required for management of a strong gag reflex or to prevent excessive use of lidocaine, the subject will be discharged only after the immediate effects of the medication have resolved to the satisfaction of the local PI and the subject will be accompanied home. Subjects have an intravenous line in place during the procedure and intravenous flumazenil is available at bedside to reverse the effects of midazolam if needed. All locally required procedures for conscious sedation will be followed.

Opiate: Intravenous fentanyl is administered to decrease cough and pain, these drugs may cause respiratory depression, sedation, hypotension, and rarely adverse reactions such as hives. Investigators will monitor blood pressure, heart rate, and pulse oximetry throughout the bronchoscopy and recovery period. Intravenous naloxone is available at bedside to reverse the effects of opiates if needed. All locally required procedures for conscious sedation will be followed.

Intravenous catheter: A small teflon tube will be inserted into the subject's vein (intravenous catheter) so that medications can be given to you through your vein. In addition, you will be asked to give a blood sample for for the collection of samples that we will analyze for research (approximately 10 mls of blood) which can usually be done by using this IV tube.

6.5. Participant Follow-up

6.5.1. Quarterly Phone Calls

After the Baseline Visit participants will be contacted by phone on a quarterly basis. Using an event ascertainment form, these calls will assess whether a participant has had an exacerbation episode within the last three months. Participants will not be contacted by phone when the timing of a quarterly follow-up coincides with an in-person visit. These contacts will provide a count of self-reported exacerbations within the SPIROMICS population.

6.6. Safety and Adverse Events

6.6.1. Participant Safety

It is expected that some participants will experience changes in disease severity, will develop comorbidities to COPD, and/or will expire during the course of participating in SPIROMICS. SPIROMICS staff will provide appropriate referrals for medical care if during study visits clinically relevant changes in disease status are found. SPIROMICS is not a treatment trial and as such will not report the above listed events as adverse or serious adverse events as they are considered the natural progression of COPD.

Adverse events that result directly from study procedures, including those risks outlined in each procedure section and any not listed in this document, will be reported to the Steering Committee and OSMB.

6.6.1.1. Procedures for Serious Adverse Events

The FDA (2009) defines a serious adverse event as an adverse event that:

- Results in the participant's death,
- Is life-threatening,
- Results in Hospitalization (initial or prolonged),
- Results in significant, persistent, or permanent change, impairment, damage, disruption, or disability in the participant's body function/structure, physical activities or quality of life,
- Results in a congenital anomaly, or
- Requires Intervention to Prevent Permanent Impairment or Damage.

If a serious adverse event (SAE) occurs during a study visit the study coordinator should first insure the participant receives any needed medical attention. If the study coordinator is notified by phone, the coordinator should confirm that the participant has received medical attention. The study coordinator should then notify the site Principal Investigator and complete the Adverse Event form found in on the study website. For serious adverse events the study coordinator should submit the form to the GIC within 48 hours of learning of the event. Study coordinators should comply with local regulations and policies when notifying the institutional IRB.

The GIC will notify the Project Officer, Steering Committee, and Observational Studies Monitoring Board within 48 hours of receiving the initial event notification.

6.6.1.2. Procedures for Adverse Events

Adverse events are undesirable experiences resulting from participation in the SPIROMICS study (FDA, 2009). Common adverse events are listed in the risks section of each procedure.

If an adverse event (AE) occurs during a study visit the study coordinator should first insure the participant receives any needed medical attention. If the study coordinator is notified by phone,

the coordinator should confirm that the participant has received medical attention. The study coordinator should then notify the site Principal Investigator and complete the Adverse Event form found in MOP 1. For adverse events the study coordinator should submit the form to the GIC within seven days of learning of the event. Study coordinators should comply with local regulations and policies when notifying the institutional IRB.

The GIC will notify the Project Officer, Steering Committee, and Observational Studies Monitoring Board within a week of receiving the initial event notification.

6.6.2. Measures to Protect the Participant

Some of the procedures performed during a SPIROMICS have known potential risks. A number of safety measures are in place to minimize these risks. During the baseline interview participants will be screened for conditions that might increase their risk while participating in these procedures, including history of heart disease, heart failure, or recent myocardial infarction. In addition, clinical center staff will discuss the risks associated with each of these procedures with participants during the screening process and before each procedure begins.

If a participant experiences a condition requiring immediate medical attention, such as a cardiac event, angina, acute hypertension the research physician will be consulted immediately and the visit terminated.

6.6.2.1. Emergencies

In the event of a life-threatening emergency a participant will require immediate transport to an acute care facility however clinical center staff may need to implement some emergency measures prior to transport. Many minor emergencies will require only onsite treatment.

6.6.2.2. Major Emergencies

Each clinical center will establish procedures for handling major medical emergencies including plans for how to transport a participant to the nearest medical facility. These procedures will define:

1) Who is in charge during an emergency
2) Who is to administer treatments
3) Who is to be notified
4) What action clinic staff is to take
5) Which reports are to be filed

Clinical centers will have access to either a physician, physician's assistant, or a registered nurse any time a participant is being interviewed or participating in a procedure. Each center has, in additional to trained personnel and emergency equipment, the phone numbers for policy and fire stations, ambulance services, and specific phone numbers or codes to alert medical teams (if applicable) posted in conspicuous places. In each participant's record contain contact information for his or her primary care physician, home or work numbers, and one or more emergency contacts.

6.6.2.3. Minor Emergencies

Minor emergencies are those not requiring emergent medical care, and can be handled at the clinical center. Common minor emergencies include syncope and dyspnea and clinic staff is trained in the appropriate response. While a minor emergency may not require physician care, study staff is to alert the research physician present to the event.

6.6.2.4. Reporting

All emergencies, major or minor, are documented. Documentation includes completing institutionally approved forms identifying the emergency as well as reporting the event to the Project Office and GIC. This form is to be completed by the person in charge at the time of the emergency, and all reports are co-signed by the clinic physician.

6.7. Clinical Endpoint Ascertainment

6.7.1. All cause mortality

6.7.1.1. Death report during follow-up call

Death is an endpoint of interest that will be analyzed as part of this phenotyping protocol. The opportunity to assess vital status will naturally occur during regularly scheduled study visits and follow-up phone calls. During the course of routine follow-up contact, if the study coordinator learns from a proxy that the participant is deceased, the coordinator should offer condolences and request that the proxy provide information regarding the circumstances of the participant's death. Records pertaining to a participant's death will be requested and sent to the GIC. Data will be reviewed and cause of death assigned to one of the following causes: respiratory, cardiovascular, cancer, other. The data will then be reviewed by at least two members of the Morbidity and Mortality Committee not associated with the institution where the death occurred and a final cause of death assigned according to the Principles of Adjudication. If the two committee members disagree on cause of death, a third member of the committee will review death records and cause of death will be assigned based on majority consensus.

6.7.1.2. National Death Index

The National Death Index will be annually searched to determine if any participants lost to follow-up have died. If a participant is found on this list, the death certificate and any associated hospitalization records will be requested, and the Death CRF submitted. The National Death Index will also be queried at the completion of the study.

6.7.2. Exacerbations

6.7.2.1. Definition

An acute exacerbation of COPD will be defined as "a sustained worsening of the patient's respiratory condition, from the stable state and beyond normal day-to-day variation, that is acute

in onset." Acute exacerbations will be graded according to the following scale:

1. Mild (requiring home management, with or without contacting a health care provider)
2. Moderate (requiring a visit to a health care provider or Emergency Department but not requiring hospitalization)
3. Severe (requiring hospitalization)

Patients will be queried at every point of contact regarding mild, moderate, and severe exacerbations and regarding the type of treatment received for each exacerbation, in particular antibiotics and/or steroids. Source documentation will be requested for severe exacerbations only (see below).

6.7.2.2. Hospitalizations

At all scheduled visits and during follow-up interviews, participants will be asked to identify any hospitalizations that have occurred since the last contact. Medical records associated with a self-reported hospitalization play a vital role by providing outside, professional assessment of a participant's health status. In particular, these records serve to identify or verify exacerbations. When a participant reports an admission to the hospital, the study coordinator will ask the participant to release the medical records from that visit for review. Records from all hospitalizations will be obtained by the local site and sent to the GIC for abstraction and review by the Morbidity and Mortality Committee.

6.7.2.3. Emergency Department Visits and Unscheduled Office Visits

Emergency Department Visits and Unscheduled Office Visits will be considered together as location of care, for these types of services may differ regionally. At all scheduled visits and during follow-up interviews, participants will be asked to identify any Emergency Department visits or unscheduled office visits that have occurred since the last contact. Participants will also be queried as to whether they were treated with antibiotics, steroids, or both as part of a scheduled office visit or received any new medical diagnoses. The purpose of these queries will be to determine whether a COPD exacerbation has occurred or notable comorbidity developed. Cardiac comorbidity and the development of cancer will be of key interest. It is at the discretion of the local PI or designated CoI whether medical records need to be obtained in order to verify occurrence of an exacerbation or notable comorbidity. Medical records related to Emergency department visits or office visits that do not result in a hospitalization will not be submitted to the GIC for abstraction and will not be adjudicated.

6.7.3. Lung Transplantation

Lung transplantation is also an endpoint of interest that will be analyzed as part of this protocol. During the course of routine follow-up contact, if the study coordinator learns that the participant has undergone a lung transplant, the study coordinator will ask the participant to release the medical records from that hospitalization which will be submitted to the GIC along with the Hospitalization CRF.

7. Statistical Plan

7.1. Sample Size and Power

Thirty-two hundred (3,200) participants will be recruited and enrolled at six clinical centers into four strata (non-smokers, smokers, mild/moderate COPD, severe COPD) as illustrated in Table 1. The sample size requirements for the study are to (1) provide an adequate number of adverse clinical events (e.g., exacerbations, all-cause mortality) over three years of follow-up and (2) sufficient precision in estimating decline in FEV_1 over three years of follow-up in order to support the study objectives of discovering novel, homogeneous subgroups of COPD and identifying potentially useful, intermediate outcomes of disease progression.

Table 1. SPIROMICS Enrollment Strata

	Non-Smokers (Stratum 1)	Smokers (Stratum 2)	Mild/Moderate COPD (Stratum 3)	Severe COPD (Stratum 4)
Smoking Status	< 1 pack-year	> 20 pack-years	> 20 pack-years	> 20 pack-years
Bronchodilator Status for Assessing Lung Function	Pre-bronchodilator	Post-bronchodilator	Post-bronchodilator	Post-bronchodilator
FEV1/FVC ratio criteria	FEV1/FVC > .7	FEV1/FVC > .7	FEV1/FVC < .7	FEV1/FVC < .7
Other Lung Function Criteria	FVC>LLN	FVC>LLN	FEV1 > 50% pred.	FEV1 < 50% pred.
Sample Size	N = 200 (6.25%)	N = 900 (28.13%)	N = 1500 (46.88%)	N = 600 (18.72%)

Data on event rates for all-cause mortality in the COPD population are available from the TORCH study, a clinical trial comparing salmeterol plus fluticasone propionate versus placebo and each therapy alone over a three-year period (Calverley, et al., 2007). Below we use the TORCH results to provide a lower bound on plausible differences between hypothetical subgroups of COPD patients with respect to morbidity/mortality for purposes of estimating power. In addition, where possible, estimates of FEV_1 change over time are derived from published findings for power analyses. Estimates of the power available for comparisons of the Mild/Moderate versus Severe COPD enrollment strata are provided for illustration purposes, as sizes of novel subgroups yet to be discovered are unknown at this time. All statistical tests will be two-sided, with Type I error alpha=0.05 after controlling for multiple testing.

For the TORCH study, all-cause, three-year mortality rates ranged from 15.2% among placebo patients to 12.6% in the combination therapy group. Results from Anthonisen et al. (1986) found

that the three-year mortality was approximately 25% for patients with FEV_1 in the range 30%-39% , and approximately 13% for those with FEV_1>50%. More recently Celli et al. (2004) reported approximately 18% three-year mortality for patients with FEV_1>36% and 30% for patients with FEV_1<36%. We conservatively power the SPIROMICS study to detect a difference in all-cause mortality, assuming a 15% rate for Severe patients versus 10% for Mild/Moderate patients. With 1,500 patients in the Mild/Moderate COPD stratum vs. 600 patients in the Severe COPD stratum, we will have 88% power for this comparison.

For the TORCH study, the annual rate of exacerbations was 1.13 per participant for placebo versus 0.85 for combination therapy. In a computer model of COPD progression based on literature surveys, Borg et al. (2004) assumed an average annual rate of moderate to severe exacerbations of approximately 1.05 among COPD patients with GOLD status I/II, and a rate of 2.05 for exacerbations among COPD patients with GOLD status III. We conservatively power the SPIROMICS study to detect a difference in annual exacerbation frequency between the Mild/Moderate and Severe COPD strata, assuming an annual rate of 1.0 in the Mild/Moderate COPD stratum vs. a rate of 1.5 in the Severe COPD stratum. Using simulation and assuming a simple Poisson log-likelihood ratio test for difference in individual counts of exacerbations in the two strata and assuming 92% retention in both strata, we obtain power in excess of 95% to detect the differences.

For decline in $FEV1_1$, the TORCH study found differences in change from baseline FEV_1 values of 0.092 (L) between the combination therapy group and placebo (95% CI = [0.075, .108]) (Calverley et al, 2007). With the proposed disease severity subgroup sample sizes and assuming attrition due to mortality ranging from 10% in the Mild/Moderate stratum to 15% in the Severe stratum, and 92% retention in those surviving in each strata, we would have approximately 95% power to detect a difference of 0.20 times the standard deviation in FEV_1 change from baseline values (using a two-sample t-test on baseline vs. Year 3 FEV_1 in the two strata).

From the earlier conservative mortality assumptions, we expect 240 (11%) or more all-cause mortality events among the 2100 COPD patients during the course of SPIROMICS. For tests of direct association of potential surrogate endpoints/markers with this outcome, we consider for simplicity a dichotomized surrogate endpoint with relative frequency 0.50 for each value of the surrogate. In order for surrogate endpoint to demonstrate promising utility for further prospective validation in the context of a clinical trial, we propose that the surrogate confer an odds ratio of 1.8 or more. The correlation structure of the potential surrogates is not known a priori, so we use a significance level of 0.001 in order to account for multiplicity of testing (corresponding to a Bonferroni correction for 50 tests). Under these assumptions and assuming retention of 92%, a contingency table test of mortality vs. the surrogate will have 77% power to reject the null hypothesis.

7.2. Interim Study Progress Reports

7.2.1. Steering Committee Reports

Monthly management reports are prepared by the GIC and provided to the Steering Committee for monitoring of study progress. Tables and figures summarizing enrollment, completion of study procedures and visits, and baseline characteristics are included.

7.2.2. Observational Studies Monitoring Board (OSMB)

Yearly data reports are prepared by the GIC and provided to the OSMB for review. In addition to the material provided in the monthly management reports, data summaries integral to the ability of the study to address its primary objectives are included.

7.3. Analysis Plan

Data analyses will be conducted in SPIROMICS with the overarching goal of achieving the two primary study objectives, namely, the identification of homogenous subgroups of COPD patients and the identification of potentially useful intermediate outcomes, both of which can inform future therapeutic clinical trials with an emphasis on trials conducted for regulatory approval. The data planned for collection in SPIROMICS are extensive and involve fairly complex data structures. Numerous specific hypotheses directed at both general study aims will be evaluated. Detailed statistical analysis plans will be developed for each specific hypothesis describing the methods to be used for data analysis and addressing issues such as missing data and multiplicity of statistical testing.

Hypothesis # 1 -- Subgroup Identification: Three different approaches will be taken to subgroup finding. The first assumes that homogeneous subgroups can be defined in terms of multiple measurement domains (including clinical, radiological, proteomic, and genomic) through the application of statistical techniques, such as hierarchical cluster analysis, described in Sections 7.4.1 and 7.4.2. Discovery of novel clustering or classification schemes is the goal of analyses conducted with this approach, therefore it is difficult to specify a priori hypotheses to be evaluated. Examples, however, can be provided, such as:

- Clusters of participants will be defined based on genome-wide genetic, epigenetic, and gene expression data that share molecular pathways of disease and are therefore useful in guiding development of targeted therapeutic approaches.
- Homogeneous subgroups of patients will be identified according to their 'sputum footprint,' defined as mucin concentration, microbiome content, and neutrophil concentration, that will provide novel groupings of disease severity and therefore be useful for targeted enrollment in future clinical trials.

Because this approach involves discovery of novel subgroups rather than confirmation of a priori hypothesized subgroups, cross-validation methods to assess the predictive power of a particular subgroup classification scheme will be invoked.

The second approach to subgroup finding posits that individualized therapies based upon a patient's genotype are possible. DNA collection followed by either genome-wide association or specific genetic tests combined with statistical analyses will allow us to evaluate this hypothesis and to continue to unravel the correlation between underlying genetics and resultant phenotypes. Several genes have been recently reported to be associated with COPD phenotypes (see, e.g., Wood, Tan, and Stocklye, 2009 Genome Medicine 1:112). Concurrently run studies (e.g., the COPDGene Study (COPDGene Investigators, 2010)) will add to this list, and SPIROMICS will

be valuable in validating newly emergent findings from these studies. An example of a specific hypothesis under this approach is:

- SPIROMICS participants with SNPs previously associated with FEV_1 decline (CHRNA3/5, EPHX1, GSTP1, and HHIP) will have faster rates of decline in FEV_1 over three years than participants without those SNPs.

The third approach to subgroup finding suggests that components of COPD syndrome can be prospectively identified that are associated with a worse prognosis, as measured by morbidity over a 3-year period, when compared to participants without those components. There are many specific examples of this general hypothesis, including the following:

- Participants with apical emphysema at baseline will have more exacerbations resulting in a hospitalization over three years than those without apical emphysema.

Hypothesis #2 – Intermediate Outcome Identification: This general hypothesis can more specifically be stated as: SPIROMICS will identify intermediate outcome measures that predict long-term clinical endpoints of morbidity. Such outcomes may be markers of short-term change (1 year) that predict long-term change (3 years), or they may be markers that change with exacerbations and are predictive of more severe long-term morbidity/mortality.

For example:

- The novel molecular methodologies employed in SPIROMICS (including T-RFLP, phylochips, and deep-sequencing) will provide candidate markers that correlate with more frequent exacerbations and are therefore predictive of long-term morbidity/mortality.

Both Hypotheses #1 and #2 (and subsequent examples) are longitudinal, and therefore differentiate SPIROMICS from other studies that are purely cross-sectional studies. They are currently viewed as co-primary hypotheses. The statistical methods planned to address these and other hypotheses are described in detail in the follow subsections.

7.4. Statistical Methods

7.4.1. Phenotype Identification for Subgroup Analyses

The SPIROMICS study will gather data from a variety of measurement domains, yielding a very large number of observations per patient. Subgroups may be defined in terms of multiple measurements, including clinical, proteomic, genomic, and radiological measurements plus combinations thereof. To identify subgroups, we will initially examine the data without regard to study design strata or clinical endpoints. Therefore no testing multiplicity issues will arise, and a variety of statistical methods can be used to examine the data for evidence of cohesive groups for which the within-group variation is smaller than the overall variation. Continuous measurements will first be regressed on covariates such as age, gender, height, and weight and design stratum to obtain residuals, which will be carried forward into the subgroup analyses. The rationale for including design stratum as a predictor is that we do not wish to recapitulate subgroups that are essentially equivalent to strata that can already be used for future trial design.

As the model-based residuals involve only the use of main stratum effects, the identified subgroups may still be correlated with the design strata.

A variety of subgroup identification procedures are described in the subsection below. Each subgroup type will be described in terms of a "cohesiveness index," which is essentially a global description of the proportion of variation in the constituent measurements that is explained by the subgroup variable. Once cohesive subgroups have been identified, each patient will be assigned to a unique subgroup, using the appropriate distance metric for the subgroup identification procedure. For subgroup identification, we conceptually distinguish between subgroups that (i) do not correlate with study design stratum/COPD severity or with clinical endpoints, but which nonetheless may be predictive of treatment response in future clinical trials, and (ii) subgroups that do correlate with strata and/or clinical endpoints. We anticipate that the most useful subgroups are likely to be at least modestly associated with design strata and/or clinical endpoints, and so we will use such associations to further prioritize the set of potentially useful subgroups. The subgroup indicators will be tested for associations with the 4-strata design indicators using $k \times 4$ contingency table analysis. In addition, ordered logistic regression modeling will be performed, with design strata ordered as Non-smokers, Smokers, Mild/Moderate COPD, Severe COPD, and the subgroup indicators as predictors. Note that multiple types of subgroups may be fit in the same model, potentially with interaction terms to identify potent combinations of subgroups of different types (e.g. a particular combination of genomic and radiological measurements). Regression procedures will also be used to identify subgroup indicators that correlate with clinical endpoints, including mortality (proportional hazards regression), exacerbations (Poisson regression), and decline in FEV_1 (linear regression).

7.4.1.1. Subgroup Identification Procedures

Subgroup identification procedures will proceed under the assumption that most univariate clinical measurements have already been examined for potential correlation with outcome and potential utility in trial design. However, the utility of ensembles of measurements, along with new genomic and radiological measurements, is unknown. Thus we will proceed with techniques that are designed for high-dimensional settings, initially performing subgroup identification only within measurement "type" (e.g. clinical).

A basic tool for subgroup identification is hierarchical cluster analysis, for which we will perform agglomerative clustering using average linkage and correlation metrics. The identification of significant clusters (i.e. subgroups) can be a difficult task, although for parametric Gaussian cluster analysis the use of criteria such as Akaike Information can be used to determine the number of clusters when the sample size exceeds the number of observations. Bayesian approaches can also be used here, with probability-based model selection techniques and Bayes factors used to distinguish between models with different cluster sizes. Recently the SigClust procedure (2008) has been introduced for testing for cluster significance when the number of observations exceeds the sample size, and we will apply the procedure for high-dimensional measurement types.

A second technique is principal component analysis, which (for mean centered data) essentially follows from singular value decomposition of the data matrices. The use of principal

components to identify subgroups and control for stratification is a mature area in genomic analysis, and we will use similar ideas in SPIROMICS. A key aspect of principal components analysis lies in testing the significance of eigenvalues, and we will use the approach detailed in Patterson et al. (2006) to consider the number of eigenvectors/PCs that should be considered in subgroup identification.

As an adjunct to subgroup identification, we will also examine the various baseline measurements to attempt direct prediction of clinical endpoints, with design strata included as covariates. Although such procedures may not immediately identify the important patient subgroups, they will provide a list of measurements that may be further examined for defining and refining subgroups. The prediction techniques that will be used will include principal components regression, support vector machines, and penalized regression (LASSO and ridge regression). Prediction accuracy will be assessed by doing repeated 10-fold cross-validation using a large number of random data splits. The relative importance of individual measurements (as predictors) will be assessed by the proportion of cross-validation sets in which the predictor appears (for techniques which include variable selection), or by the average standardized coefficient across the cross-validation sets. Sets of important measurements will be further analyzed for evidence of subgroups, and considered in combination with previously identified subgroups.

7.4.2. Additional issues in genomic data analysis

Data from microarray datasets or other -omics technology datasets will follow standard manufacturer protocols for data generation. To obtain high quality measurements, we will use software that is considered standard for each platform, with a publication record to support its use. Examples include GCRMA (Wu et al, 2004) for Affymetrix expression arrays, or several packages in R/Bioconductor for expression analysis and genotype calling. The potential for batch effects will be considered and handled via regression methods for clinical site, as well as further correction for modest batch effects that may only be apparent from projections of high-dimensional data to low dimensions. We will employ global batch effect correction procedures such as ComBat (Johnson et al, 2007).

For simple exploratory analyses of genomic data we will employ standard two-way cluster analysis and dimensional reduction techniques (such as principal components) to visualize -omics expression measurements and provide initial views of the data structure. We will also employ iterative techniques, such as tight clustering (Tseng & Wong Biometrics, 2005), and randomization approaches designed to test the strength of cluster relationships. Pairwise correlation matrices will be examined across the genes (or a filtered set) and across arrays to identify unexpected features that are not reflected in the clustering. In certain analyses we will act to reduce the weight of genes that have high correlation with other genes, so as to appropriately reduce the influence of highly correlated groups of genes/samples that may otherwise dominate the final clustering. Grouping of genes with various profiles will also be accomplished using Self-Organizing Maps and graphed using various software packages. We will employ standard software for analysis of microarrays and other -omics platforms. Examples of the standard software include Treeview/Cluster, Genespring, and JMP Genomics. A variety of pathway analysis procedures will also be employed, including Gene Set Enrichment Analysis, SAFE, SAFE-GUI, GoMiner, and GenMAPP.

Genomics data are often highly multivariate, and the complicated data structures enable "sharing" of information across portions of the data to improve the power of hypothesis testing. For differential expression analysis, we will employ Significance Analysis of Microarrays (SAM) or similar software, which applies a shrinkage factor to penalize the significance of genes with low expression. Empirical Bayesian techniques can have similar effects to increase power for detecting significant genes in microarray experiments, compared to standard significance testing. For error rate control, resampling methods will be employed to identify statistically significant relationships in -omics data while controlling for numerous hypothesis tests, while reflecting covariance relationships in the data without explicit modeling. Among the simplest examples are controlling Type I (false-positive) error in the discovery of differentially expressed genes, or the discovery and characterization of genetic pathways correlated with clinical parameters. Analysis of genetic association data also greatly benefits from this approach, because positive correlation among hypothesis tests is very common, and thus approaches such as the Bonferroni correction may be unacceptably conservative. Bootstrap methods resample with replacement, and are thought to better represent draws from the alternative hypothesis (if indeed the alternative holds). Both permutation and bootstrapping will be used to control family-wise error rates and false discovery rates (FDR). Permutation methods for controlling the FDR have advantages in precise error control. Error control will be provided via the Benjamini-Hochberg step-down procedure when parametric tests are used and permutation is not feasible (e.g. when the sample size is too small). Cross-validation will also be performed in assessing prediction accuracy for complicated statistical models where standard theory may not apply. Key considerations include the relative proportions of the data to be used for training models and testing them, and whether it is more important to estimate the relevant quantities (such as prediction error) with little bias or little variance.

The data gathered in SPIROMICS may include single nucleotide polymorphism (SNP) genotypes, perhaps in candidate genes or regions suggested by other studies**. Association mapping of clinical outcomes will be carried out using individual SNP testing approaches, as well as simple haplotype-based approaches augmented by SNP imputation using the MACH software (Scott et al., (2007) Science 316:1341-1345). The statistical models to be used will depend on the type of data representing the trait of interest. For example, for a binary trait, such as presence or absence of a clinical biomarker, logistic regression or a chi-square test may be used. For ordinal or time-to-event responses, such as disease severity or time to first exacerbation, multinomial regression or a proportional hazard/odds model will be performed. For continuous traits, such as lung density or lung function, simple linear regression will be performed. Significance of results will employ permutation-based testing as described above to control family-wise error and false discovery rates.

7.4.3. Intermediate Outcome Identification and Initial Validation

Validating biomarkers or surrogate endpoints is quite challenging, especially if the goal is to satisfy the Prentice criteria for surrogate validity (Prentice, 1989). The Prentice criteria demand two conditions of a surrogate *S* for a primary endpoint *T*: (1) that *S* and *T* be correlated (i.e., not independent) and (2) that a test of the null hypothesis of no treatment effect on *S* is equivalent to a test of the null hypothesis of no treatment effect on *T*. In applying the Prentice criteria,

candidate surrogates are first screened relative to condition (1) by examining the correlation between the candidate surrogates and clinical endpoints of interest. The primary clinical endpoints in SPIROMICS are all-cause mortality, exacerbations, and decline in FEV_1, over three years. In addition, baseline measures such as initial FEV_1 will be used as secondary measures for surrogate candidate discovery. At the second stage, a test of condition (2) is carried out. With novel surrogate candidates, such as those anticipated for SPIROMICS, sets of completed clinical trials are likely not available. The approach planned here is to view differences among disease severity categories and controls as analogues of treatment group differences, and proceed with screening candidate surrogates using the methods of Daniels and Hughes (1987) for condition (2). The design of SPIROMICS is observational, involving no randomized conditions. Thus any findings of association between clinical endpoints and candidate surrogates will not establish causality of disease severity on the biomarker (or vice versa). However, association of a potential surrogate (or changes in the surrogate) with clinical endpoints occurring prospectively in the study will offer powerful evidence of the potential utility of the surrogate for future trial design.

The identification and validation process consists of a two-part screening step in which each of a pool of intermediate outcomes is examined for (a) correlation with respect to the four study design strata defined above and (b) correlation with the clinical endpoints assessed after three years of follow-up. For step (a), the term "correlation" will refer to any association between the intermediate outcome and the design strata, with no order restrictions. For step (b), it is generally understood that the most useful relationships between surrogates and clinical endpoints are monotone, therefore test statistics that are powerful for evaluation of monotone relationships are favored. Intermediate outcomes are handled as univariate measures, such as change from baseline to Year 1 values of a continuous measurement or presence/absence of a biological or clinical marker assessed at Year 1. Each intermediate outcome will be regressed on baseline covariates thought *a priori* to be important predictors, including, gender, height, weight, and clinical site. Linear models will be used for both continuous and dichotomous outcomes to produce residuals, which form the basis for the correlation tests. Least squares estimation will be employed at this step, because the focus is on examining correlations between the residuals and disease severity, after adjusting for baseline covariates, and not on prediction. The model residuals for the pool of intermediate outcomes will then be analyzed for significant trends with respect to design stratum using permutation to control the family-wise error rates for the number of potential surrogates (Westfall and Young, 1993). This approach will keep the entire set of residuals intact, permuting with respect to the clinical endpoints, and benefit from any positive correlation among intermediate outcomes in order to avoid overly stringent significance thresholds.

Candidate markers will also be identified based on previous studies or findings in the literature and considered for evaluation in SPIROMICS. Methods such as examining signal-to-noise ratios will be employed for this evaluation. For example, decline in lung density measured by computed tomography (CT) has been shown to track disease progression in emphysema patients (Stolk, et al., 2007). The signal-to-noise ratio for lung densitometry was found to be 2.5-fold that of decline in lung function or gas diffusion based on 144 COPD patients followed over 30 months.

8. Data Handling and Record Keeping

8.1. Data Collection

Data are collected at the clinic visits and through follow-up telephone interviews.

8.2. Data Entry

Data collection for SPIROMICS is primarily through direct data entry by clinic staff using the SPIROMICS web-based data management system (DMS). The clinical database resides on servers located in a secure, climate controlled server facility at UNC. The use of web-based data collection, entry, and processing allows for real-time data edits at the time of data entry and the generation of real-time status reports and data queries for monitoring study data by clinic personnel. In the event that internet service is temporarily unavailable at a clinical center, the data can be entered in local mode, with data transfers completed once internet service is resumed.

Reading Center data are transferred via secure FTP to the GIC on a regular basis throughout the study, and are merged with the clinical database.

8.3. Case Report Forms and Source Documents

Paper copies of electronic case report forms are included on the study website (http://www.cscc.unc.edu/spir/)

8.4. Confidentiality and Security

The SPIROMICS clinical database is housed in a secure, climate controlled server facility at UNC. The CSCC has in place an IT Security Plan as required by NIH contracts and grants. The plan documents standard operating procedures required to secure the CSCC network and databases, including management, operational and technical controls. As part of the plan, the principle of least access privilege for study files is implemented. Included in the plan are a risk assessment, a system continuity plan, and a disaster recovery plan.

Data confidentiality and security measures are applied at all levels of SPIROMICS data acquisition, transfer and storage, and applied to all study agencies, including the clinical centers, the Radiology Center, the GIC, and any central laboratories and reading centers engaged for the study. The SPIROMICS DMS meets exacting data management standards of confidentiality, as well as HIPAA requirements. Beyond the password-controlled access to the study equipment and the DMS, data collected at the clinical centers are encrypted by the system and can only be decrypted for display on-screen by authorized study personnel. Personal identifiers are collected on separate forms. The UNC GIC is responsive to data confidentiality requirements originating from providers of medical care or IRBs, as needed to enable the work of the clinical centers. It is the goal of the SPIROMICS study to collect all data electronically however, should paper data

collection forms be used they will be retained at secure locations at the clinical centers until the Steering Committee acts on recommendations from the UNC GIC to dispose of such records (e.g., after incremental data closure). The secure storage and disposition of hard copy records at clinical centers will follow institutional procedures at each site.

As standard practice, output mailed to a clinical center identifies participants only by ID number. Printed material containing confidential information is discarded through supervised loading, transportation, and storage using a chain of custody control process, until the material can be recycled into paper pulp.

Personally identifying information will be collected in SPIROMICS including name, address and address history, phone number, date of birth, dates of medical procedures, and social security number. These data will be stored on a secure server at the SPIROMICS GIC following the procedures described above.

8.5. Records Retention

8.5.1. GIC

The GIC will comply with all local and federal regulations in maintaining study related documentation. This documentation includes financial records, supporting documents, and all records related to the award. NHLBI policy states that these must be kept for at least three years after study closure (NHLBI, 2009). Please see Section 8.4 for more information on GIC security measures.

8.5.2. Biospecimen Storage

The UNC Biospecimen Processing Facility (BSP) is not a general repository in that all samples that are stored are the property of each particular study. These samples are not available without permission from the study PI to non-study investigators. The BSP collects no PHI information on any samples that come into the lab. The BSP retains indefinitely data relating to specimen processing, such as time from collection to processing, DNA yield, volume of plasma, quality checks, etc.

Regarding the destruction of samples, it is the BSP's policy that participants retain their rights to have their samples removed from the repository inventory at any time and have no further analytical disbursements performed. The withdrawal request must be made to the originating-PI. This individual PI will transmit a signed, dated written request to the BSP Facility's managers (acting as honest broker) who will identify the link between the participant ID and the repository ID and generate a removal/destruction request for all samples and records associated with a specific repository ID. Samples already released to approved requesting investigators according to the BSP repository's approved guidelines cannot be returned or destroyed. In addition data generated prior to a participants request for sample removal will not be destroyed.

The only other time the BSP destroys samples is at the request of the study PI when a study participant turns out to be ineligible, in accordance with the IRB documentation. At this point

both the sample and any data associated with it are destroyed in the same manner as described above.

8.5.3. Radiology Center

The Principal Investigator is required by University of Iowa and federal regulations to maintain records of all correspondence relating to the use of human subjects in research. Copies of the application for approval, notice of final approval and notice of continuing review must be maintained in the investigator's records. All records of human subject research are subject to inspection by federal authorities. Copies of all research records must be kept for three years after the close of the study.

An investigator may retain records either in their original form or by means of microfilm, microfiche, photocopies, or other accurate reproductions of the original records. If copies are used, however, they must be legible and the investigator is required to assure that such reproductions are true and accurate copies of the original. When reproduction techniques (e.g., microfilming) are used, a reader and photocopying equipment should be readily available. If written notes, erasure marks, or other changes are not apparent on the reproduction, a notation of this fact should be clear on the reproduction of the record, and the original record should be retained for the time required.

The Human Subjects Office maintains records of all protocols. These records include copies of the ongoing research, minutes, voting records and correspondence regarding every study that has been submitted for review.

Electronic records will be maintained in a readily retrievable form for the entire time they are retained regardless of status to prevent system migration problems. For example, if a new computer system (hardware, software, etc.) is implemented that uses records from an older computer system, the records will be converted to the new computer system's format or maintained in some other format that can still be readily retrieved.

8.5.4. Clinical Sites

The secure storage and disposition of hard copy records at clinical centers will comply with local institutional procedures as well as federal regulations.

9. Study Monitoring, Auditing, and Inspecting

9.1. Study Monitoring Plan

The GIC will conduct monitoring visits to the clinical centers and the Radiology Center to assess compliance with study protocol and MOPs, to help identify and resolve problems, and to verify correspondence between the study data and clinic records. Site visits will begin during the first year of patient recruitment.

The objectives for such visits include:

1. Assess adherence to the protocol, organization, and currency of study documentation and files, and accuracy and completeness of data collection and management.
2. Provide re-training and re-certification when appropriate, and discussing issues related to protocol interpretation, study procedures, etc.
3. Discuss any issues identified by quality control and study management reports.
4. Monitor study forms against source data for completeness and accuracy.
5. Ensure accurate and timely reporting of serious adverse events (SAEs).

Visits to the sites will include:

1. A written agenda is prepared for each site visit, including the activities completed at every visit and special topics as appropriate (if data quality analyses prior to the visit indicate deficiencies). The agenda will be distributed, and agreed to in advance.
2. A meeting held with the investigator and clinic coordinator at the start of the visit to review the plan for the visit and discuss any sensitive issues.
3. A debriefing meeting held at the end of the visit.
4. A constructive, written site-visit report will be provided in a timely manner.

Site visits will also be made to the Radiology Center and the central laboratories and processing facilities to review the internal quality control procedures, image, record, or specimen handling and storage, and to review management procedures.

9.2. Auditing and Inspecting

9.2.1. Clinical Center QA/QC

Clinic staff will attend annual central trainings conducted by the GIC. New staff members will be trained by certified clinic staff, visiting study monitor, and/or web-based training from the GIC. Data quality will further be assured by adhering to the study protocol, including the quality control/quality assurance procedures outlined for each assessment. These instructions, along with step-by-step descriptions of study procedures, can be found in the Manuals of Procedure.

9.2.2. GIC QA/QC

9.2.2.1. Training

The GIC will conduct annual trainings, the first of which will be held prior to the start of enrollment. The GIC will maintain a database of staff members with their corresponding data collector ID, certification (recertification) date, and procedures for which they are certified. New staff members will receive certification via web-based videoconference or monitoring visit (please see Section 9.1 for more details on monitoring visits).

9.2.2.2. Data Checks

Edit checks performed during web-based data entry at the clinical centers include range checks, correct execution of skip patterns, and across-variable consistency checks. In addition, the GIC will conduct complex across-form and across-visit data checks on a monthly basis to identify problem areas and assess data quality. These analyses may include but are not limited to:

1. Comparisons among clinical centers and among equipment at clinical centers to identify protocol violations, differences in interpretation, failure of standardization of methodology or equipment, and malfunction of measurement devices.
2. Descriptive statistics on selected variables by technician, to identify differences in the application or interpretation of the study protocol.
3. Tabulations and listings of incomplete or inconsistent responses on data collection forms; tabulations and listings of expected forms not received in a timely manner; and tabulations of clinical center error rates in data entry.
4. Analyses of digit preference for clinical measurements, investigation of correlations between variables and other evidence suggesting inadequate or fraudulent data collection
5. Selective collection of repeated measurements for quality control purposes. We suggest that measurements be repeated on a randomly-selected 5% sample of study participants and only for specific measurements

9.2.2.3. Equipment Checks and Calibration

In order to ensure accurate and consistent data it is essential that site equipment be in working order and correctly calibrated. Therefore it is important that sites monitor how often equipment is checked and calibrated on at least an annual basis unless checks and calibrations are recommended more frequently.

9.2.2.4. Radiology Center QC

Prior to participant enrollment CT scanners at each site will be calibrated using the COPDGene Lung Phantom (CTP674, The Phantom Lab). Each scanning site will have a phantom designated to the site for the duration of the study. Initially, the phantom will be scanned at the Radiology Center and then sent to the sites for scanning on the scanners for use in the study. The data will then be sent back to the Radiology Center for analysis. The inclusion of scanners will be based on a combination of results comparison to known reference values of that particular phantom as well as a comparison to a database of reference values obtained using the COPDGene Lung Phantom on scanners of the same make and model. Each CT scanner must be approved prior to participant scanning and monthly calibration checks using the COPDGene Lung phantom will be run throughout the scanning portion of the study.

Quality assurance of CT will be performed at each step of the data collection phase. The CT technologists will undergo an online training on the scanning protocol for the study. They will be responsible for completion of this training and will sign off on the training as well as indicate that they followed the study protocol on each Scan Acquisition Case Report Form (CRF). The CT scan will be visually inspected by two separate entities; the local clinical radiologist as well as the Radiology Center. The scans will be inspected for adequate inspiration, absence of motion artifact and inclusion of all parts of the chest. The Research Assistant at the Radiology Center

will be trained to assess the completeness of the scan, compliance with protocol, adequacy of inspiration, and presence of motion artifact. Any adverse finding will be reported directly to the imaging sites and GIC from the Radiology Center within 5 business days of scan receipt.

The Research Assistant performing the image analysis will follow a semi-automated process of analysis with a series of checklists and perform minimal manual editing as needed. Internal quality control of the image analysis will occur on an ongoing basis. A randomly selected **5%** of scans will be reanalyzed using the same software.

9.2.2.5. Laboratory QC

The UNC Biospecimen Processing Facility (BSP) has both an established QA/QC Policy and Manual of Procedures for insuring the quality of all samples processed and stored in the lab. The BSP requires staff members to undergo annual reviews of BSP internal policy and to maintain certification on all regulatory training courses.

Standard BSP procedure states that all reagents are appropriately labeled and stored. Similarly buffers are labeled with dates and are remade a necessary. Recipes for buffers are maintained in a notebook in the lab.

Maintenance procedures for laboratory equipment are fully specified in the laboratory protocols or in manufacturers' manuals referenced in the protocols. Equipment is maintained on a regular basis and records of these checks and equipment performance are maintained in the lab. The laboratory protocol also fully specifies the sources of calibration standards and quality control materials, the procedures used to prepare and store calibration standards and quality control materials, to guarantee the stability of the material and the accuracy of the assay.

To assure sample and data quality the BSP has established re-analysis cut points for samples with results outside of expected parameters. In addition, every six months the staff checks that the location of at least 10% of samples in the freezers and cold boxes match the sample tracking system.

For further details on BSP QA/QC policies please see Manual of Operations.

9.2.2.6. Repeatability and Replicate Study

9.2.2.6.1.Repeatability

The entire clinic visit will be repeated on 100 (3%) volunteers to determine reliability of measurement procedures. All baseline study-related procedures and questionnaires, including the CT scan, will be re-administered and new samples of blood, urine, saliva, and sputum will be collected. Field center staff will process these biospecimen samples according to protocol.

Each site will recruit 16-17 volunteers at a uniform rate over the study period to participate in the Repeatability Study. The repeatability study will occur only during the recruitment period of SPIROMICS. Sites will need to recruit 1 to 2 participants per month. Selection of participants

will reflect the four strata with each site recruiting at least 2 healthy controls, 3 smokers, 6 mild/moderate cases of COPD, and 6 severe cases of COPD. Selection within these groups will be random.

Details on the implementation of the repeatability study and data analysis are available in MOP 7 – Quality Assurance and Quality Control.

9.2.2.6.2.Blinded Replicate

A replicate sample is obtained by either drawing one to two additional tubes of blood or by dividing a urine sample into separate containers. The replicate samples are then processed using the same method as for the original samples. Investigators and laboratory staff analyzing and processing the samples should be unable to distinguish original samples from replicate samples. Over the entire study, replicate samples will be obtained on 5% of each specimen type (n = 160 at baseline, n=151 at year 1, and n=134 at year 3 for each specimen). Nine participants will be needed to provide a complete set of 9 QC replicate specimens (8 tubes of blood and 1 urine specimen), unless urine is collected from a participant providing a blood specimen replicate. In general only one additional tube of blood will be taken for a replicate sample from a participant. If a site needs to "catch up" to ensure a 5% sample of each replicate is obtained, up to two replicate samples blood can be drawn from one participant, while remaining under the 7 tablespoon limit. At baseline and year 1 just under half of the participants (45%) will contribute to the pool of replicate samples. In year 3 approximately 40% of participants will contribute to the replicate samples.

Details on the implementation of the replicate study and data analysis are available in MOP 7 – Quality Assurance and Quality Control.

9.2.2.7. Closure Checks to 'freeze' database

Periodically the study's consolidated database is subjected to closure checks for completeness and accuracy of data collection and processing. These checks are performed on a "frozen" version of the database defined by a specific time cut point, and precede the use of data for publication. Typical closure checks include classifying the universe of IDs, assuring that all expected forms were received and all queries were resolved, examining the consistency of items across forms and visits, and checking distributions of key variables for possible errors. Current plans entail closing the database in waves, one per examination year so that investigators will have access to interim results for study monitoring, review, and publication.

10. Study Administration

10.1. Organization and Participating Centers

- University of California at Los Angeles
- University of Michigan
    - Subsite: Temple University

- Columbia University
    - Subsite: Johns Hopkins University
    - Subsite: University of Iowa
- University of California at San Francisco
    - Subsite: National Jewish Health
- University of Utah
    - Subsite: University of Illinois at Chicago
- Wake Forest University Health Sciences
    - Subsite: University of Alabama at Birmingham
- Genomics and Informatics Center (GIC); University of North Carolina at Chapel Hill
    - Biospecimen Core
- Radiology Center
- PFT Reading Center
- Project Office

10.1.1. SPIROMICS Study Clinical Centers Participating in the Bronchoscopy Substudy (Section 6.4.8)

- University of California at Los Angeles
- University of Michigan (Note: The Wayne State subsite will not participate in the bronchoscopy substudy)
- Columbia University (Note: The Johns Hopkins subsite will not participate in the bronchoscopy substudy)
- University of California at San Francisco
- University of Utah
- Wake Forest University Health Sciences

10.2. Committee Structure

10.2.1. Steering Committee

- Ancillary Studies Subcommittee
- Quality Control Subcommittee
- Publications Subcommittee
- Mortality and Morbidity Classification Subcommittee
- Imaging Subcommittee
- Pulmonary Function Tests Subcommittee
- Exacerbations Subcommittee
- Other Specimens Subcommittee
- Questionnaires Subcommittee
- Sputum Subcommittee
- Data Sharing Subcommittee

10.2.2. Ancillary Studies Policy

The charge of the ancillary studies subcommittee is:

- To ensure that ancillary studies do not compromise the parent study in any way
- To ensure that the costs of ancillary studies are adequately supported by the ancillary study
- To maximize the quality of the science of ancillary studies
- To encourage ancillary studies and to maximize the overall scientific yield of SPIROMICS

An ancillary study involves the collection of new data, either directly from participants or from previously collected samples, images, or other sources (e.g., medical records). The aims of an ancillary study are not necessarily the same as the primary aims of the parent study. A SPIROMICS ancillary study is one that derives funding from other than SPIROMICS contract funds.

10.2.3. Observational Studies Monitoring Board (OSMB)

An OSMB is constituted to provide an annual evaluation of the study with recommendations to the NHLBI regarding:

A. Participant safety, burden, confidentiality and any other matter pertaining to protection of the study participants;

B. Study performance in terms of recruitment and retention, implementation of procedures and questionnaires, follow-up for events, and all aspects of quality control; and

C. Study productivity in terms of significant research results in addressing the primary study aims:

a) Identify homogeneous subgroups of COPD patients for targeted enrollment in future therapeutic clinical trials
b) Identify and validate intermediate biological or clinical outcomes for use as clinical trial endpoints

The Board meets in person on an annual basis with members of the study Steering Committee (others investigators if needed). The GIC provides materials to inform the Board of SPIROMICS progress, and the investigators provide presentations and respond to any concerns addressed by the Board.

The Board has the responsibility of reviewing all Ancillary Study proposals to determine whether the Ancillary Study could provide harm to the conduct of the main study. The Ancillary Study review by the Board can be done during the annual Board meeting, or by email review. An Executive Secretary for the Board is an NHLBI staff member not associated with SPIROMICS, who provides for all interaction between the Study and the Board.

The following NHLBI website describes the responsibilities of OSMBs:
http://www.nhlbi.nih.gov/funding/policies/osmb_inst.htm

10.3. Funding Source and Conflicts of Interest

10.3.1. Funding Source

National Heart, Lung, and Blood Institute, National Institutes of Health

10.4. Study Timetable

Task	Start	Complete	Completed in Study Month
Phase 1: Development (Yr. 1, Mos. 1-9)			
Protocol development	15Apr09	31Jan10	11
Design data collection forms	01Jun09	31Jan10	11
MOP	01Jun09	28Feb10	12
OMB Submission	03Mar10	04Jun10	15
OSMB Meeting (review protocol)	29Sep09	01Jul10	17
Domain vocabularies identified		31Jul09	6
IRB approvals	19Dec09	30Jun10	16
Data entry system		28Jun10	16
Central Training @ GIC		15Mar10	13
Pilot test baseline clinic exam	01Jun10	28Jun10	16
Quarterly Progress Reports			4,7, 10, 13, 16
Steering Committee Meetings			1, 2, 3, 4, 5, 6, 7, 8, 9
Phase 2: Enrollment and Clinic Exams (Yr. 1, Mo. 10 through Yr. 6, Mo. 6)			
Report on inclusion of women and minorities			12
Annual Report			12
Patient recruitment	1Jun10	15Mar13	51
Baseline clinic exams	1Jun10	31Mar13	51
OSMB Meeting			12,24,36,48,60,72
2nd clinic exam	01Jun11	31Mar14	63
3rd (long-term follow-up) clinic exams	01Jun13	31Mar16	87
Data editing/querying/dbase updates	01Jun10		Ongoing
Conduct site monitoring visits	15Jul10	31Jun15	75
Quarterly Progress Reports			19,22,24, etc.
Annual Quality Control Report			12,24,36,48,60,72
Steering Committee Meeting			Biannually w/ monthly calls
Analysis of baseline data	01Sep10		Ongoing
Analysis of follow-up clinic data	01Sep11		Ongoing
Analysis of long-term outcomes; identification and validation of intermediate outcomes	01Sep13		Ongoing
Phase 3: Close out (Yr. 7, Mos. 7-12)			
Final data editing/query resolution		3Mar16	87
OSMB Meeting			86
Steering Committee Meeting			Biannually w/ monthly calls
Database lock		30Apr16	85
Deliver data to NHLBI		31May16	86
Create LADs		30Jun16	87
Complete final data analysis		30Jun16	87
Submit final priority manuscripts		30Jun16	87
Store or destroy paper records		30Jun16	87
Permanent specimen storage		31May16	86

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