Discovery & Identification of biomarkers of Chronic Obstructive Pulmonary Disease Onset using a Case-control study design & Translational systems biology-Outline study plan

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Objective

It is well known that a major cause of Chronic Obstructive Pulmonary Disease (COPD) is repeated long-term exposure to tobacco smoke[1]. Philip Morris International is currently engaged in an effort to develop novel tobacco products with the potential to reduce the risk of tobacco-related diseases. COPD takes an average 20-25 years to develop. In the absence of appropriate biomarkers, it would be necessary to conduct studies for at least this duration in order to assess the impact of novel products on COPD prevalence. In an effort to significantly reduce this assessment timeframe for every novel product, it is necessary to identify biomarkers which change on exposure to tobacco smoke and precede clinical onset of COPD. Once identified, validated and qualified, these biomarkers may be used for assessing novel tobacco products through the comparison of these biomarkers in smokers of conventional tobacco products and novel, modified risk tobacco products.

Need For This Study

COPD is an area of focus for the international respiratory medical community, with a number of large academic – industry sponsored studies to identify biomarkers[2-5]. However, these studies all have therapeutic intervention in established disease as their primary focus. In 2001, the American Thoracic Society (ATS) and the European Respiratory Society (ERS) published a set of guidelines for the diagnosis of COPD[6]. Known as the Global initiative for chronic Obstructive Lung Disease (GOLD), these guidelines are used to categorize COPD patients into GOLD Stage 1-4 depending on the severity of disease (with GOLD stage 4 being the most severe). Many of the major pharmaceutical companies are interested in discovering and developing treatment options for COPD, and the current pipeline of drugs consist of bronchodilators (which give some relief to the obstructed airways in COPD) and anti-inflammatory treatments (Figure 1). There is therefore an obvious and applicable need for biomarkers which distinguish between the different GOLD stages, in order to test the efficacy of these drugs in patients with established disease (Figure 2).
In our quest to develop novel tobacco products with the potential to reduce disease, established COPD GOLD stage 1 is already too advanced in the pathogenesis between smoke exposure and COPD. It is therefore necessary to conduct a study in which we will analyse the biological networks that are differentially disturbed when a smoker is exposed to conventional cigarette smoke and compare these to the biological networks in matched healthy smokers (to determine which biological networks are perturbed by smoke exposure, but do not result in COPD), matched ex-smokers (to determine if any have reversed) and matched never-smokers (to ascertain baseline levels of the biological networks). Through this evaluation, we hope to understand the key biological networks linking smoke exposure to the onset and development of the early stages of COPD. Once understood, we hope to identify pertinent markers of these networks, which can then be further developed and validated as Biomarkers of COPD onset.
These biomarkers of COPD onset can then be used in comparative studies, in which smokers of conventional cigarettes are asked to switch to novel products. A comparison of biomarker levels would then provide significant evidence as to whether the novel products might reduce COPD onset.

Study Design

The current study represents a unique combination of an epidemiological case-control study design with a translational systems biology approach. The study is designed to integrate measurements of pre-clinical and clinical parameters to identify biological network disturbances associated with early disease development. It is thus uniquely positioned to identify biomarkers of COPD onset for use in comparative clinical assessments of novel tobacco products with the potential to reduce smoking-related disease.
The study is a non-interventional, observational case-control design study.
The case-group consists of 60 smokers* with GOLD stage I or stage IIa COPD, and is matched by age, gender, smoking history (pack-years; where appropriate) to 3 control groups: 60 healthy never-smokers, 60 healthy ex-smokers and 60 healthy current smokers*.

The Inclusion Criteria for this study are:

- Provision of written signed informed consent which includes a genetic consent
- Ability to comply with study procedures
- Males and females aged 40-70 years inclusive
- Have a body mass index (BMI) between 18 and 35 kg/m^3 inclusive and a minimum body weight of 50kg
- Have a normal physical examination, and have normal laboratory values. 12-lead ECG and vital signs (blood pressure, heart rate and respiratory rate), unless the Investigator considers an abnormality to be not clinically significant
- Ability to perform reproducible spirometery according to the ATS and ERS guidelines
- Be able to produce a minimum of 0.1g sputum sample after induction with inhaled hypertonic saline

Additional Inclusion Criteria for the COPD group are:

- A clinical diagnosis of COPD according to the GOLD guidelines
- Current smokers* with a ≥ 10 pack-year smoking history
- Demonstration of a post-bronchodilator ratio between FEV_1 and FVC of ≤70% and FEV_1 ≥50%

Additional Inclusion Criteria for the Smoker group are:

- Current smokers* with a defined smoking history of ≥10 pack years
- Have normal lung function by post bronchodilator FEV_1 ≥80% predicted normal, with no evidence of airway obstruction with FEV_1/FVC ratio ≥70%

Additional Inclusion Criteria for the never-smoker group are:

- Have never smoked tobacco products
- Have normal lung function by post bronchodilator FEV1 ≥80% predicted normal, with no evidence of airway obstruction with FEV_1/FVC ratio ≥70%
- Have a sputum eosinophilia <2% and a sputum neutrophilia of <80% from the sample collected at visit 1 (to exclude allergic airway disease).

The study is being conducted in the United Kingdom, and has been approved by a UK National Health Service (NHS) Ethics Committee. Although purely observational in nature, with no investigational product, the study is being conducted under Good Clinical Practice with participant care in line with the 2001 Declaration of Helsinki.

Due to the number of biological samples (summarised in box 4) and physiological assessments (summarised in box 5), each participant is required to attend 4 visits to the clinic. All 4 visits for each participant are scheduled in a 4-week period.

** All current smokers are given Smoking cessation advice and counselling at visits 1 and at the follow-up consultation, by certified level 1 smoking cessation advisors. Subjects who express an interest in giving-up smoking are referred to the NHS stop smoking services for continuing support, including advice on nicotine dependence and discussions on medications and other nicotine replacement therapy products.*

Figure 1: The Key pathologies of COPD

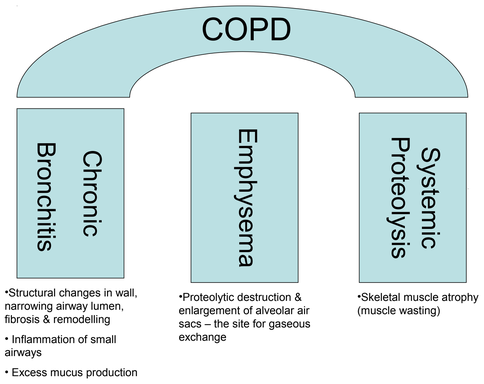
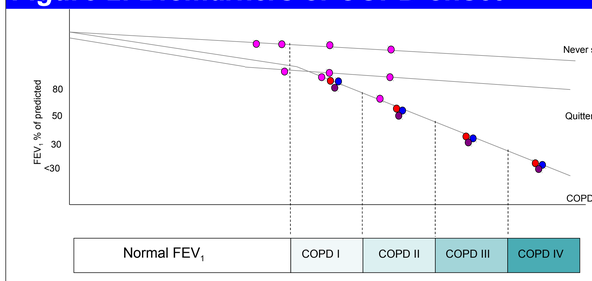


Figure 2: Biomarkers of COPD onset



COPD is diagnosed by an irreversible decline in FEV_1 lower than 80% of predicted. It is generally accepted that if a smoker quits before the onset of disease, the rate of FEV_1 decline can revert back to the rate of a never smoker, albeit with a lower % of predicted than a never-smoker. The current study (●) focuses on biomarkers of disease onset, by comparing 3 matched control groups to a group of COPD patients with GOLD stage 1 / 2 disease. Other large studies e.g. SPIROMICS[2] (●), CBQC[3] (●) and ECLIPSE[4] (●) focus on biomarkers of disease progression through the differing GOLD stages. COPDgene[5] is a large multi-pharmaceutical company sponsored study which looks at genetic susceptibility.

Biological samples

In order to determine the biological networks that are perturbed by exposure to cigarette smoke, a detailed transcriptomic and proteomic analysis will be carried out on biological samples from these subjects. Biomarkers with known associations with established COPD will also be measured in all samples.

Since COPD represents a chronic inflammatory condition of the small airways, the differential in biological activity in these these airways would provide the best representation. According to the 2002 ERS guidelines, the best representation of the airways in COPD is the analysis of induced sputum[7]. Hence the primary biological sample in this study is sputum that has been induced by inhalation of hypertonic saline. A list of the samples and reasons for their collection are given the table below:

Induced Sputum	Proteomics	Best representation of the inflammatory state of the small airways. Contains cells of interest and protein mediators
Whole Blood (Lymphocytes)	Transcriptomics Proteomics Lipidomics	Identify correlations with data identified from the sputum. Blood represents the ideal and most convenient matrix for large-scale biomarker measurements
Nasal fluid	Proteomics	A number of respiratory researchers have shown strong correlations between inflammation in the lung & nose. May represent a convenient matrix for large-scale studies
Nasal scrapes	Transcriptomics Proteomics	May represent a convenient matrix for large-scale studies if correlations are seen with the sputum
Nasal lavage	Transcriptomics Proteomics	May represent a convenient matrix for large-scale studies if correlations are seen with the sputum

Clinical Measurements

The table below shows the clinical/physiological measurements that are being made in the current study in order to define the subjects' characteristics. It is indeed possible that some of these measurements may themselves be shown to be suitable as biomarkers of COPD onset.

1	High-resolution (HR) CT-Scan	High resolution image of airways. Possibility of quantifying emphysema and inflammation.
2	Full lung function including gas transfer measurement (TLCO)	Pre- and post-bronchodilator measurements. Scope is greater than FEV_1 alone and is more sensitive to subtle changes in the small airways compared to FEV_1 spirometery. TLCO is able to detect oedema in the small airways which may be a very early indicator of COPD, even in the absence of a decline in FEV_1.
3	Differential (fractional) exhaled Nitric Oxide (NO)	FeNO is the best validated gaseous constituent of exhaled breath and is used to assess airway inflammation in clinical practice.
4	Exercise Tolerance	Some studies indicate that impaired lung function, even before a decline in FEV_1 can be recorded, manifests as a reduced tolerance to exercise.
5	Acoustic imaging (Stethographics®)	A novel non-invasive technology which uses multiple sound detectors on the thoracic area of the subject. Subtle changes in frequency and amplitude correspond to pathological patterns in the airways. In this study, this technology will be validated against HR-CT and FEV_1 measurements.
6	Impulse Oscillometry	Measurement of respiratory impedance of the central and peripheral airways.
7	Exhaled Breath Temperature	Increases in airway inflammation results in subtle but measurable increases in exhaled breath temperature.

Translational sample analysis

The primary biological sample of interest in this study is the induced sputum, which best represents the pathophysiology of the small airways. Several researchers have attempted to identify COPD severity biomarkers in the sputum, using shotgun 2D electrophoresis methodologies[9]. Although the study numbers were very small (n=9) and the methodology relatively insensitive, there have been some positive findings. It is our intention to use a targeted Mass Spectrometery (MS)-based proteomics approach. The use of this approach allows for the detection of low-abundant proteins, and is exclusively hypothesis-driven using prior information to generate validated MS assays for the detection and quantification of peptide candidates in complex samples.

Prior information will be sought from the literature, some preliminary work from sputum samples of a pilot study and from the information gained from our COPD mouse model, in which the 4 groups in the current study have been modelled. Those proteins found to be differentially expressed in the smokers who have COPD versus the never-smoker and healthy smoker groups would provide leads for potential biomarkers. Given the epidemiology of smoking cessation in COPD, those potential biomarkers that seem to follow a 'reversal trend' in the ex-smoker group would provide further confidence in their utility as biomarkers of disease onset.

Assaying methodologies for any potential biomarker leads would then be developed for independent confirmation of their identity and quantity.

A comparison of the differential protein expressions in the sputum will be made with the proteome of the white blood cells and nasal samples, to offer the possibility of less complex sampling in future studies.

The results from the transcriptomics, from the biological samples will be analysed using computational approaches and compared to published biological networks and those built internally through pre-clinical experimental and data mining activities.

The results from the clinical/physiological measurements will be integrated with the molecular results that are obtained, and a combined physiological/proteomic/transcriptomic signature will be generated as potential biomarkers of COPD onset.

Future Directions

If potential biomarker signatures are discovered in this study, it will be necessary to conduct the steps outlined below:

- Analytical development of methodologies to measure biomarkers in the biomarker signature
- Analytical validation of those methodologies
- Replication of the findings in an independent cohort
- Clinical qualification of the biomarker signature

In medical science, there is an emerging consensus, although not full agreement, as to how biomarkers should be validated and qualified for clinical use. It is our interest to have an active part in this discussion/debate through presentation and publication. It is inevitably a cross-industry exercise to develop an accepted framework for biomarker validation and qualification.

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