Statistical Analysis Plan

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1 Abbreviations and Definitions

Abbreviation	Description
AAT	Alpha-1antitrypsin
AATD	Alpha-1 antitrypsin deficiency
Aα-Val360	Fragment from neutrophil elastase cleavage of elastin at Aα-Val360 site
AcPGP	Acetylated Proline-Glycine-Proline
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
AUC	Area under the curve
BAL	Bronchoalveolar lavage
bid	Twice (2 times) a day
C1M	MMP-derived collagen type I breakdown fragment
C6M	MMP-derived collagen type VI breakdown fragment
CAT	COPD assessment test
Cat g	Cathepsin G
CF	Cystic fibrosis
CFR	United States Code of Federal Regulations
CMP	Complete metabolic profile
CMV	Cytomegalovirus
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
CRO	Clinical Research Organization
CT	Computerized or computed tomography
CYP2C9	Cytochrome P450 2C9
DCC	Data Coordinating Center
DES	Desmosine
dL	Decilitre
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
ECG	Electrocardiogram
eCRF	Electronic case report form
EL-CG	Elastin degradation mediated by Cathepsin G
EL-NE	Elastin degradation mediated by NE
EL-P3	Elastin like polypeptide 3
EOT	End of treatment
EP-3	Elastin peptide 3
FAS	Full Analysis Set
FEV1	Forced expiratory volume in 1 second
FEV1/FVC	Forced expiratory volume in 1 second divided by the forced vital capacity
FVC	Forced vital capacity
FIB-4	Fibrosis-4
FU	Follow-up
FVC	Forced vital capacity
g	Gram
GCP	Good Clinical Practice
h	Hour
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus

Abbreviation	Description
hsCRP	High-sensitivity C-reactive protein
HRT	Hormone replacement therapy
HSV	Herpes simplex virus
Hy's Law	a rule of thumb that a patient is at high risk of a fatal drug-induced liver injury (DILI) if given a medication that causes hepatocellular injury (not cholestatic injury) with jaundice
IC50	the concentration of an inhibitor where the response (or binding) is reduced by half
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL-1β	Interleukin 1 beta
IL-6	interleukin-6
IL-8	interleukin-8
IND	Investigational new drug
INR	International normalized ratio
IRB	Institutional Review Board
IWRS	Interactive web response system
Ki	Inhibitory constant
kPa	kiloPascal, a unit of force
LSM	Liver stiffness measurement
LTB4	Leukotriene b4
MedDRA	Medical Dictionary for Regulatory Activities
mGy	milliGray, a unit of radiation
mg/dL	Milligrams per decilitre
mIU	Milli International Units
MMP	Matrix metalloproteinase
MMRC	Modified medical research council
MOP	Methods of procedures
MPO	Myeloperoxidase
MS	Millisecond
mSV	milliSever
NCATS	National Center for Advancing Translational Science
NCT	National Clinical Trial
NE	Neutrophil elastase
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NSAID	Non-steroidal anti-inflammatory drug
Pa	Pascal
PD	Pharmacodynamics
PI	Principal investigator
Pi*ZZ	Alpha-1 ZZ genotype
Pi*SZ	Alpha-1 SZ genotype
Pi*Null, Null	Alpha-1 Null genotype
PGP	P-glycoprotein
PKS	Pharmacokinetics Set
PK	Pharmacokinetics
PPS	Per Protocol Set
PR	time from the onset of the P wave to the start of the QRS complex on ECG
PR3	Proteinase 3

Abbreviation	Description
PROs	Patient reported outcomes
PRO-C6	Released C-terminal pro-peptide of type VI collagen
QRS	Represents ventricular depolarization on ECG
QT	The duration of ventricular depolarization and repolarization on ECG
QTc	Corrected QT interval
QTcF	QTc by Fridericia's correction method
QTcV	QTc by Van de Water's correction formula
RANTES	Regulated on activation, normal T cell expressed and secreted
RBC	Red blood cell
SAE	Serious adverse event
SAF	Safety Set
SAP	Statistical Analysis Plan
SAS	Statistical analysis software
SGRQ-C	St. George's Respiratory Questionnaire compact version
SoA	Schedule of Activities
SOB	Shortness of breath
SOP	Standard operating procedure
SOBQ	Shortness of breath questionnaire
Sp02	Pulse oximetry
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Total bilirubin
ug	Microgram
ULN	Upper limit of normal
uM	Micromolar
UPs	Unanticipated problems
VZV	Varicella zoster virus
WBC	White blood cell
Wk.	Week

2 Introduction

2.1 Preface

This is a Phase 2, multicenter, double-blind, randomized (1:1), placebo-controlled, 12-week, proof-of-concept study to evaluate the safety and tolerability as well as the mechanistic effect of oral administration of Alvelestat (MPH966) in subjects with confirmed AATD defined as Pi*ZZ, Pi*SZ, Pi*null, or another rare phenotype/genotype known to be associated with either low (serum AAT level <11 µM or <57.2 mg/dL) or functionally impaired AAT including “F” or “I” mutations.

2.2 Purpose

The purpose of this Statistical Analysis Plan (SAP) is to provide detailed descriptions of the statistical methods, data derivations and data displays for the ATALANTa study; to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

3 Study Objectives

3.1 Primary Objectives:

- To evaluate the effect of Alvelestat (MPH966) administered twice daily (bid) for 12 weeks on blood markers of neutrophil elastase activity
- To evaluate the safety and tolerability of Alvelestat (MPH966) administered twice daily (bid) for 12 weeks

3.2 Secondary Objectives

- To evaluate the effect of Alvelestat (MPH966) on other blood pharmacodynamic markers of neutrophil activation and elastase activity
- To evaluate the effect of Alvelestat (MPH966) on blood biomarkers of lung tissue degradation
- To evaluate the effect of Alvelestat (MPH966) on biomarkers of inflammation in blood

3.3 Exploratory Objectives

- To evaluate the effect of Alvelestat (MPH966) on respiratory symptoms, breathlessness, and health status
- To evaluate PK efficacy relationships in AATD
- To evaluate the effect of Alvelestat (MPH966) on neutrophil activation, elastase, and inflammatory activity in lung. This was a secondary outcome but now exploratory depending on data available for analyses due to sputum and BAL being optional.

3.4 General Study Design and Plan

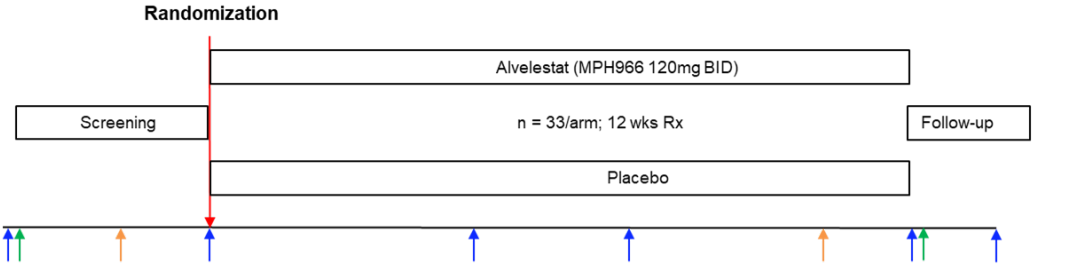
This will be a multicenter, double-blind, randomized, placebo-controlled trial. One dose of Alvelestat (MPH966), 120 mg bid, will be tested against placebo. These doses are based on PK and PD modelling of Alvelestat (MPH966) inhibition of human neutrophil elastase.

The participants will be those patients with the AATD at risk for emphysema (Pi*ZZ, Pi*SZ, Pi*null, or another rare phenotype/genotype known to be associated with either low [serum AAT level <11 µM or <57.2 mg/dL] or functionally impaired AAT including “F” or “I” mutations)

Following a screening period of up to 4 weeks, patients will be dosed with study treatment or placebo for 12 weeks, with a subsequent 4-week follow-up period for safety and determination of the offset of the mechanistic effect of MPH966 on the primary endpoint (desmosine/isodesmosine).

The study is divided into 3 phases: a screening phase, treatment phase, and washout phase as detailed below:

Figure 1: Study Schematic



Visit	1	2	3	4	5	6	7	8	9	10	11	12
Week	-4 to 0	-1	0	+1	+2	+4	+6	+8	+10	+11	+12	+16
Description	Screen	Bronch	Randomization	Safety	Safety	4-week interim	Safety	8-week interim	Safety and Pre-bronch	Bronch	Study End	Post-IP Washout

Red arrow = Randomization
Blue arrow = Blood (biomarker) collection
Green arrow = Sputum collection
Orange arrow = BAL collection

- **A screening period** from Week -4 to Day -1. Baseline safety assessments and measurement of DES in blood, sputum, and BAL (in those who consent to bronchoscopy) will occur prior to randomization and treatment allocation.

- **A treatment period** consisting of Alvelestat (MPH966) 120mg bid or placebo for 12 weeks. Safety assessments including tests of liver function, ECG, hematology, and chemistries will be assessed at the week 1 and week 2 visits and then bi-weekly for the remainder of the 12-week treatment period. Blood will be collected at the Week 4, 8, and 12 visit for biomarker analysis. Sputum will be collected at the week 12 visit. Follow-up BAL will be collected at week 11.

- **A washout period** from Week 12 to Week 16. Blood will be collected for biomarker analysis at week 16.

3.5 Inclusion-Exclusion Criteria and General Study Population

3.5.1 Inclusion Criteria

Participants are eligible to be included in the study only if ALL of the following criteria apply:

Type of Participant and Disease Characteristics

1. Capable of giving signed informed consent as described in Appendix 3, which includes compliance with the requirements and restrictions listed in the informed consent form and in this protocol
2. Age ≥18 and ≤80 years
3. Patients with a confirmed diagnosis of AATD: Pi*ZZ, Pi*SZ, Pi*null, or another rare phenotype/genotype known to be associated with either low (serum AAT level <11 μM or <57.2 mg/dL) or functionally impaired AAT including “F” or “I” mutations.
4. FEV1 ≥25% predicted
5. Patients will be eligible if they are either a) are not currently receiving augmentation treatment and have not received augmentation in the 12 weeks prior to screening or b) have received weekly infusions of augmentation at 60 mg/kg for at least 12 weeks prior to screening and intend to continue augmentation through the study period.
6. Male or female sex
   a. Male participants must agree to use a highly effective contraception as detailed in Appendix 5 during the treatment period and for at least 4 days after the last dose of study treatment and refrain from donating sperm during this period
   b. Female participants are eligible to participate if not pregnant; not breastfeeding; and at least one of the following conditions is met:
      i. Not a woman of childbearing potential as defined in Appendix 5
      OR
      ii. A woman of childbearing potential who agrees to follow the contraceptive guidance in Appendix 5. During the treatment phase and for at least 4 days after the last dose of study medication.

3.5.2 Exclusion Criteria

Participants are excluded from the study if ANY of the following criteria apply:

Excluded Medical Conditions

1. Subjects with Pi*MZ, Pi*FM, Pi*MS, Pi*SS, or other AATD phenotypes/genotypes not known to be independently associated with emphysema.
2. Any clinically diagnosed lung disease other than COPD such as diffuse interstitial lung diseases, cystic fibrosis, or clinically significant bronchiectasis as determined by the Investigator
3. Acute exacerbation of underlying lung disease requiring oral steroids and/or antibiotics within 4 weeks of baseline
4. Acute or chronic hepatitis, including hepatitis B, hepatitis C (positive serologies, including hepatitis B and C antibody)
5. HIV infection or other immunodeficiency or with an absolute neutrophil count ≤1.0 × 109/L
6. Abnormal liver biochemistry (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) >1.5 × upper limit of normal or total bilirubin > upper limit of normal (unless Gilbert’s disease with normal conjugated bilirubin)
7. Any of the following laboratory abnormalities are present at baseline:
   a. Platelet count <150×109/L
   b. Serum albumin ≤ 3.5 g/dL
   c. INR ≥1.2
   d. CPK ≥ ULN.

8. History or current evidence of cirrhosis (on biopsy or imaging), esophageal varices, ascites or hepatic encephalopathy.
9. Evidence of other forms of chronic liver disease based on diagnostic testing as per the guidelines (i.e. autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson's disease, Hemochromatosis or iron overload).
10. Patients with nonalcoholic fatty liver disease (NAFLD) as diagnosed by any imaging modality (or use of drugs associated with NAFLD for more than 2 weeks in the year prior to screening).
11. Subjects with a history of significant alcohol consumption for a period of more than 3 consecutive months within 1 year prior to screening, defined as average of >20g/ day in female subjects and >30g/ day in male subjects.
12. Fibrosis-4 (FIB-4) score >3.25
13. Any of the following cardiovascular conditions within 6 months prior to the screening visit:
    a. Myocardial infarction or unstable angina
    b. Coronary artery bypass surgery, balloon angioplasty, percutaneous coronary intervention, or carotid revascularization procedure
    c. Uncontrolled hypertension
    d. Stroke or transient ischemic attack
14. Congestive heart failure (New York Heart Association III/IV) with left ventricular ejection fraction < 40%
15. Any clinically significant 12-lead electrocardiogram abnormalities at screening or baseline, including corrected QT interval by Fridericia's correction method >450 ms or history of significant cardiac dysrhythmia, including long QT syndrome
16. History of cancer within the last 5 years, except for well-treated basal cell carcinoma and squamous cell carcinoma of the skin
17. Other documented comorbidities or laboratory abnormalities that in the opinion of the Investigator could affect the outcome of the study assessments, participant safety, or ability of the participant to comply with the requirements of the protocol

<u>Excluded Prior/Concomitant Therapy</u>

18. Daily use of prednisone (>10mg daily), or other systemic glucocorticoids at comparable or higher equivalent dose, or use of other immunosuppressant therapies are prohibited
19. Immunomodulating monoclonal antibodies within 6 months prior to screening are prohibited
20. Daily use of non-steroidal anti-inflammatory drugs (NSAIDs) is prohibited. Daily use of acetaminophen up to 2 g per day and aspirin up to 325 mg per day is permitted.
21. Initiation of drugs known for hepatotoxic potential within the 28 days prior to screening including but not limited to: statins, NSAIDS, amoxicillin/clavulanate, PDE inhibitors (theophylline, roflumilast), and anti-epileptics. Subjects on established treatment for more than 28 days prior to screening will not be excluded. Requirement for medications mainly metabolized by CYP2C9 and with narrow therapeutic index (eg, warfarin, phenytoin) is prohibited

<u>Excluded Prior/Concurrent Clinical Study Experience</u>

22. Participation in any clinical investigation using medical devices or non-biologic treatments within 4 weeks or 5 half-lives of the drug (whichever is longer) prior to the initial dosing (or longer if required by local regulations) is prohibited
23. Participation in any clinical investigation using biologic treatment within 6 months of screening is prohibited
24. Previous participation in a gene therapy study for AATD at any time is prohibited

<u>Other Exclusions</u>

25. History of hypersensitivity to Alvelestat (MPH966) or any of its excipients or the class of neutrophil elastase inhibitors
26. Known hypersensitivity to medications used in the study procedures (e.g. midazolam, fentanyl, and lidocaine for bronchoscopy)

3.5.3 Sample Size

Over the course of the study, target enrolment is 66 with the goal of 60 completers. Participants will be randomized in a 1:1 ratio to receive one of the following: 120mg Alvelestat (MPH966) BID or placebo. Randomization will be stratified by AAT genotype/phenotype.

The study will be powered for within-individual change in plasma desmosine/isodesmosine (DES) as the primary endpoint at Week 12 compared to baseline. A sample size of 30 participants in the MPH966 treatment group will give us >90% power to detect a 15% mean decrease of plasma DES from baseline to 12 weeks follow-up in patients treated with MPH966 (within subject change) using a two-sided paired t-test at α=0.05. The calculation is based on an assumed correlation value of 0.5, baseline mean DES value of 0.466 and standard deviation of 0.09. This 15% mean decrease has been observed in trials of standard dose augmentation versus placebo (Ma S, 2013). A recent study of double dose versus single dose augmentation revealed a within subject decrease in plasma DES from 0.42 +/-0.03 ng/ml to 0.38 +/- 0.03 ng/ml (Campos MA, 2019). We will have >99% power to detect a comparable difference in DES in those who have Alvelestat added to augmentation if we assume their observed SD=0.03 and 85% power if we double their assumed SD, i.e., SD=0.06. Power will not be enough (~50%) to detect this difference if we use our original SD value of 0.9.

For comparing the mean change in DES between the two groups (placebo vs. MPH966), and assuming that there is no placebo effect over 12 weeks, a two-sided two sample t-test at α=0.05 with a sample size of 60 (30 per group) reveals that we will have 82% power to detect a 15% difference in mean change in plasma DES.

By including a 10% attrition rate over 12 weeks, the required sample size is inflated to 66 (33 per group) to achieve the estimated powers. If we observe attrition rate less than we expected, we will have more statistical power to detect the expected differences. As one interim analysis will be conducted only for safety concerns (see Data Safety and Monitoring Plan), no adjustments to the Type I error are considered for estimating statistical powers.

All participants will be centrally randomized 1:1 to the 120 mg and placebo arms using an interactive web response system (IWRS). A unique number will be assigned to each participant and will be linked to randomization numbers using a randomization list produced by the DCC. These randomization numbers will be linked to the 2 treatment regimens, and the randomization scheme will be stratified according to genotype (Pi*ZZ, Pi*SZ, Pi*(Null) (Null))

3.6 Treatment Administration and Blinding

Treatments Administered

Study Treatment Name:	Alvelestat 120 mg	Placebo
Dosage Formulation:	Tablet	Tablet

Unit Dose Strength(s) / Dosage Level(s):	4 × 30 mg Alvelestat	4 × 30 mg Placebo
Route of Administration	Oral	Oral
Dosing Instructions:	To be taken bid, 12 hours apart and with water	
Packaging and Labeling:	Study treatment will be provided in boxes. Each box will contain treatment for 7 days and will be labeled as required per U.S. regulations.	
Manufacturer:	Mereo BioPharma 4 Ltd., 1 Cavendish Place, London, W1G 0QF, UK	

This is double-blind study. The investigators, participants, Mereo and NCATS will remain blinded to the study treatment allocation until the end of the study. The randomization list will be kept secured from the study team, investigators, and participants throughout the conduct of the study and until unblinding is authorized by the DCC. The DSMB will be unblinded according to the DSMB charter.

The IWRS will be programmed with blind-breaking instructions. The study blind may be broken for a medical emergency in which the knowledge of the specific blinded study treatment will affect the immediate management of the participant's condition. In this case, the DCC, the NCATS Medical Monitor, and Mereo must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and case report form (CRF), as applicable.

3.7 Study Procedures and Flowchart

Study procedures and their timing are summarized in the Schedule of events (SOA) – see Figure 2.

Figure 2: SCHEDULE OF ACTIVITIES (SOA)

Visit #	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
Visit Description	Pre-bronch/ Screen	Baseline bronch	Randomize	Safety F/U	Safety F/U	4 week F/U	Safety F/U	8 week F/U	Safety F/U and Pre-bronch	End of study bronch	Study End	Washout End
Week (from randomization)	-4 to 0	-1	0	+1	+2	+4	+6	+8	+10	+11	+12	+16
Randomization			X									
Baseline history	X											
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X
Interim history and adverse event recording		X	X	X	X	X	X	X	X	X	X	X
Blood draw (routine/safety)	X			X	X	X	X	X	X		X	X
Blood draw (biomarkers)	X		X			X		X			X	X
Blood (PK)			X@	X	X	X		X	X		X	
Pregnancy test	X		X	X	X	X	X	X	X		X	X
ECG	X		X@	X	X	X		X	X		X	X
Sputum biomarkers	X										X	
Spiro- pre and post BD			X			X					X	X
Spiro – post BD	X								X			
PROs#			X			X					X	X
Bronchoscopy		X								X		
Phone contact		X (day after)								X (day after)		
Electronic diary	X		X	X	X	X	X	X	X	X	X	X

#Patient reported outcomes to include Modified Medical Research Council questionnaire (MMRC), COPD assessment test (CAT), St George Respiratory Questionnaire (SGRQ), San Diego Shortness of Breath Questionnaire; @ pre-dose and between 1 and 2 hours post-dose

4 Endpoints

4.1 Primary Efficacy Endpoints

Within-individual % change from baseline in plasma desmosine/isodesmosine at end of treatment with MPH966

4.2 Primary Safety Endpoints

1. Numbers and % of subjects who experience at least 1 treatment-emergent adverse event
2. Adverse events of special interest (liver function abnormalities, corrected QT interval/cardiac, infections, and neutropenia)

4.3 Secondary Efficacy Endpoints

Blood pharmacodynamic markers of neutrophil activation and elastase activity. Change from baseline in the following outcomes at end of the treatment within patient and compared to placebo:

1. Plasma desmosine/isodesmosine (only change compared to placebo; within-individual change is the primary outcome)
2. Plasma Aa-Val-360
3. Serum NE activity
4. Plasma proteinase 3
5. Plasma cathepsin B
6. Plasma CRP
7. Plasma IL-6
8. Plasma IL-8
9. Plasma IL-1b
10. Plasma RANTES
11. Plasma LTB4
12. Plasma MMP9
13. Plasma MMP12
14. Plasma MPO
15. Plasma PGP
16. Plasma PK/PD (LCG/DDS)

The above list was revised from the original protocol as a result of lack of data from sputum and BAL collections due to restrictions during the COVID pandemic. Data on NE may not be available on all subjects.

4.4 Exploratory Endpoints

1. Change from baseline in pre-and post-bronchodilator forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC (total and percent predicted), and maximal mid-expiratory flow at end of treatment . Data may not be sufficient to perform this analyses due lack of spirotmetry data.
2. Change from baseline in St. George's Respiratory Questionnaire (SGRQ) (overall and per domain), COPD Assessment Test (CAT), Modified Medical Research Council

Questionnaire (MMRC), San Diego Breath Questionnaire (SOBQ) and daily symptom scores

3. PK/pharmacodynamic (PD) relationship with efficacy biomarkers in blood and sputum. Due to the COVID pandemic, protocol was revised to make sputum collection optional. Only correlation of PK/PD with blood will be done.

4.5 Study Variables

4.5.1 Key Screening Assessments

FIB-4 Score

The FIB-4 test combines age with 3 standard biochemical values (platelets, ALT, and AST) as a non-invasive test to assess fibrosis and enable exclusion of participants who may be at greater risk of liver AEs (https://www.mdcalc.com/fibrosis-4-fib-4-index-liver-fibrosis). The following formula will be used:

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST Level (U/L)}}{\text{Platelet Count } (10^9/L) \times \sqrt{\text{ALT (U/L)}}}$$

Using a cut-off value of 1.45, a FIB-4 score <1.45 has been shown to have a negative predictive value of 90% for advanced fibrosis. In contrast, a FIB-4 >3.25 would have a 97% specificity and a positive predictive value of 65% for advanced fibrosis[42]. FIB-4 index has been shown to be superior to other scoring systems for differentiating between advanced and mild fibrosis.

4.5.2 Blood, Sputum, and Bronchoalveolar Lavage Biomarkers

Blood

Blood will be collected as per the SOA, and samples will be collected and processed as described by the MOP.

Sputum (this is optional due to COVID as reflected in the revised protocol Version 1.4)

Sputum will be collected from induced samples (refer to the MOP for processing details). Sputum induction will not be undertaken if the baseline FEV_1 for the procedure is less than 1 L. Further details of sputum induction will be included in the MOP. Sputum induction is considered an aerosol generating procedure (AGP) and sites should use local guidance on conducting AGP as part of clinical research in the COVID-19 era.

Bronchoalveolar Lavage (BAL) (this is optional due to COVID as reflected in the revised protocol)

BAL will be collected in the subset of participants who agree to participate in the bronchoscopy sub-study. Samples will be collected at two time points as per the SOA, and samples will be collected and processed as described by the MOP. Bronchoscopy and BAL is considered an aerosol generating procedure (AGP) and sites should use local guidance on conducting AGP as part of clinical research in the COVID-19 era.

Blood, sputum, and BAL efficacy biomarkers (see Table 1) will be taken at the time points as detailed in the SoA to evaluate their association with the observed mechanism of action responses to neutrophil elastase inhibition, including response of neutrophil elastase activity, inflammation, and lung damage to Alvelestat (MPH966).

The samples will be collected and processed as described in the MOP.

Samples may be stored for a maximum of 60 months following the end of study at a facility selected by the Sponsor to enable further analysis of biomarker responses to Alvelestat (MPH966).

Table 1 Biomarkers of Efficacy Collected in Blood (list below has been revised to exclude biomarkers from Sputum and BAL)

Biomarker	Collected at Visit Number (see Figure 1 for week corresponding to visit number)
Plasma desmosine/isodesmosine	1, 3, 6, 8, 11, 12
Plasma Aa-Val-360	1, 3, 6, 8, 11, 12
Serum NE activity	1, 3, 6, 8, 11, 12
Plasma proteinase 3	3, 11
Plasma cathepsin B	3, 11
Plasma CRP	3, 11
Plasma IL-6	3, 11
Plasma IL-8	3, 11
Plasma IL-1b	3, 11
Plasma RANTES	3, 11
Plasma LTB4	3, 11
Plasma MMP9	3, 11
Plasma MMP12	3, 11
Plasma MPO	3, 11
Plasma PGP	1, 3, 11
Plasma PK/PD (LCG/DDS)	3, 4, 5, 6, 8, 9, 11, 12

In addition, samples will be stored and analysis may be performed on additional biomarker variants thought to play a role in neutrophil elastase–induced lung damage to evaluate their association with observed neutrophil activation responses to Alvelestat (MPH966).

Biomarker data are reported as concentration results, measured using a specific assay with a working range defined by the two limits: Lower limit of quantification (LLOQ) and Upper limit of quantification (ULOQ). Values which fall below the LLOQ or above the ULOQ are reported as <LLOQ * dilution factor (dilution factor: if sample diluted and concentration measured still below LLOQ) and > ULOQ * dilution factor, respectively. In case of censored values (values below the LLOQ and/or values above the ULOQ), the values, reported as <LLOQ will be imputed as 0.5*LLOQ and values reported as >ULOQ will be imputed as 1.5*ULOQ.

4.5.3 Functional and Quality of Life Instruments

- **SGRQ-C**

The St. George's Respiratory Questionnaire (SGRQ-C) will be administered at time points as detailed in the SoA. This is a questionnaire that measures health impairment in patients with COPD. It consists of 3 parts: a symptom score, an activity score, and an impact score. A total score is also produced.

- **COPD Assessment Test (CAT)**

The CAT is an 8 item questionnaire used to report health status in COPD. It will be administered at time points as shown in the SOA.

- **Modified Medical Research Council (MMRC)**

The MMRC will be administered at time points as detailed in the SoA and is used to measure dyspnea on a 5-point scale.

- **San Diego Breath Questionnaire (SOBQ)**

The SOBQ assesses patient reported dyspnea. The SOBQ will be administered at time points as detailed in the SoA.

- **Pulmonary Function Testing/Spirometry**

Pulmonary function testing (FEV_1, FVC, FEV_1/FVC, and maximal mid-expiratory flow) will be performed at the times specified in the SoA according to ATS guidelines. Further details on the spirometry procedure can be found in the Specific MOP. Spirometry is considered an aerosol generating procedure (AGP) and sites should use local guidance on conducting AGP as part of clinical research in the COVID-19 era.

4.5.4 Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

- **Physical Examinations**

    - A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, integumentary, and neurological systems. Height and weight will also be measured and recorded.
    - A brief physical examination will include, at a minimum, assessments of the lungs, heart, abdomen, and skin indicating normal vs abnormal
    - Investigators should pay special attention to clinical signs related to previous serious illnesses.

- **Vital Signs**

Vital signs will be measured in a semi-supine position after 5 minutes of rest and will include temperature, systolic and diastolic blood pressure, heart rate, respiratory rate, and pulse oximetry.

    - Oral or tympanic temperature.

- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g., television, cell phones).
- Pulse oximetry will be measured using a sensor placed on the participant's fingertip or earlobe. Oxygen saturation (SpO2) will be recorded.

- **Electrocardiograms**

  - 12-lead ECG will be obtained as outlined in the SoA using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. Refer to the Protocol for QTc withdrawal criteria and additional QTc readings that may be necessary.
  - Single ECGs will be performed at all scheduled time points, with the exception of when a change from baseline occurs that meets drug discontinuation criteria. In this situation, a triplicate measurement is required to confirm the finding and for discontinuation decisions.
  - When a triplicate ECG is required, 3 consecutive ECG tracings should be taken in a 30-minute period with at least 5 minutes between each ECG.
  - QTc value will be calculated using the Fridericia formula ($QTc=QT/3\sqrt{RR}$).

- **Clinical Safety Laboratory Assessments**

  - The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
  - All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 28 days after the last dose of study treatment should be repeated until the values return to normal or baseline, with a frequency based on the judgement of the Site Investigator. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the DCC notified.
  - The monitoring schedule proposed for a case of acute liver function abnormalities or worsening of a liver function abnormality is reported is listed in the Protocol (Liver Test Abnormalities).
  - Clinically significant liver function abnormalities should be reported as an AE/AESI.
  - All protocol-required laboratory assessments must be conducted in accordance with the MOP and the SOA.

5 Analysis Sets

For purposes of analysis, the following populations are defined in Table 2. Subjects to be included in the analysis sets will be done prior to breaking the blind.

Table 2: Definition of Analysis Sets

Population	Description
Enrolled Set	The Enrolled Set will include all participants who sign the ICF.
Randomized Set	The Randomized Set will include all subjects who signed the ICF and were subsequently randomized into the study, regardless of study treatment administration.
Full Analysis Set (FAS)	The Full Analysis Set will serve as the primary population for the analysis of efficacy and will consist of all randomized subjects who took at least 1 dose of double-blind study treatment and have at least 1 evaluable change from baseline in plasma desmosine / isodesmosine levels. Subjects will be analysed according to randomized treatment.
Per-Protocol Set (PPS)	The Per-Protocol Set includes all participants from the Full Analysis Set who have been treated according to the protocol and fulfil the following criteria: 1. All inclusion/exclusion criteria satisfied 2. Absence of major protocol violations with respect to factors likely to affect the efficacy of treatment where the nature of protocol violation will be defined before breaking the blind 3. Adequate study medication compliance – to be determined before breaking the blind. Subjects will be analysed according to randomized treatment.
Safety Set (SAF)	All randomized participants who take at least 1 dose of study treatment. Participants will be analysed according to treatment taken.
PK Set (PKS)	The PK Set will include all participants in the Safety Set who have at least 1 evaluable serum concentration.

6 General Aspects for Statistical Analyses

6.1 General Methods

Database lock will occur once all subjects complete the final study visit (Safety follow up at Week 16) or terminate early from the study. All analyses will be performed after data lock and cleaning. Statistical analysis will be performed using SAS® version 9.4 or higher. The main population for efficacy analysis will be the Full Analysis Set; supportive analyses will also be performed using the Per-Protocol Set.

Continuous data will be presented using descriptive summaries (e.g., mean, standard deviation, minimum, maximum, median, lower quartile, and upper quartile). Categorical variables will be presented by the number of observations and relative (%) frequency.

Unless otherwise stated, baseline value for any variable will be the last value taken prior to the first dose of study medication. In general, all data will be listed, sorted by site, treatment and subject, and when appropriate by visit number within subject. All summary tables will be structured with a column for Active, Placebo and Total, and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

As a Phase II study, no adjustments for Type I errors (see Section 6.6) will be done. All tests will be 2-sided and conducted at the 5% level. Confidence intervals will be 2-sided 95% confidence intervals.

Biomarker data are reported as concentration results, measured using a specific assay with a working range defined by the two limits: Lower limit of quantification (LLOQ) and Upper limit of quantification (ULOQ). Values which fall below the LLOQ or above the ULOQ are reported as <LLOQ * dilution factor (dilution factor: if sample diluted and concentration measured still below LLOQ) and > ULOQ * dilution factor, respectively. All results for the above biomarker parameters, if collected, will be summarised on the respective populations by descriptive statistics stratified by treatment and time-point, and including the geometric mean and coefficient of variation. Changes in the biomarker concentrations from baseline (Study Day 1 pre-dose) will be also summarised at each post-baseline time-point. In the summary table, the frequency (n, %) of values below the LLOQ and above the ULOQ, respectively, will be included. In case of values below the LLOQ and/or values above the ULOQ, the values, reported as <LLOQ will be imputed as 0.5*LLOQ and values reported as >ULOQ will be imputed as 1.5*ULOQ.

6.2 Key Definitions

Table 3: Definitions of Terminology

Terminology	Definition
Baseline value	defined as the last non-missing value prior to first study treatment dose
End of Treatment (EOT) Visit	visit refers to the final day of study treatment administration including cases of premature discontinuation of the study treatment.
End of Treatment (EOT) Value	Last non-missing post-baseline value under study treatment.
Treatment Period	duration that a subject is under treatment in the study between the date of first study treatment intake and the date of last study treatment intake.
Study duration (in days), time to day of last visit or the day of study termination	Calculated as: *Date of Safety follow-up (FU) visit (or EOT visit in case of premature discontinuation) – Date of Baseline (Day 0) + 1.*
Study Completion and Withdrawal	• A subject will be defined as “completed the study” if subject completes the follow-up at Week 16 – either having completed, all or partially, the protocol defined visits before Week 16. • Termination/withdrawal at a different time point will be considered as study discontinuation/withdrawal.
EndPoint value	considered the value at Week 12 or EOT value (last non-missing post-baseline value under treatment)
Relative change from baseline	defined as percentage change from baseline Calculated as: *[(Post-baseline value – Value at baseline)/ Value at baseline]x100%*

Absolute change from baseline	Calculated as: *Absolute change = Post-baseline value – Value at baseline*
Efficacy Offset Period	Change from Week 12 to end of study follow-up at Week 16

6.3 Covariates and Subgroups

Prespecified covariates have been provided in the above efficacy analyses. Covariates to be included in the primary and secondary efficacy analyses on change in the biomarkers are baseline values, age and smoking history. Although we have stratified randomization by genotype, we will not include genotype as a covariate in the main analyses. Depending on available data, we may be able to compare outcomes between genotypes, but this will be exploratory in nature.

Due to the change in protocol to drop augmentation therapy from the exclusion criterion, subgroup analyses between those enrolled with and without augmentation will be performed as secondary analyses.

6.4 Missing Data

Missingness will be assessed and tabulated by treatment group along with reasons for missingness to assess any differential missingness between the treatment arms. Patients impacted by COVID-19 that are related to study discontinuations, missing assessments, and missing visits due to COVID-19 will be created, sorted by patient number, randomized treatment and discontinuation date or visit, as applicable. All data will be listed and sorted by treatment and patient number within treatment.

The efficacy analyses relating to change from baseline will be based on all the available post-baseline measurements. For sensitivity analyses, we will perform a best-case (i.e., replacing missing by best value) and worst-case (i.e., replacing missing with worst value) scenario analyses with respect to the primary outcome and if the results match with the primary analyses, we will stop additional sensitivity analyses.

6.5 Multi-center Studies

This study was a multi-center study. Data will be analyzed by combining data from all study centers. No investigation of center effects is planned.

6.6 Multiple Testing

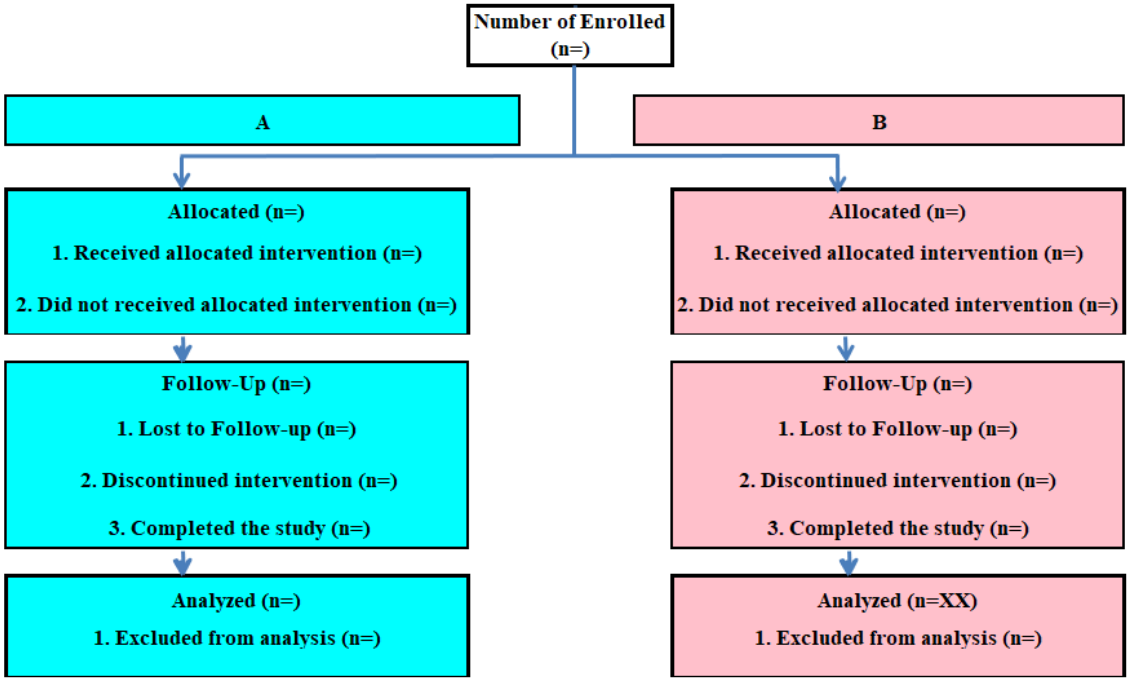
As a signal-seeking study, unless otherwise stated, all statistical tests will be 2-sided and conducted at the 5% level. All presented confidence intervals will be 2-sided 95% confidence intervals. No adjustment for multiplicity will be made for the primary and secondary efficacy variables.

7 Demographic, Other Baseline Characteristics, Medication and Protocol Deviations

7.1 Subject Disposition

The following flowchart (Figure 3) will be used to summarize the subject disposition in the study and by treatment. Numbers will be obtained based on the following CRFs: Inclusion/Exclusion, Randomization, Study Treatment Termination or Completion, and Study Termination or Completion.

Figure 3: Disposition of Enrolled Participants by Treatment Group



Listings of the following will be provided.

- screen failures and reasons for failures
- discontinuation from study drug with reasons
- early discontinuation from study participation with reasons

7.2 Demographic and Baseline Variables

Demographic characteristics will be based on the information provided at the screening Visit.

The characteristics will be presented by treatment arm, genotype and overall:

- Age (years) at study entry - continuous
- Sex - categorical
- Childbearing potential status (female subjects only) - categorical
- Race - categorical
- Height (cm) at baseline - continuous
- Weight (kg) at baseline - continuous
- Alcohol use history - categorical
- Smoking history - categorical/ continuous (packs/year)

7.3 Medical History and Concomitant Diseases

Medical history will be defined as any clinically significant past medical conditions that ended before screening. Medical history will be summarised for the SAF by treatment arm and overall with number and percentage of subjects with at least one medical history item, and number and percentage of subjects by system organ class (SOC) and preferred term (PT). The terms will be sorted by descending frequency across all subjects of SOC and then, within SOC, by descending frequency across all subjects for PT.

Relevant AATD medical history will be based on the information provided at the screening visit. The characteristics will be presented by treatment arm and overall for the SAF. The following will be tabulated:

- Genotype of AATD
- Alpha-1 level (mg/dl)
- COPD (yes/no)
- Liver Disease (yes/no)
- Cirrhosis (yes/no)
- Augmentation Therapy (yes/no)
    - Continue augmentation therapy for study duration (yes/no)
- Asthma (yes/no)
- Lung cancer (yes/no)
- Bronchiectasis (yes/no)
- CV issue in last 6 months (yes/no)
- Severe/uncontrolled CHF (yes/no)
- Supplemental oxygen use (yes/no)
  If yes on supplemental oxygen
    - amount prescribed (≤1 liter/min, 2 liters/min, 3 liters/min, 4 liters/min, ≥5 liters/min)
    - duration per day (<12 hrs/day vs 12-23 hrs/day)
    - years prescribed (0-2, 3-5, 6+)
    - Screening or Historical FEV1% predicted
- AECOPD within the last year (yes/no)
  If yes,
    - Number of exacerbations
    - Number of hospitalizations related to AECOPD

Medical History ongoing at start of study will be summarised separately.

7.4 Medications

Prior medications will be defined as medications started 30 days or more prior to screening visit and stopped any day prior to administration of the first dose of study treatment. Concomitant medications will be defined as medications started within 30 days prior to screening visit and ongoing at or began during or after the administration of the first dose of study treatment. Specific list of medications checked (yes/no) at screening and in follow-up visits are:

- SABA
- SAMA
- LABA
- LAMA
- ICS
- Roflumilast
- AAT augmentation
- Azithromycin
- Theophylline
- NSAIDS
- Acetaminophen
- Warfarin/Coumadin
- other anticoagulant
- Clopidogrel
- Aspirin

These medications will be summarized by treatment and by visit.

7.5 Protocol Deviations

Protocol deviations will be summarized by treatment, site, and type of deviation; deviations that resulted in adverse and serious adverse events; and protocol deviations that resulted in termination.

A raw listing of all protocol deviations will be provided by treatment, site, subject ID, deviation date, resulting in AE, SAE, and termination; deviation description; reason for the deviation; deviation category; effect on product stability; and deviation resolution.

Finally, any subjects enrolled but not randomized will also be excluded from the analyses.

8 Efficacy Analyses

Analyses on all efficacy outcomes will be based on the 12-week period after randomization. Time periods included are baseline, Week 4, Week 8 and Week 12. Safety outcomes will include through Week 16.

Actual values, absolute change in actual values and the relative (percent) change from baseline will be summarised with descriptive statistics for continuous variables overall and by treatment arms. Relative (percentage) change will be calculated by:

[(Week or EndPoint value – Baseline Value) / Baseline value)] x 100%.

Baseline value will be computed as the last non-missing value prior to first study treatment dose at baseline visit (V1).

EndPoint will be computed as:

- Value at Week 12 for the completers (regardless of treatment compliance) or
- EOT value must be last non-missing post-baseline value for those that discontinue treatment before Week 12.

8.1 Primary Efficacy Endpoint Analyses:

The primary outcome is the relative change from baseline at week 12 in plasma desmosine/isodesmosine levels within treatment group. Within individual relative (percent) change from baseline in plasma desmosine/isodesmosine levels will be analyzed using Mixed Model Repeated Measures (MMRM). Relative change (percent) from baseline will be considered the response variable, with time as main independent fixed effect variable and baseline levels, augmentation, age, and smoking history (yes/no) as a fixed effect covariates, using an unstructured covariance matrix to model the within-subject errors. Available data from all time points will be included. The least squared estimates of the mean at each Week and EndPoint will be generated. [Note: Originally, it was planned to use sputum strata and FEV1 as covariates in the model. However, due to the restrictions of the pandemic, there was limited opportunity to collect the required sputum, and therefore will not be included in the analyses.] The analysis will be conducted using the SAS 'proc mixed', with the below specific code:

```
proc mixed data=Data; by TRTP;
class SUBJID TRTP AVISIT AUGMENTATION SMOKING;
model PCTCHG = BASE AVISIT AUGMENTATION  AGE SMOKING;
ddfm=KENWARDROGER;
repeated AVISIT/ subject=SUBJID type=UN;
lsmeans AVISIT / pdiff;
run;
```

where PCTCHG is the relative (percent) change from baseline, BASE are baselines values, TRT is treatment arm, AVISIT is Weeks, AGE is age at enrollment, SMOKING is yes/no at enrollment and AUGMENTATION (yes/no) at enrollment. If imbalances are found between treatment groups in baseline characteristics deemed to be clinically important in influencing the relative change in the outcome, sensitivity analyses will be performed by including these variables as covariates in the above model. P-value<0.05 for the mean difference at Week 12 will be deemed significant. Estimate including 95% confidence interval of the least squares mean difference will also be provided.

If any issues related to convergence occur and cannot be resolved within the 'proc mixed', then alternative procedures will be considered (such as 'proc glimmix'). Other covariance structures will also be considered if convergence is an issue with unstructured covariance. Transformation of data or alternative distribution for the model will be considered should there be evidence of nonnormality of the data.

8.2 Secondary Efficacy Endpoint Analyses:

Percent change from baseline in the following outcomes at end of the treatment within patient and compared to placebo:

1. Plasma desmosine/isodesmosine (only relative change compared to placebo; within-individual change is the primary outcome)
2. Plasma Aa-Val-360
3. Serum NE activity
4. Plasma proteinase 3
5. Plasma cathepsin B
6. Plasma CRP
7. Plasma IL-6
8. Plasma IL-8
9. Plasma IL-1b

10. Plasma RANTES
11. Plasma LTB4
12. Plasma MMP9
13. Plasma MMP12
14. Plasma MPO
15. Plasma PGP
16. Plasma PK/PD (LCG/DDS)

The same approach, i.e., mixed modelling, will be used to analyse the change from baseline within and between treatments for the secondary outcomes using available data based on the schedule of collection and analyses. The analysis will be conducted using the SAS 'proc mixed', with the specific code given below. For each outcome, p-value<0.05 for pairwise comparisons of the least squared means between treatment groups will indicate treatment effect. This will be done for each outcome.

```
proc mixed data=Data;
class SUBJID TRTP AVISIT SMOKING;
model PCTCHG = BASE TRT|AVISIT FEV1 AGE SMOKING;
ddfm=KENWARDROGER;
repeated AVISIT/ subject=SUBJID type=UN;
lsmeans TRTP*AVISIT / pdiff;
run;
```

8.3 Exploratory Efficacy Endpoint Analyses:

The following exploratory efficacy endpoints will be analysed using MMRM. For data that may not follow a normal distribution, we will consider either using an appropriate transformation to approximate normality or using the generalized linear model (e.g., for categorical outcomes) utilizing the generalized estimating equations or generalized linear mixed model with random intercept to address correlation of repeated measures from the same subject. Appropriate distribution based on the outcome will be chosen to fit the chosen model.

- Change from baseline in pre-and post-bronchodilator forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC (total and percent predicted), and maximal mid-expiratory flow at end of treatment. The analyses of this outcome will be dependent on the amount of spirometry data available.
- Change from baseline in St. George's Respiratory Questionnaire (SGRQ) (total and subscales), COPD Assessment Test (CAT), Modified Medical Research Council Questionnaire (MMRC), San Diego Breath Questionnaire (SOBQ) and daily symptom scores

Pearson (or Spearman rank) correlation of select biomarkers shown to be responsive to treatment with time-matched drug concentrations and PK parameters, if available, will be used to evaluate exposure-response relationship.

8.4 Analysis of Pharmacokinetics

Sparse PK sampling will be performed before and after study treatment dosing as detailed in the SoA and MOP. Blood samples of approximately 3 mL will be collected for measurement of blood concentrations ofAlvelestat (MPH966) as specified in the SoA. Instructions for the

collection and handling of biological samples will be provided by the DCC. The actual date and time (24-hour clock time) of each sample willbe recorded.

8.5 Correlation of PK Exposure with Efficacy (PD) and Safety

Correlation of select biomarkers (shown to have significant treatment effect) with time-matched drug concentrations and PK parameters, if available, may be used to evaluate exposure-response relationship.

The safety correlation analysis with drug concentration will be done for the safety parameters shown to be sensitive to Alvelestat exposure. The comparison of exposure will be done between groups with non-clinically significant changes and clinically significant changes in safety parameters using graphic and statistical methods where data allow.

8.5.1 Presentation of Concentration Data

8.5.1.1 Handling of Missing Data

Plasma concentrations below the lower limit of quantification (LLOQ) of the assay are designated as NQ (not quantifiable) in the PK data and will be replaced by half value of LLOQ in listings. Values above the ULOQ will be replaced by 1.5*ULOQ. This approach will be used for non-PK data as well.

Samples taken far outside the sampling windows and any other protocol deviation related to plasma concentration measurements will be discussed but will be excluded from summary tables. Missing values due to bad sample or unable to measure will also be excluded for that visit. Other non-missing data from the same subject will be included in the analyses.

8.5.1.2 Listing, Presentation and Summary of PK Data

The following presentations of subject plasma concentration data will be provided for the Alvelestat (MPH966) treatment group for the PK Set:

- A listing including subject, week/time point treatment and plasma concentrations
- A summary table of plasma concentrations at each week/time point (n; Geometric mean, SD, coefficient of variation (CV)% calculated as 100% × SD/mean, minimum, median and maximum)
- A figure with individual visits 1 to 12 from pre-dose through 1-2h post-dose plasma concentration-time profiles on both linear and log-linear scale (Y-axis is the concentrations; X-axis is the time e.g. Weeks 1, 4, 8, 12)
- A figure with geometric mean ± SD plasma concentration-time profiles presented on both linear and log-linear scales (Y-axis is the Visit x pre dose (or visit x 1-2 hours post dose concentrations – 2 separate plots; X-axis is the time e.g. Weeks 1, 4, 8, 12)

Nominal time points and visit number (planned) will be used for summary and geometric mean concentration graphical presentations. The time of collection of the pre-dose sample will be set to zero. Mean plasma concentrations will not be presented in the

summary or graphics for the time points with 50% or more of the actual values are below the LLOQ or missing.

9 Safety Analyses

All analyses described in this section will be performed on the SAF and will be presented by treatment arm and overall. The results will be descriptive in nature. All data will be summarized and listed.

Safety will be assessed on the basis of exposure, compliance, AEs, laboratory parameters, vital signs and physical examination.

9.1 Extent of Exposure

The duration of exposure will be expressed as the time in days from the first dose of treatment through to last treatment day (inclusive). Duration of exposure will be summarized for the SAF using summary statistics for continuous variables.

9.2 Treatment Compliance

From baseline and at subsequent visits while the patient is being treated with the study treatment, the patient will be directed to bring back all unused tablets. Compliance will be checked by calculating number of pills taken/expected pills to be taken by the Investigator during those visits and recorded on the eCRF.

The number and percentage of compliant patients will be presented for the SAF by study visit, where compliant is defined as percentage compliance between 80.0% and 100.0% inclusive. The following percentage compliance categories will be presented:<80.0%; ≥80.0% to ≤100.0%; >100.0%. Descriptive statistics for treatment compliance will also be presented.

9.3 Safety Outcomes

9.3.1 Primary Safety Outcomes

The primary safety outcomes of interest are (1) numbers and % of subjects who experience at least 1 treatment-emergent adverse event and (2) adverse events of special interest (liver function abnormalities, corrected QT interval/cardiac, infections, and neutropenia). AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Summaries will be by system organ class and preferred term. We will also present data separately by augmentation.

Treatment-emergent AEs (TEAEs) are defined as any AE occurring on or after the first dose of study medication. Listing will be sorted by treatment arm, subject ID, chronologically (start and end dates) and then alphabetically by SOC, PT and then verbatim description. The listing will include all the distinct levels of SOC, PT and the verbatim Investigator description reported in the study. A flag per subject will be included to indicate whether the patient received COVID-19 vaccination. If a participant experiences the same preferred term multiple times, the event will be counted only once overall and by the greatest severity. The frequency and incidence of TEAEs will be presented by system organ class and preferred term for each treatment group (number and percentage of participants experiencing at least 1 AE per preferred term as well as the number of observed events per preferred term). Separate tables will be presented by severity and by relationship to study drug. All AEs will be presented in a comprehensive listing

including participant number, treatment regimen, day of onset, severity, seriousness, action taken, outcome, relationship to treatment, onset/stop, and duration. Details of SAEs and AEs leading to withdrawal will be listed separately.

The frequency and incidence of adverse events of acute exacerbations of COPD will be presented (number and percentage of participants experiencing at least 1 AE per preferred term as well as the number of observed events per preferred term) and overall. Events that started after treatment initiation (TEAEs) up until Week 12 will only be presented, meaning that events commencing after the end of treatment and during the safety follow up period will not be summarized.

AESIs, including liver function abnormalities, ECG/cardiac events, infections, and neutropenia, will be tabulated and summarized by treatment group and overall.

9.3.2 Secondary Safety Outcomes

For secondary safety outcomes,

- Physical examination results will be listed by subject ID and body system. ECG parameters will be summarized as observed values and change from baseline by treatment group and study visit.
- Abnormal findings (“normal”, “abnormal, not clinically significant”, and “abnormal, clinically significant”) will be summarized by the number and percentage within each category and change from baseline will be summarized by shift tables.
- Laboratory parameters will be summarized as observed values and change from baseline by treatment group and study visit.
- Vital signs (temperature, SBP, DBP, heart rate, respiration rate, oxygen saturation) and ECG results will be summarized as observed values and change from baseline by treatment group and study visit.

9.3.3 Exploratory Safety Outcomes

Safety correlation analysis with drug concentration will be done for the safety parameters shown to be sensitive to Alvelestat exposure. The comparison of exposure will be done between groups with non-clinically significant changes and clinically significant changes in safety parameters using graphic and statistical methods where data allow.

10 Changes from Analysis Planned in the Protocol

In Version 1.3 of the protocol, augmentation therapy was removed from the list of not allowed treatment. Sample size calculation was revised but sample size remained the same. Also, stratified analyses by augmentation therapy will be performed.

Due to the risk during the COVID pandemic, spirometry as well as sputum and BAL collections were changed to optional. Consequently, all analyses using spirometry, sputum biomarkers, and BAL biomarker outcomes and data will no longer be performed in this study.

Primary analysis in the protocol is based on ANCOVA with outcome being the percent change at Week 12 from baseline. However, to minimize the effect of missing data and to maximize the information from all visits, the primary analysis is now based on fitting a mixed model using change from baseline at all available follow-up visits as outcome with the assumption of missing at random for missing data.

11 Interim Analyses

No interim analysis for efficacy is planned for this study. An interim safety analysis was conducted after 30 subjects have completed the study and presented to the DSMB.

12 References

Ma S, Lin YY, He J, Rouhani FN, Brantly M, Turino GM. Alpha-1 antitrypsin augmentation therapy and biomarkers of elastin degradation. COPD 2013;10:473-81.

Campos MA, Geraghty P, Holt G, et al. The Biological Effects of Double-Dose Alpha-1 Antitrypsin Augmentation Therapy. A Pilot Clinical Trial. Am J Respir Crit Care Med 2019;200:318-26.

13 Reporting Conventions

P-values will be reported to 3 decimal places; p-values less than 0.0005 will be reported as "<0.001" and p-values greater than 0.9995 will be reported as ">0.999".

The mean, standard deviation, and other statistics will be reported to 1 decimal place greater than the original data. The minimum and maximum will use the same number of decimal places as the original data. Proportions will be presented as 2 decimal places; values greater than zero but <0.005 will be presented as "<0.01".

Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

14 Listing of Tables, Figures, and Listings

14.1 Listing of Tables

Table Number	Table Name	Analysis Set
	Patient Disposition	Enrolled Set
	Protocol Deviations	Randomized Set
	Protocol Deviations Related to COVID-19	Randomized Set
	Reasons for Exclusion from Analysis Sets	
	Subject Demographic and Baseline Characteristics	Safety Set (SAF)
	Subject Demographic and Baseline Characteristics	Full Analysis Set (FAS)
	Subject Demographic and Baseline Characteristics	Per-Protocol Set (PPS)
	Medical History	Safety Set (SAF)
	Ongoing Medical History	Safety Set (SAF)
	AATD Medical History	Safety Set (SAF)
	Prior Medication	Safety Set (SAF)
	Concomitant Medication	Safety Set (SAF)
	Concomitant COPD Medication	Safety Set (SAF)
	Treatment Compliance	Safety Set (SAF)
	Absolute and Relative Change from Baseline in Plasma Desmosine/Isodesmosine up to End of Treatment: Primary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma Desmosine/Isodesmosine up to End of Treatment: Primary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Blood Aα-Val^{360} levels up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Serum NE activity up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma proteinase 3 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma cathepsin B up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma CRP up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma IL-6 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)

Table Number	Table Name	Analysis Set
	Absolute and Relative Change from Baseline in Plasma IL-8 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma IL-1b up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma RANTES up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma LTB4 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma MMP9 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma MMP12 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Plasma MPO up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Full Analysis Set (FAS)
	Absolute and Relative Change from Baseline in Blood Aα-Val^{360} levels up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Serum NE activity up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma proteinase 3 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma cathepsin B up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma CRP up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma IL-6 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma IL-8 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma IL-1b up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma RANTES up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma LTB4 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)

	Absolute and Relative Change from Baseline in Plasma MMP9 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma MMP12 up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Absolute and Relative Change from Baseline in Plasma MPO up to End of Treatment: Secondary Efficacy Analysis (MMRM)	Per-Protocol Set (PPS)
	Summary PK Concentrations for Alvelestat (MPH966)	PK Set
	Overall Summary of Adverse Events	Safety Set (SAF)
	Summary of Treatment Emergent Adverse Events (TEAEs) by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Treatment Related Treatment Emergent Adverse Events (TEAEs) by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Serious Treatment Emergent Adverse Events (TEAEs) by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Treatment Related Serious Treatment Emergent Adverse Events (TEAEs) by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Treatment Emergent Adverse Events (TEAEs) Leading to Permanent Withdrawal of Study Drug by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Adverse Events of Special Interest (AESIs) by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Adverse Events Leading to Death by System Organ Class and Preferred Term	Safety Set (SAF)
	Summary of Treatment Emergent Adverse Events (TEAEs) by System Organ Class, Preferred Term and Maximum Intensity	Safety Set (SAF)
	Summary of Adverse Events of Acute Exacerbations of COPD up until Week 12 by System Organ Class and Preferred Term	Safety Set (SAF)
	Deaths, Listing	Safety Set (SAF)

Table Number	Table Name	Analysis Set
	Summary of Hematology Laboratory Parameters	Safety Set (SAF)
	Summary of Clinical Chemistry Laboratory Parameters	Safety Set (SAF)
	Summary of Urinalysis Laboratory Parameters	Safety Set (SAF)
	Summary of Vital Sign Parameters	Safety Set (SAF)
	Summary of Electrocardiogram (ECG) Parameters	Safety Set (SAF)
	Summary of Study Drug Compliance	Safety Set (SAF)

14.2Listing of Figures

Figure Number	Figure Name	Analysis Set
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma Desmosine/Isodesmosine at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma Desmosine/Isodesmosine at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma Desmosine/Isodesmosine at Scheduled Study Visits	Per-Protocol Set (PPS)
	Individual Absolute Values in Plasma Desmosine/Isodesmosine at Scheduled Study Visits	Per-Protocol Set (PPS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma Aa-Val-360 at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values Plasma Aa-Val-360 at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Serum NE activityat Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Blood Serum NE activityat Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma proteinase 3 levels at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma proteinase 3 levels at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma cathepsin B levels at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma cathepsin B levels at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma CRP levels at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma CRP levels at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma IL-6 at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma IL-6 at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma IL-8 at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma IL-8 levels at Scheduled Study Visits	Full Analysis Set (FAS)

Figure Number	Figure Name	Analysis Set
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma IL-1b at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma IL-1b at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma RANTES at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma RANTES at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma LTB4 at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma LTB4 at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma MMP9 at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma MMP9 at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma MMP12 at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma MMP12 at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma MPO at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma MPO at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Plasma PGP at Scheduled Study Visits	Full Analysis Set (FAS)
	Individual Absolute Values in Plasma PGP at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Markers of Pulmonary Function at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in Markers of Pulmonary Function at Scheduled Study Visits	Per-Protocol Set (PPS)
	Mean (± SE) Absolute Values and % Change from Baseline in SOBQ at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in MMRC at Scheduled Study Visits	Full Analysis Set (FAS)
	Mean (± SE) Absolute Values and % Change from Baseline in St George's Respiratory Questionnaire at Scheduled Study Visits	Per-Protocol Set (PPS)

Figure Number	Figure Name	Analysis Set
	Individual plasma concentration Profiles for Alvelestat (MPH966) vs Time by Treatment – Linear Scale	PK Set
	Individual plasma concentration Profiles for Alvelestat (MPH966) vs Time by Treatment – Log-Linear Scale	PK Set
	Geometric Mean (± SD) plasma concentration Profile for Alvelestat (MPH966) vs Time by Treatment – Linear Scale	PK Set
	Geometric Mean (± SD) plasma concentration Profile for Alvelestat (MPH966) vs Time by Treatment – Log-Linear Scale	PK Set
	Box-plot of plasma concentration for Alvelestat (MPH966) vs Time (pre-dose and by visit post-dose) by Treatment	PK Set

14.3Listings

Listing Number	Listing Name	Analysis Set
	Patient Disposition – Screen Failures	Enrolled Set
	Patient Disposition	Randomized Set
	Patients Impacted by COVID-19 Related Study Discontinuations	Randomized Set
	Missing Assessments Due to COVID-19	Randomized Set
	Missing Visits Due to COVID-19	Randomized Set
	Protocol Deviations	Randomized Set
	Protocol Deviations Related to COVID-19 and Related Measures	Randomized Set
	Inclusion/ Exclusion Criteria	Randomized Set
	Exclusion from Analysis Sets	
	Demographics	Randomized Set
	Pregnancy Test	Randomized Set
	Medical History (Prior and Ongoing)	Safety Set (SAF)
	AATD Medical History	Safety Set (SAF)
	Alcohol History	Randomized Set
	Smoking History	Randomized Set
	Prior and Concomitant Medications	Safety Set (SAF)
	Randomization and Treatment Administration	Safety Set (SAF)
	Drug Accountability	Safety Set (SAF)
	Primary Efficacy Endpoint: Plasma Desmosine/Isodesmosine	Full Analysis Set (FAS)
	Primary Efficacy Endpoint: Plasma Desmosine/Isodesmosine, Blood Neutrophil Elastase Activity and Blood Aα-Val^{360}	Full Analysis Set (FAS)
	Secondary Efficacy Endpoint: Plasma Aa-Val-360 Serum NE activity, Plasma proteinase 3, Plasma cathepsin B,Plasma CRP, Plasma IL-6, Plasma IL-8, Plasma IL-1b, Plasma RANTES, Plasma LTB4, Plasma MMP9,Plasma MMP12, Plasma MPO, Plasma PGP, Plasma PK/PD (LCG/DDS)	Full Analysis Set (FAS)
	Exploratory Efficacy Endpoints: Absolute and Relative Change from Baseline in Indicators of Pulmonary Function	Full Analysis Set (FAS)
	Exploratory Efficacy Endpoints: Absolute Change from Baseline in St George's Respiratory Questionnaire	Full Analysis Set (FAS)
	Exploratory Efficacy Endpoints: Absolute Change from Baseline in SOBQ	Full Analysis Set (FAS)
	Exploratory Efficacy Endpoints: Absolute Change from Baseline in MMRC	Full Analysis Set (FAS)
	Exploratory Efficacy Endpoints: Absolute Change from Baseline in CAT	Full Analysis Set (FAS)
	Pharmacokinetic Endpoints: Listing of PK Plasma Concentration data	PK Set

Listing Number	Listing Name	Analysis Set
	Listing of all Adverse Events	Safety Set (SAF)
	Listing of Hematology Laboratory Parameters	Safety Set (SAF)
	Listing of Clinical Chemistry Laboratory Parameters	Safety Set (SAF)
	Listing of Vital Signs	Safety Set (SAF)
	Listing of Electrocardiogram (ECG) Parameters	Safety Set (SAF)
	Listing of Physical Examination	Safety Set (SAF)
	Listing of Serious Treatment Emergent Adverse Events (Serious TEAEs)	Safety Set (SAF)
	Listing of Non-Treatment Emergent Serious Adverse Events (SAEs)	Safety Set (SAF)
	Listing of Treatment Emergent Adverse Events (TEAEs) Leading to Permanent Withdrawal of Study Drug	Safety Set (SAF)
	Listing of Treatment Emergent Adverse Events (TEAEs) with a Preferred Term of Headache	Safety Set (SAF)
	Listing of Treatment Emergent Adverse Events (TEAEs) of COPD or exacerbation of COPD	Safety Set (SAF)
	Listing of Patients with at Least One Clinically Significant Clinical Chemistry Laboratory Parameter	Safety Set (SAF)
	Listing of Patients with at Least One Clinically Significant Hematology Laboratory Parameter	Safety Set (SAF)