BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-042526
Article Type:	Protocol
Date Submitted by the Author:	09-Jul-2020
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Keywords:	Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Chronic airways disease < THORACIC MEDICINE, Emphysema < THORACIC MEDICINE, Computed tomography < RADIOLOGY & IMAGING

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FOOTPRINTS® study: rationale and methodology of a 3-year longitudinal observational study to phenotype patients with COPD

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Short title: Emphysema progression biomarker study

Keywords: Protocols & guidelines, chronic airways disease, emphysema, computed tomography

Word count: 4053/4000

ABSTRACT

Introduction

A better understanding is needed of the different phenotypes that exist for patients with chronic obstructive pulmonary disease (COPD), their relationship with the pathogenesis of COPD, and how they may affect disease progression. Biomarkers, including those associated with emphysema, may assist in characterising patients and in predicting and monitoring the course of disease. The FOOTPRINTS® study (Study 352.2069; NCT02719184) aims to identify biomarkers associated with emphysema, over a 3-year period.

Methods and analysis

The FOOTPRINTS® study is a prospective, longitudinal, multinational (12 countries), multicentre (51 sites) biomarker study, which has enrolled a total of 463 ex-smokers, including subjects without airflow limitation (as defined by the 2015 Global Initiative for Chronic Obstructive Lung Disease [GOLD] strategy report), patients with COPD across the GOLD stages 1 to 3, and patients with COPD and alpha1-antitrypsin deficiency. The study has an observational period lasting 156 weeks that includes seven site visits and additional phone interviews. Biomarkers in blood and sputum, imaging data (computed tomography and magnetic resonance), clinical parameters, medical events of special interest and safety are being assessed at regular visits. Disease progression based on biomarker values and COPD phenotypes are being assessed using multivariate statistical prediction models.

Ethics and dissemination

The study protocol was approved by the authorities and ethics committees/institutional review boards of the respective institutions where applicable; written informed consent has been obtained from all study participants. Ethics committee approval was obtained for all participating sites prior to enrolment of the study participants. The study results will be reported in peer-reviewed publications.

Strengths and limitations of this study

- The study helps to address an unmet need to understand different COPD phenotypes, and to expand on our understanding of biomarkers that are associated with emphysema, and hence COPD, in a 3-year longitudinal setting.
- A subset of patients with alpha1-antitrypsin deficiency provides information on an important but rarely studied subpopulation of patients that present with earlier onset and faster progression of emphysema.
- A regular sampling schedule is employed, with frequent visits and biomarker sample collections to allow early detection of changes, if present.
- Magnetic resonance imaging and sputum collection only occur at certain study sites, and not necessarily at all planned times; hence, the smaller subsets of patients may not be representative of the entire population.
- Patients with the newly defined PRISm phenotype are not included, nor are current smokers or healthy subjects who have never smoked, thus limiting the control groups for reference. Potential exists for patient selection bias at site and patient level.

Lay summary

Patients with chronic obstructive pulmonary disease (COPD) have different individual characteristics that may affect how the disease progresses and how they respond to different treatments. Damage to the air sacs of the lungs (called emphysema) is one of the main components that contributes to irreversible progression of COPD. We have therefore decided to focus on how different patient characteristics could be linked to emphysema and, as a result, COPD.

This study, termed the FOOTPRINTS® study, has enrolled 463 ex-smokers, including ex-smokers without COPD, patients with COPD, and a subset of patients with COPD who also have a genetic alpha1-antitrypsin deficiency that has a known link to emphysema. Patients are being assessed over 3 years, which includes seven study centre visits and additional phone calls. A range of assessments are made at each visit, including the analysis of biofluid samples such as blood for specific biological markers, lung imaging, clinical laboratory tests and safety. The results are then analysed to understand how certain markers could be used to help characterise patients with COPD.

INTRODUCTION

Airflow limitation is a key characteristic of chronic obstructive pulmonary disease (COPD).[1,2] It is caused by small airways disease, such as obstructive bronchiolitis, and structural changes related to parenchymal destruction (emphysema).[1,2] Small airways disease and structural changes can occur alone or in combination with one another, with the degree of severity and relative contributions varying between individuals.[1]

COPD is associated with inflammation and tissue damage, which is thought to be linked to – among other aspects – an imbalance between neutrophil serine proteases (NSPs) and their inhibitors.[3,4] The serine protease inhibitor alpha1-antitrypsin (A1AT) inactivates NSPs such as neutrophil elastase (NE), proteinase 3 (PR3) and cathepsin G (Cat G), to protect lung tissue against protease-mediated damage.[5,6] To date, A1AT deficiency (A1ATD) is the only underlying gene defect that has been identified as a cause for COPD.[7] It is associated with a higher risk of developing emphysema that tends to occur at a younger age and progresses faster than for patients without A1ATD.[5,8,9] Subjects with the most severe A1ATD are usually homozygous for the ZZ allele (ZZ).[5] The resulting emphysema among patients with A1ATD is thought to be caused primarily by increased NSP activity in the lungs.[9]

NE and PR3 are responsible for the degradation of critical components of the extracellular matrix, including elastin and fibronectin, and can produce other key features of COPD, such as mucous hypersecretion.[3] When quantified, NE and PR3, as well as other proteases and biomarkers, have been associated with poorer

disease outcomes in patients with COPD, such as incidence of exacerbations and higher risk of mortality.[10-13]

In the era of precision-based patient care, there is a need for a more in-depth understanding of COPD patient phenotypes, pathogenesis and progression.[14,15] Biomarkers may assist in characterising patients, and in predicting and monitoring the course of disease, in order to better enable the development of drugs aimed at slowing COPD progression.[14-16] In fact, there are encouraging signs that therapies that modify NSP imbalance (e.g. augmentation therapy for A1ATD) appear to alter emphysema progression, at least when considering blood and radiological biomarkers of disease.[17,18] Since quantitative computed tomography (CT) is a well-established method for documenting the presence, extent and progression of emphysema, it is potentially useful for assessing these emerging biomarkers.[19-22]

This prospective, longitudinal study is investigating biomarkers in different biofluids (whole blood, serum, plasma and induced sputum), imaging parameters assessed by CT and magnetic resonance imaging (MRI), as well as clinical parameters potentially associated with emphysema, over a 3-year period. It is hoped that correlation of lung destruction biomarkers with CT densitometry findings should increase our understanding of the underlying pathophysiology of emphysema progression. The insights generated may support the development of future treatments that could slow or halt disease progression in patients with COPD. Here we report the methodology of the FOOTPRINTS® study, as described in the study protocol (version 5.0; date: 29 May 2018).

Objectives

The primary objective of this longitudinal study is to explore whether any of the biomarkers assessed in patients with COPD, patients with COPD plus A1ATD and subjects without airflow limitation (controls; as defined by the 2015 Global Initiative for Chronic Obstructive Lung Disease [GOLD] strategy report),[23] or specific patterns thereof, are correlated with COPD progression, particularly emphysema progression, over 156 weeks.

Further objectives, to be assessed over 156 weeks, include:

- To assess the correlation of soluble biomarkers of inflammation and lung tissue destruction with COPD severity, presence of emphysema and disease progression.
- To determine the concentration and activity of proteases in induced sputum, and to assess potential biomarkers of lung tissue destruction in sputum and blood, from patients with different severities of COPD (including a subset of patients with A1ATD) and subjects without airflow limitation (controls).
- To assess the correlation of physiological biomarkers and functional imaging biomarkers with COPD severity, the presence of emphysema and disease progression, and to evaluate MRI-based imaging biomarkers with respect to their feasibility.
- To identify COPD phenotypes and define their risk for, and rate of, disease progression.

METHODS AND ANALYSIS

Study design

The FOOTPRINTS study® (registered on 21 March 2016) is a multinational, prospective, longitudinal, biomarker study in subjects without airflow limitation, patients with COPD, and patients with COPD and A1ATD, taking place at 51 sites across 12 countries (Belgium, Canada, Denmark, Finland, Germany, Japan, the Republic of Korea, Poland, Spain, Sweden, United Kingdom, and the United States). A complete list of study sites can be found at https://clinicaltrials.gov/ct2/show/NCT02719184.

The study consists of two consecutive periods (a screening period lasting up to 28-days and an observational period lasting 156 weeks) and includes seven site visits and additional phone interviews (figure 1). The observational period is completed at Visit 7 or at the discontinuation visit, which is regarded as study completion. Patients are considered to be in the observational phase only after completing Visit 2. All subjects who prematurely discontinue the study during the observational phase are followed for vital status and COPD exacerbation status (for patients with COPD and for patients with COPD and A1ATD only) via phone contact until 156 weeks after Visit 2.

Calculation of sample size, and power calculations for annual decline in forced expiratory volume in 1 second and 15th percentile density assessed by CT, are provided in Supplementary File 1 (text 1 and tables 1 and 2).

Inclusion and exclusion criteria

Male and female participants who have signed an informed consent form consistent with the International Conference on Harmonisation Good Clinical Practice (GCP) guidelines prior to participation have been included, provided the following were applicable:

- Aged 40–70 years (30–70 years for patients with COPD and A1ATD).
- Ex-smokers for ≥9 months with a smoking history of ≥20 pack-years (≥10 pack-years for patients with COPD and A1ATD).
- Body mass index (BMI) 18–35 kg/m^2 (≤30 kg/m^2 in the MRI subset).
- Able to perform all study-related procedures.
- Required to have been on stable therapy for 4 weeks prior to Visit 1.

Participants were excluded if any of the following were applicable:

- Significant pulmonary disease (other than COPD) or other significant medical condition, for example, rheumatoid arthritis, severe liver disease or psoriasis.
- Documented history of asthma.
- Any respiratory tract infection or COPD exacerbation within 4 weeks prior to Visit 1 or during the screening period prior to Visit 2, if it was not possible to meet the rescheduling rules.
- Presence of an immunocompromising condition (e.g. HIV or history of hepatitis B or C).
- Any treatment with phosphodiesterase-4 inhibitors and maintenance treatment with methylxanthines within 3 months or 6 half-lives prior to Visit 1, and until Visit 2; patients were permitted to initiate treatment following Visit 2.
- Patients with COPD (with and without A1ATD) with newly added anti-inflammatory treatment (either respiratory or non-respiratory) or change in any therapy within 4 weeks prior to Visit 1 and during the screening period between Visit 1 and Visit 2; patients were permitted to initiate treatment following Visit 2.
- Any current or planned A1AT augmentation therapy.

- Permitted and restricted medications and therapy are detailed in Supplementary File 1 (table 3).
- Pregnant or lactating females.
- Previous participation in this study or participation in a parallel interventional clinical trial within 6 weeks prior to Visit 1 or during the study.

Patient population and recruitment

The study is ongoing; however, enrolment is complete. In total, 463 patients were enrolled, who are described in more detail in table 1. Strategies for achieving adequate participant enrolment and retainment are described in Supplementary File 1 (text 1).

The study started on 22 July 2016 when the first subject was screened, and the planned end date for the study is 29 March 2021.

Table 1. FOOTPRINTS® study participants: all enrolled subjects

Patient group	Rationale
383 patients with COPD, including: • 123 patients with mild COPD[a] • 130 patients with moderate COPD[b] • 130 patients with severe COPD[c]	Included to provide data on increased lung protease levels and the contribution that this may have on the development and progression of emphysema in the absence of A1ATD.
18 patients with COPD and A1ATD[d]	Included as they present with an earlier onset of emphysema and a faster decline in lung function, as well as a faster change in lung density.[8]
62 ex-smokers without airflow limitation	Included to provide a comparison for biomarkers between subjects with and without airflow limitation. Subjects were required to be healthy based on a complete medical history and to have: FEV_1 ≥80% of predicted normal and post-bronchodilator. FEV_1/forced vital capacity ≥lower limit of normal. A mean post DL_{CO} ≥70% of predicted normal at Visit 1.
Total enrolled: 463 subjects	

[a]Defined as GOLD stage 1, FEV_1 ≥80% predicted [23]; [b]defined as GOLD stage 2, FEV_1 ≥50–<80% predicted [23]; [c]defined as GOLD stage 3, FEV_1 ≥30–<50% predicted [23]; [d]defined as having a diagnosis of COPD and a documented A1ATD of ZZ genotype prior to Visit 2.
A1ATD, alpha1-antitrypsin deficiency; COPD, chronic obstructive pulmonary disease; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; FEV_1, forced expiratory volume in 1 second; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Main outcome measures

The main outcome measures are change in lung density (assessed by CT), lung function decline and number, duration and severity of exacerbations over the course of the study.

Planned analyses and assessments

Variables to be analysed include a range of soluble biomarkers, imaging biomarkers and clinical parameters, as well as medical events of special interest (MESI) and safety assessments. The pre-specified biomarkers being assessed are summarised in table 2, and the biomarker sampling schedule and overall trial design are illustrated in figure 1.

Table 2. Summary of biomarker assessments

Variables	Biomarker assessment
Pre-specified soluble biomarkers	
Blood	• Total and differential cell counts • DNA analyses (to determine A1AT genotype)
Induced sputum*	• Neutrophil elastase activity • Proteinase 3 activity • Cat-G activity
Serum/plasma	• Tissue turnover biomarkers (including neutrophil elastase-specific elastin fragment) • Other protease-generated neoepitopes (including Cat-S cleaved decorin) • Soluble form of receptor for advanced glycation end products • Surfactant protein D • Lysyl oxidase like 2 • Biomarkers of systemic inflammation (including IL-6, high-sensitivity C-reactive protein, white blood cell count and fibrinogen)
Imaging biomarkers	

CT scanning	• Airway morphology
	  ○ Mean lumen diameter
	  ○ % wall area

	○ Mean segmental and sub-segmental airway wall thickness ○ Square root of wall area of bronchus with internal perimeter of 10 mm (pi10) • Emphysema (on inspiratory CT) ○ PD15 of the CT histogram ○ PD15 adjusted for inspired lung volume ○ % lung voxels with attenuation ≤–950 HU (LAA-950) • Air trapping (on expiratory CT) ○ % lung voxels with attenuation ≤–856 HU (LAA-856) • Registration-based parenchyma measurements ○ % normal lung, % emphysema and % small airway disease
MRI	• Conducted at 1.5 Tesla in a subset of subjects without airflow limitation and in patients with COPD and without A1ATD to assess functional lung parameters, including pulmonary blood flow
Clinical parameters/assessments	• Spirometry to assess FEV_1 and FVC • DL_{CO} and DL_{CO} per unit of alveolar volume • Body plethysmography (functional residual capacity, total lung capacity, inspiratory capacity, inspiratory vital capacity, residual volume, expiratory reserve volume) • Pulse oximetry • Symptom questionnaires (modified Medical Research Council Dyspnoea Scale, COPD Assessment Test, St. George's Respiratory Questionnaire) • 6-minute walk test • Body mass index and BODE index • Adverse events and MESI

*Collected at selected sites.

A1AT, alpha1-antitrypsin; A1ATD, alpha1-antitrypsin deficiency; Cat, cathepsin; COPD, chronic obstructive pulmonary disease; CT, computed tomography; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HI, Hounsfield Units; IL-6, interleukin-6; LAA, low attenuation areas; MESI, medical events of special interest; MRI, magnetic resonance imaging; PD15, lung density at the 15th percentile point of the CT histogram.

Blood and sputum are being collected at Visits 2–7 for analysis of pre-specified

soluble biomarkers, and blood is also being collected at Visit 1 to perform a genetic

test for A1ATD (figure 1). Total and differential cell counts in blood, which are being assessed within the safety laboratory assessments, are also being assessed as biomarkers. Sputum will be induced to generate sputum samples, including cell slides to assess total and differential cell count and supernatant to assess soluble biomarker levels (table 2).

Chest CT scans are being conducted annually at Visits 2, 5, 6 and 7, following a specific low-dose protocol in order to minimise radiation exposure at each visit. MRI evaluation of the pulmonary vasculature and right ventricle is conducted in a subset of patients without airflow limitation and patients with COPD (not in patients with COPD and A1ATD) at Visits 2, 4, 5, 6 and 7. MESI include respiratory disorders such as pneumonia, pulmonary fibrosis, bronchiectasis and COPD exacerbations, as well as any other disorders (e.g. cardiovascular, metabolic/digestive and neurological/psychiatric) reported at the investigator's discretion. COPD exacerbations are defined as new or increased lower respiratory events or symptoms related to the underlying COPD with duration ≥3 days. Assessment of safety is carried out at Visits 1, 5, 6 and 7 unless otherwise indicated, including physical examination (plus assessment of height [Visit 1 only] and weight), vital signs (blood pressure and pulse rate), safety laboratory parameters (from blood; assessed at every site visit [Visits 1–7]) and electrocardiogram. Adverse events associated with any study procedure and MESI are collected at every site visit (Visits 1–7; MESI also assessed via telephone contact between visits; figure 1). All adverse events are followed up until they have resolved, been sufficiently characterised, or no further information can be obtained.

Rescheduling of clinic visits

Any deviations from the planned visit schedule are being documented. Rescheduling of certain clinic visits was permitted to promote participant retention. Clinic visits that involve a lung function test may be rescheduled twice within the permitted time windows due to violation of lifestyle restrictions. Subjects should refrain from strenuous activity for at least 12 hours prior to lung function testing (and throughout the testing period); and should avoid cold temperatures, environmental smoke, dust or areas with strong odours (e.g. perfumes). Sputum induction at Visit 2 can be rescheduled by up to 3 days; at all subsequent visits, it should not be repeated if the subject is unable to produce sputum or if the sputum sample quality is not acceptable. If chest CT and MRI (where applicable) cannot be performed on the day of the scheduled visit, the imaging assessment can be rescheduled up to 14 days after the visit. If a patient experiences a COPD exacerbation or respiratory tract infection during the screening period, Visit 2 will be postponed until 4 weeks following recovery from the exacerbation or infection, and the screening period may be extended up to 8 weeks.

Statistical analysis

Biomarker assessments and clinical outcomes measures are evaluated by regression models for repeated measures using respective point estimates and CI estimates. The correlation between individual biomarkers, and between biomarkers and other clinical outcome measures, is being assessed. Multivariate statistical prediction models for disease progression based on biomarker values and COPD phenotypes are being developed and examined. Interim analyses will also be conducted by the trial team once data from the 52-week and 104-week assessments

are available for ≥70% of subjects entered into the study. The purpose of this is to provide information on the use of MRI and soluble biomarker assessments.

Information on steering committee

A scientific steering committee, Emphysema Progression Biomarker Study (FOOTPRINTS® study) Steering Committee, was established in January 2017 to discuss and evaluate key scientific data in the field of respiratory medicine, and to provide independent advice and recommendations to optimise and strengthen the FOOTPRINTS® biomarker study. The steering committee comprises 13 external and internal scientific experts in respiratory medicine and chest radiology (Supplementary File 1; table 4), and is conducted in adherence to industry regulations on the cooperation of the pharmaceutical industry with the medical professions.[24-26]

Data collection and management

An interactive response technology system was used to track subject enrolment, and to register clinic visits and phone calls. A central laboratory facility is handling all standard safety blood laboratory analyses. Spirometry is performed using an external vendor's equipment to allow for centralised readings. Electrocardiograms are recorded for about a 10-second duration after the subject has rested for at least 5 minutes in a supine position, with electrode placement performed according to the Wilson method (precordial leads) and Goldberger and Einthoven (limb leads). Pulse oximetry will be performed to measure oxygen saturation; all recordings will be made using the same pulse oximetry measurement device per site on the same finger of the subject. Body plethysmography, DL_{CO}, the 6-minute walk test (6MWT) and sputum induction and processing (at selected sites only) are performed according to international accepted guidelines as follows. Body plethysmography and DL_{CO}

measurement are being performed according to American Thoracic Society/European Respiratory Society guidelines, after salbutamol administration.[27] The 6MWT is being conducted according to ATS guidelines.[28] Inspiratory and expiratory CT scans are being performed according to the specifications provided in Supplementary File 1 (table 5). Sputum induction and processing are performed according to the sputum manual issued by the vendor for sputum assessment and analysis.

All samples collected for pre-specified biomarker assessments are prepared by the site according to instructions given in the investigator site file until shipment to the central contract research organisation. Additional blood samples (whole blood, plasma and serum) will be taken at Visits 2 to 7 for future unspecified analyses, while potential leftovers from pre-specified blood analysis and induced sputum will also be stored and used for this purpose; these samples will be stored for up to 15 years after the final study report has been signed. Biomarkers are analysed by the sponsor or contractors of the sponsor. Data management and statistical evaluation are conducted by the sponsor or contractors of the sponsor. Source documents are filed at the investigator's site; these data are transferred to the electronic case report form. Missing data may be imputed if necessary.

A clinical trial monitor, appointed by the sponsor, ensures the good running of the study and directs the clinical study team in the preparation, conduct, and reporting of the study, in accordance with the sponsor's standardised operating procedures, GCP guidelines, and current legislation.

Medical information obtained for individual subjects during the study is considered confidential and this will be ensured by using subject identification code numbers.

Further information on confidentiality is detailed in the informed consent form (Supplementary File 2).

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study is being performed in accordance with the International Conference on Harmonisation Tripartite Guideline for GCP, the Japanese GCP regulations and local regulations. The protocol was approved by the authorities and IEC/IRB of the respective institutions where applicable, which included study sites in Belgium, Canada, Denmark, Finland, Germany, Japan, the Republic of Korea, Poland, Spain, Sweden, United Kingdom and the United States. In the respective countries, the ethics approval preceded the study participant's enrolment. Written informed consent is obtained from all study participants. Notably, any protocol amendments will be initiated only after all required legal documentation has been reviewed and/or approved by the respective IRB/IEC, as applicable.

DISCUSSION

COPD is characterised by progressive airflow limitation and a decline in lung function. It has also been demonstrated that emphysema, a major cause of airflow limitation in COPD, is independently associated with a rapid decline in lung function.[29,30] Current pharmacological treatment options for COPD and emphysema do not prevent the progressive decline of FEV_1 – the most commonly used marker of disease severity and progression in COPD.[1,31,32] However, FEV_1

is poorly correlated with symptoms and some other measures of disease progression.[1,33]

The heterogeneity of COPD extends to a molecular level, a concept familiar in asthma, where endotype-directed therapy is now well accepted;[34] hence there is a need for biomarkers that can assist with diagnosis, risk stratification and assessment of therapeutic interventions.[35,36] A recent systematic review by Fermont *et al.* reported that biomarkers and clinical parameters including 6MWT, fibrinogen, C-reactive protein, white cell count and interleukin-6, are associated with clinical outcomes in COPD and worth further investigation.[10] A systematic review of CT density as a radiological biomarker came to similar conclusions.[37] It is anticipated that biomarkers may facilitate a better understanding of the prognosis of COPD, help to guide treatment options and allow for a greater degree of precision medicine as treatment options.[35]

So far, few studies have addressed the potential of biomarkers to predict the longitudinal outcomes of COPD, and this has been assessed for a limited number of biomarkers only. The COPDGene® study, which has enrolled over 10,000 smoking (with or without COPD) and non-smoking subjects, is predominantly focused on identifying genetic determinants for COPD susceptibility,[38] while the UK-based British Lung Foundation early COPD study is currently enrolling smokers with normal lung function or mild lung function abnormalities to assess the very early stages of COPD development.[39] The Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) study [40] and the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS) [41] have enrolled subjects with COPD and smoking/non-smoking control subjects, similar to the COPDGene®

study. Other longitudinal studies include the German COPD and SYstemic consequences-COmorbidities NETwork (COSYCONET) study [42] and the Canadian Cohort Obstructive Lung Disease (CanCOLD) study.[43] All of these studies plan to analyse a range of soluble biomarkers and clinical outcomes, and conduct CT imaging to help identify COPD phenotypes.[39-45]

This prospective, longitudinal study was designed to investigate, over a 3-year period, biomarkers in different biofluids, imaging biomarkers and clinical parameters that may be associated with emphysema. A key strength of the FOOTPRINTS® study is that it actively recruited a subset of patients with A1ATD. This is an important but rarely studied subpopulation of patients, which has not been actively recruited for in other longitudinal studies, such as the COPDGene®,[38] ECLIPSE,[40] SPIROMICS [41] and UK early COPD cohort studies.[45] Patients with A1ATD present with earlier onset of emphysema, and could help us to better understand possible markers for emphysema progression, in particular the role of NSP imbalance conditional upon A1AT levels.[9] While A1ATD is associated with a higher risk of emphysema and COPD, increased levels of serum A1AT occur in response to inflammation.[46] Interestingly, increased levels of A1AT have also been associated with worse outcomes in patients with COPD. A recent analysis of data from the Hokkaido COPD cohort study examined the association of circulating A1AT levels with the clinical course of COPD patients without A1ATD and reported that higher A1AT levels were associated with worse outcomes, including more emphysema, a worse systemic inflammation status and higher 10-year mortality.[46] Our findings in patients with A1ATD could help us to further understand the complex relationship between A1AT levels and COPD disease progression.

Compared with the COPDGene® study, in which assessments were scheduled at baseline, 5 years and 10 years (ongoing),[38,44,47] the FOOTPRINTS® study has more frequent visits and biomarker sample collections, similar to the ECLIPSE[40] and SPIROMICS [41] studies. In particular, the regular collection of sputum is a major asset of the FOOTPRINTS® study; sputum collection is scheduled to occur more frequently than in both the COPDGene® [44] and SPIROMICS [41] studies, and with a similar frequency to the ECLIPSE [40] study, with four collections scheduled in the first year of the study, and subsequent collections scheduled at Year 2 and Year 3. Another key strength and differentiator of the FOOTPRINTS® study is that annual chest CT scans are being performed; compared to the other longitudinal studies discussed here, and to the best of our knowledge, the FOOTPRINTS® study will provide the most regular CT imaging data. This, in addition to the frequent biomarker sampling, will allow for more robust measures of short- to medium-term decline, critical for informing clinical trials of emphysema targeted medicinal products.[40,41] The FOOTPRINTS® study is also assessing a wider range of non-genetic biomarkers and clinical parameters across the entire study population compared with the COPDGene® study, which is focused primarily on genetic analyses of DNA samples.[38,44] For example, the FOOTPRINTS® study is assessing NE, PR3 and Cat G activity, which could help us to further understand the role of these biomarkers in patients with COPD, including those with A1ATD and poorer disease prognosis.[9] In addition, FOOTPRINTS® is conducting MRI assessments, which could be valuable in improving our understanding of changes in vasculature and perfusion in the lung that may precede changes in emphysema.[48] Due to the small number of patients included in the subset analysis and the

challenges associated with obtaining meaningful MRI data, these findings are expected to be limited.

Although the FOOTPRINTS® study is not focusing on frequent exacerbators, it is anticipated that a considerable number of adverse events, medical events of special interest and hospital admissions will be detected during the 3 years of follow-up. The wide range of biomarkers measured in sputum and blood may help identify subjects at risk for such events. In addition, the longitudinal biosampling in FOOTPRINTS® will offer a unique opportunity to study the impact of exacerbations on the evolution of pathogenic processes.

It is anticipated that the results of the FOOTPRINTS® study will complement, as well as expand on, the data generated in other longitudinal studies, such as COPDGene®,[38] ECLIPSE [40] and SPIROMICS.[41] It is envisaged that the results of this study will increase our understanding of COPD phenotypes and the underlying pathophysiology of emphysema progression, which may be of use when developing drugs to reduce COPD advancement. In addition, biomarkers associated with ongoing emphysematous destruction of lung parenchyma may be identified, which could assist in predicting and monitoring patients' COPD disease course.

A potential limitation of the FOOTPRINTS® study is that sputum collection and MRI only occur at certain study sites. As such, the smaller subset of subjects may not be sufficiently large to represent the entire population. In addition, there is potential for selection bias at site and patient levels, as the study is non-randomised and all participants are required to be ex-smokers. Note that current smokers were not included because they can cause high variability in the outcome if they change their smoking status; since disease progression occurs despite smoking cessation,

ex-smokers were considered to be a more appropriate population. A further limitation is that patients with the newly defined preserved ratio impaired spirometry (PRISm) phenotype [38,49,50] and healthy subjects who have never smoked are not included.

Finally, an inherent challenge in studying biomarker levels in patients with COPD is the variability in biomarker levels over time. For example, a study by Dickens *et al.* looked at variability in levels of biomarkers over 3 months in subjects with COPD. At Month 3, fibrinogen was a highly repeatable biomarker, with levels within 25% of their baseline values for 89% of study participants, yet only 21% of patients had levels of C-reactive protein within 25% of baseline values.[36] In the current study, the lack of a healthy never-smoker control group limits the opportunity to compare the extent of biomarker fluctuations over time. The reasons for variability over time are poorly understood, but the repeatability of biomarkers should be considered when selecting for clinical applications.[36] Therefore, longitudinal assessment of biomarkers is planned, to help understand the longitudinal stability of biomarker-based phenotypes.

The study findings will be reported in due course at research conferences and in peer-reviewed journals. The study protocol and clinical study data will be available to be shared after publication of the primary manuscript in a peer-reviewed journal. These data will be available upon reasonable request; further details will be provided in the primary manuscript.

CONCLUSION

Biomarkers may help to characterise patients with COPD, allow for better monitoring and prediction of the disease course, and enable an increased understanding of COPD itself. Subsequently, this should help us to develop drugs to reduce disease

progression. The FOOTPRINTS® longitudinal study is investigating biomarkers in biofluids, imaging biomarkers and clinical parameters associated with emphysema over a 3-year period, to increase the understanding of COPD patient phenotypes, pathogenesis and disease progression.

ACKNOWLEDGEMENTS

Vicki Cronin, PhD, at MediTech Media provided editorial assistance in the development of this manuscript, funded by Boehringer Ingelheim, and Harald Koegler, MD, assisted with reviewing the manuscript. The authors would like to thank all the patients and healthy volunteers for participating in this trial, and also extend their thanks to the investigators and study staff.

COMPETING INTERESTS

J. Crapo is a Co-Prinicipal Investigator for the COPDGene® study, and has received grants from NIH/NHLBI. A. Gupta, M. Beck, B. Langellier, C. Ittrich, F. Risse and C. Diefenbach are employees of Boehringer Ingelheim. D. A. Lynch has received grants from NHLBI, and personal fees from Boehringer Ingelheim, Parexel, Siemens and Veracyte. In addition, D. A. Lynch has a patent for ‘systems and methods for classifying severity of COPD’ pending. H. Watz has received grants and personal fees from Boehringer Ingelheim, and personal fees from AstraZeneca, Chiesi, GSK, Menarini and Novartis. J. Vogel-Claussen has received grants and personal fees from Boehringer Ingelheim and Novartis, and grants from GSK and Siemens Healthineers. A.M. Turner has received personal fees, payment for educational talks

and expenses payments for work on the FOOTPRINTS steering committee from Boehringer Ingelheim, grants and personal fees from AstraZeneca, CSL Behring and Grifols Biotheraputics, and grants, personal fees and non-finanacial support from Chiesi. R. Mroz has received personal fees from AstraZeneca, Boehringer Ingelheim, GSK, Merck Sharp & Dohme, Mundipharma, Novartis and Roche. W. Janssens has received grants from AstraZeneca and Chiesi. A. Ludwig-Sengpiel reports receipt of a study fee for involvement in conducting the FOOTPRINTS study.

FUNDING OF STUDY

This study was funded and sponsored by Boehringer Ingelheim. The trial clinical monitor for this study was Markus Beck (Boehringer Ingelheim Pharma GmbH & Co. KG, Dept. Clinical Research Germany/Respiratory Diseases, Birkendorfer Str. 65, 88397 Biberach an der Riss, Germany; Phone: +49 (0) 7351 / 54 96 803).

AUTHORS' CONTRIBUTIONS

J. Crapo, A. Gupta, D. A. Lynch, J. Vogel-Claussen, H. Watz, M. Beck, C. Ittrich, F. Risse and C. Diefenbach contributed towards the overall concept and design of the original study protocol. B. Langellier, C. Diefenbach, M. Beck and C. Ittrich led the writing of the protocol. J. Crapo, A. Gupta, D. A. Lynch, J. Vogel-Claussen, H. Watz, M. Beck, B. Langellier, C. Ittrich, F. Risse and C. Diefenbach provided critical input at all stages. All authors have contributed towards the development of the manuscript, and have read and approved the final manuscript.

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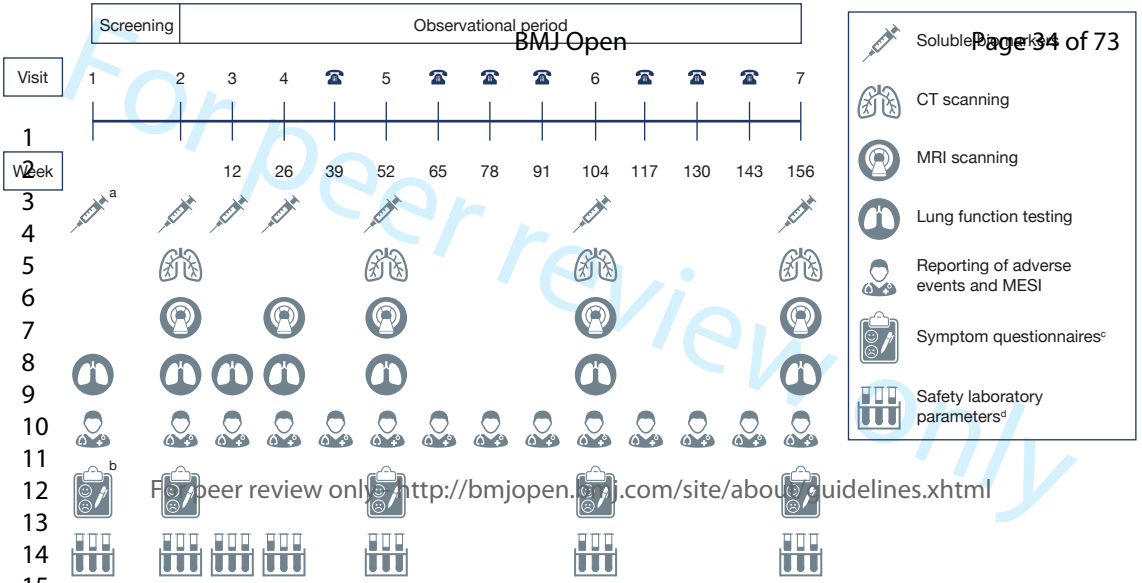
Figure 1. Overview of trial design

[a]A1ATD analysis only; this occurs at Visit 1 only. [b]mMRC only. [c]Symptom questionnaires include the CAT, mMRC and SGRQ. The CAT, mMRC and SGRQ are being conducted in patients with COPD and with COPD plus A1AT deficiency only. [d]A full panel of haematology, blood chemistry, and coagulation parameters is performed at Visits 1, 2, 5, 6 and 7, with a reduced panel comprising of haematology, differential automatic cell counts, fibrinogen, highly sensitive C-reactive protein and creatinine performed at Visits 3 and 4.

A1ATD, alpha1-antitrypsin deficiency; CAT, COPD assessment test; COPD, chronic obstructive pulmonary disease; CT, computed tomography; MESI, medical events of special interest; mMRC, modified Medical Research Council dyspnoea scale; MRI, magnetic resonance imaging; SGRQ, St. George's Respiratory Questionnaire.



Supplementary Text 1. Participant enrolment and retention, sample size calculation, and power calculations for annual decline in forced expiratory volume in 1 second and 15th percentile density assessed by CT

To achieve adequate participant enrolment, a recruitment plan was set up prior to the start of recruitment; this detailed the number of subjects expected at each site across the different study populations, and was based on feedback during site feasibility assessments. This plan was monitored constantly during the recruitment phase to track planned versus actual enrolment numbers. Local approaches to facilitate recruitment, such as advertising, were also used. To promote participant retention, a comprehensive strategy was set up during the course of the study. This included maintaining frequent contact with study participants (especially from Visits 5 to 7, where visits are 1 year apart), for example via phonecalls and greetings cards, as well as awareness training for site staff.

As this study is exploratory in nature, sample size calculations are focused on achieving the desired precision for biomarker estimates and relevant clinical assessments in all COPD patient groups except for the A1ATD group. This study planned to enrol 125 patients with mild COPD, 125 with moderate COPD and 125 with severe COPD – by assuming a ≤20% dropout rate after 3 years, at least 100 subjects per group are expected to contribute to the analysis by the end of the study period.

For annual decline in forced expiratory volume in 1 second (FEV_1), available information reports standard deviations (SDs) ranging from about 20 mL/year [1] to 60 mL/year.[2] Using this information and considering a planned 100 subjects in each group, supplementary table 1 reports the coverage probability for the half-width of a 95% confidence interval (CI) for annual FEV_1 decline for a given SD. For

example, if the observed SD is 50 mL/year, and the true half-width of the CI is 11 mL/year, with 100 subjects, the probability that the observed CI covers the true CI is 93.8%. The reported SD on annual decline in 15th percentile density (PD15) ranges from 3.4 g/L [3] to 5.5 g/L.[4] Using the same assumptions as for FEV_1 decline, we report the coverage probability of a true CI for a given SD and half-width of the CI for 100 subjects (supplementary table 2).

In addition, with 100 subjects in each group, and allowing for up to 15 covariates, a partial Pearson correlation coefficient of 0.30 can be declared significant with 80% power between any two continuous variables (e.g. FEV_1 and a new biomarker) within the group.

Supplementary Table 1. Coverage probability of 95% CIs for FEV_1 annual decline based on 100 subjects

SD	Half-width of a 95% CI									
	6	7	8	9	10	11	12	13	14	15
25	0.998	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
30	0.557	0.993	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
35	0.026	0.557	0.983	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
40	<0.001	0.048	0.557	0.97	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
45	<0.001	<0.001	0.072	0.557	0.954	>0.999	>0.999	>0.999	>0.999	>0.999
50	<0.001	<0.001	0.003	0.097	0.557	0.938	0.998	>0.999	>0.999	>0.999
55	<0.001	<0.001	<0.001	0.006	0.122	0.557	0.921	0.996	>0.999	>0.999
60	<0.001	<0.001	<0.001	<0.001	0.011	0.146	0.557	0.905	0.993	>0.999
65	<0.001	<0.001	<0.001	<0.001	<0.001	0.018	0.168	0.557	0.889	0.988
70	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.026	0.189	0.557	0.873

CI, confidence interval; FEV_1, forced expiratory volume in 1 second; SD, standard deviation.

Supplementary Table 2. Coverage probability of 95% CIs for PD15 annual decline based on 100 subjects

SD	Half-width of a 95% CI										
	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00	1.05
2.5	0.938	0.998	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
3.0	0.146	0.557	0.905	0.993	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
3.5	0.001	0.026	0.189	0.557	0.873	0.983	0.999	>0.999	>0.999	>0.999	>0.999
4.0	<0.001	<0.001	0.005	0.048	0.226	0.557	0.846	0.970	0.997	>0.999	>0.999
4.5	<0.001	<0.001	<0.001	<0.001	0.011	0.072	0.257	0.557	0.822	0.954	0.993
5.0	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.021	0.097	0.284	0.557	0.801
5.5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	0.033	0.122	0.306
6.0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.011	0.048

CI, confidence interval; PD15, lung density at the 15th percentile point of the CT histogram; SD, standard deviation.

Supplementary Table 3. Overview of permitted and restricted medications/therapy

Drug class	Prior to Visit 1	Between Visits 1 and 2	Observational period
Corticosteroid – nasal/ocular	Permitted	Permitted	Permitted
Corticosteroid – oral, inhaled*	Permitted†	Permitted (stable dose)‡	Permitted
Corticosteroid – i.v., i.m.	Permitted†	Not permitted‡	Permitted
SABA – inhaled§	Permitted†	Permitted (stable dose)‡	Permitted
LABA – inhaled§	Permitted†	Permitted (stable dose)‡	Permitted
SAMA – inhaled	Permitted†	Permitted (stable dose)‡	Permitted
LAMA – inhaled§	Permitted†	Permitted (stable dose)‡	Permitted
Leukotriene modifier	Permitted†	Permitted (stable dose)‡	Permitted
Cromolyn sodium / nedocromil sodium	Permitted†	Permitted (stable dose)‡	Permitted
Methylxanthines	Not permitted‖	Not permitted	Permitted
PDE-4 inhibitors	Not permitted‖	Not permitted	Permitted

Immunomodulators (e.g. methotrexate)	Not permitted‖	Not permitted	Permitted
Immunotherapy (e.g. subcutaneous or sublingual)	Permitted†	Permitted	Permitted
Biologic antagonists (e.g. omalizumab)	Not permitted‖	Not permitted	Not permitted
A1AT augmentation therapy	Not permitted	Not permitted	Permitted
Other investigational drugs	Not permitted	Not permitted	Not permitted

*For patients taking stable maintenance OCS with a total daily dose of ≤20 mg prednisone or equivalent.

†Allowed as stable dose for at least 4 weeks before Visit 1.

‡If unstable (e.g. in case of exacerbations), extend screening period.

§Includes fixed dose combination therapy.

‖Allowed before Visit 1 with a washout period of 3 months or 6 half-lives whichever is greater.

A1AT, alpha1-antitrypsin; LABA, long-acting β2-adrenergic agonist; LAMA, long-acting anticholinergic; OCS, oral corticosteroids; PDE-4, phosphodiesterase-4; SABA, short-acting β2-adrenergic agonist; SAMA, short-acting anticholinergic

Supplementary Table 4. Steering committee members

Scientific expert	Affiliation	Role
Prof. James Crapo	National Jewish Health, Denver (CO), USA	Chair/Advisor/Discussant
Prof. David Lynch	National Jewish Health, Denver (CO), USA	Advisor/Discussant
Dr. Henrik Watz	Pulmonary Research Institute, Lungen Clinic Grosshansdorf GmbH, Grosshansdorf, Germany	Advisor/Discussant
Prof. Pascal Chanez	CHU Marseille – Hôpital Nord, Marseille, France	Advisor/Discussant
Prof. Gaetan Deslee	CHU Reims – Hôpital Maison Blanche, Reims, France	Advisor/Discussant
Dr. Alice Turner	Centre for Translational Inflammation Research, Birmingham, UK	Advisor/Discussant
Dr. Richard Casaburi	Los Angeles Biomedical Research Institute, Torrance (CA), USA	Advisor/Discussant
Prof. Kazuhisa Asai	Osaka University Graduate School of Medicine, Osaka, Japan	Advisor/Discussant
Dr. Harald Koegler	Boehringer Ingelheim	Medical Project Lead
Dr. Markus Beck	Boehringer Ingelheim	Participant/Discussant
Dr. Claudia Diefenbach	Boehringer Ingelheim	Participant/Discussant
Dr. Frank Risse	Boehringer Ingelheim	Participant/Discussant
Dr Abhya Gupta	Boehringer Ingelheim	Participant/Discussant

Supplementary Table 5. CT scan protocol in inspiration and expiration

Scan coverage	Lung apices to costophrenic recesses
Scanning mode	Helical
Scanning plane	Axial
Dose modulation	On
Detector configuration	64 x 0.625 mm (GE VCT Discovery STE, VCT Discovery 750HD 64 slice) 64 x 0.5 mm (Philips Brilliance 64 slice) (64)32 x 0.6 mm (Siemens Definition DS, Sensation 64 slice) (128)64 x 0.6 mm (Siemens Definition Edge, AS+ 128 slice, Flash 128 slice, Somatom Force)
kVp	120 kVp
mA/mAs	GE (750HD, Discovery STE): Target noise index: 66.5, Maximum mA limit 100 Philips (Brilliance 64): Reference mAs – 46 mAs (128 mA) Siemens (effective mA): Sensation 64, Definition (DS/AS 64 slice)/(AS+ 128 slice/Flash 128 slice), Edge, Force – Reference mAs 35 mAs
Pitch	GE (Discovery 750HD, Discovery STE) – 0.984 Philips (Brilliance 64) – 0.923 Siemens – 1.0
Rotation time	0.5 s
Reconstructions	
Non-iterative	GE (750HD, Discovery STE): Standard, Bone Philips (Brilliance 64): B, D (YB)

	Siemens (Sensation 64, Definition DS/AS/AS+/Flash 64/128): B31f, B45f (B46f) Siemens (Somatom Force): Bf40d, Qr44d Siemens (Somatom Definition Edge): Bf37f, Bv45f
Iterative	GE (750HD, Discovery STE): Standard ASIR 40%, Bone ASIR 40%, Standard ASIR 100% Philips (Brilliance 64): B iDose L3, D (YB) iDose L3, B iDose L7 Siemens (Sensation 64, Definition DS/AS/AS+/Flash 64/128): I31f SAFIRE 2, I44f SAFIRE 2, I31f SAFIRE 5 Siemens (Somatom Force): Bf40d ADMIRE 2, Qr44d ADMIRE 2, Bf40d ADMIRE 5 Siemens (Somatom Definition Edge): Bf37 f2, Br54 f2, Bf37 f5
Thickness	GE (Discovery 750HD, Discovery STE) – 0.625 Philips (Brilliance 64) – 0.67 Siemens – 0.75 mm
Interval	0.5 mm
DFOV	Lungs (no more than a 1 cm margin outside of the lungs on either side of the scan, where the lungs are the widest in the chest)
HD mode	GE (750HD, Discovery STE): OFF Philips (Brilliance 64): n/a Siemens (Sensation 64, Definition Edge/DS/AS/AS+/Flash 64/128, Somatom Force): n/a

DFOV, display field of view; HD, high definition.

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2. Bhatt SP, Bodduluri S, Hoffman EA*, et al.* Computed tomography measure of lung at risk and lung function decline in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2017;196(5):569-76.
3. Coxson HO, Dirksen A, Edwards LD*, et al.* The presence and progression of emphysema in COPD as determined by CT scanning and biomarker expression: a prospective analysis from the ECLIPSE study. *Lancet Respir Med* 2013;1(2):129-36.
4. Dirksen A, Piitulainen E, Parr D*, et al.* Exploring the role of CT densitometry: a randomised study of augmentation therapy in α1-antitrypsin deficiency. *Eur Respir J* 2009;33(6):1345-53.

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INFORMATION AND CONSENT FORM FOR PATIENTS WITH COPD (including Sputum assessment)

STUDY TITLE: Observational study in healthy subjects and patients with COPD to assess the relationship between clinical, imaging and biomarker measurements, and progression of emphysema over three years.

PROTOCOL No.: 352.2069 FOOTPRINTS™

SUBJECT No.: _____

EudraCT No.: NA

SPONSOR: Boehringer Ingelheim Pharma GmbH & Co. KG
Birkendorfer Straße 65, 88397 Biberach an der Riss, Germany

STUDY DOCTOR: Name, address, telephone number

Dear Study Participant,

You are being asked to participate in this observational study because:

- You are a 40 to 70 year old ex-smoker, having quit smoking more than 9 months and you have been diagnosed with Chronic Obstructive Pulmonary Disease (COPD)

  Or

- You are a 30 to 70 year old ex-smoker, having quit smoking more than 9 months you have an alpha 1-antitrypsin (A1AT) deficiency phenotype zz and you have been diagnosed with COPD.

This is a 3 year observational study to investigate if there are biomarkers, specific for chronic obstructive pulmonary disease (COPD) and especially emphysema. COPD is a common cause of lung problems and is most often due to smoking. At first, COPD may cause no symptoms or only mild symptoms. As the disease gets worse, symptoms usually become more severe. People with COPD often have symptoms such as cough, abnormal sputum production and shortness of breath, due to narrowing and blockage of the breathing tubes through which air flows in and out of the lungs. Apart from smoking cessation, there is no treatment that can prevent the progression of the disease. Destruction of lung tissue (called emphysema) is a characteristic of COPD involving damage to the air sacs (alveoli) in the lungs which are needed for gas exchange. As a result, the body does not get the oxygen it needs. Many patients with COPD, but not all, develop emphysema at some point. Biomarkers are medical signs, which can be measured, such as blood tests, imaging

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and lung tests. Biomarkers identified in this study could contribute to discovery of new drugs for treatment of COPD and associated lung tissue destruction.

Please read the following information carefully. It contains important information to help you decide whether to participate in this research study. The study staff will have a detailed discussion with you to inform you about the study and the possible benefits and risks of your participation. Ask questions about anything that is not clear at any time. You may take home an unsigned copy of this information to think about and discuss with your family, friends or family doctor before you make your decision to participate or not.

After reading and discussing the information you should know:

- Why this research study is being done;
- What will happen during the study;
- Any possible benefits to you;
- The possible risks to you;
- Other options you could choose instead of being in this study;
- How your personal information / health information will be treated during the study and after the study is over, and which data privacy rights you have;
- Whether being in this study could involve any cost to you; and
- What to do if you have problems or questions about this study.

Participation in this study is voluntary (your choice). If you join this study, you can still stop at any time. You have the right not to sign this consent form. If you do not sign, you cannot take part in this research study. If you decide to participate, you will be asked to sign and date at the end of this form.

PURPOSE OF THE STUDY

The purpose of this study is to evaluate biomarkers correlating to the development and progression of the COPD disease, in particular the emphysema. This study is an observational study. The study does not include administration of any investigational medicinal product and you are allowed to continue and change your standard medication during the study.

COPD is a common disease of lower airways, which significantly affects the quality of life. The disease progresses over time and eventually results in complete disability. Despite major advances in COPD treatment, none of existing drugs can stop the progression of the disease, nor lung tissue destruction (called emphysema), which occurs in every 5th patient with COPD. Also, there are no reliable biological markers (biomarkers) of the disease in sputum and blood, which would allow for earlier recognition of COPD, its progression and response to treatment other than lung function tests. Such biomarkers would also contribute to faster discovery of new drugs for COPD. This study is undertaken to collect data on various biomarkers in induced sputum and blood as well as markers derived from imaging techniques (like chest CT or MRI) in patients with different degrees of COPD and of healthy subjects, who serve as control group. It will also be checked if any of these markers reflect COPD severity or presence or progression of emphysema. This research can lead to the discovery of new diagnostics and medicines and improve the treatment of COPD including associated emphysema in the future.

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We estimate that approximately <enter number of participants assigned to your OPU> people will participate in this study in <insert country of OPU> and approximately 455 participants worldwide.

This study has been approved by <insert applicable local authorities, if required, otherwise this statement can be deleted>.

DESCRIPTION OF THE STUDY

The study is multi-national and involves approximately 12 participating countries. It consists of 2 consecutive periods, a screening and an observational period. The screening period lasts up to 28 days. The observational period lasts 3 years and includes 6 clinic visits and 7 additional phone contacts.

FLOW CHART OF THE STUDY

Study Periods	**Screening Period**	**Observational Period**												
Visit	**1**	**2**	**3**	**4**	☎	**5**	☎	☎	☎	**6**	☎	☎	☎	**7**
Weeks			**12**	**26**	**39**	**52**	**65**	**78**	**91**	**104**	**117**	**130**	**143**	**156**
Interview MESI Check	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination & Vital Signs	X					X				X				X
Blood collection for safety and biomarkers	X	X	X	X		X				X				X
Urine collection (smoking status)	X	X	X	X		X				X				X
Questionnaires	X	X				X				X				X
Pulse oximetry	X	X		X		X				X				X
Lung function testing	X	X	X	X		X				X				X
Review of the diary		X	X	X	X	X	X	X	X	X	X	X	X	X
Chest CT		X				X				X				X
MRI (optional)		X		X		X				X				X
Induced sputum		X	X	X		X				X				X
Exercise testing (6 MWT)	X	X								X				X

STUDY PROCEDURES

Pre-study procedures

Before any study-related procedures are performed, you will be asked to read and sign this consent form to confirm that you wish to participate. The study will be explained to you. You can ask questions and if you agree to take part in the study, you need to sign the consent form.
By signing the Informed Consent you confirm your participation in this study.

Please note, that you are also asked to agree to collection of unspecified blood and sputum samples for biobanking. A biobank is a place where samples are long term stored until they are needed for

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further research. This is part of this study. Please read the respective descriptive sections in this document.

Study procedures taking place at each visit

At each clinic visit or scheduled phone visit

- You will be asked about your overall health and changes in medication.
- Please use your reminder diary and note your respiratory symptoms, hospitalization or doctor's visits and all medication you are taking.

Study procedures at the screening visit (Visit 1)

- The study will be introduced to you.
- You will be asked for your demographics, such as sex, race, year of birth.
- Your medical and smoking history will be reviewed.
- You will have a complete physical examination, which will include measurements of blood pressure, pulse rate, temperature, weight, and height.
- An electrocardiography (ECG) will be performed. The ECG is a painless test which measures the electrical activity of the heart.
- Blood (approximately 15 ml or 3 teaspoons) will be drawn from a vein in your arm for different laboratory tests, including a genetic test to look whether you have a genetic variation called alpha 1-antitrypsin deficiency (A1ATD). A1ATD is a genetic disorder that causes defective production of alpha 1-antitrypsin (A1AT). There are several forms and degrees of the deficiency. The most common abnormal genes are the S and Z alleles. In this study only patients with the zz-gene mutation can participate. Severe A1ATD can lead to liver disease and emphysema.
- A urine sample will be taken for nicotine testing.
- For all women of child bearing potential a urine pregnancy test will be done.
- You will complete the mMRC (Modified Medical Research Council) questionnaire.
- You will have a lung function test before and after taking salbutamol/albuterol [OPU to adapt locally]. You will be asked to blow hard into the mouthpiece of the measuring device. The test determines how well your lungs work. The procedure will be explained in detail before the test.
- You will have a lung diffusion test called D_{LCO} after taking salbutamol/albuterol [OPU to adapt locally]: this lung diffusion test measures how well the lungs exchange gases. This is an important part of lung function testing, because the major function of the lungs is to allow oxygen to "diffuse" or pass into the blood from the lungs, and to allow carbon dioxide to "diffuse" from the blood into the lungs. You breathe in (inhale) air containing a very small amount of carbon monoxide, a gas existing in the atmosphere but given to you in a higher concentration. You hold your breath for 10 seconds, and then rapidly blow it out (exhale). The exhaled gas is tested to determine how much of the carbon monoxide gas was absorbed during the breath. The remaining gas will easily be eliminated from your body by normal breathing.
- A 6-minutes walking exercise test will be done. You will be asked to walk as far as possible for 6 minutes and the distance will be measured. You will get sufficient time to recover from the walking test.
- Pulse oximetry: this test measures how much oxygen your blood is carrying. By using a small device put on your forefinger your blood oxygen level can be checked.

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- You get a reminder diary to write down if your health conditions have gotten worse, if you experienced symptoms and all medication that you are taking.

The results of the tests and/or questions at the screening visit will help the study team to decide whether you can continue in this study. If these tests show that you are eligible to participate, you will be able to continue the study. If you do not meet the eligibility criteria, you will not be able to continue.

Even if you are eligible for the study, your participation cannot be guaranteed. At the moment when the maximum number of subjects who can be included is reached, you will not be able to enter the observational phase of the study.

Visit 1 will last approximately 3 - 4 hours.

You will be scheduled to return for Visit 2 within the next 28 days.

Study procedures in the observational period (Visits 2 to 7)

You will be scheduled to return to the clinic for 6 observational period visits. All visits during the observational period will be scheduled to begin between 06:00 and 09:00 in the mornings. Please come fasted to the visits. Please do not have anything to eat or drink, except water, for 6 hours before the visit. This is very important to get good laboratory measurements.

- First at each visit you will be asked about your medication intake, your diet and life style and your smoking status.
- You will have a complete physical examination, which will include measurements of blood pressure, pulse rate, temperature, weight, and respiratory rate at visits 5, 6 and 7.
- For all women of child bearing potential a urine pregnancy test will be performed at visits 2, 4, 5, 6 and 7.
- You will complete at visits 2, 5, 6 and 7 three questionnaires prior to pulmonary function testing. The questionnaires contain questions about your health, the breathing and COPD status: All questionnaires together will take approximately 60 minutes to complete.
- Lung function testing (each performed twice), D_{LCO} and pulse oximetry will be performed at each clinic visit, except at visit 3.
- Body plethysmography will be performed at each clinic visit, except at visit 3. You sit in a small, airtight room known as a body box. You breathe against a mouthpiece. Clips are put on your nose to shut off your nostrils. As your chest moves while you breathe, it changes the pressure and amount of air in the room and against the mouthpiece. From these changes an accurate measure of the amount of air in your lungs can be determined.
- Between lung function tests salbutamol/albuterol [OPU to adapt locally] will be administered.
- Induced sputum will be collected at each clinic visit. Sputum induction is a procedure to help you cough up secretions from your lungs more easily. The principle is to create extra moisture in the airways of the lungs by inhaling a saline enriched aerosol, which will help loosen the sputum deep in your lungs. The sputum sample will be examined further. In case you are not able to produce an acceptable sputum sample at Visit 2 and at the Visit 2 retest you will continue in the study, but no more sputum induction will be performed in the study.
- Blood samples for safety laboratory testing, and biomarker assessments will be drawn at each clinic visit (about 90 ml or 9 tablespoons).

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- A urine sample for nicotine testing will be taken at each clinic visit.
- Your reminder diary will be reviewed at each visit.
- An ECG will be performed at visits 5, 6 and 7.
- A chest computer tomography (CT), a radiologic imaging method, enabling a detailed look into your body, will be performed at visits 2, 5, 6 and 7 between 1h and 4h after salbutamol/albuterol [OPU to adapt locally] administration for the lung function testing.
- The 6-minute walk test will be performed at visits 2, 6 and 7 after the lung function testing.

Visits 2 – 7 will last approximately 4 – 7 hours. Some visit procedures like induced sputum and/or chest CT can be rescheduled to another day (within 14 days of the original visit), e.g. if you have time constraints. In this case you will be administered with salbutamol/albuterol [OPU to adapt locally] prior to the assessment(s).

Telephone contacts

Additionally, you will have 7 telephone contacts between clinic visits 4, 5, 6 and 7 to follow up and collect information by telephone interviews. Your reminder diary helps you to remember your respiratory symptoms, any COPD exacerbations (COPD flare up with symptoms worsened suddenly), hospitalization or health care visits, and all medication you were taking.
Please remember to inform your study team prior to scheduled telephone interviews in case you have been hospitalized. Telephone calls might take about 30 minutes.

Study completion

Visit 7 or the discontinuation visit, in case you terminate the study early, will mark the end of the study and you have completed the study.

Early discontinuation

If you terminate the study during the observational period for any reason, you will be asked to come to the clinic for a discontinuation visit at the time of your next scheduled visit. You will undergo the procedures of this particular visit as outlined in the flowchart. If you do not agree to undergo all procedures of the next scheduled visit, you are asked to do at least the safety assessments.

Women who become pregnant will be discontinued from further study participation and will undergo the procedures for safety assessments like for example blood collection and physical examination.

Phone calls after early discontinuation

After you have discontinued the study early, the study team will contact you for further follow up, if you agree. You will be asked if you have experienced COPD exacerbations. If your discontinuation takes place between:

Discontinuation date between Visit 2 and 5, three telephone calls:

1. First call: 52 weeks after your Visit 2
2. Second call: 104 weeks after your Visit 2
3. Third call: 156 weeks after Visit 2

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Discontinuation date between Visit 5 and 6, two telephone calls:

1. First call: 104 weeks after your Visit 2
2. Second call: 156 weeks after your Visit 2

Discontinuation date between Visit 6 and 7, one telephone call:

1. Call: 156 weeks after Visit 2

YOUR RESPONSIBILITIES

- While participating in this study, you should not take part in another medication research study. You must tell your study doctor if you have been in another research study in the past 6 weeks or are currently in another research study.
- You must follow the study instructions provided by the study staff, come to all scheduled study visits and be reasonably available for the scheduled telephone visits.
- Prior to and during the lung function assessments you have to stay in in the building where the measurements are performed.
- You must tell the study doctor about all prescription and non-prescription drugs, herbal preparations and food supplements that you are taking or planning to take.
- Please refrain from strenuous activity for at least 12 hours prior to lung function testing, also avoid cold temperatures, environmental smoke, dust or areas with strong odors (e.g. perfumes).
- Please do not donate blood during the observational period of the study.
- You must fast, not have anything to eat or drink, except water, for 6 hours before all study visits.
- You might be asked to provide an additional sputum sample prior to Visit 2 that will be used for quality assurance of sputum processing. This sputum sample will be taken as described above.

POTENTIAL BENEFITS

This study is an observational study with no change in your usual medication treatment. There will be no direct benefit for you, except for the benefit which derives from the extensive examinations and periodic monitoring of your health or disease status.

RISKS AND/OR DISCOMFORTS

Your participation in this study requires standard medical procedures which are well known to patients with respiratory diseases. The examinations include procedures like physical examination, blood sampling, ECGs, lung function testing, D_{LCO} measurements, bodyplethysmography, chest CTs and sputum induction.

Computed tomography

A CT scan will be done at four visits. This is a painless test, a type of X-ray exam, which shows detailed lung structures. You will be asked to strip to the waist and lie on the narrow table that will slide through a hole in the center of the CT scanner. You have to stay still during the procedure and follow the instructions when to hold your breath. The actual CT scanning takes less than 30 seconds and the entire process is usually completed within 15 minutes.

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The scientific unit of measurement for radiation dose is the millisievert (mSv). Chest CT scans involve exposure to radiation equivalent to 20 chest x-rays (approximately 4 millisievert). Although the amount of radiation exposure is higher than a typical x-ray, the risk of harmful effects from a single exam is small. Persons are exposed to radiation from natural sources in the environment all the time. These natural "background" doses vary from area to area. The natural background radiation is 2.4 mSv per year. The additional risk of developing a fatal cancer from a single 4 mSv exposure in a person 50 years old is approximately 1 in 5000 or 0.02%. If it were to occur, it could take many years or decades for you to develop cancer related to this study. The latent period for cancer is estimated to be 6 to 10 years for blood borne cancers (leukemia, lymphoma) and 10 to 25 years for solid organ cancers. Please keep in mind that the risk from all sources of radiation is cumulative over a lifetime.

The chest CT is a very sensitive diagnostic method and reveals lung nodules in approximately 20% of people. The chest CT scan may detect lung abnormalities much earlier than other diagnostic methods, which may be life-saving in subjects at risk of lung cancer (e.g. smokers). Such finding causes anxiety and triggers further diagnostic work-up to confirm or reject malignancy. While an early diagnosis of lung cancer is critical for successful treatment, most lung nodules accidentally found on the chest CT scan turn out to be benign.

ECG

You may experience skin irritation from the ECG electrode pads or pain when pads are removed.

Lung function measurement (spirometry and diffusing capacity of the lungs for carbon monoxide)

Risks and discomforts associated with lung function testing may include shortness of breath, dizziness, or headache during the breathing tests. Should this occur, you may receive treatment.

6-minute walk test

The 6-minute walk test measures the distance that you can quickly walk on a flat, hard surface in a period of 6 minutes. To prevent any risk to your health you will be constantly supervised by specially trained medical personnel and your health will be monitored. The test may be stopped if you are unable to continue safely in the opinion of the supervising personnel. After the test you will have enough time to recover and to leave the office/clinic in good health.

Sputum induction

Sputum induction is a painless and safe procedure. Rarely, it may cause transient wheeze or chest tightness. Let your study doctor know immediately if you feel any of these symptoms at any time during the induction procedure. These symptoms could be quickly relieved by inhaling an appropriate drug. The procedure is performed after inhalation of salbutamol/albuterol [OPU to adapt locally] that helps open up the air passages. Side-effects of salbutamol/albuterol [OPU to adapt locally] are rare and further details can be found below.

Laboratory tests

Blood draw may cause some discomfort or mild pain, as well as redness or bruising at the site of puncture. In rare cases the puncture site can also become infected or nerves may be damaged, inducing long lasting abnormal sensations (paresthesia), impaired sensation of touch and persistent pain.

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A total blood volume of about 500mL will be withdrawn over a period of about 3 years. Approximately 90 mL will be drawn per time point (Visit 2 – 7). The estimated blood loss will not impact your health.

Bodyplethysmography

Some people may feel uncomfortable in the small plethysmography chamber called body box. Tell the study staff if you feel uncomfortable in any way during the test and keep in mind that you can open the door from the inside at any time.

Salbutamol/Albuterol [OPU to adapt locally]

Salbutamol/Albuterol [OPU to adapt locally] is a short-acting bronchodilator used to treat narrowed airways like in asthma or COPD. It is taken by the inhaled route for direct effect on bronchial smooth muscle. In general, salbutamol/albuterol [OPU to adapt locally] is well tolerated even at large doses. In this study, salbutamol/albuterol [OPU to adapt locally] is used only at clinic visit days in combination with some diagnostic tests like PFTs, imaging assessments or induced sputum. It is not administered for the treatment of a disease.

The most common side effects are shaking of fingers, anxiety, headache, muscle cramps, dry mouth, and palpitation (awareness of heart beat). Other symptoms may include increase in heart rate, irregular heartbeat, flushing, myocardial ischemia (reduced blood supply of the heart muscle) (rare), and disturbances of sleep and behavior. Rarely occurring, but of importance, are allergic reactions like unexpected narrowing of the airways (can be life threatening in rare cases), rapid swelling of the facial skin rarely with life threatening breathing difficulties, skin hives with itching and redness, decrease in blood pressure, and collapse. High doses or prolonged use may cause hypokalemia (low levels of potassium in your blood).

ALTERNATIVES

This study is for research purposes only. Your participation is voluntary. The alternative is not to participate in the study.

Please talk to the study doctor about your options before you decide whether you will take part in this study.

NEW INFORMATION ABOUT THE STUDY

During the study, you will be notified of changes to study procedures, which may affect your health or willingness to participate. You may be asked to sign a new consent form that shows that you have been informed of changes.

INFORMATION ON BIRTH CONTROL

You cannot participate in this study if you are pregnant or plan to become pregnant due to the planned radiologic procedures. If you should nevertheless become pregnant or you think you could be pregnant during the study, it is important for you to tell the study doctor or study staff prior to each clinic visit. In case of pregnancy, you will be removed from the study procedures and you will be followed up by phone calls.

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WITHDRAWAL FROM THE STUDY PARTICIPATION

Your participation in this study is voluntary. You may choose to leave this study completely at any time. Your decision will not result in any penalty or loss of benefits to which you are otherwise entitled. Leaving the study will not affect your future medical care.
All data and samples that had already been collected up to the time of withdrawal of your consent, including data and samples gathered at any of your final assessments, will still be used to ensure the correct completion and documentation of the trial and comply with applicable law.

Your study doctor might decide to stop your study participation early without your consent when, in the study doctor's judgment, it is in your best health interest to do so or under certain circumstances listed below:

- Your inability to participate as instructed.
- Study cancellation by the sponsor or [if applicable regulatory authority/EC].
- Or for other unforeseen reasons that make it necessary to stop your participation in the study.

If you are removed from the study, the study doctor will explain to you why you were removed.

CONFIDENTIALITY / PRIVACY AND DATA SHARING

Use of your personally identifiable information

The part of your personal information that directly identifies you such as your name, address, or birth date will remain at the study site and can be accessed by the study doctor and other people at the site who are assisting with the study or your care. This information may also be checked at the study site by the

- sponsor, or the sponsor's representatives (including monitors hired by the sponsor through a service provider),
- ethics review board/committee that reviewed the ethical aspects of this study, and/or
- [Please adapt or delete if not applicable] domestic or foreign regulatory agencies such as the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) that approve medicines.

These persons check that the study is carried out correctly at the study site. They are bound by a duty of confidentiality.

Use of your coded data

Your personally identifiable information and health information collected for the purpose of the study as well as the samples will be labelled with a unique code number. Coded data may also include data/information such as images (e.g. CTs/MRTs). The code number will be used in place of your name and other information that directly and easily identifies you, for example, address and birth date. Only the study site will have the link between your personal information and the coded data. This link will not be provided to the sponsor; only your coded data such as medical data, biomarker data, images and all other information collected in the study will be sent to the sponsor and/or contractors of the sponsor. The sponsor and/or contractors of the sponsor will take measures to protect the confidentiality and security of your coded data and your privacy in accordance with current law.

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The sponsor and other members of the Boehringer Ingelheim Group of Companies and those working with them such as their associates, collaborators, research partners, assignees, licensees and designees, and its/their affiliated companies and agents, and other individuals and organizations, may use your coded data for the following purposes:

- Keep it electronically and analyze it to understand the study and the study results;
- Share it with domestic or foreign regulatory agencies worldwide such as the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), that approve medicines;
- Share it with ethics review boards/committees worldwide, or steering committee that checks whether the study was run properly.
- Analyse it to improve the quality of this study and other clinical trials

The coded data may be transferred within your country or to other countries for analysis. Where the data protection rules in other countries are not as strict as the rules in your country, the sponsor will adopt appropriate measures to provide an adequate level of protection according to EU law.

Use of anonymized data for additional research

Anonymized data refers to data from which the subject cannot be identified by the recipient of the information. Anonymizing data is one of the strongest safeguards for the protection of subjects' identity.

The sponsor may give scientists and medical researchers in other companies, research organizations, or academic institutions access to anonymized data for further research and education beyond the disease investigated in this study. This may include research looking into improving patient care or quality and efficiency in conducting clinical studies in general. Details on the anonymization and data sharing process are set forth in the Boehringer Ingelheim "Policy on Transparency and Publication of Clinical Study Data" available at: http://trials.boehringer-ingelheim.com.

Information and correction rights

You have the right to review which personal data the trial site and sponsor store about you. You can also request that incorrect personal data is corrected or that processing is restricted. You can request a copy of the contractual safeguards implementing adequate protection of personal data if your data is shared outside the EU/EEA.

In order to exercise your rights please contact the study site [if applicable: and its data protection officer (ADD EMAIL)] who will align with the sponsor. You can also ask to receive the personal information you have provided for the study in a standardized electronic format or to have them transmitted to another person of your choice. You can also contact your local data protection authority in case of questions or concerns about the handling of your personal data. In some cases, your rights can be limited under applicable laws, especially where they conflict with the conduct of the study and mandatory archiving requirements. In this case you will be informed accordingly.

If you have signed an Information and Consent Form for the optional DNA banking sample, all the information provided in this form under section "Confidentiality / Privacy and Data Sharing" your rights under data protection laws and the information on how to exercise them applies as well to the optional DNA banking consent form.

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Clinical study websites and publication

A description of this clinical study will be available on http://www.clinicaltrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

The results of the study will be published on Boehringer Ingelheim' s study web site (http://trials.boehringer-ingelheim.com) and may also appear in other clinical study/study registries in countries in which the study is conducted. The results will not include information that can identify you.

The results of the study may also be published in a professional journal or presented at scientific meetings. Your identity will not be disclosed in those presentations.

USE OF SAMPLES IN THIS STUDY

Blood and sputum will be collected for pre-specified as well as for unspecified analyses. Urine samples will also be collected. All samples collected during the study as described under the section "Study Procedures" will be coded and sent to the central laboratory (except pregnancy test). A part of the analyses will be done at the central laboratory. In addition, samples will be further distributed to Boehringer Ingelheim and contractors of Boehringer Ingelheim for analyses.

Urine samples

Urine samples will be collected from all patients for the purpose of nicotine testing and (if applicable) additionally for pregnancy testing in women. Pregnancy testing will be done at the site and the sample will be discarded right after testing. Urine samples for nicotine testing will be shipped to and analyzed by the central laboratory and neither kept nor used for any future analyses.

Pre-specified biomarker assessment

The pre-specified analyses done in blood samples comprise safety laboratory testing, testing of A1ATD and biomarker assessment. Pre-specified biomarkers will also be analysed in sputum samples. All blood and sputum samples will be collected to gain a better understanding of COPD and the influence of A1ATD. Those biomarkers include but are not limited to markers of inflammation and tissue destruction, which have been reported and/or hypothesized to be indicative of physiological and pathophysiological changes in the lungs. In addition investigations aiming at identification of new biomarkers will be done.

The DNA sample used to determine A1ATD and left over of other biomarker samples for prespecified analyses will be destroyed once this analysis has been completed.

Left over sputum samples that were not used for the pre-specified biomarker analyses will be transferred to the biobank (see section "Unspecified biomarker assessment").

Unspecified biomarker assessment (Biobanking)

Since knowledge in the biomarker field is steadily increasing, we also ask you to consent to the collection of samples for unspecified biomarker analyses. These samples including left over sputum samples described above will be stored up to 15 years after the final study report has been written to enable these additional explorative investigations

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Approximately 50 ml blood will be collected per visit during the observational period. All analyses aim at increasing our understanding of COPD and other respiratory diseases. This may help to develop new therapies for COPD in the future. Samples will be sent to Boehringer Ingelheim or a storage CRO for long term storage.

The future research on your samples will not affect your present medical care. Reports from any future research with your samples and data will not be given to you or your doctor. Summaries of the research results may be published in scientific journals, on the internet, in data repositories or presented at meetings for other researchers, so that other doctors and researchers can find out about the results. In any case, your identity will not be disclosed in any publications or presentations.
Subjects who donate her/his samples to BI do not retain any property rights to the materials such as samples and their derivatives and the related data.

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COMPENSATION / COSTS

[Amend in accordance with local legal requirements or insurance.]
This study is funded by the sponsor. The sponsor has signed a contract with <insert CRO, applicable> to conduct this study. The sponsor <CRO on behalf of CRO> will pay the study doctor's and/or institution's for his/her expenses, time and effort to conduct this study. The study doctor and the institution/Hospital have no other financials ties to Boehringer Ingelheim.

There will be no additional costs to you for your participation in this study. All study procedures including lab work, tests, and doctor visits are provided to you free of charge by the sponsor, Boehringer Ingelheim, and will not be billed to you or your insurance carrier as long as you are participating in the study. You will receive <enter amount and/or a description of a payment schedule> to cover out-of-pocket expenses such as meals and parking for visits that are required as part of the study.

The sponsor will be owner of the study results. If commercial products or other valuable discoveries result from research using your samples and/or data, these products and discoveries may be owned, patented, licensed, or otherwise developed for commercial sale by sponsor, other researchers, or companies. If this should occur, you will not receive any financial benefits or compensation or other proprietary interest from any commercial products or discoveries that may result from such research.

INJURY

You will receive necessary medical treatment in the event that an injury results because of your participation in this study. Financial compensation for lost wages, disability or discomfort due to an injury is not generally available. You do not give up any legal rights by signing this form. You do not release the sponsor, institution, study doctor or their agents from any liability for negligence by signing this form.

EMERGENCY CONTACT / ETHICS CONTACT

If you have questions concerning the conduct of the study, or for any other reason you may contact Dr. _____ at _____ or the Study Coordinator _____ at _____.

In case of an emergency, please go to the nearest hospital emergency department and inform your study doctor as soon as possible.

If you have any questions about your rights as a study subject, please contact your family doctor, lawyer, or write to the committee that reviewed the ethical aspects of this study at: <insert ethics committee name and contact here>

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DECLARATION OF INFORMED CONSENT

STUDY SUBJECT No.: _____

My signature on this consent form means that:

- I understand that I am being asked to participate in a research study entitled: Observational study in healthy subjects and patients with COPD to assess the relationship between clinical, imaging and biomarker measurements, and progression of emphysema over three years.
- I have had this study explained to me by _____.
- I have read each page of this document.
- I have had all of my questions answered fully and to my satisfaction.
- I was given sufficient time to think in peace and quiet and decide whether to participate.
- I have been told that my participation is voluntary and I can withdraw at any time without giving any reasons.
- I agree to the collection, storage, processing, transfer and use of my personal data and biological samples (blood, urine and sputum) as explained in the above information.
- I agree that blood will be collected for biobanking and will be used for future unspecified analyses.
- I agree that left over of my sputum samples can be stored for future unspecified analyses.
- I agree that further, today unspecified analysis of my biological samples can be done during and after the study.
- I hereby expressly declare and agree that I transfer all rights of ownership of the collected samples and sample-related data to Boehringer Ingelheim Pharma (GmbH & Co. KG) so that the same may use them as described in this Informed Consent Form.
- I voluntarily consent to participate in this study.
- I will be given a signed copy of this consent document for my records

It is important that your personal doctor is aware that you are in a research study because you are undergoing examinations that could affect your health. With your permission, we will notify him/her that you are taking part in this study.

I consent to my personal doctor being notified that I am taking part in this study.

❑ YES, I agree. ❑ NO, I don't agree.

I agree that in case I discontinue the study early, the study team can contact me as described above, for further follow up by telephone.

❑ YES, I agree. ❑ NO, I don't agree to be contacted after early discontinuation.

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I consent that in case I die during the time of this study my study doctor is allowed to contact my family members to ask after the circumstances and that he can review my relevant medical records.

❑ YES ❑ NO

Name of Study Participant (*please print*)	**Consent Signature of Study Participant**	**Date** (dd mmm yyyy)
Name of Person Obtaining Consent (*please print*)	**Signature of Person Obtaining Consent**	**Date** (dd mmm yyyy)

STATEMENT OF INVESTIGATOR / STUDY DOCTOR:

I certify that I have explained to the above individual(s) the nature and purpose of the study and the possible benefit and risks associated with participation. I have answered any questions that have been raised and the potential study participant has received a copy of this signed consent document.

I acknowledge my responsibility for the care and well-being of the above study participant, to respect the rights and wishes of the participant, and to conduct the study according to applicable Good Clinical Practice guidelines and regulations.

Investigator Name (*please print*)	**Investigator Signature**	**Date** (dd mmm yyyy)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Reported on page number (section)
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3 (Abstract: Introduction).
	2b	All items from the World Health Organization Trial Registration Data Set	Please see table on pages 10–15.
Protocol version	3	Date and version identifier	7 (Introduction).
Funding	4	Sources and types of financial, material, and other support	22 (Acknowledgements); 23 (Funding of study).
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	22 (Competing Interests); 23 (Authors' contributions); 38 (Supplementary Table 4. Steering committee members).
	5b	Name and contact information for the trial sponsor	23 (Funding of study).

	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23 (Authors' contributions).
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14 (Information on steering committee); 14/15 (Data collection and management); 23 (Authors' contributions); 38 (Supplementary Table 4. Steering committee members).
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7 (Introduction); 8 (Objectives); summary of interventions is not applicable for this observational biomarker study.
	6b	Explanation for choice of comparators	Not applicable for this observational biomarker study.
Objectives	7	Specific objectives or hypotheses	8 (Objectives).
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8/9 (Study design); 11 (Patient population and recruitment); 28 (Figure 1. Overview of trial design); 29 (Table 1. FOOTPRINTS study participants); 32/33 (Supplementary Text 1) – describes study as exploratory.
Methods: Participants, interventions, and outcomes			

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8–9 (Study design).
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9–11 (Inclusion and exclusion criteria).
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Not applicable as study was observational biomarker study. Planned analyses and assessments are detailed on pages 11–12.
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Not applicable as an observational biomarker study. Details on study discontinuation are provided on page 9 (Study design) and rescheduling of clinic visits on page 13.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Not applicable. Further details provided on page 14–15 on registering clinic visits and phone calls are provided in the “data collection and management” section.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	36/37 (Supplementary Table 3. Overview of permitted and restricted medications/therapy).

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8 (Objectives); 11 (Main outcome measures); 11–12 Planned analyses and assessments);
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9 (Study design); 28 (Figure 1. Overview of trial design);
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	32 (Supplementary text 1); 34 (Supplementary Table 1); 35 (Supplementary Table 2)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	32/33 (Supplementary Text 1)
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Not applicable (observational biomarker study).

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Not applicable.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Not applicable.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Not applicable.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Not applicable.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14/15 (Data collection and management); 39/40 (Supplementary Table 5. CT scan protocol in inspiration and expiration)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9 (Study design) provides information on discontinuation; 13 (Rescheduling of clinic visits); 32/33 (Supplementary text 1).

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14/15 (Data collection and management)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13 (Statistical analysis)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13 (Statistical analysis)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Note, analysis population will be described in future publications. 15 (Data collection and management) for handling of missing data.
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	No DMC was implemented for the FOOTPRINTS study, as it is a non-interventional, purely observational biomarker study.

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13 (Statistical analysis); details on stopping guidelines are not applicable for this study (observational biomarker study) and access to study results is generally not restricted to a dedicated group since there are no blinded data.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12 (Planned analyses and assessments)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15 (Data collection and management)
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4 (Ethics and dissemination); 16 (ETHICS APPROVAL AND CONSENT TO PARTICIPATE)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16 (ETHICS APPROVAL AND CONSENT TO PARTICIPATE).
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4 (Ethics and dissemination); 9/10 (Inclusion and exclusion criteria)

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15/16 (Data collection and management)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22 (Competing interests); Financial disclosure is not a prerequisite for this type of study, so this information was not collected for all principal investigators.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	21 (Discussion)
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable (observational biomarker study)
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	21 (Discussion)
	31b	Authorship eligibility guidelines and any intended use of professional writers	22 (Acknowledgments); 23 (AUTHORS' CONTRIBUTIONS)

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	21 (Discussion)
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided in the Supplementary. Described on page 4 (Ethics and dissemination); 9 (Inclusion and exclusion criteria)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	14/15 (Data collection and management)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

WHO Trial Registration Data Set (Version 1.3.1)	Reported on page number (section)
1. Primary Registry and Trial Identifying Number Name of Primary Registry, and the unique ID number assigned by the Primary Registry to this trial.	3 (Abstract).
2. Date of Registration in Primary Registry Date when trial was officially registered in the Primary Registry.	9 (Study design).
3. Secondary Identifying Numbers Other identifiers besides the Trial Identifying Number allocated by the Primary Registry, if any. These include: - The Universal Trial Number (UTN) - Identifiers assigned by the sponsor (record Sponsor name and Sponsor-issued trial number (e.g. protocol number)) - Other trial registration numbers issued by other Registries (both Primary and Partner Registries in the WHO Registry Network, and other registries) - Identifiers issued by funding bodies, collaborative research groups, regulatory authorities, ethics committees / institutional review boards, etc. - All secondary identifiers will have 2 elements: an identifier for the issuing authority (e.g. NCT, ISRCTN, ACTRN) plus a number. There is no limit to the number of secondary identifiers that can be provided.	3 (Abstract).
4. Source(s) of Monetary or Material Support Major source(s) of monetary or material support for the trial (e.g. funding agency, foundation, company, institution).	23 (FUNDING OF STUDY).
5. Primary Sponsor The individual, organization, group or other legal entity which takes responsibility for initiating, managing and/or financing a study. The Primary Sponsor is responsible for ensuring that the trial is properly registered. The Primary Sponsor may or may not be the main	23 (FUNDING OF STUDY).

funder.	
6. Secondary Sponsor(s) Additional individuals, organizations or other legal persons, if any, that have agreed with the primary sponsor to take on responsibilities of sponsorship. A secondary sponsor may have agreed to: - take on all the responsibilities of sponsorship jointly with the primary sponsor; or form a group with the Primary Sponsor in which the responsibilities of sponsorship are allocated among the members of the group; or act as the Primary Sponsor's legal representative in relation to some or all of the trial sites.	Not applicable.
7. Contact for Public Queries Email address, telephone number and postal address of the contact who will respond to general queries, including information about current recruitment status. "Note: The information provided in here is functional and not personal, it is recommended to provide institutional and not personal information. By providing this information the registrant consents that the information provided can or may be published on a public website. Once provided the information cannot be redacted or anonymized as a result of new privacy legislation such as the European General Data Protection Regulation (GDPR)".	2 (Corresponding author); 23 (FUNDING OF STUDY).
8. Contact for Scientific Queries There must be clearly assigned responsibility for scientific leadership to a named Principal Investigator. The PI may delegate responsibility for dealing with scientific enquiries to a scientific contact for the trial. This scientific contact will be listed in addition to the PI.	2 (Corresponding author); 23 (FUNDING OF STUDY).

"Note: The information provided in here is functional and not personal, it is recommended to provide institutional and not personal information. By providing this information the registrant consents that the information provided can or may be published on a public website. Once provided the information cannot be redacted or anonymized as a result of new privacy legislation such as the European General Data Protection Regulation (GDPR)". The contact for scientific queries must include: Name and title, email address, telephone number, postal address and affiliation of the Principal Investigator, and; Email address, telephone number, postal address and affiliation of the contact for scientific queries about the trial (if applicable). The details for the scientific contact may be generic (that is, there does not need to be a named individual): e.g. a generic email address for research team members qualified to answer scientific queries.	
9. Public Title Title intended for the lay public in easily understood language.	'FOOTPRINTS' study included throughout.
10. Scientific Title Scientific title of the study as it appears in the protocol submitted for funding and ethical review. Include trial acronym if available.	'FOOTPRINTS' study included throughout.
11. Countries of Recruitment The countries from which participants will be, are intended to be, or have been recruited at the time of registration.	9 (Study design).
12. Health Condition(s) or Problem(s) Studied Primary health condition(s) or problem(s) studied (e.g., depression, breast cancer, medication error). If the study is conducted in healthy human volunteers belonging to the target population of the intervention (e.g. preventive or screening	6–7 (Introduction); 8 (Objectives).

interventions), enter the particular health condition(s) or problem(s) being prevented.	
13. Intervention(s) For each arm of the trial record a brief intervention name plus an intervention description.	Not applicable as observational biomarker study.
14. Key Inclusion and Exclusion Criteria Inclusion and exclusion criteria for participant selection, including age and sex. Other selection criteria may relate to clinical diagnosis and co-morbid conditions; exclusion criteria are often used to ensure patient safety. If the study is conducted in healthy human volunteers not belonging to the target population (e.g. a preliminary safety study), enter "healthy human volunteer".	9–10 (Inclusion and exclusion criteria).
15. Study Type	9 (Study design).
16. Date of First Enrollment Anticipated or actual date of enrolment of the first participant.	11 (Patient population and recruitment).
17. Sample Size Sample Size consists of: Number of participants that the trial plans to enrol in total. Number of participants that the trial has enrolled.	32/33 (Supplementary Text 1) 29 (Table 1. FOOTPRINTS® study participants: all enrolled subjects)
18. Recruitment Status	11 (Patient population and recruitment).
19. Primary Outcome(s) Outcomes are events, variables, or experiences that are measured because it is believed that they may be influenced by the intervention. The Primary Outcome should be the outcome used in sample size	8 (Objectives); 11–12 (Planned analyses and assessments) details specifics on measurement methods and timepoints.

calculations, or the main outcome(s) used to determine the effects of the intervention(s). Most trials should have only one primary outcome. For each primary outcome provide: The name of the outcome (do not use abbreviations) The metric or method of measurement used (be as specific as possible) The timepoint(s) of primary interest	
20. Key Secondary Outcomes Secondary outcomes are outcomes which are of secondary interest or that are measured at timepoints of secondary interest. A secondary outcome may involve the same event, variable, or experience as the primary outcome, but measured at timepoints other than those of primary interest. As for primary outcomes, for each secondary outcome provide: The name of the outcome (do not use abbreviations) The metric or method of measurement used (be as specific as possible) The timepoint(s) of interest	8 (Objectives); 11–12 (Planned analyses and assessments) details specifics on measurement methods and timepoints.
21. Ethics Review The ethics review process information of the trial record in the primary register database. It consists of: Status (possible values: Not approved, Approved, Not Available) Date of approval Name and contact details of Ethics committee(s)	16 (ETHICS APPROVAL AND CONSENT TO PARTICIPATE); Trial record can be found at https://clinicaltrials.gov/ct2/show/NCT02719184; List of ethics committees (name/contact details) provided for purposes of submission (not to be included in the publication)
22. Completion date Date of study completion: The date on which the final data for a clinical study were collected (commonly referred to as, "last subject, last visit").	11 (Patient population and recruitment).
23. Summary Results It consists of:	Not applicable – trial ongoing.

Date of posting of results summaries Date of the first journal publication of results URL hyperlink(s) related to results and publications Baseline Characteristics: Data collected at the beginning of a clinical study for all participants and for each arm or comparison group. These data include demographics, such as age and sex, and study-specific measures. Participant flow: Information to document the progress and numbers of research participants through each stage of a study in a flow diagram or tabular format. Adverse events: An unfavorable change in the health of a participant, including abnormal laboratory findings, and all serious adverse events and deaths that happen during a clinical study or within a certain time period after the study has ended. This change may or may not be caused by the intervention being studied. Outcome measures: A table of data for each primary and secondary outcome measure and their respective measurement of precision (eg a 95% confidence interval) by arm (that is, initial assignment of participants to arms or groups) or comparison group (that is, analysis groups), including the result(s) of scientifically appropriate statistical analyses that were performed on the outcome measure data, if any. URL link to protocol file(s) with version and date Brief summary	
24. IPD sharing statement Statement regarding the intended sharing of deidentified individual clinical trial participant-level data (IPD). Should indicate whether or not IPD will be shared, what IPD will be shared, when, by what mechanism, with whom and for what types of analyses. It consists of: Plan to share IPD (Yes, No) Plan description	21 (Discussion).

BMJ Open

FOOTPRINTS® study protocol: rationale and methodology of a 3-year longitudinal observational study to phenotype patients with COPD

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-042526.R1
Article Type:	Protocol
Date Submitted by the Author:	01-Dec-2020
Complete List of Authors:	Crapo, James; National Jewish Health, Gupta, Abhya; Boehringer Ingelheim International GmbH, TA Inflammation Med Lynch, David; National Jewish Health, Department of Radiology Vogel-Claussen, Jens; Hannover Medical School, Diagnostic and Interventional Radiology; Biomedical research in endstage and obstructive lung disease Hannover (BREATH) Watz, Henrik; LungenClinic Grosshansdorf GmbH, Pulmonary Research Institute; Airway Research Center North (ARCN) Turner, Alice; University of Birmingham, Institute of Applied Health Research Mroz, Robert; Medical University of Bialystok, 2nd Department of Lung Diseases and Tuberculosis Janssens, Wim; KU Leuven University Hospitals Leuven, Laboratory of Respiratory Diseases and Thoracic surgery, Department of Respiratory Medicine Ludwig-Sengpiel, Andrea; KLB Gesundheitsforschung Lübeck GmbH Beck, Markus; Boehringer Ingelheim Pharma GmbH and Co KG Biberach, Clinical Research Langellier, Bérengère; Boehringer Ingelheim France SAS, Medical Affairs Ittrich, Carina; Boehringer Ingelheim Pharma GmbH and Co KG Biberach, Global Department of Biostatistics and Data Sciences Risse, Frank; Boehringer Ingelheim Pharma GmbH and Co KG Biberach, Translational Medicine and Clinical Pharmacology Diefenbach, Claudia; Boehringer Ingelheim Pharma GmbH and Co KG Biberach, Translational Medicine and Clinical Pharmacology
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Respiratory medicine
Keywords:	Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Chronic airways disease < THORACIC MEDICINE, Emphysema < THORACIC MEDICINE, Computed tomography < RADIOLOGY & IMAGING

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FOOTPRINTS® study protocol: rationale and methodology of a 3-year longitudinal observational study to phenotype patients with COPD

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Short title: Emphysema progression biomarker study

Keywords: Protocols & guidelines, chronic airways disease, emphysema, computed tomography

Word count: 4365/4000

ABSTRACT

Introduction

A better understanding is needed of the different phenotypes that exist for patients with chronic obstructive pulmonary disease (COPD), their relationship with the pathogenesis of COPD, and how they may affect disease progression. Biomarkers, including those associated with emphysema, may assist in characterising patients and in predicting and monitoring the course of disease. The FOOTPRINTS® study (Study 352.2069; NCT02719184) aims to identify biomarkers associated with emphysema, over a 3-year period.

Methods and analysis

The FOOTPRINTS® study is a prospective, longitudinal, multinational (12 countries), multicentre (51 sites) biomarker study, which has enrolled a total of 463 ex-smokers, including subjects without airflow limitation (as defined by the 2015 Global Initiative for Chronic Obstructive Lung Disease [GOLD] strategy report), patients with COPD across the GOLD stages 1 to 3, and patients with COPD and alpha1-antitrypsin deficiency. The study has an observational period lasting 156 weeks that includes seven site visits and additional phone interviews. Biomarkers in blood and sputum, imaging data (computed tomography and magnetic resonance), clinical parameters, medical events of special interest and safety are being assessed at regular visits. Disease progression based on biomarker values and COPD phenotypes are being assessed using multivariate statistical prediction models.

Ethics and dissemination

The study protocol was approved by the authorities and ethics committees/institutional review boards of the respective institutions where applicable, which included study sites in Belgium, Canada, Denmark, Finland, Germany, Japan, Korea, Poland, Spain, Sweden, United Kingdom and the United States; written informed consent has been obtained from all study participants. Ethics committee approval was obtained for all participating sites prior to enrolment of the study participants. The study results will be reported in peer-reviewed publications.

Trial registration number

NCT02719184.

Strengths and limitations of this study

- The study helps to address an unmet need to understand different COPD phenotypes, and to expand on our understanding of biomarkers that are associated with emphysema, and hence COPD, in a 3-year longitudinal setting.
- A subset of patients with alpha1-antitrypsin deficiency provides information on an important but rarely studied subpopulation of patients that present with earlier onset and faster progression of emphysema.
- A regular sampling schedule is employed, with frequent visits and biomarker sample collections to allow early detection of changes, if present.
- Magnetic resonance imaging and sputum collection only occur at certain study sites, and not necessarily at all planned times; hence, the smaller subsets of patients may not be representative of the entire population.

- Patients with the newly defined PRISm phenotype are not included, nor are current smokers or healthy subjects who have never smoked, thus limiting the control groups for reference.

INTRODUCTION

Airflow limitation is a key characteristic of chronic obstructive pulmonary disease (COPD).[1,2] It is caused by small airways disease, such as obstructive bronchiolitis, and structural changes related to parenchymal destruction (emphysema).[1,2] Small airways disease and structural changes can occur alone or in combination with one another, with the degree of severity and relative contributions varying between individuals.[1]

COPD is associated with inflammation and tissue damage, which is thought to be linked to – among other aspects – an imbalance between neutrophil serine proteases (NSPs) and their inhibitors.[3,4] The serine protease inhibitor alpha1-antitrypsin (A1AT) inactivates NSPs such as neutrophil elastase (NE), proteinase 3 (PR3) and cathepsin G (Cat G), to protect lung tissue against protease-mediated damage.[5,6] Although a number of gene defects have been associated with emphysema,[7] A1AT deficiency (A1ATD) is the most well established as a cause for COPD.[8] It is associated with a higher risk of developing emphysema that tends to occur at a younger age and progresses faster than for patients without A1ATD.[5,9,10] Subjects with the most severe A1ATD are usually homozygous for the ZZ allele (ZZ).[5] The resulting emphysema among patients with A1ATD is thought to be caused primarily by increased NSP activity in the lungs.[10]

NE and PR3 are responsible for the degradation of critical components of the extracellular matrix, including elastin and fibronectin, and can produce other key features of COPD, such as mucous hypersecretion.[3] When quantified, levels of NE, PR3 or their specific elastin degradation products have been associated with poorer disease outcomes in patients with COPD, such as incidence of exacerbations and higher risk of mortality.[11-13] Other proteases and biomarkers, including desmosine, fibrinogen and C-reactive protein, have also been associated with poorer disease outcomes in patients with COPD.[13-15]

In the era of precision-based patient care, there is a need for a more in-depth understanding of COPD patient phenotypes, pathogenesis and progression.[16,17] Biomarkers may assist in characterising patients, and in predicting and monitoring the course of disease, in order to better enable the development of drugs aimed at slowing COPD progression.[16-18] In fact, there are encouraging signs that therapies that modify NSP imbalance (e.g. augmentation therapy for A1ATD) appear to alter emphysema progression, at least when considering blood and radiological biomarkers of disease.[19,20] Since quantitative computed tomography (CT) is a well-established method for documenting the presence, extent and progression of emphysema, it is potentially useful for assessing these emerging biomarkers.[21-24]

This prospective, longitudinal study is investigating biomarkers in different biofluids (whole blood, serum, plasma and induced sputum), imaging parameters assessed by CT and magnetic resonance imaging (MRI), as well as clinical parameters potentially associated with emphysema, over a 3-year period. It is hoped that correlation of lung destruction biomarkers with CT densitometry findings should increase our understanding of the underlying pathophysiology of emphysema

progression. The insights generated may support the development of future treatments that could slow or halt disease progression in patients with COPD. Here we report the methodology of the FOOTPRINTS® study, as described in the study protocol (version 5.0; date: 29 May 2018).

Objectives

The primary objective of this longitudinal study is to explore whether any of the biomarkers assessed in patients with COPD, patients with COPD plus A1ATD and subjects without airflow limitation (controls; as defined by the 2015 Global Initiative for Chronic Obstructive Lung Disease [GOLD] strategy report),[25] or specific patterns thereof, are correlated with COPD progression, particularly emphysema progression, over 156 weeks.

Further objectives, to be assessed over 156 weeks, include:

- To assess the correlation of soluble biomarkers of inflammation and lung tissue destruction with COPD severity, presence of emphysema and disease progression.
- To determine the concentration and activity of proteases in induced sputum, and to assess potential biomarkers of lung tissue destruction in sputum and blood, from patients with different severities of COPD (including a subset of patients with A1ATD) and subjects without airflow limitation (controls).
- To assess the correlation of physiological biomarkers and functional imaging biomarkers with COPD severity, the presence of emphysema and disease progression, and to evaluate MRI-based imaging biomarkers with respect to their feasibility.

- To identify COPD phenotypes and define their risk for, and rate of, disease progression.

METHODS AND ANALYSIS

Study design

The FOOTPRINTS study® (registered on 21 March 2016) is a multinational, prospective, longitudinal, biomarker study in subjects without airflow limitation, patients with COPD, and patients with COPD and A1ATD, taking place at 51 sites across 12 countries (Belgium, Canada, Denmark, Finland, Germany, Japan, Korea, Poland, Spain, Sweden, United Kingdom, and the United States). A complete list of study sites is detailed in Supplementary File 1 (table 1).

The study consists of two consecutive periods (a screening period lasting up to 28-days and an observational period lasting 156 weeks) and includes seven site visits and additional phone interviews (figure 1). The observational period is completed at Visit 7 or at the discontinuation visit, which is regarded as study completion. Patients are considered to be in the observational phase only after completing Visit 2. All subjects who prematurely discontinue the study during the observational phase are followed for vital status and COPD exacerbation status (for patients with COPD and for patients with COPD and A1ATD only) via phone contact until 156 weeks after Visit 2.

Calculation of sample size, and power calculations for annual decline in forced expiratory volume in 1 second and 15th percentile density assessed by CT, are provided in Supplementary File 1 (text 1 and tables 2 and 3).

Inclusion and exclusion criteria

Male and female participants who have signed an informed consent form consistent with the International Conference on Harmonisation Good Clinical Practice (GCP) guidelines prior to participation have been included, provided the following were applicable:

- Aged 40–70 years (30–70 years for patients with COPD and A1ATD).
- Ex-smokers for ≥9 months with a smoking history of ≥20 pack-years (≥10 pack-years for patients with COPD and A1ATD).
- Body mass index (BMI) 18–35 kg/m^2 (≤30 kg/m^2 in the MRI subset).
- Able to perform all study-related procedures.
- Required to have been on stable therapy for 4 weeks prior to Visit 1.

Participants were excluded if any of the following were applicable:

- Significant pulmonary disease (other than COPD) or other significant medical condition, for example, rheumatoid arthritis, severe liver disease or psoriasis.
- Documented history of asthma, including during childhood.
- Any respiratory tract infection or COPD exacerbation within 4 weeks prior to Visit 1 or during the screening period prior to Visit 2, if it was not possible to meet the rescheduling rules.
- Presence of an immunocompromising condition (e.g. HIV or history of hepatitis B or C).
- Any treatment with phosphodiesterase-4 inhibitors and maintenance treatment with methylxanthines within 3 months or 6 half-lives prior to Visit 1, and until Visit 2; patients were permitted to initiate treatment following Visit 2.

- Patients with COPD (with and without A1ATD) with newly added anti-inflammatory treatment (either respiratory or non-respiratory) or change in any therapy within 4 weeks prior to Visit 1 and during the screening period between Visit 1 and Visit 2; patients were permitted to initiate treatment following Visit 2.
- Any prior, current or planned A1AT augmentation therapy.
- Permitted and restricted medications and therapy are detailed in Supplementary File 1 (table 4).
- Pregnant or lactating females.
- Previous participation in this study or participation in a parallel interventional clinical trial within 6 weeks prior to Visit 1 or during the study.

Patient population and recruitment

The study is ongoing; however, enrolment is complete. In total, 463 patients were enrolled, who are described in more detail in table 1. Strategies for achieving adequate participant enrolment and retainment are described in Supplementary File 1 (text 1).

The study started on 22 July 2016 when the first subject was screened, and the planned end date for the study is 29 March 2021.

Table 1. FOOTPRINTS® study participants: all enrolled subjects

Patient group	Rationale
383 patients with COPD, including: • 123 patients with mild COPD[a] • 130 patients with moderate COPD[b] • 130 patients with severe COPD[c]	Included to provide data on increased lung protease levels and the contribution that this may have on the development and progression of emphysema in the absence of A1ATD.
18 patients with COPD and A1ATD[d]	Included as they present with an earlier onset of emphysema and a faster decline in lung function, as well as a faster change in lung density.[9]
62 ex-smokers without airflow limitation	Included to provide a comparison for biomarkers between subjects with and without airflow limitation. Subjects were required to be healthy based on a complete medical history and to have: FEV_1 ≥80% of predicted normal and post-bronchodilator. FEV_1/forced vital capacity ≥lower limit of normal. A mean post DL_{CO} ≥70% of predicted normal at Visit 1.
Total enrolled: 463 subjects	

[a]Defined as GOLD stage 1, FEV_1 ≥80% predicted [25]; [b]defined as GOLD stage 2, FEV_1 ≥50–<80% predicted [25]; [c]defined as GOLD stage 3, FEV_1 ≥30–<50% predicted [25]; [d]defined as having a diagnosis of COPD and a documented A1ATD of ZZ genotype prior to Visit 2.
A1ATD, alpha1-antitrypsin deficiency; COPD, chronic obstructive pulmonary disease; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; FEV_1, forced expiratory volume in 1 second; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

Main outcome measures

The main outcome measures are change in lung density (assessed by CT), lung function decline and number, duration and severity of exacerbations over the course of the study.

Planned analyses and assessments

Variables to be analysed include a range of soluble biomarkers, imaging biomarkers and clinical parameters, as well as medical events of special interest (MESI) and safety assessments. The pre-specified biomarkers being assessed are summarised in table 2, and the biomarker sampling schedule and overall trial design are illustrated in figure 1.

Table 2. Summary of biomarker assessments

Variables	Biomarker assessment
Pre-specified soluble biomarkers	
Blood	• Total and differential cell counts • DNA analyses (to determine A1AT genotype)
Induced sputum*	• Neutrophil elastase activity • Proteinase 3 activity • Cat-G activity
Serum/plasma	• Tissue turnover biomarkers (including neutrophil elastase-specific elastin fragment) • Other protease-generated neoepitopes (including Cat-S cleaved decorin) • Soluble form of receptor for advanced glycation end products • Surfactant protein D • Lysyl oxidase like 2 • Biomarkers of systemic inflammation (including IL-6, high-sensitivity C-reactive protein, white blood cell count and fibrinogen)
Imaging biomarkers	

CT scanning	• Airway morphology
	  ○ Mean lumen diameter
	  ○ % wall area

	○ Mean segmental and sub-segmental airway wall thickness ○ Square root of wall area of bronchus with internal perimeter of 10 mm (pi10) • Emphysema (on inspiratory CT) ○ PD15 of the CT histogram ○ PD15 adjusted for inspired lung volume ○ % lung voxels with attenuation ≤–950 HU (LAA-950) • Air trapping (on expiratory CT) ○ % lung voxels with attenuation ≤–856 HU (LAA-856) • Registration-based parenchyma measurements ○ % normal lung, % emphysema and % small airway disease
MRI	• Conducted at 1.5 Tesla in a subset of subjects without airflow limitation and in patients with COPD and without A1ATD to assess functional lung parameters, including pulmonary blood flow
Clinical parameters/assessments	• Spirometry to assess FEV_1 and FVC • DL_{CO} and DL_{CO} per unit of alveolar volume • Body plethysmography (functional residual capacity, total lung capacity, inspiratory capacity, inspiratory vital capacity, residual volume, expiratory reserve volume) • Pulse oximetry • Symptom questionnaires (modified Medical Research Council Dyspnoea Scale, COPD Assessment Test, St. George's Respiratory Questionnaire) • 6-minute walk test • Body mass index and BODE index • Adverse events and MESI

*Collected at selected sites.

A1AT, alpha1-antitrypsin; A1ATD, alpha1-antitrypsin deficiency; Cat, cathepsin; COPD, chronic obstructive pulmonary disease; CT, computed tomography; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HI, Hounsfield Units; IL-6, interleukin-6; LAA, low attenuation areas; MESI, medical events of special interest; MRI, magnetic resonance imaging; PD15, lung density at the 15th percentile point of the CT histogram.

Blood and sputum are being collected at Visits 2–7 for analysis of pre-specified

soluble biomarkers, and blood is also being collected at Visit 1 to perform a genetic

test for A1ATD (figure 1). Total and differential cell counts in blood, which are being assessed within the safety laboratory assessments, are also being assessed as biomarkers. Sputum will be induced to generate sputum samples, including cell slides to assess total and differential cell count and supernatant to assess soluble biomarker levels (table 2).

Chest CT scans are being conducted annually at Visits 2, 5, 6 and 7, following a specific low-dose protocol in order to minimise radiation exposure at each visit. A COPDGene® phantom [26] is being used before the first patient scan and then bi-monthly to monitor the stability of CT measurements for each scanner. MRI evaluation of the pulmonary vasculature and right ventricle is conducted in a subset of patients without airflow limitation and patients with COPD (not in patients with COPD and A1ATD) at Visits 2, 4, 5, 6 and 7. MESI include respiratory disorders such as pneumonia, pulmonary fibrosis, bronchiectasis and COPD exacerbations, as well as any other disorders (e.g. cardiovascular, metabolic/digestive and neurological/psychiatric) reported at the investigator's discretion. COPD exacerbations are defined as new or increased lower respiratory events or symptoms related to the underlying COPD with duration ≥3 days. Assessment of safety is carried out at Visits 1, 5, 6 and 7 unless otherwise indicated, including physical examination (plus assessment of height [Visit 1 only] and weight), vital signs (blood pressure and pulse rate), safety laboratory parameters (from blood; assessed at every site visit [Visits 1–7]) and electrocardiogram. Adverse events associated with any study procedure and MESI are collected at every site visit (Visits 1–7; MESI also assessed via telephone contact between visits; figure 1). All adverse events are followed up until they have resolved, been sufficiently characterised, or no further information can be obtained.

Rescheduling of clinic visits

Any deviations from the planned visit schedule are being documented. Rescheduling of certain clinic visits was permitted to promote participant retention. Clinic visits that involve a lung function test may be rescheduled twice within the permitted time windows due to violation of lifestyle restrictions. Subjects should refrain from strenuous activity for at least 12 hours prior to lung function testing (and throughout the testing period); and should avoid cold temperatures, environmental smoke, dust or areas with strong odours (e.g. perfumes). Sputum induction at Visit 2 can be rescheduled by up to 3 days; at all subsequent visits, it should not be repeated if the subject is unable to produce sputum or if the sputum sample quality is not acceptable. If chest CT and MRI (where applicable) cannot be performed on the day of the scheduled visit, the imaging assessment can be rescheduled up to 14 days after the visit. If a patient experiences a COPD exacerbation or respiratory tract infection during the screening period, Visit 2 will be postponed until 4 weeks following recovery from the exacerbation or infection, and the screening period may be extended up to 8 weeks.

Statistical analysis

Biomarker assessments and clinical outcomes measures are evaluated by regression models for repeated measures using respective point estimates and CI estimates. The correlation between individual biomarkers, and between biomarkers and other clinical outcome measures, is being assessed. Multivariate statistical prediction models for disease progression based on biomarker values and COPD phenotypes are being developed and examined. Interim analyses will also be conducted by the trial team once data from the 52-week and 104-week assessments

are available for ≥70% of subjects entered into the study. The purpose of this is to provide information on the use of MRI and soluble biomarker assessments.

Information on steering committee

A scientific steering committee, Emphysema Progression Biomarker Study (FOOTPRINTS® study) Steering Committee, was established in January 2017 to discuss and evaluate key scientific data in the field of respiratory medicine, and to provide independent advice and recommendations to optimise and strengthen the FOOTPRINTS® biomarker study. The steering committee comprises 13 external and internal scientific experts in respiratory medicine and chest radiology (Supplementary File 1; table 5), and is conducted in adherence to industry regulations on the cooperation of the pharmaceutical industry with the medical professions.[27-29]

Data collection and management

An interactive response technology system was used to track subject enrolment, and to register clinic visits and phone calls. A central laboratory facility is handling all standard safety blood laboratory analyses. Spirometry is performed using an external vendor's equipment to allow for centralised readings. Electrocardiograms are recorded for about a 10-second duration after the subject has rested for at least 5 minutes in a supine position, with electrode placement performed according to the Wilson method (precordial leads) and Goldberger and Einthoven (limb leads). Pulse oximetry will be performed to measure oxygen saturation; all recordings will be made using the same pulse oximetry measurement device per site on the same finger of the subject. Body plethysmography, DL_{CO}, the 6-minute walk test (6MWT) and sputum induction and processing (at selected sites only) are performed according to international accepted guidelines as follows. Body plethysmography and DL_{CO}

measurement are being performed according to American Thoracic Society/European Respiratory Society guidelines, after salbutamol administration.[30] The 6MWT is being conducted according to ATS guidelines.[31] Inspiratory and expiratory CT scans are being performed according to the specifications provided in Supplementary File 1 (table 6). Sputum induction and processing are performed according to the sputum manual issued by the vendor for sputum assessment and analysis.

All samples collected for pre-specified biomarker assessments are prepared by the site according to instructions given in the investigator site file until shipment to the central contract research organisation. Additional blood samples (whole blood, plasma and serum) will be taken at Visits 2 to 7 for future unspecified analyses, while potential leftovers from pre-specified blood analysis and induced sputum will also be stored and used for this purpose; these samples will be stored for up to 15 years after the final study report has been signed. Biomarkers are analysed by the sponsor or contractors of the sponsor. Data management and statistical evaluation are conducted by the sponsor or contractors of the sponsor. Source documents are filed at the investigator's site; these data are transferred to the electronic case report form. Missing data may be imputed if necessary.

A clinical trial monitor, appointed by the sponsor, ensures the good running of the study and directs the clinical study team in the preparation, conduct, and reporting of the study, in accordance with the sponsor's standardised operating procedures, GCP guidelines, and current legislation.

Medical information obtained for individual subjects during the study is considered confidential and this will be ensured by using subject identification code numbers.

Further information on confidentiality is detailed in the informed consent form (Supplementary File 2).

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

ETHICS AND DISSEMINATION

This study is being performed in accordance with the International Conference on Harmonisation Tripartite Guideline for GCP, the Japanese GCP regulations and local regulations. The study protocol has received approvals from the following IEC/IRBs: **Belgium:** Ethische commissie onderzoek UZ / KU Leuven; **Canada:** Hamilton Integrated Research Ethics Board, Ontario; Research Ethics Board University of Cardiology and Pneumology Institute of Quebec; Health Research Ethics Board, University of Alberta; University of Saskatchewan Biomedical Research Ethics Board; **Denmark:** De Videnskabsetiske Komitéer for Region Hovedstaden, Hillerød; **Finland:** Varsinais-Suomen sairaanhoitopiiri Eettinen toimikunta; **Germany:** Ethikkommission Landesärztekammer Hessen, Frankfurt; Ethikkommission Schleswig-Holstein, Bad Segeberg; Ethikkommission der Medizinischen Hochschule Hannover, Hannover; **Japan:** The IRB of Osaka City University Hospital, Osaka; The IRB of Kishiwada City Hospital, Kishiwada; The IRB of Showa University Hospital, Shinagawa; The IRB of Kagoshima University Hospital, Kagoshima; The IRB of Showa University Hospital, Shinagawa; **Republic of Korea:** The Catholic University of Korea, Eunpyeong St. Mary's Hospital IRB, Seoul; Seoul Metropolitan Government Seoul National University Boramae Medical; Korea University Guro Hospital IRB, Seoul; Konkuk University Medical Center IRB, Seoul; **Poland:**

Bioethics Committee at Regional Medical Chamber, Bialystok; **Spain:** CEIm Hospital Clínic, Barcelona; CEIC Illes Balears, Palma; CEIC Hospital Universitari Vall Hebrón, Barcelona; CEIC-Parc de Salut Mar, Barcelona; CEIC – Hospital de Bellvitge, Barcelona; **Sweden:** Regionala etikprövningsnämnden i Lund, Lund; **United Kingdom:** North West – Greater Manchester East Research Ethics Committee; **United States:** University of Alabama at Birmingham Western Institutional Review Board, Puyallup; Baylor College of Medicine Institutional Review Board for Baylor, Houston; University of California San Diego UCSD Human Research Protection Program, La Jolla; Temple University Hospital Western Institutional Review Board (WIRB), Puyallup; University of Utah Health Sciences Center University of Utah IRB, Salt Lake City; University of California Los Angeles Western Institutional Review Board, Puyallup; Chesapeake IRB, Columbia; Advarra, Inc., Columbia; University of Iowa Human Subjects Office/Institutional Review Board, Iowa City; Johns Hopkins Institutional Review Board Reed Hall - B 130, Baltimore; National Jewish Health Western Institutional Review Board, Puyallup.

In the respective countries, the ethics approval preceded the study participant's enrolment. Written informed consent is obtained from all study participants. Notably, any protocol amendments will be initiated only after all required legal documentation has been reviewed and/or approved by the respective IRB/IEC, as applicable.

The study findings will be reported in due course at research conferences and in peer-reviewed journals. The study protocol and clinical study data will be available to be shared after publication of the primary manuscript in a peer-reviewed journal. These data will be available upon reasonable request; further details will be provided in the primary manuscript.

DISCUSSION

COPD is characterised by progressive airflow limitation and a decline in lung function. It has also been demonstrated that emphysema, a major cause of airflow limitation in COPD, is independently associated with a rapid decline in lung function.[32,33] Current pharmacological treatment options for COPD and emphysema do not prevent the progressive decline of FEV_1 – the most commonly used marker of disease severity and progression in COPD.[1,34,35] However, FEV_1 is poorly correlated with symptoms and some other measures of disease progression.[1,36]

The heterogeneity of COPD extends to a molecular level, a concept familiar in asthma, where endotype-directed therapy is now well accepted;[37] hence there is a need for biomarkers that can assist with diagnosis, risk stratification and assessment of therapeutic interventions.[38,39] A recent systematic review by Fermont *et al.* reported that biomarkers and clinical parameters including 6MWT, fibrinogen, C-reactive protein, white cell count and interleukin-6, are associated with clinical outcomes in COPD and worth further investigation.[14] A systematic review of CT density as a radiological biomarker came to similar conclusions.[40] It is anticipated that biomarkers may facilitate a better understanding of the prognosis of COPD, help to guide treatment options and allow for a greater degree of precision medicine as treatment options.[38]

So far, few studies have addressed the potential of biomarkers to predict the longitudinal outcomes of COPD, and this has been assessed for a limited number of biomarkers only. The COPDGene® study, which has enrolled over 10,000 smoking (with or without COPD) and non-smoking subjects, is predominantly focused on

identifying genetic determinants for COPD susceptibility,[41] while the UK-based British Lung Foundation early COPD study is currently enrolling smokers with normal lung function or mild lung function abnormalities to assess the very early stages of COPD development.[42] The Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) study [43] and the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS) [44] have enrolled subjects with COPD and smoking/non-smoking control subjects, similar to the COPDGene® study. Other longitudinal studies include the German COPD and SYstemic consequences-COmorbidities NETwork (COSYCONET) study [45] and the Canadian Cohort Obstructive Lung Disease (CanCOLD) study.[46] All of these studies plan to analyse a range of soluble biomarkers and clinical outcomes, and conduct CT imaging to help identify COPD phenotypes.[42-48]

This prospective, longitudinal study was designed to investigate, over a 3-year period, biomarkers in different biofluids, imaging biomarkers and clinical parameters that may be associated with emphysema. A key strength of the FOOTPRINTS® study is that it actively recruited a subset of patients with A1ATD. This is an important but rarely studied subpopulation of patients, which has not been actively recruited for in other longitudinal studies, such as the COPDGene®,[41] ECLIPSE,[43] SPIROMICS [44] and UK early COPD cohort studies.[48] Patients with A1ATD present with earlier onset of emphysema, and could help us to better understand possible markers for emphysema progression, in particular the role of NSP imbalance conditional upon A1AT levels.[10] While A1ATD is associated with a higher risk of emphysema and COPD, increased levels of serum A1AT occur in response to inflammation.[49] Interestingly, increased levels of A1AT have also been associated with worse outcomes in patients with COPD. A recent analysis of data

from the Hokkaido COPD cohort study examined the association of circulating A1AT levels with the clinical course of COPD patients without A1ATD and reported that higher A1AT levels were associated with worse outcomes, including more emphysema, a worse systemic inflammation status and higher 10-year mortality.[49] Our findings in patients with A1ATD could help us to further understand the complex relationship between A1AT levels and COPD disease progression.

Compared with the COPDGene® study, in which assessments were scheduled at baseline, 5 years and 10 years (ongoing),[41,47,50] the FOOTPRINTS® study has more frequent visits and biomarker sample collections, similar to the ECLIPSE[43] and SPIROMICS [44] studies. In particular, the regular collection of sputum is a major asset of the FOOTPRINTS® study; sputum collection is scheduled to occur more frequently than in both the COPDGene® [47] and SPIROMICS [44] studies, and with a similar frequency to the ECLIPSE [43] study, with four collections scheduled in the first year of the study, and subsequent collections scheduled at Year 2 and Year 3. Another key strength and differentiator of the FOOTPRINTS® study is that annual chest CT scans are being performed; compared to the other longitudinal studies discussed here, and to the best of our knowledge, the FOOTPRINTS® study will provide the most regular CT imaging data. This, in addition to the frequent biomarker sampling, will allow for more robust measures of short- to medium-term decline, critical for informing clinical trials of emphysema targeted medicinal products.[43,44] The FOOTPRINTS® study is also assessing a wider range of non-genetic biomarkers and clinical parameters across the entire study population compared with the COPDGene® study, which is focused primarily on genetic analyses of DNA samples.[41,47] For example, the FOOTPRINTS® study is assessing NE, PR3 and Cat G activity, which could help us to further understand the

role of these biomarkers in patients with COPD, including those with A1ATD and poorer disease prognosis.[10] In addition, FOOTPRINTS® is conducting MRI assessments, which could be valuable in improving our understanding of changes in vasculature and perfusion in the lung that may precede changes in emphysema.[51] Due to the small number of patients included in the subset analysis and the challenges associated with obtaining meaningful MRI data, these findings are expected to be limited.

Although the FOOTPRINTS® study is not focusing on frequent exacerbators, it is anticipated that a considerable number of adverse events, medical events of special interest and hospital admissions will be detected during the 3 years of follow-up. The wide range of biomarkers measured in sputum and blood may help identify subjects at risk for such events. In addition, the longitudinal biosampling in FOOTPRINTS® will offer a unique opportunity to study the impact of exacerbations on the evolution of pathogenic processes.

It is anticipated that the results of the FOOTPRINTS® study will complement, as well as expand on, the data generated in other longitudinal studies, such as COPDGene®,[41] ECLIPSE [43] and SPIROMICS.[44] It is envisaged that the results of this study will increase our understanding of COPD phenotypes and the underlying pathophysiology of emphysema progression, which may be of use when developing drugs to reduce COPD advancement. In addition, biomarkers associated with ongoing emphysematous destruction of lung parenchyma may be identified, which could assist in predicting and monitoring patients' COPD disease course.

A potential limitation of the FOOTPRINTS® study is that sputum collection and MRI only occur at certain study sites. As such, the smaller subset of subjects may not be

sufficiently large to represent the entire population. In addition, there is potential for selection bias at site and patient levels, as the study is non-randomised and all participants are required to be ex-smokers. Note that current smokers were not included because smoking cessation can cause high variability in outcomes, particularly lung density measurements.[52,53] Given that disease progression occurs despite smoking cessation, ex-smokers were considered to be a more appropriate population. A further limitation is that patients with the newly defined preserved ratio impaired spirometry (PRISm) phenotype [41,54,55] and healthy subjects who have never smoked are not included.

Finally, an inherent challenge in studying biomarker levels in patients with COPD is the variability in biomarker levels over time. For example, a study by Dickens *et al.* looked at variability in levels of biomarkers over 3 months in subjects with COPD. At Month 3, fibrinogen was a highly repeatable biomarker, with levels within 25% of their baseline values for 89% of study participants, yet only 21% of patients had levels of C-reactive protein within 25% of baseline values.[39] In the current study, the lack of a healthy never-smoker control group limits the opportunity to compare the extent of biomarker fluctuations over time. The reasons for variability over time are poorly understood, but the repeatability of biomarkers should be considered when selecting for clinical applications.[39] Therefore, longitudinal assessment of biomarkers is planned, to help understand the longitudinal stability of biomarker-based phenotypes.

In summary, biomarkers may help to characterise patients with COPD, allow for better monitoring and prediction of the disease course, and enable an increased understanding of COPD itself. Subsequently, this should help us to develop drugs to

reduce disease progression. The FOOTPRINTS® longitudinal study is investigating biomarkers in biofluids, imaging biomarkers and clinical parameters associated with emphysema over a 3-year period, to increase the understanding of COPD patient phenotypes, pathogenesis and disease progression.

ACKNOWLEDGEMENTS

Vicki Cronin, PhD, at MediTech Media provided editorial assistance in the development of this manuscript, funded by Boehringer Ingelheim, and Harald Koegler, MD, assisted with reviewing the manuscript. The authors would like to thank all the patients and healthy volunteers for participating in this trial, and also extend their thanks to the investigators and study staff.

COMPETING INTERESTS

J. Crapo is a Co-Prinicipal Investigator for the COPDGene® study, and has received grants from NIH/NHLBI. A. Gupta, M. Beck, B. Langellier, C. Ittrich, F. Risse and C. Diefenbach are employees of Boehringer Ingelheim. D. A. Lynch has received grants from NHLBI, and personal fees from Boehringer Ingelheim, Parexel, Siemens and Veracyte. In addition, D. A. Lynch has a patent for ‘systems and methods for classifying severity of COPD’ pending. H. Watz has received grants and personal fees from Boehringer Ingelheim, and personal fees from AstraZeneca, Chiesi, GSK, Menarini and Novartis. J. Vogel-Claussen has received grants and personal fees from Boehringer Ingelheim and Novartis, and grants from GSK and Siemens Healthineers. A. M. Turner has received personal fees, payment for educational talks

and expenses payments for work on the FOOTPRINTS steering committee from Boehringer Ingelheim, grants and personal fees from AstraZeneca, CSL Behring and Grifols Biotheraputics, and grants, personal fees and non-finanacial support from Chiesi. R. M. Mroz has received personal fees from AstraZeneca, Boehringer Ingelheim, GSK, Merck Sharp & Dohme, Mundipharma, Novartis and Roche. W. Janssens has received grants from AstraZeneca and Chiesi. A. Ludwig-Sengpiel reports receipt of a study fee for involvement in conducting the FOOTPRINTS study.

FUNDING OF STUDY

This study was funded and sponsored by Boehringer Ingelheim. The trial clinical monitor for this study was Markus Beck (Boehringer Ingelheim Pharma GmbH & Co. KG, Dept. Clinical Research Germany/Respiratory Diseases, Birkendorfer Str. 65, 88397 Biberach an der Riss, Germany; Phone: +49 (0) 7351 / 54 96 803).

AUTHORS' CONTRIBUTIONS

J. Crapo, A. Gupta, D. A. Lynch, J. Vogel-Claussen, H. Watz, M. Beck, C. Ittrich, F. Risse and C. Diefenbach contributed towards the overall concept and design of the original study protocol. A. M. Turner also contributed towards the study design by providing advice on the inclusion criteria for patients with alpha1-antitrypsin deficiency. B. Langellier, C. Diefenbach, M. Beck and C. Ittrich led the writing of the protocol. J. Crapo, A. Gupta, D. A. Lynch, J. Vogel-Claussen, H. Watz, M. Beck, B. Langellier, C. Ittrich, F. Risse and C. Diefenbach provided critical input at all stages. H. Watz, A. M. Turner, R. M. Mroz, W. Janssens and A. Ludwig-Sengpiel are principal investigators of the study and were involved in enrolling patients, as well as documenting patient information in the trial database. All authors have contributed

towards the development of the manuscript, and have read and approved the final manuscript.

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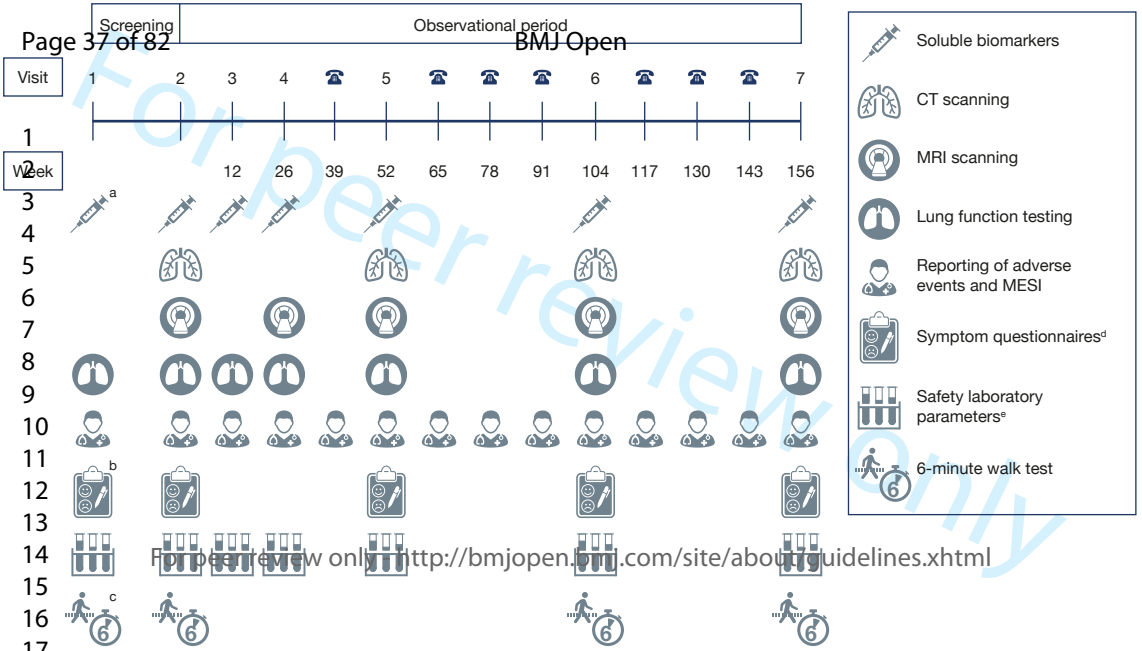
Figure legend

Figure 1. Overview of trial design

[a]A1ATD analysis only; this occurs at Visit 1 only. [b]mMRC only. [c]At Visit 1, the 6-minute walk test is being performed to train the patients for the procedure. [d]Symptom questionnaires include the CAT, mMRC and SGRQ. The CAT, mMRC and SGRQ are being conducted in patients with COPD and with COPD plus A1AT deficiency only. [e]A full panel of haematology, blood chemistry, and coagulation parameters is performed at Visits 1, 2, 5, 6 and 7, with a reduced panel comprising of haematology, differential automatic cell counts, fibrinogen, highly sensitive C-reactive protein and creatinine performed at Visits 3 and 4.

A1ATD, alpha1-antitrypsin deficiency; CAT, COPD assessment test; COPD, chronic obstructive pulmonary disease; CT, computed tomography; MESI, medical events of special interest; mMRC, modified Medical Research Council dyspnoea scale; MRI, magnetic resonance imaging; SGRQ, St. George's Respiratory Questionnaire.



Supplementary text 1. Participant enrolment and retention, sample size calculation, and power calculations for annual decline in forced expiratory volume in 1 second and 15th percentile density assessed by CT

To achieve adequate participant enrolment, a recruitment plan was set up prior to the start of recruitment; this detailed the number of subjects expected at each site across the different study populations, and was based on feedback during site feasibility assessments. This plan was monitored constantly during the recruitment phase to track planned versus actual enrolment numbers. Local approaches to facilitate recruitment, such as advertising, were also used. To promote participant retention, a comprehensive strategy was set up during the course of the study. This included maintaining frequent contact with study participants (especially from Visits 5 to 7, where visits are 1 year apart), for example via phonecalls and greetings cards, as well as awareness training for site staff.

As this study is exploratory in nature, sample size calculations are focused on achieving the desired precision for biomarker estimates and relevant clinical assessments in all COPD patient groups except for the A1ATD group. This study planned to enrol 125 patients with mild COPD, 125 with moderate COPD and 125 with severe COPD – by assuming a ≤20% dropout rate after 3 years, at least 100 subjects per group are expected to contribute to the analysis by the end of the study period.

For annual decline in forced expiratory volume in 1 second (FEV_1), available information reports standard deviations (SDs) ranging from about 20 mL/year [1] to 60 mL/year.[2] Using this information and considering a planned 100 subjects in each group, supplementary table 1 reports the coverage probability for the half-width of a 95% confidence interval (CI) for annual FEV_1 decline for a given SD. For

example, if the observed SD is 50 mL/year, and the true half-width of the CI is 11 mL/year, with 100 subjects, the probability that the observed CI covers the true CI is 93.8%. The reported SD on annual decline in 15th percentile density (PD15) ranges from 3.4 g/L [3] to 5.5 g/L.[4] Using the same assumptions as for FEV_1 decline, we report the coverage probability of a true CI for a given SD and half-width of the CI for 100 subjects (supplementary table 2).

In addition, with 100 subjects in each group, and allowing for up to 15 covariates, a partial Pearson correlation coefficient of 0.30 can be declared significant with 80% power between any two continuous variables (e.g. FEV_1 and a new biomarker) within the group.

Supplementary table 1. Study sites

Study site and address
United States, Alabama
University of Alabama at Birmingham Birmingham, Alabama, United States, 35294
United States, California
University of California San Diego San Diego, California, United States, 92103-8415
The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center Torrance, California, United States, 90502
United States, Colorado
National Jewish Health Denver, Colorado, United States, 80206
United States, Iowa
University of Iowa Hospitals and Clinics Iowa City, Iowa, United States, 52242
United States, Maryland
Johns Hopkins University Baltimore, Maryland, United States, 21224
United States, Massachusetts
Brigham and Women's Hospital Boston, Massachusetts, United States, 02115

Study site and address
United States, Pennsylvania
Temple University Hospital Philadelphia, Pennsylvania, United States, 19140
United States, Texas
Baylor College of Medicine Houston, Texas, United States, 77030
Diagnostics Research Group San Antonio, Texas, United States, 78229
United States, Utah
University of Utah Health Sciences Center Salt Lake City, Utah, United States, 84108
Belgium
Brussels – UNIV St-Pierre Brussels, Belgium, 1000
UZ Leuven Leuven, Belgium, 3000
Canada, Alberta
University of Alberta Hospital (University of Alberta) Edmonton, Alberta, Canada, T6G 1Z1
Canada, Ontario
Saint Joseph's Healthcare Hamilton, Ontario, Canada, L8N 4A6
McMaster University Medical Centre Hamilton, Ontario, Canada, L8N 4K1

Study site and address
Canada, Quebec
McGill University Health Centre (MUHC) Montreal, Quebec, Canada, H4A 3J1
Canada, Saskatchewan
Royal University Hospital Saskatoon, Saskatchewan, Canada, S7N 0W8
Canada
IUCPQ (Laval University) Quebec, Canada, G1V 4G5
Denmark
Aarhus University Hospital, Skejby Aarhus N, Denmark, 8200
Gentofte Hospital Hellerup, Denmark, 2900
Hvidovre Hospital Hvidovre, Denmark, 2650
Finland
HYKS Keuhkosairauksien tutkimusyksikkö Helsinki, Finland, 00029
TYKS, Keuhkosairauksien klinikka, Turku Turku, Finland, 20520
Germany
IKF Pneumologie GmbH & Co. KG Frankfurt, Germany, 60596

Study site and address
Pneumologisches Forschungsinstitut an der LungenClinic Grosshansdorf GmbH Grosshansdorf, Germany, 22927
Fraunhofer ITEM Hannover, Germany, 30625
KLB Gesundheitsforschung Lübeck GmbH Lübeck, Germany, 23552
Japan
Kagoshima University Medical And Dental Hospital Kagoshima, Kagoshima, Japan, 890-8520
Showa University Fujigaoka Hospital Kanagawa, Yokohama, Japan, 227-8501
Kishiwada City Hospital Osaka, Kishiwada, Japan, 596-8501
Osaka City University Hospital Osaka, Osaka, Japan, 545-8586
Showa University Hospital Tokyo, Shinagawa-ku, Japan, 142-8666
Republic of Korea
Konkuk University Medical Center Seoul, Republic of Korea, 05030
SMG-SNU Boramae Medical Center Seoul, Republic of Korea, 07061
Korea University Guro Hospital Seoul, Republic of Korea, 08308

Study site and address
The Catholic University of Korea, Eunpyeong St. Mary's Hospital Seoul, Republic of Korea, 22711
Poland
Respiratory Medicine Centre, private prac., Bialystok Bialystok, Poland, 15044
University Clinical Center, Gdansk Gdansk, Poland, 80 952
Institute of Tuberculosis & Lung Disease, Warsaw Warsaw, Poland, 01138
Spain
Hospital del Mar Barcelona, Spain, 08003
Hospital Vall d'Hebron Barcelona, Spain, 08035
Hospital Clínic de Barcelona Barcelona, Spain, 08036
Hospital de Bellvitge L'Hospitalet de Llobregat, Spain, 08907
Hospital Son Espases Palma de Mallorca, Spain, 07010
Hospital Quirónsalud Madrid Pozuelo de Alarcón, Spain, 28223
Sweden
Skånes universitetssjukhus, Lund Lund, Sweden, 221 85

Study site and address
United Kingdom
Queen Elizabeth Hospital Birmingham, United Kingdom, B15 2GW
Glenfield Hospital Leicester, United Kingdom, LE3 9QP
Royal Free Hospital London, United Kingdom, NW3 2PF
Medicines Evaluation Unit Manchester, United Kingdom, M23 9QZ

Supplementary table 2. Coverage probability of 95% CIs for FEV_1 annual decline based on 100 subjects

SD	Half-width of a 95% CI									
	6	7	8	9	10	11	12	13	14	15
25	0.998	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
30	0.557	0.993	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
35	0.026	0.557	0.983	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
40	<0.001	0.048	0.557	0.97	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
45	<0.001	<0.001	0.072	0.557	0.954	>0.999	>0.999	>0.999	>0.999	>0.999
50	<0.001	<0.001	0.003	0.097	0.557	0.938	0.998	>0.999	>0.999	>0.999
55	<0.001	<0.001	<0.001	0.006	0.122	0.557	0.921	0.996	>0.999	>0.999
60	<0.001	<0.001	<0.001	<0.001	0.011	0.146	0.557	0.905	0.993	>0.999
65	<0.001	<0.001	<0.001	<0.001	<0.001	0.018	0.168	0.557	0.889	0.988
70	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.026	0.189	0.557	0.873

CI, confidence interval; FEV_1, forced expiratory volume in 1 second; SD, standard deviation.

Supplementary table 3. Coverage probability of 95% CIs for PD15 annual decline based on 100 subjects

SD	Half-width of a 95% CI										
	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00	1.05
2.5	0.938	0.998	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
3.0	0.146	0.557	0.905	0.993	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
3.5	0.001	0.026	0.189	0.557	0.873	0.983	0.999	>0.999	>0.999	>0.999	>0.999
4.0	<0.001	<0.001	0.005	0.048	0.226	0.557	0.846	0.970	0.997	>0.999	>0.999
4.5	<0.001	<0.001	<0.001	<0.001	0.011	0.072	0.257	0.557	0.822	0.954	0.993
5.0	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	0.021	0.097	0.284	0.557	0.801
5.5	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	0.033	0.122	0.306
6.0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.011	0.048

CI, confidence interval; PD15, lung density at the 15th percentile point of the CT histogram; SD, standard deviation.

Supplementary table 4. Overview of permitted and restricted medications/therapy

Drug class	Prior to Visit 1	Between Visits 1 and 2	Observational period
Corticosteroid – nasal/ocular	Permitted	Permitted	Permitted
Corticosteroid – oral, inhaled*	Permitted†	Permitted (stable dose)‡	Permitted
Corticosteroid – i.v., i.m.	Permitted†	Not permitted‡	Permitted
SABA – inhaled§	Permitted†	Permitted (stable dose)‡	Permitted
LABA – inhaled§	Permitted†	Permitted (stable dose)‡	Permitted
SAMA – inhaled	Permitted†	Permitted (stable dose)‡	Permitted
LAMA – inhaled§	Permitted†	Permitted (stable dose)‡	Permitted
Leukotriene modifier	Permitted†	Permitted (stable dose)‡	Permitted
Cromolyn sodium / nedocromil sodium	Permitted†	Permitted (stable dose)‡	Permitted
Methylxanthines	Not permitted‖	Not permitted	Permitted
PDE-4 inhibitors	Not permitted‖	Not permitted	Permitted

Immunomodulators (e.g. methotrexate)	Not permitted‖	Not permitted	Permitted
Immunotherapy (e.g. subcutaneous or sublingual)	Permitted†	Permitted	Permitted
Biologic antagonists (e.g. omalizumab)	Not permitted‖	Not permitted	Not permitted
A1AT augmentation therapy	Not permitted	Not permitted	Permitted
Other investigational drugs	Not permitted	Not permitted	Not permitted

*For patients taking stable maintenance OCS with a total daily dose of ≤20 mg prednisone or equivalent.

†Allowed as stable dose for at least 4 weeks before Visit 1.

‡If unstable (e.g. in case of exacerbations), extend screening period.

§Includes fixed dose combination therapy.

‖Allowed before Visit 1 with a washout period of 3 months or 6 half-lives whichever is greater.

A1AT, alpha1-antitrypsin; LABA, long-acting β2-adrenergic agonist; LAMA, long-acting anticholinergic; OCS, oral corticosteroids; PDE-4, phosphodiesterase-4; SABA, short-acting β2-adrenergic agonist; SAMA, short-acting anticholinergic.

Supplementary table 5. Steering committee members

Scientific expert	Affiliation	Role
Prof. James Crapo	National Jewish Health, Denver (CO), USA	Chair/Advisor/Discussant
Prof. David Lynch	National Jewish Health, Denver (CO), USA	Advisor/Discussant
Dr. Henrik Watz	Pulmonary Research Institute, Lungen Clinic Grosshansdorf GmbH, Grosshansdorf, Germany	Advisor/Discussant
Prof. Pascal Chanez	CHU Marseille – Hôpital Nord, Marseille, France	Advisor/Discussant
Prof. Gaetan Deslee	CHU Reims – Hôpital Maison Blanche, Reims, France	Advisor/Discussant
Dr. Alice Turner	Centre for Translational Inflammation Research, Birmingham, UK	Advisor/Discussant
Dr. Richard Casaburi	Los Angeles Biomedical Research Institute, Torrance (CA), USA	Advisor/Discussant
Prof. Kazuhisa Asai	Osaka University Graduate School of Medicine, Osaka, Japan	Advisor/Discussant
Dr. Harald Koegler	Boehringer Ingelheim	Medical Project Lead
Dr. Markus Beck	Boehringer Ingelheim	Participant/Discussant
Dr. Claudia Diefenbach	Boehringer Ingelheim	Participant/Discussant
Dr. Frank Risse	Boehringer Ingelheim	Participant/Discussant
Dr Abhya Gupta	Boehringer Ingelheim	Participant/Discussant

Supplementary table 6. CT scan protocol in inspiration and expiration

Scan coverage	Lung apices to costophrenic recesses
Scanning mode	Helical
Scanning plane	Axial
Dose modulation	On
Detector configuration	64 x 0.625 mm (GE VCT Discovery STE, VCT Discovery 750HD 64 slice) 64 x 0.5 mm (Philips Brilliance 64 slice) (64)32 x 0.6 mm (Siemens Definition DS, Sensation 64 slice) (128)64 x 0.6 mm (Siemens Definition Edge, AS+ 128 slice, Flash 128 slice, Somatom Force)
kVp	120 kVp
mA/mAs	GE (750HD, Discovery STE): Target noise index: 66.5, Maximum mA limit 100 Philips (Brilliance 64): Reference mAs – 46 mAs (128 mA) Siemens (effective mA): Sensation 64, Definition (DS/AS 64 slice)/(AS+ 128 slice/Flash 128 slice), Edge, Force – Reference mAs 35 mAs
Pitch	GE (Discovery 750HD, Discovery STE) – 0.984 Philips (Brilliance 64) – 0.923 Siemens – 1.0
Rotation time	0.5 s
Reconstructions	
Non-iterative	GE (750HD, Discovery STE): Standard, Bone Philips (Brilliance 64): B, D (YB)

	Siemens (Sensation 64, Definition DS/AS/AS+/Flash 64/128): B31f, B45f (B46f) Siemens (Somatom Force): Bf40d, Qr44d Siemens (Somatom Definition Edge): Bf37f, Bv45f
Iterative	GE (750HD, Discovery STE): Standard ASIR 40%, Bone ASIR 40%, Standard ASIR 100% Philips (Brilliance 64): B iDose L3, D (YB) iDose L3, B iDose L7 Siemens (Sensation 64, Definition DS/AS/AS+/Flash 64/128): I31f SAFIRE 2, I44f SAFIRE 2, I31f SAFIRE 5 Siemens (Somatom Force): Bf40d ADMIRE 2, Qr44d ADMIRE 2, Bf40d ADMIRE 5 Siemens (Somatom Definition Edge): Bf37 f2, Br54 f2, Bf37 f5
Thickness	GE (Discovery 750HD, Discovery STE) – 0.625 Philips (Brilliance 64) – 0.67 Siemens – 0.75 mm
Interval	0.5 mm
DFOV	Lungs (no more than a 1 cm margin outside of the lungs on either side of the scan, where the lungs are the widest in the chest)
HD mode	GE (750HD, Discovery STE): OFF Philips (Brilliance 64): n/a Siemens (Sensation 64, Definition Edge/DS/AS/AS+/Flash 64/128, Somatom Force): n/a

DFOV, display field of view; HD, high definition.

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4. Dirksen A, Piitulainen E, Parr D*, et al.* Exploring the role of CT densitometry: a randomised study of augmentation therapy in α1-antitrypsin deficiency. *Eur Respir J* 2009;33(6):1345-53.

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INFORMATION AND CONSENT FORM FOR PATIENTS WITH COPD (including Sputum assessment)

STUDY TITLE: Observational study in healthy subjects and patients with COPD to assess the relationship between clinical, imaging and biomarker measurements, and progression of emphysema over three years.

PROTOCOL No.: 352.2069 FOOTPRINTS™

SUBJECT No.: _____

EudraCT No.: NA

SPONSOR: Boehringer Ingelheim Pharma GmbH & Co. KG
Birkendorfer Straße 65, 88397 Biberach an der Riss, Germany

STUDY DOCTOR: Name, address, telephone number

Dear Study Participant,

You are being asked to participate in this observational study because:

- You are a 40 to 70 year old ex-smoker, having quit smoking more than 9 months and you have been diagnosed with Chronic Obstructive Pulmonary Disease (COPD)

  Or

- You are a 30 to 70 year old ex-smoker, having quit smoking more than 9 months you have an alpha 1-antitrypsin (A1AT) deficiency phenotype zz and you have been diagnosed with COPD.

This is a 3 year observational study to investigate if there are biomarkers, specific for chronic obstructive pulmonary disease (COPD) and especially emphysema. COPD is a common cause of lung problems and is most often due to smoking. At first, COPD may cause no symptoms or only mild symptoms. As the disease gets worse, symptoms usually become more severe. People with COPD often have symptoms such as cough, abnormal sputum production and shortness of breath, due to narrowing and blockage of the breathing tubes through which air flows in and out of the lungs. Apart from smoking cessation, there is no treatment that can prevent the progression of the disease. Destruction of lung tissue (called emphysema) is a characteristic of COPD involving damage to the air sacs (alveoli) in the lungs which are needed for gas exchange. As a result, the body does not get the oxygen it needs. Many patients with COPD, but not all, develop emphysema at some point. Biomarkers are medical signs, which can be measured, such as blood tests, imaging

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and lung tests. Biomarkers identified in this study could contribute to discovery of new drugs for treatment of COPD and associated lung tissue destruction.

Please read the following information carefully. It contains important information to help you decide whether to participate in this research study. The study staff will have a detailed discussion with you to inform you about the study and the possible benefits and risks of your participation. Ask questions about anything that is not clear at any time. You may take home an unsigned copy of this information to think about and discuss with your family, friends or family doctor before you make your decision to participate or not.

After reading and discussing the information you should know:

- Why this research study is being done;
- What will happen during the study;
- Any possible benefits to you;
- The possible risks to you;
- Other options you could choose instead of being in this study;
- How your personal information / health information will be treated during the study and after the study is over, and which data privacy rights you have;
- Whether being in this study could involve any cost to you; and
- What to do if you have problems or questions about this study.

Participation in this study is voluntary (your choice). If you join this study, you can still stop at any time. You have the right not to sign this consent form. If you do not sign, you cannot take part in this research study. If you decide to participate, you will be asked to sign and date at the end of this form.

PURPOSE OF THE STUDY

The purpose of this study is to evaluate biomarkers correlating to the development and progression of the COPD disease, in particular the emphysema. This study is an observational study. The study does not include administration of any investigational medicinal product and you are allowed to continue and change your standard medication during the study.

COPD is a common disease of lower airways, which significantly affects the quality of life. The disease progresses over time and eventually results in complete disability. Despite major advances in COPD treatment, none of existing drugs can stop the progression of the disease, nor lung tissue destruction (called emphysema), which occurs in every 5th patient with COPD. Also, there are no reliable biological markers (biomarkers) of the disease in sputum and blood, which would allow for earlier recognition of COPD, its progression and response to treatment other than lung function tests. Such biomarkers would also contribute to faster discovery of new drugs for COPD. This study is undertaken to collect data on various biomarkers in induced sputum and blood as well as markers derived from imaging techniques (like chest CT or MRI) in patients with different degrees of COPD and of healthy subjects, who serve as control group. It will also be checked if any of these markers reflect COPD severity or presence or progression of emphysema. This research can lead to the discovery of new diagnostics and medicines and improve the treatment of COPD including associated emphysema in the future.

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We estimate that approximately <enter number of participants assigned to your OPU> people will participate in this study in <insert country of OPU> and approximately 455 participants worldwide.

This study has been approved by <insert applicable local authorities, if required, otherwise this statement can be deleted>.

DESCRIPTION OF THE STUDY

The study is multi-national and involves approximately 12 participating countries. It consists of 2 consecutive periods, a screening and an observational period. The screening period lasts up to 28 days. The observational period lasts 3 years and includes 6 clinic visits and 7 additional phone contacts.

FLOW CHART OF THE STUDY

Study Periods	Screening Period	Observational Period												
Visit	**1**	**2**	**3**	**4**	☎	**5**	☎	☎	☎	**6**	☎	☎	☎	**7**
Weeks			**12**	**26**	**39**	**52**	**65**	**78**	**91**	**104**	**117**	**130**	**143**	**156**
Interview MESI Check	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination & Vital Signs	X					X				X				X
Blood collection for safety and biomarkers	X	X	X	X		X				X				X
Urine collection (smoking status)	X	X	X	X		X				X				X
Questionnaires	X	X				X				X				X
Pulse oximetry	X	X		X		X				X				X
Lung function testing	X	X	X	X		X				X				X
Review of the diary		X	X	X	X	X	X	X	X	X	X	X	X	X
Chest CT		X				X				X				X
MRI (optional)		X		X		X				X				X
Induced sputum		X	X	X		X				X				X
Exercise testing (6 MWT)	X	X								X				X

STUDY PROCEDURES

Pre-study procedures

Before any study-related procedures are performed, you will be asked to read and sign this consent form to confirm that you wish to participate. The study will be explained to you. You can ask questions and if you agree to take part in the study, you need to sign the consent form.
By signing the Informed Consent you confirm your participation in this study.

Please note, that you are also asked to agree to collection of unspecified blood and sputum samples for biobanking. A biobank is a place where samples are long term stored until they are needed for

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further research. This is part of this study. Please read the respective descriptive sections in this document.

Study procedures taking place at each visit

At each clinic visit or scheduled phone visit

- You will be asked about your overall health and changes in medication.
- Please use your reminder diary and note your respiratory symptoms, hospitalization or doctor's visits and all medication you are taking.

Study procedures at the screening visit (Visit 1)

- The study will be introduced to you.
- You will be asked for your demographics, such as sex, race, year of birth.
- Your medical and smoking history will be reviewed.
- You will have a complete physical examination, which will include measurements of blood pressure, pulse rate, temperature, weight, and height.
- An electrocardiography (ECG) will be performed. The ECG is a painless test which measures the electrical activity of the heart.
- Blood (approximately 15 ml or 3 teaspoons) will be drawn from a vein in your arm for different laboratory tests, including a genetic test to look whether you have a genetic variation called alpha 1-antitrypsin deficiency (A1ATD). A1ATD is a genetic disorder that causes defective production of alpha 1-antitrypsin (A1AT). There are several forms and degrees of the deficiency. The most common abnormal genes are the S and Z alleles. In this study only patients with the zz-gene mutation can participate. Severe A1ATD can lead to liver disease and emphysema.
- A urine sample will be taken for nicotine testing.
- For all women of child bearing potential a urine pregnancy test will be done.
- You will complete the mMRC (Modified Medical Research Council) questionnaire.
- You will have a lung function test before and after taking salbutamol/albuterol [OPU to adapt locally]. You will be asked to blow hard into the mouthpiece of the measuring device. The test determines how well your lungs work. The procedure will be explained in detail before the test.
- You will have a lung diffusion test called D_{LCO} after taking salbutamol/albuterol [OPU to adapt locally]: this lung diffusion test measures how well the lungs exchange gases. This is an important part of lung function testing, because the major function of the lungs is to allow oxygen to "diffuse" or pass into the blood from the lungs, and to allow carbon dioxide to "diffuse" from the blood into the lungs. You breathe in (inhale) air containing a very small amount of carbon monoxide, a gas existing in the atmosphere but given to you in a higher concentration. You hold your breath for 10 seconds, and then rapidly blow it out (exhale). The exhaled gas is tested to determine how much of the carbon monoxide gas was absorbed during the breath. The remaining gas will easily be eliminated from your body by normal breathing.
- A 6-minutes walking exercise test will be done. You will be asked to walk as far as possible for 6 minutes and the distance will be measured. You will get sufficient time to recover from the walking test.
- Pulse oximetry: this test measures how much oxygen your blood is carrying. By using a small device put on your forefinger your blood oxygen level can be checked.

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- You get a reminder diary to write down if your health conditions have gotten worse, if you experienced symptoms and all medication that you are taking.

The results of the tests and/or questions at the screening visit will help the study team to decide whether you can continue in this study. If these tests show that you are eligible to participate, you will be able to continue the study. If you do not meet the eligibility criteria, you will not be able to continue.

Even if you are eligible for the study, your participation cannot be guaranteed. At the moment when the maximum number of subjects who can be included is reached, you will not be able to enter the observational phase of the study.

Visit 1 will last approximately 3 - 4 hours.

You will be scheduled to return for Visit 2 within the next 28 days.

Study procedures in the observational period (Visits 2 to 7)

You will be scheduled to return to the clinic for 6 observational period visits. All visits during the observational period will be scheduled to begin between 06:00 and 09:00 in the mornings. Please come fasted to the visits. Please do not have anything to eat or drink, except water, for 6 hours before the visit. This is very important to get good laboratory measurements.

- First at each visit you will be asked about your medication intake, your diet and life style and your smoking status.
- You will have a complete physical examination, which will include measurements of blood pressure, pulse rate, temperature, weight, and respiratory rate at visits 5, 6 and 7.
- For all women of child bearing potential a urine pregnancy test will be performed at visits 2, 4, 5, 6 and 7.
- You will complete at visits 2, 5, 6 and 7 three questionnaires prior to pulmonary function testing. The questionnaires contain questions about your health, the breathing and COPD status:
  All questionnaires together will take approximately 60 minutes to complete.
- Lung function testing (each performed twice), D_{LCO} and pulse oximetry will be performed at each clinic visit, except at visit 3.
- Body plethysmography will be performed at each clinic visit, except at visit 3. You sit in a small, airtight room known as a body box. You breathe against a mouthpiece. Clips are put on your nose to shut off your nostrils. As your chest moves while you breathe, it changes the pressure and amount of air in the room and against the mouthpiece. From these changes an accurate measure of the amount of air in your lungs can be determined.
- Between lung function tests salbutamol/albuterol [OPU to adapt locally] will be administered.
- Induced sputum will be collected at each clinic visit. Sputum induction is a procedure to help you cough up secretions from your lungs more easily. The principle is to create extra moisture in the airways of the lungs by inhaling a saline enriched aerosol, which will help loosen the sputum deep in your lungs. The sputum sample will be examined further. In case you are not able to produce an acceptable sputum sample at Visit 2 and at the Visit 2 retest you will continue in the study, but no more sputum induction will be performed in the study.

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- Blood samples for safety laboratory testing, and biomarker assessments will be drawn at each clinic visit (about 90 ml or 9 tablespoons).
- A urine sample for nicotine testing will be taken at each clinic visit.
- Your reminder diary will be reviewed at each visit.
- An ECG will be performed at visits 5, 6 and 7.
- A chest computer tomography (CT), a radiologic imaging method, enabling a detailed look into your body, will be performed at visits 2, 5, 6 and 7 between 1h and 4h after salbutamol/albuterol [OPU to adapt locally] administration for the lung function testing.
- The 6-minute walk test will be performed at visits 2, 6 and 7 after the lung function testing.

Visits 2 – 7 will last approximately 4 – 7 hours. Some visit procedures like induced sputum and/or chest CT can be rescheduled to another day (within 14 days of the original visit), e.g. if you have time constraints. In this case you will be administered with salbutamol/albuterol [OPU to adapt locally] prior to the assessment(s).

Telephone contacts

Additionally, you will have 7 telephone contacts between clinic visits 4, 5, 6 and 7 to follow up and collect information by telephone interviews. Your reminder diary helps you to remember your respiratory symptoms, any COPD exacerbations (COPD flare up with symptoms worsened suddenly), hospitalization or health care visits, and all medication you were taking.
Please remember to inform your study team prior to scheduled telephone interviews in case you have been hospitalized. Telephone calls might take about 30 minutes.

Study completion

Visit 7 or the discontinuation visit, in case you terminate the study early, will mark the end of the study and you have completed the study.

Early discontinuation

If you terminate the study during the observational period for any reason, you will be asked to come to the clinic for a discontinuation visit at the time of your next scheduled visit. You will undergo the procedures of this particular visit as outlined in the flowchart. If you do not agree to undergo all procedures of the next scheduled visit, you are asked to do at least the safety assessments.

Women who become pregnant will be discontinued from further study participation and will undergo the procedures for safety assessments like for example blood collection and physical examination.

Phone calls after early discontinuation

After you have discontinued the study early, the study team will contact you for further follow up, if you agree. You will be asked if you have experienced COPD exacerbations. If your discontinuation takes place between:

Discontinuation date between Visit 2 and 5, three telephone calls:

1. First call: 52 weeks after your Visit 2
2. Second call: 104 weeks after your Visit 2

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3. Third call: 156 weeks after Visit 2

Discontinuation date between Visit 5 and 6, two telephone calls:
1. First call: 104 weeks after your Visit 2
2. Second call: 156 weeks after your Visit 2

Discontinuation date between Visit 6 and 7, one telephone call:
1. Call: 156 weeks after Visit 2

YOUR RESPONSIBILITIES

- While participating in this study, you should not take part in another medication research study. You must tell your study doctor if you have been in another research study in the past 6 weeks or are currently in another research study.
- You must follow the study instructions provided by the study staff, come to all scheduled study visits and be reasonably available for the scheduled telephone visits.
- Prior to and during the lung function assessments you have to stay in in the building where the measurements are performed.
- You must tell the study doctor about all prescription and non-prescription drugs, herbal preparations and food supplements that you are taking or planning to take.
- Please refrain from strenuous activity for at least 12 hours prior to lung function testing, also avoid cold temperatures, environmental smoke, dust or areas with strong odors (e.g. perfumes).
- Please do not donate blood during the observational period of the study.
- You must fast, not have anything to eat or drink, except water, for 6 hours before all study visits.
- You might be asked to provide an additional sputum sample prior to Visit 2 that will be used for quality assurance of sputum processing. This sputum sample will be taken as described above.

POTENTIAL BENEFITS

This study is an observational study with no change in your usual medication treatment. There will be no direct benefit for you, except for the benefit which derives from the extensive examinations and periodic monitoring of your health or disease status.

RISKS AND/OR DISCOMFORTS

Your participation in this study requires standard medical procedures which are well known to patients with respiratory diseases. The examinations include procedures like physical examination, blood sampling, ECGs, lung function testing, D_{LCO} measurements, bodyplethysmography, chest CTs and sputum induction.

Computed tomography

A CT scan will be done at four visits. This is a painless test, a type of X-ray exam, which shows detailed lung structures. You will be asked to strip to the waist and lie on the narrow table that will slide through a hole in the center of the CT scanner. You have to stay still during the procedure

and follow the instructions when to hold your breath. The actual CT scanning takes less than 30 seconds and the entire process is usually completed within 15 minutes.
The scientific unit of measurement for radiation dose is the millisievert (mSv). Chest CT scans involve exposure to radiation equivalent to 20 chest x-rays (approximately 4 millisievert). Although the amount of radiation exposure is higher than a typical x-ray, the risk of harmful effects from a single exam is small. Persons are exposed to radiation from natural sources in the environment all the time. These natural "background" doses vary from area to area. The natural background radiation is 2.4 mSv per year. The additional risk of developing a fatal cancer from a single 4 mSv exposure in a person 50 years old is approximately 1 in 5000 or 0.02%. If it were to occur, it could take many years or decades for you to develop cancer related to this study. The latent period for cancer is estimated to be 6 to 10 years for blood borne cancers (leukemia, lymphoma) and 10 to 25 years for solid organ cancers. Please keep in mind that the risk from all sources of radiation is cumulative over a lifetime.
The chest CT is a very sensitive diagnostic method and reveals lung nodules in approximately 20% of people. The chest CT scan may detect lung abnormalities much earlier than other diagnostic methods, which may be life-saving in subjects at risk of lung cancer (e.g. smokers). Such finding causes anxiety and triggers further diagnostic work-up to confirm or reject malignancy. While an early diagnosis of lung cancer is critical for successful treatment, most lung nodules accidentally found on the chest CT scan turn out to be benign.

ECG

You may experience skin irritation from the ECG electrode pads or pain when pads are removed.

Lung function measurement (spirometry and diffusing capacity of the lungs for carbon monoxide)

Risks and discomforts associated with lung function testing may include shortness of breath, dizziness, or headache during the breathing tests. Should this occur, you may receive treatment.

6-minute walk test

The 6-minute walk test measures the distance that you can quickly walk on a flat, hard surface in a period of 6 minutes. To prevent any risk to your health you will be constantly supervised by specially trained medical personnel and your health will be monitored. The test may be stopped if you are unable to continue safely in the opinion of the supervising personnel. After the test you will have enough time to recover and to leave the office/clinic in good health.

Sputum induction

Sputum induction is a painless and safe procedure. Rarely, it may cause transient wheeze or chest tightness. Let your study doctor know immediately if you feel any of these symptoms at any time during the induction procedure. These symptoms could be quickly relieved by inhaling an appropriate drug. The procedure is performed after inhalation of salbutamol/albuterol [OPU to adapt locally] that helps open up the air passages. Side-effects of salbutamol/albuterol [OPU to adapt locally] are rare and further details can be found below.

Laboratory tests

Blood draw may cause some discomfort or mild pain, as well as redness or bruising at the site of puncture. In rare cases the puncture site can also become infected or nerves may be damaged,

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inducing long lasting abnormal sensations (paresthesia), impaired sensation of touch and persistent pain.
A total blood volume of about 500mL will be withdrawn over a period of about 3 years. Approximately 90 mL will be drawn per time point (Visit 2 – 7). The estimated blood loss will not impact your health.

Bodyplethysmography

Some people may feel uncomfortable in the small plethysmography chamber called body box. Tell the study staff if you feel uncomfortable in any way during the test and keep in mind that you can open the door from the inside at any time.

Salbutamol/Albuterol [OPU to adapt locally]

Salbutamol/Albuterol [OPU to adapt locally] is a short-acting bronchodilator used to treat narrowed airways like in asthma or COPD. It is taken by the inhaled route for direct effect on bronchial smooth muscle. In general, salbutamol/albuterol [OPU to adapt locally] is well tolerated even at large doses. In this study, salbutamol/albuterol [OPU to adapt locally] is used only at clinic visit days in combination with some diagnostic tests like PFTs, imaging assessments or induced sputum. It is not administered for the treatment of a disease.
The most common side effects are shaking of fingers, anxiety, headache, muscle cramps, dry mouth, and palpitation (awareness of heart beat). Other symptoms may include increase in heart rate, irregular heartbeat, flushing, myocardial ischemia (reduced blood supply of the heart muscle) (rare), and disturbances of sleep and behavior. Rarely occurring, but of importance, are allergic reactions like unexpected narrowing of the airways (can be life threatening in rare cases), rapid swelling of the facial skin rarely with life threatening breathing difficulties, skin hives with itching and redness, decrease in blood pressure, and collapse. High doses or prolonged use may cause hypokalemia (low levels of potassium in your blood).

ALTERNATIVES

This study is for research purposes only. Your participation is voluntary. The alternative is not to participate in the study.

Please talk to the study doctor about your options before you decide whether you will take part in this study.

NEW INFORMATION ABOUT THE STUDY

During the study, you will be notified of changes to study procedures, which may affect your health or willingness to participate. You may be asked to sign a new consent form that shows that you have been informed of changes.

INFORMATION ON BIRTH CONTROL

You cannot participate in this study if you are pregnant or plan to become pregnant due to the planned radiologic procedures. If you should nevertheless become pregnant or you think you could be pregnant during the study, it is important for you to tell the study doctor or study staff prior to each clinic visit. In case of pregnancy, you will be removed from the study procedures and you will be followed up by phone calls.

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WITHDRAWAL FROM THE STUDY PARTICIPATION

Your participation in this study is voluntary. You may choose to leave this study completely at any time. Your decision will not result in any penalty or loss of benefits to which you are otherwise entitled. Leaving the study will not affect your future medical care.
All data and samples that had already been collected up to the time of withdrawal of your consent, including data and samples gathered at any of your final assessments, will still be used to ensure the correct completion and documentation of the trial and comply with applicable law.

Your study doctor might decide to stop your study participation early without your consent when, in the study doctor's judgment, it is in your best health interest to do so or under certain circumstances listed below:

- Your inability to participate as instructed.
- Study cancellation by the sponsor or [if applicable regulatory authority/EC].
- Or for other unforeseen reasons that make it necessary to stop your participation in the study.

If you are removed from the study, the study doctor will explain to you why you were removed.

CONFIDENTIALITY / PRIVACY AND DATA SHARING

Use of your personally identifiable information

The part of your personal information that directly identifies you such as your name, address, or birth date will remain at the study site and can be accessed by the study doctor and other people at the site who are assisting with the study or your care. This information may also be checked at the study site by the

- sponsor, or the sponsor's representatives (including monitors hired by the sponsor through a service provider),
- ethics review board/committee that reviewed the ethical aspects of this study, and/or
- [Please adapt or delete if not applicable] domestic or foreign regulatory agencies such as the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) that approve medicines.

These persons check that the study is carried out correctly at the study site. They are bound by a duty of confidentiality.

Use of your coded data

Your personally identifiable information and health information collected for the purpose of the study as well as the samples will be labelled with a unique code number. Coded data may also include data/information such as images (e.g. CTs/MRTs). The code number will be used in place of your name and other information that directly and easily identifies you, for example, address and birth date. Only the study site will have the link between your personal information and the coded data. This link will not be provided to the sponsor; only your coded data such as medical data, biomarker data, images and all other information collected in the study will be sent to the sponsor and/or contractors of the sponsor. The sponsor and/or contractors of the sponsor will take measures to protect the confidentiality and security of your coded data and your privacy in accordance with current law.

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The sponsor and other members of the Boehringer Ingelheim Group of Companies and those working with them such as their associates, collaborators, research partners, assignees, licensees and designees, and its/their affiliated companies and agents, and other individuals and organizations, may use your coded data for the following purposes:

- Keep it electronically and analyze it to understand the study and the study results;
- Share it with domestic or foreign regulatory agencies worldwide such as the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), that approve medicines;
- Share it with ethics review boards/committees worldwide, or steering committee that checks whether the study was run properly.
- Analyse it to improve the quality of this study and other clinical trials

The coded data may be transferred within your country or to other countries for analysis. Where the data protection rules in other countries are not as strict as the rules in your country, the sponsor will adopt appropriate measures to provide an adequate level of protection according to EU law.

Use of anonymized data for additional research

Anonymized data refers to data from which the subject cannot be identified by the recipient of the information. Anonymizing data is one of the strongest safeguards for the protection of subjects' identity.

The sponsor may give scientists and medical researchers in other companies, research organizations, or academic institutions access to anonymized data for further research and education beyond the disease investigated in this study. This may include research looking into improving patient care or quality and efficiency in conducting clinical studies in general. Details on the anonymization and data sharing process are set forth in the Boehringer Ingelheim "Policy on Transparency and Publication of Clinical Study Data" available at: http://trials.boehringer-ingelheim.com.

Information and correction rights

You have the right to review which personal data the trial site and sponsor store about you. You can also request that incorrect personal data is corrected or that processing is restricted. You can request a copy of the contractual safeguards implementing adequate protection of personal data if your data is shared outside the EU/EEA.

In order to exercise your rights please contact the study site [if applicable: and its data protection officer (ADD EMAIL)] who will align with the sponsor. You can also ask to receive the personal information you have provided for the study in a standardized electronic format or to have them transmitted to another person of your choice. You can also contact your local data protection authority in case of questions or concerns about the handling of your personal data. In some cases, your rights can be limited under applicable laws, especially where they conflict with the conduct of the study and mandatory archiving requirements. In this case you will be informed accordingly.

If you have signed an Information and Consent Form for the optional DNA banking sample, all the information provided in this form under section "Confidentiality / Privacy and Data Sharing" your rights under data protection laws and the information on how to exercise them applies as well to the optional DNA banking consent form.

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Clinical study websites and publication

A description of this clinical study will be available on http://www.clinicaltrials.gov, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

The results of the study will be published on Boehringer Ingelheim' s study web site (http://trials.boehringer-ingelheim.com) and may also appear in other clinical study/study registries in countries in which the study is conducted. The results will not include information that can identify you.

The results of the study may also be published in a professional journal or presented at scientific meetings. Your identity will not be disclosed in those presentations.

USE OF SAMPLES IN THIS STUDY

Blood and sputum will be collected for pre-specified as well as for unspecified analyses. Urine samples will also be collected. All samples collected during the study as described under the section "Study Procedures" will be coded and sent to the central laboratory (except pregnancy test). A part of the analyses will be done at the central laboratory. In addition, samples will be further distributed to Boehringer Ingelheim and contractors of Boehringer Ingelheim for analyses.

Urine samples

Urine samples will be collected from all patients for the purpose of nicotine testing and (if applicable) additionally for pregnancy testing in women. Pregnancy testing will be done at the site and the sample will be discarded right after testing. Urine samples for nicotine testing will be shipped to and analyzed by the central laboratory and neither kept nor used for any future analyses.

Pre-specified biomarker assessment

The pre-specified analyses done in blood samples comprise safety laboratory testing, testing of A1ATD and biomarker assessment. Pre-specified biomarkers will also be analysed in sputum samples. All blood and sputum samples will be collected to gain a better understanding of COPD and the influence of A1ATD. Those biomarkers include but are not limited to markers of inflammation and tissue destruction, which have been reported and/or hypothesized to be indicative of physiological and pathophysiological changes in the lungs. In addition investigations aiming at identification of new biomarkers will be done.

The DNA sample used to determine A1ATD and left over of other biomarker samples for prespecified analyses will be destroyed once this analysis has been completed.

Left over sputum samples that were not used for the pre-specified biomarker analyses will be transferred to the biobank (see section "Unspecified biomarker assessment").

Unspecified biomarker assessment (Biobanking)

Since knowledge in the biomarker field is steadily increasing, we also ask you to consent to the collection of samples for unspecified biomarker analyses. These samples including left over sputum samples described above will be stored up to 15 years after the final study report has been written to enable these additional explorative investigations

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Approximately 50 ml blood will be collected per visit during the observational period. All analyses aim at increasing our understanding of COPD and other respiratory diseases. This may help to develop new therapies for COPD in the future. Samples will be sent to Boehringer Ingelheim or a storage CRO for long term storage.

The future research on your samples will not affect your present medical care. Reports from any future research with your samples and data will not be given to you or your doctor. Summaries of the research results may be published in scientific journals, on the internet, in data repositories or presented at meetings for other researchers, so that other doctors and researchers can find out about the results. In any case, your identity will not be disclosed in any publications or presentations. Subjects who donate her/his samples to BI do not retain any property rights to the materials such as samples and their derivatives and the related data.

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COMPENSATION / COSTS

[Amend in accordance with local legal requirements or insurance.]
This study is funded by the sponsor. The sponsor has signed a contract with <insert CRO, applicable> to conduct this study. The sponsor <CRO on behalf of CRO> will pay the study doctor's and/or institution's for his/her expenses, time and effort to conduct this study. The study doctor and the institution/Hospital have no other financials ties to Boehringer Ingelheim.

There will be no additional costs to you for your participation in this study. All study procedures including lab work, tests, and doctor visits are provided to you free of charge by the sponsor, Boehringer Ingelheim, and will not be billed to you or your insurance carrier as long as you are participating in the study. You will receive <enter amount and/or a description of a payment schedule> to cover out-of-pocket expenses such as meals and parking for visits that are required as part of the study.

The sponsor will be owner of the study results. If commercial products or other valuable discoveries result from research using your samples and/or data, these products and discoveries may be owned, patented, licensed, or otherwise developed for commercial sale by sponsor, other researchers, or companies. If this should occur, you will not receive any financial benefits or compensation or other proprietary interest from any commercial products or discoveries that may result from such research.

INJURY

You will receive necessary medical treatment in the event that an injury results because of your participation in this study. Financial compensation for lost wages, disability or discomfort due to an injury is not generally available. You do not give up any legal rights by signing this form. You do not release the sponsor, institution, study doctor or their agents from any liability for negligence by signing this form.

EMERGENCY CONTACT / ETHICS CONTACT

If you have questions concerning the conduct of the study, or for any other reason you may contact Dr. _____ at _____ or the Study Coordinator _____ at _____.

In case of an emergency, please go to the nearest hospital emergency department and inform your study doctor as soon as possible.

If you have any questions about your rights as a study subject, please contact your family doctor, lawyer, or write to the committee that reviewed the ethical aspects of this study at: <insert ethics committee name and contact here>

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DECLARATION OF INFORMED CONSENT

STUDY SUBJECT No.: _____

My signature on this consent form means that:

- I understand that I am being asked to participate in a research study entitled: Observational study in healthy subjects and patients with COPD to assess the relationship between clinical, imaging and biomarker measurements, and progression of emphysema over three years.
- I have had this study explained to me by _____.
- I have read each page of this document.
- I have had all of my questions answered fully and to my satisfaction.
- I was given sufficient time to think in peace and quiet and decide whether to participate.
- I have been told that my participation is voluntary and I can withdraw at any time without giving any reasons.
- I agree to the collection, storage, processing, transfer and use of my personal data and biological samples (blood, urine and sputum) as explained in the above information.
- I agree that blood will be collected for biobanking and will be used for future unspecified analyses.
- I agree that left over of my sputum samples can be stored for future unspecified analyses.
- I agree that further, today unspecified analysis of my biological samples can be done during and after the study.
- I hereby expressly declare and agree that I transfer all rights of ownership of the collected samples and sample-related data to Boehringer Ingelheim Pharma (GmbH & Co. KG) so that the same may use them as described in this Informed Consent Form.
- I voluntarily consent to participate in this study.
- I will be given a signed copy of this consent document for my records

It is important that your personal doctor is aware that you are in a research study because you are undergoing examinations that could affect your health. With your permission, we will notify him/her that you are taking part in this study.

I consent to my personal doctor being notified that I am taking part in this study.

❑ YES, I agree. ❑ NO, I don't agree.

I agree that in case I discontinue the study early, the study team can contact me as described above, for further follow up by telephone.

❑ YES, I agree. ❑ NO, I don't agree to be contacted after early discontinuation.

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I consent that in case I die during the time of this study my study doctor is allowed to contact my family members to ask after the circumstances and that he can review my relevant medical records.

❑ YES ❑ NO

Name of Study Participant (*please print*)	**Consent Signature of Study Participant**	**Date** (dd mmm yyyy)
Name of Person Obtaining Consent (*please print*)	**Signature of Person Obtaining Consent**	**Date** (dd mmm yyyy)

STATEMENT OF INVESTIGATOR / STUDY DOCTOR:

I certify that I have explained to the above individual(s) the nature and purpose of the study and the possible benefit and risks associated with participation. I have answered any questions that have been raised and the potential study participant has received a copy of this signed consent document.

I acknowledge my responsibility for the care and well-being of the above study participant, to respect the rights and wishes of the participant, and to conduct the study according to applicable Good Clinical Practice guidelines and regulations.

Investigator Name (*please print*)	**Investigator Signature**	**Date** (dd mmm yyyy)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Reported on page number (section)
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3 (Abstract: Introduction).
	2b	All items from the World Health Organization Trial Registration Data Set	Please see table on pages 10–15.
Protocol version	3	Date and version identifier	7 (Introduction).
Funding	4	Sources and types of financial, material, and other support	22 (Acknowledgements); 23 (Funding of study).
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	22 (Competing Interests); 23 (Authors' contributions); 38 (Supplementary Table 4. Steering committee members).
	5b	Name and contact information for the trial sponsor	23 (Funding of study).

	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23 (Authors' contributions).
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14 (Information on steering committee); 14/15 (Data collection and management); 23 (Authors' contributions); 38 (Supplementary Table 4. Steering committee members).
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7 (Introduction); 8 (Objectives); summary of interventions is not applicable for this observational biomarker study.
	6b	Explanation for choice of comparators	Not applicable for this observational biomarker study.
Objectives	7	Specific objectives or hypotheses	8 (Objectives).
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8/9 (Study design); 11 (Patient population and recruitment); 28 (Figure 1. Overview of trial design); 29 (Table 1. FOOTPRINTS study participants); 32/33 (Supplementary Text 1) – describes study as exploratory.
Methods: Participants, interventions, and outcomes			

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8–9 (Study design).
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9–11 (Inclusion and exclusion criteria).
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Not applicable as study was observational biomarker study. Planned analyses and assessments are detailed on pages 11–12.
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Not applicable as an observational biomarker study. Details on study discontinuation are provided on page 9 (Study design) and rescheduling of clinic visits on page 13.
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Not applicable. Further details provided on page 14–15 on registering clinic visits and phone calls are provided in the “data collection and management” section.
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	36/37 (Supplementary Table 3. Overview of permitted and restricted medications/therapy).

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8 (Objectives); 11 (Main outcome measures); 11–12 Planned analyses and assessments);
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9 (Study design); 28 (Figure 1. Overview of trial design);
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	32 (Supplementary text 1); 34 (Supplementary Table 1); 35 (Supplementary Table 2)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	32/33 (Supplementary Text 1)
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Not applicable (observational biomarker study).

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Not applicable.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Not applicable.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Not applicable.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Not applicable.
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14/15 (Data collection and management); 39/40 (Supplementary Table 5. CT scan protocol in inspiration and expiration)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9 (Study design) provides information on discontinuation; 13 (Rescheduling of clinic visits); 32/33 (Supplementary text 1).

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14/15 (Data collection and management)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13 (Statistical analysis)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13 (Statistical analysis)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Note, analysis population will be described in future publications. 15 (Data collection and management) for handling of missing data.
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	No DMC was implemented for the FOOTPRINTS study, as it is a non-interventional, purely observational biomarker study.

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13 (Statistical analysis); details on stopping guidelines are not applicable for this study (observational biomarker study) and access to study results is generally not restricted to a dedicated group since there are no blinded data.
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12 (Planned analyses and assessments)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15 (Data collection and management)
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	4 (Ethics and dissemination); 16 (ETHICS APPROVAL AND CONSENT TO PARTICIPATE)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16 (ETHICS APPROVAL AND CONSENT TO PARTICIPATE).
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4 (Ethics and dissemination); 9/10 (Inclusion and exclusion criteria)

	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15/16 (Data collection and management)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22 (Competing interests); Financial disclosure is not a prerequisite for this type of study, so this information was not collected for all principal investigators.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	21 (Discussion)
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Not applicable (observational biomarker study)
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	21 (Discussion)
	31b	Authorship eligibility guidelines and any intended use of professional writers	22 (Acknowledgments); 23 (AUTHORS' CONTRIBUTIONS)

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	21 (Discussion)
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided in the Supplementary. Described on page 4 (Ethics and dissemination); 9 (Inclusion and exclusion criteria)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	14/15 (Data collection and management)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

WHO Trial Registration Data Set (Version 1.3.1)	Reported on page number (section)
1. Primary Registry and Trial Identifying Number Name of Primary Registry, and the unique ID number assigned by the Primary Registry to this trial.	3 (Abstract).
2. Date of Registration in Primary Registry Date when trial was officially registered in the Primary Registry.	9 (Study design).
3. Secondary Identifying Numbers Other identifiers besides the Trial Identifying Number allocated by the Primary Registry, if any. These include: - The Universal Trial Number (UTN) - Identifiers assigned by the sponsor (record Sponsor name and Sponsor-issued trial number (e.g. protocol number)) - Other trial registration numbers issued by other Registries (both Primary and Partner Registries in the WHO Registry Network, and other registries) - Identifiers issued by funding bodies, collaborative research groups, regulatory authorities, ethics committees / institutional review boards, etc. - All secondary identifiers will have 2 elements: an identifier for the issuing authority (e.g. NCT, ISRCTN, ACTRN) plus a number. There is no limit to the number of secondary identifiers that can be provided.	3 (Abstract).
4. Source(s) of Monetary or Material Support Major source(s) of monetary or material support for the trial (e.g. funding agency, foundation, company, institution).	23 (FUNDING OF STUDY).
5. Primary Sponsor The individual, organization, group or other legal entity which takes responsibility for initiating, managing and/or financing a study. The Primary Sponsor is responsible for ensuring that the trial is properly registered. The Primary Sponsor may or may not be the main	23 (FUNDING OF STUDY).

funder.	
6. Secondary Sponsor(s) Additional individuals, organizations or other legal persons, if any, that have agreed with the primary sponsor to take on responsibilities of sponsorship. A secondary sponsor may have agreed to: - take on all the responsibilities of sponsorship jointly with the primary sponsor; or form a group with the Primary Sponsor in which the responsibilities of sponsorship are allocated among the members of the group; or act as the Primary Sponsor's legal representative in relation to some or all of the trial sites.	Not applicable.
7. Contact for Public Queries Email address, telephone number and postal address of the contact who will respond to general queries, including information about current recruitment status. "Note: The information provided in here is functional and not personal, it is recommended to provide institutional and not personal information. By providing this information the registrant consents that the information provided can or may be published on a public website. Once provided the information cannot be redacted or anonymized as a result of new privacy legislation such as the European General Data Protection Regulation (GDPR)".	2 (Corresponding author); 23 (FUNDING OF STUDY).
8. Contact for Scientific Queries There must be clearly assigned responsibility for scientific leadership to a named Principal Investigator. The PI may delegate responsibility for dealing with scientific enquiries to a scientific contact for the trial. This scientific contact will be listed in addition to the PI.	2 (Corresponding author); 23 (FUNDING OF STUDY).

"Note: The information provided in here is functional and not personal, it is recommended to provide institutional and not personal information. By providing this information the registrant consents that the information provided can or may be published on a public website. Once provided the information cannot be redacted or anonymized as a result of new privacy legislation such as the European General Data Protection Regulation (GDPR)". The contact for scientific queries must include: Name and title, email address, telephone number, postal address and affiliation of the Principal Investigator, and; Email address, telephone number, postal address and affiliation of the contact for scientific queries about the trial (if applicable). The details for the scientific contact may be generic (that is, there does not need to be a named individual): e.g. a generic email address for research team members qualified to answer scientific queries.	
9. Public Title Title intended for the lay public in easily understood language.	'FOOTPRINTS' study included throughout.
10. Scientific Title Scientific title of the study as it appears in the protocol submitted for funding and ethical review. Include trial acronym if available.	'FOOTPRINTS' study included throughout.
11. Countries of Recruitment The countries from which participants will be, are intended to be, or have been recruited at the time of registration.	9 (Study design).
12. Health Condition(s) or Problem(s) Studied Primary health condition(s) or problem(s) studied (e.g., depression, breast cancer, medication error). If the study is conducted in healthy human volunteers belonging to the target population of the intervention (e.g. preventive or screening	6–7 (Introduction); 8 (Objectives).

interventions), enter the particular health condition(s) or problem(s) being prevented.	
13. Intervention(s) For each arm of the trial record a brief intervention name plus an intervention description.	Not applicable as observational biomarker study.
14. Key Inclusion and Exclusion Criteria Inclusion and exclusion criteria for participant selection, including age and sex. Other selection criteria may relate to clinical diagnosis and co-morbid conditions; exclusion criteria are often used to ensure patient safety. If the study is conducted in healthy human volunteers not belonging to the target population (e.g. a preliminary safety study), enter "healthy human volunteer".	9–10 (Inclusion and exclusion criteria).
15. Study Type	9 (Study design).
16. Date of First Enrollment Anticipated or actual date of enrolment of the first participant.	11 (Patient population and recruitment).
17. Sample Size Sample Size consists of: Number of participants that the trial plans to enrol in total. Number of participants that the trial has enrolled.	32/33 (Supplementary Text 1) 29 (Table 1. FOOTPRINTS® study participants: all enrolled subjects)
18. Recruitment Status	11 (Patient population and recruitment).
19. Primary Outcome(s) Outcomes are events, variables, or experiences that are measured because it is believed that they may be influenced by the intervention. The Primary Outcome should be the outcome used in sample size	8 (Objectives); 11–12 (Planned analyses and assessments) details specifics on measurement methods and timepoints.

calculations, or the main outcome(s) used to determine the effects of the intervention(s). Most trials should have only one primary outcome. For each primary outcome provide: The name of the outcome (do not use abbreviations) The metric or method of measurement used (be as specific as possible) The timepoint(s) of primary interest	
20. Key Secondary Outcomes Secondary outcomes are outcomes which are of secondary interest or that are measured at timepoints of secondary interest. A secondary outcome may involve the same event, variable, or experience as the primary outcome, but measured at timepoints other than those of primary interest. As for primary outcomes, for each secondary outcome provide: The name of the outcome (do not use abbreviations) The metric or method of measurement used (be as specific as possible) The timepoint(s) of interest	8 (Objectives); 11–12 (Planned analyses and assessments) details specifics on measurement methods and timepoints.
21. Ethics Review The ethics review process information of the trial record in the primary register database. It consists of: Status (possible values: Not approved, Approved, Not Available) Date of approval Name and contact details of Ethics committee(s)	16 (ETHICS APPROVAL AND CONSENT TO PARTICIPATE); Trial record can be found at https://clinicaltrials.gov/ct2/show/NCT02719184; List of ethics committees (name/contact details) provided for purposes of submission (not to be included in the publication)
22. Completion date Date of study completion: The date on which the final data for a clinical study were collected (commonly referred to as, "last subject, last visit").	11 (Patient population and recruitment).
23. Summary Results It consists of:	Not applicable – trial ongoing.

Date of posting of results summaries Date of the first journal publication of results URL hyperlink(s) related to results and publications Baseline Characteristics: Data collected at the beginning of a clinical study for all participants and for each arm or comparison group. These data include demographics, such as age and sex, and study-specific measures. Participant flow: Information to document the progress and numbers of research participants through each stage of a study in a flow diagram or tabular format. Adverse events: An unfavorable change in the health of a participant, including abnormal laboratory findings, and all serious adverse events and deaths that happen during a clinical study or within a certain time period after the study has ended. This change may or may not be caused by the intervention being studied. Outcome measures: A table of data for each primary and secondary outcome measure and their respective measurement of precision (eg a 95% confidence interval) by arm (that is, initial assignment of participants to arms or groups) or comparison group (that is, analysis groups), including the result(s) of scientifically appropriate statistical analyses that were performed on the outcome measure data, if any. URL link to protocol file(s) with version and date Brief summary	
24. IPD sharing statement Statement regarding the intended sharing of deidentified individual clinical trial participant-level data (IPD). Should indicate whether or not IPD will be shared, what IPD will be shared, when, by what mechanism, with whom and for what types of analyses. It consists of: Plan to share IPD (Yes, No) Plan description	21 (Discussion).