A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients With Mild to Moderate COPD With Chronic Bronchitis

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ionispharma.com

2855 Gazelle Court
Carlsbad, CA 92010

(760) 931-9200

Official Title:	**A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis**
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IONIS PHARMACEUTICALS, INC.

ION-827359-CS2

A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis

2 February 2021

Protocol Amendment 3

EudraCT No: 2020-000210-15

Trial Sponsor:

Ionis Pharmaceuticals
2855 Gazelle Court
Carlsbad, CA 92010
Phone: 760-931-9200
Fax: [redacted]

Key Sponsor Contact:

Dr. [redacted]
[redacted]
Phone: [redacted]
Fax: [redacted]

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Protocol Amendment 3

EudraCT No: 2020-000210-15

Clinical Phase: 2a

A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis

Protocol History

Original Protocol: 21 January 2020

Protocol Amendment 1: 28 May 2020

Protocol Amendment 2: 10 August 2020

Sponsor
Ionis Pharmaceuticals, Inc.
Carlsbad, CA 92010

See electronic signature and date attached at end of document

[redacted], M.D.
[redacted]

Confidentiality Statement

This document contains confidential information of Ionis Pharmaceuticals, Inc. that must not be disclosed to anyone other than the recipient study staff and members of the independent ethics committee, institutional review board, or authorized regulatory agencies. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

PROTOCOL SIGNATURE PAGE

Protocol Number:	ION-827359-CS2
Protocol Title:	A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis
Amendment:	Protocol Amendment 3
Date:	2 February 2021

I hereby acknowledge that I have read and understand the attached clinical protocol, entitled "A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with CB" dated 2 February 2021, and agree to conduct the study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

_____
Investigator's Signature

_____ _____
Investigator's Name *(please print)* Date (DD Month YYYY)

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PROTOCOL AMENDMENT

Protocol Number: ION-827359-CS2

Protocol Title: A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis

Amendment Number: 3

Amendment Date: 2 February 2021

The major purpose of this amendment is to help prevent any potential bronchospasm after inhalation of Study Drug.

A list of changes to the protocol are below:

Protocol Section	Description of Change	Rationale
Section 6.1.3 Treatment Period	The following is **deleted** from Table 1 (Withhold times for bronchodilators): LABA (e.g., formoterol or salmeterol) 24 hours Ultra-LABA (e.g., indacaterol, vilanterol, or olodaterol 36 hours LAMA (e.g., tiotropium, umeclidinium, aclidinium, or glycopyrronium) 36 – 48 hours The following is **added**: The usual dose of bronchodilators should not be taken the morning of the visit, until after spirometry is performed and prior to dosing (if applicable) with the study medication. Albuterol (salbutamol) 2 puffs, should be dosed prior to study drug both for in-clinic and at-home dosing.	To help prevent any potential bronchospasm
Appendix A Schedule of Procedures	**Footnote 2 (vital signs) currently reads:** [2] BP, heart rate (HR), respiratory rate (RR), temperature **Will be amended to:** [2] BP, heart rate (HR), respiratory rate (RR), temperature**, and pulse oximetry**	Correct an omission.
Appendix A Schedule of Procedures	**Footnote g added for spirometry on Day 1:** Perform spirometry (pre-Study Drug) pre and 20-30 minutes post albuterol dose (2 puffs) and then perform spirometry 1 and 2 hours post-Study Drug dosing.	Collect baseline post-albuterol spirometry

PROTOCOL SYNOPSIS

Protocol Title	A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis
Study Phase	2a
Indication	Chronic Bronchitis (CB)
Primary Objective	To evaluate the efficacy of ION-827359 in patients with mild to moderate CB over 13 weeks of treatment
Secondary Objectives	• To evaluate the effect of ION-827359 on symptoms of CB • To evaluate the effect of ION-827359 on quality of life (QoL) in COPD patients with CB • To evaluate the pharmacokinetics of ION-827359 in COPD patients with CB • To evaluate the safety and tolerability of ION-827359 compared to placebo
Exploratory Objectives	• To assess changes in lung volumes (RV, FRC) • To assess changes in lung mucins (total, MUC5AC, and MUC5B) • To assess changes in regional lung ventilation in low dose high resolution CT scanning (HRCT) scans • To determine mRNA target engagement and inflammatory biomarkers • To evaluate the dose response of ION-827359
Study Design	Double-blind, placebo-controlled, multi-center study
Number of Patients	Approximately 180 patients
Study Population	**Inclusion Criteria** 1. Must have given written informed consent (signed and dated) and any authorizations required by local law and be able to comply with all study requirements 2. Males or females. Aged 40–70 inclusive at the time of informed consent 3. Females must be non-pregnant and non-lactating, and either surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) or post-menopausal (defined as 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the post-menopausal range for the laboratory involved)

PROTOCOL SYNOPSIS (Continued)

Study Population (Continued)	**Inclusion Criteria Continued** Males must be surgically sterile or abstinent*, if engaged in sexual relations with a female of child-bearing potential, the subject must be using a condom in addition to a highly effective contraceptive method by the partner from the time of signing the informed consent form until at least 10 weeks after the last dose of Study Drug (ION-827359 or placebo) * Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception 4. BMI < 35.0 kg/m^2 5. Patients with a diagnosis of COPD as defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS) • Ability to perform acceptable and reproducible spirometry • Post-bronchodilator (4 puffs of albuterol) spirometry at Screening demonstrating the following • FEV_1/FVC ratio of < 0.70 • $FEV_1 \geq 50\%$ and $\leq 90\%$ of predicted normal 6. Clinically stable COPD in the 4 weeks prior to Screening (Visit 1) 7. Current and former smokers with smoking history of ≥ 20 pack years 8. Meet SGRQ definition of CB (See Section 6.1.1) 9. CAT score of ≥ 10 **Exclusion Criteria** 1. Clinically significant abnormalities in medical history (e.g., previous acute coronary syndrome within 6 months of Screening, major surgery within 3 months of Screening) or physical examination 2. Screening laboratory results as follows, or any other clinically significant abnormalities in screening laboratory values that would render a subject unsuitable for inclusion • Urine protein/creatinine (P/C) ratio ≥ 0.3 mg/mg. In the event of P/C ratio above this threshold eligibility may be confirmed by a quantitative total urine protein measurement of < 300 mg/24 hr

PROTOCOL SYNOPSIS (Continued)

Study Population (Continued)	**Exclusion Criteria (Continued)** • Positive test (including trace) for blood on urinalysis. In the event of a positive test eligibility may be confirmed with urine microscopy showing ≤ 5 red blood cells per high power field • ALT, AST, bilirubin, alkaline phosphatase, serum creatinine, BUN > 1.5 × upper limit of normal (ULN) • Platelet count < LLN • Serum potassium > 5.2 mmol/L • Estimated GFR< 60 mL/min (as determined by the Cockcroft-Gault Equation for creatinine clearance) 3. Any active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1 4. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator 5. Active infection with human immunodeficiency virus (HIV), hepatitis C or hepatitis B 6. Uncontrolled hypertension (BP > 160/100 mm Hg) 7. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix that has been successfully treated. Patients with a history of other malignancies that have been treated with curative intent and which have no recurrence within 5 years may also be eligible if approved by the PI and reviewed by the Sponsor Medical Monitor 8. Treatment with another investigational drug, biological agent, or device within 1 month of Screening, or 5 half-lives of investigational agent, whichever is longer 9. Previous treatment with an oligonucleotide (including siRNA) within 4 months of screening if single dose received, or within 12 months of screening if multiple doses received 10. Clinically important pulmonary disease other than COPD 11. Asthma as a primary or main diagnosis according to the Global Initiative for Asthma (GINA) guidelines (GINA 2011) or other accepted guidelines. Patients with a past medical history of asthma (e.g. childhood or adolescence) may be included 12. Treatment with systemic corticosteroids and/or antibiotics, and/or hospitalization for a COPD exacerbation within 4 weeks prior to enrolment (Visit 1)

PROTOCOL SYNOPSIS (Continued)

Study Population (Continued)	**Exclusion Criteria (Continued)** 13. Acute upper or lower respiratory infection requiring antibiotics or antiviral medication within 4 weeks prior to enrolment (Visit 1) 14. Long term oxygen therapy (LTOT) 15. Patients participating in, or scheduled for, an intensive (active) COPD rehabilitation program (patients who are in the maintenance phase of a rehabilitation program are eligible to take part) 16. Recent history of, or current drug or alcohol abuse 17. Concomitant medication restrictions: Oral anticoagulants, oral steroids (e.g. prednisone or Medrol), theophylline, chronic azithromycin, or roflumilast 18. Have any other conditions, which, in the opinion of the Investigator would make the subject unsuitable for inclusion, or could interfere with the subject participating in or completing the Study 19. A positive PCR test for SARS-CoV-2 at any time prior to randomization
Treatment Groups	**Patients will be randomized 2:2:1:1 to treatment with:** • ION-827359 37.5 mg (1.5 mL) once per week • ION-827359 75mg (3 mL) once per week • Placebo (1.5 mL) once per week • Placebo (3 mL) once per week
Study Drug Dosage and Administration	ION-827359 (25mg/mL) and placebo will be supplied in stoppered glass vials. A total of 1.5 mL or 3 mL will be placed into a Pari eFlow® nebulizer and administered by oral inhalation once weekly.
[redacted]	[redacted]
Study Visit Schedule and Procedures	Blood and urine samples will be collected regularly throughout the study for safety, PK, and PD analyses. Appendix B shows a list of analytes required for the study. The safety of ION-827359 will be monitored in an ongoing fashion throughout the trial. **Screening and Baseline: Study Days -28 to -1** Laboratory and other study procedures will be performed to assess eligibility during the Screening Period. Patients will also receive a SARS-CoV-2 PCR test based on nasal/oral swab. Patients will be given an electronic diary to record their baseline daily symptoms starting on Day -7.

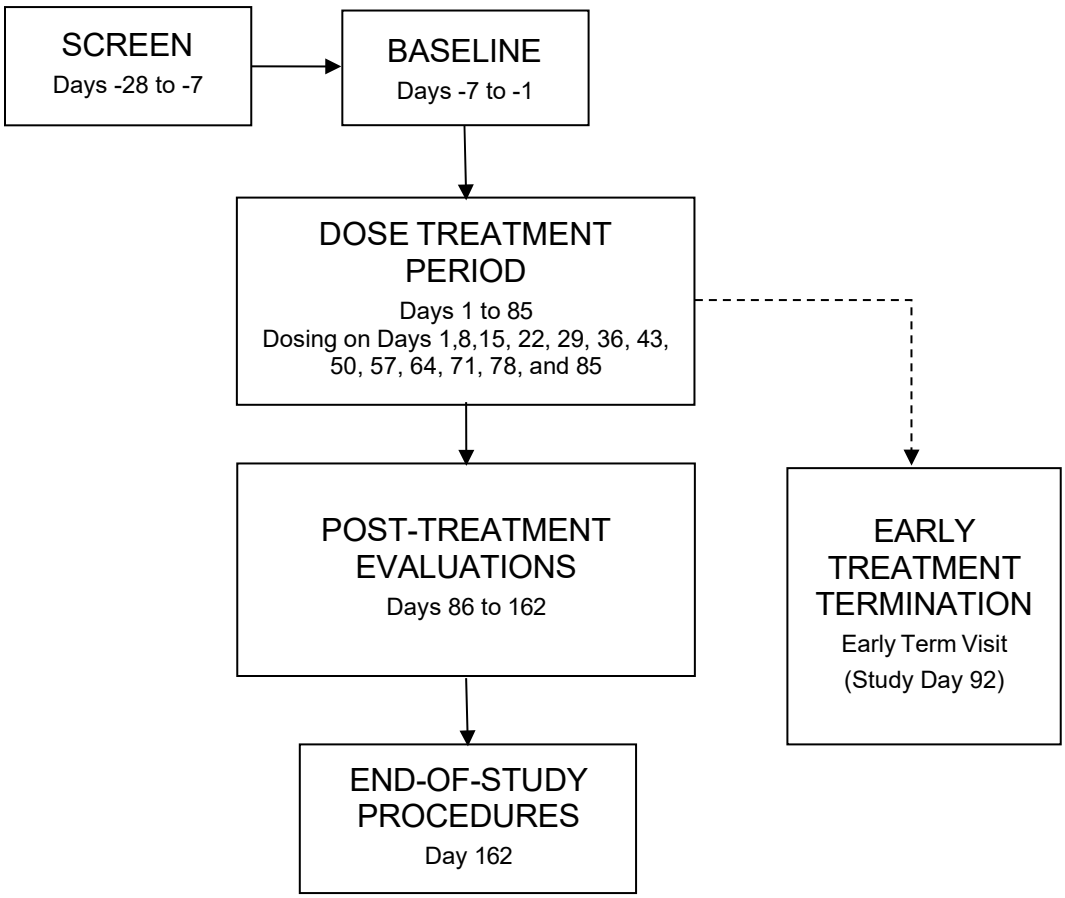
PROTOCOL SYNOPSIS (Continued)

Study Visit Schedule and Procedures (Continued)	**Treatment: Study Days 1 to 85** Eligible patients will be randomized 2:2:1:1 to ION-827359 (37.5 or 75 mg) or matching placebos. Patients will receive nebulized doses of Study Drug once weekly starting on Study Day 1. Both in-clinic and at-home dosing of ION-827359 will be performed. Patients will record their symptoms in a daily electronic diary and return for study visits to include monitoring and spirometry. There are 3 subset studies: 1. A subset of up to about 24 patients will have PK profile sampling performed after the first and last dose 2. A subset of up to about 60 patients will have low dose inspiratory and expiratory HRCT scans to assess regional lung ventilation performed before and post-treatment 3. A subset of up to about 18 patients will undergo bronchoscopy and BAL for target engagement and inflammatory biomarkers before and post-treatment A patient may be enrolled in a maximum of 1 subset study **Post-Treatment: Study Days 86 to 162** Patients are to return to the Study Center for post-treatment visits on Study Days 92, 120 and 162. Patients that discontinue treatment are encouraged to remain in the study for the Post-Treatment Evaluation Period and will conduct procedures outlined at Study Day 92 visit upon discontinuation of Study Drug. Patients will continue their stable COPD medication regimen throughout the study (screening treatment and post-treatment periods).
Primary Endpoints	Change from Baseline to the primary time point (defined as the average of Weeks 13 and 14) in FEV_1 compared to placebo
Secondary Endpoints	• Change from Baseline in the E-RS (evaluating respiratory symptoms) daily symptom diary to the primary time point • Change from Baseline in the CAT to the Week 14 time point • Change from Baseline in SGRQ to the Week 14 time point • Change from Baseline in post-bronchodilator FEV_1 • Pharmacokinetics • Incidence and severity of treatment-emergent adverse events (TEAE) • Abnormal findings in laboratory assessments, ECG, and vital signs

PROTOCOL SYNOPSIS (Continued)

Exploratory Endpoints	• Change from Baseline in sputum mucins (total, MUC5AC, and MUC5B) • Change from Baseline in ENaC mRNA levels from bronchial brushings • Change from Baseline in inflammatory biomarkers from bronchoalveolar lavage • Change from Baseline in FRC (functional respiratory capacity) and RV (residual volume) • Change from Baseline in regional lung volumes as measured by HRCT
Statistical Considerations	The sample size assumptions for the primary endpoint include: • The increase in FEV_1 from Baseline to the primary time point in the ION-827359 treatment group is 100 mL; no change in the placebo group • Standard deviation of change in FEV_1 is 220 mL • Significance level (alpha) of 0.05 (2-sided test) With the above assumptions, a sample size of 174 patients (58 patients in each ION-827359 treatment groups and 29 patients in each placebo group) will provide a power of at least 80% for the primary comparison (pooled ION-827359 treatment groups vs. pooled placebo groups). Approximately 180 patients will be enrolled in this trial to account for a 3% dropout rate.
Sponsor	Ionis Pharmaceuticals

STUDY DESIGN AND TREATMENT SCHEMA



STUDY GLOSSARY

Abbreviation	Definition
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)
aPTT	activated partial thromboplastin time
ASO	antisense oligonucleotide
AST	aspartate aminotransferase
ATS	American Thoracic Society
AUCt	area under the plasma concentration-time curve from time zero to time t
BAL	bronchoalveolar lavage
βhCG	beta-subunit of human chorionic gonadotropin (pregnancy test)
BP	blood pressure
BUN	blood urea nitrogen
C	centigrade
C5a	complement factor C5a (activated complement split product)
CAT	COPD assessment test
CB	chronic bronchitis
CL	systemic clearance
CMV	cytomegalovirus
COPD	chronic obstructive pulmonary disease
CS	clinically significant
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
dL	deciliter
DLCO	diffusing capacity
ECG	electrocardiogram
eCRF	electronic Case Report Form
eGFR	estimated glomerular filtration rate
ENaC	epithelial sodium channel
ERS	European Respiratory Society
E-RS	EXACT respiratory symptoms
FAS	Full Analysis Set
FEV_1	forced expiratory volume in 1 second
FRC	functional residual capacity
FSH	follicle stimulating hormone
FVC	forced vital capacity
g	gram
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HRCT	high resolution CT scanning
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee

IgM	immunoglobulin M
IL-5	interleukin-5
IL-8	interleukin-8
INR	international normalized ratio
IRB	Institutional Review Board
ION-827359	antisense inhibitor of ENaC
kg	kilogram
m^2	square meter
MAD	multiple-ascending dose
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA™	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mm	millimeter
mRNA	messenger ribonucleic acid
NCI	National Cancer Institute
NCS	not clinically significant
NOAEL	no adverse effect level
on study	the patient is 'on study' from signing of the informed consent until their last study visit
P/C	urine protein/creatinine
pH	measure of the acidity or basicity of a solution
PK	pharmacokinetic(s)
PPS	Per Protocol Set
PT	prothrombin time
QoL	quality of life
RNase H1	an ubiquitous endonuclease that specifically hydrolyzes the RNA strand in RNA/DNA hybrids
RV	residual volume
SAD	single-ascending dose
SAE	serious adverse event
SAP	Statistical Analysis Plan
SGRQ	St. George's Respiratory Questionnaire
siRNA	small interfering ribonucleic acid
TLC	total lung capacity
Study Day 1	defined as the first day Study Drug product is administered to the patient
Study Drug	ION-827359 or placebo
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
T_{max}	time to maximal concentration
ULN	upper limit of normal
WBC	white blood cell
WOCBP	woman of child-bearing potential

1. OBJECTIVES AND ENDPOINTS

1.1. Objectives

1.1.1. Primary Objective

To evaluate the effect of ION-827359 on forced expiratory volume in 1 second (FEV_1) in patients with mild to moderate chronic obstructive pulmonary disease (COPD) with chronic bronchitis (CB)

1.1.2. Secondary Objectives

- To evaluate the effect of ION-827359 on symptoms of CB
- To evaluate the effect of ION-827359 on quality of life (QoL)in patients of CB
- To evaluate the pharmacokinetics (PK) of ION-827359 in patients with CB
- To evaluate the safety and tolerability of ION-827359 compared to placebo

1.1.3. Additional/Exploratory Objectives

- Assess changes in PD markers
- Assess changes in lung volumes
- Assess changes in lung ventilation
- Assess target engagement

1.2. Study Endpoints

1.2.1. Primary Endpoint

Change from Baseline to the primary time point (defined as the average of Weeks 13 and 14) in FEV_1 compared to placebo

1.2.2. Secondary Endpoints

- Change from Baseline in the EXACT respiratory symptoms (E-RS) (evaluating respiratory symptoms) daily symptom diary to the primary time point
- Change from Baseline in the COPD assessment test (CAT) to the Week 14 time point
- Change from Baseline in St. George's Respiratory Questionnaire (SGRQ) to the Week 14 time point
- Change from Baseline in post-bronchodilator FEV_1
- Pharmacokinetics (Section 1.2.3.1)
- Safety Endpoints
  - Incidence and severity of treatment-emergent adverse events (TEAE)
  - Abnormal findings in laboratory assessments, electrocardiogram (ECGs), and vital signs

1.2.3. Exploratory Endpoints

The tertiary and PD endpoints include:

- Change from Baseline in sputum mucins (total, MUC5AC, and MUC5B)
- Change from Baseline in epithelial sodium channel (ENaC) messenger ribonucleic acid (mRNA) levels from bronchial brushings
- Change from Baseline in inflammatory biomarkers from bronchoalveolar lavage (BAL)
- Change from Baseline in functional respiratory capacity (FRC) and residual volume (RV)
- Change from Baseline in regional lung volumes as measured by high resolution CT scanning (HRCT)

1.2.3.1. Pharmacokinetic (PK) Endpoints

Plasma exposure over time will be summarized using data from PK profile samples collected following the first and last dose in a subset of patients, as well as trough samples collected in all patients throughout the study. In addition, potential PK/PD correlation on relevant biomarkers may be evaluated.

2. BACKGROUND AND RATIONALE

2.1. Overview of Disease

Chronic Bronchitis (CB) is an inflammatory disease of the bronchi, especially the central bronchi, and is characterized by excessive mucus secretion. Classically this is defined as significant sputum production for at least 3 months for at least 2 successive years. Abnormalities in mucus production and properties are critical to the pathophysiology of the disease. Patients suffer from varying degrees of chronic cough, sputum expectoration, and dyspnea. CB is 1 of the 2 underlying diagnoses for COPD, the other being emphysema. COPD is characterized by progressive and incompletely reversible airflow obstruction.

Chronic Bronchitis is quite common in the general population, with a wide range of 3.4%-22.0% of the adult population. This variability is caused largely by the varying definitions of CB (Kim and Criner 2015). It appears that CB affects approximately 10 million individuals in the U.S., interestingly including significant number of individuals without a diagnosis of COPD (de Oca et al. 2012). Among COPD patients those with CB have greater smoking exposure, more symptoms, and worse general health than the general COPD population. In the COPD Gene study the prevalence of CB in the COPD population was 26.2% using the classical definition and 39% using a definition based on the SGRQ (de Oca et al. 2012).

Airway mucus obstruction appears to play an important role in the pathogenesis of chronic inflammation and infection that drives the disease progression in COPD (Fahy and Dickey 2010). Multiple studies have demonstrated that CB in COPD patients is associated with accelerated lung function decline, increased risk of exacerbations, and increased mortality. As a

result of the increased exacerbation rates in CB there are greater number of emergency room visits and hospitalizations. In a large cross-sectional study of COPD patients in Belgium the number of exacerbations was 2.08 per year for the CB group vs. 1.05 in the non-CB group (Corhay et al. 2013). Some 37.3% of patients with CB had 2 or more exacerbations per year as compared to 14.2% without CB. Similar trends were seen in the COPD Gene and SPIROMICS studies. As exacerbation rates are high in CB, an emphasis on reduction of these rates has been perused. Roflumilast is a phosphodiesterase-4 (PDE4) inhibitor which has been shown to reduce exacerbation rates only in patients with CB, but not in a general COPD population. Bronchodilators remain the mainstay of treatment for all patients with COPD. However, no therapy has been demonstrated to reduce the mortality rate in COPD or decrease the rate of progression of the disease. There remains a significant unmet medical need for additional therapeutic interventions.

2.2. Therapeutic Rationale

Chronic Bronchitis is a disease of chronic mucus hypersecretion which predisposes patients to lower respiratory infections and is linked to disease progression and mortality. The study by Kesimer also found that airway mucin concentrations are linked to COPD severity (Kesimer et al. 2018) and may be a biomarker of CB. The airway in CB has been demonstrated to be dehydrated as a result of dysregulated ion transport along with an increase in goblet cells and mucus production (Graeber et al. 2013). A recent study suggested that the mucus hyperconcentration along with airway adherence is more important than the loss of mucous transport in bronchitic airway pathology (Livraghi-Butrico et al. 2017). This adherent mucus blocks airflow, which is in part responsible for reduced lung function, but more importantly is the site of bacterial airway infections. Furthermore, the mucus plugs themselves are pro-inflammatory, probably through local epithelial hypoxia which induces interleukin-1 (IL-1) and interleukin-8 (IL-8) secretion. It has been demonstrated that cigarette smoke induces ENaC activity, which results in sodium absorption from the airway and thereby hyper-concentrated airway mucus (Downs et al. 2013). It has been demonstrated that a potent and selective ENaC inhibitor with a long duration of action can indeed increase airway surface liquid (ASL) height and airway hydration (Astrand et al. 2015). In a woodsmoke murine model, an ENaC ASO resulted in a decrease in airway mucus and inflammation (Ionis data on file). Additionally, delivery of the EnaC ASO in a NEDD4L mouse model of cystic fibrosis resulted in a decrease in mucus gene expression (Gob5, Muc5ac) and a decrease in neutrophilic inflammation (Crosby et al. 2017). There was significant improvement in airway hyperreactivity. When ION-827359 was dosed in healthy volunteers there was a decrease in mucus mRNA including Gob5 and MUC5AC. Therefore, ION-827359 may be effective in treating an underlying defect in CB, specifically the increased mucus production and the hyper-concentrated mucus which result in impaired airflow and recurrent infections.

2.3. ION-827359

2.3.1. Mechanism of Action

[redacted]

[redacted] Maximal antisense-mediated reduction of target mRNA levels is typically greater than 90% of control levels in sensitive tissues (Crooke and Bennett 1996; Zhang et al. 2010). Furthermore, reduction in target mRNA levels using this approach correlates directly with a subsequent reduction in target protein levels.

2.3.2. Chemistry

[redacted] The phosphorothioate modifications impart *in vivo* stability to the molecule by providing resistance to nucleases but also confer a substantial PK benefit by increasing the binding to plasma proteins, preventing renal excretion of ASOs (Geary et al. 2008). [redacted].

Two (2) of these segments, the 3 nucleotides at both the 5′ and 3′ ends, contain constrained ethyl or cEt modifications to the sugar moieties (Seth et al. 2008). [redacted] Uniformly modified ASOs are not capable of eliciting RNase H1-induced catalytic cleavage of complementary mRNA (Monia et al. 1993). The gapmer design provides greatly improved metabolic stability and affinity to ASOs and maintains RNase H1-mediated antisense activity.

2.3.3. Preclinical Experience

Detailed information concerning the preclinical studies conducted with ION-827359 can be found in the Investigator's Brochure.

2.3.4. Clinical Experience

The safety and tolerability of ION-827359 has been evaluated in a Phase 1 clinical trial in 64 healthy volunteers. Please refer to the Investigator's Brochure for a detailed description. This study was a single-ascending dose (SAD) / multiple-ascending dose (MAD) study with 4 cohorts of 8 patients in each part. Doses in the SAD study ranged from 3 mg to 100 mg nebulized. In the MAD study the doses ranged from 10 mg to 75 mg with 5 doses over 4 weeks,

except for the final cohort where 10 doses were given. ION-827359 was well-tolerated without any serious adverse event (SAEs) or adverse events (AE) of concern. There were also no changes in clinical or laboratory measures, including no evidence of hyperkalemia. There is currently on-going a MAD trial in adult patients with cystic fibrosis.

[redacted]

2.5. Benefit-Risk Assessment

Detailed information concerning the benefit-risk assessment of ION-827359 can be found in the Investigator's Brochure. However, there are no known risks associated with ENaC inhibition in the lung. Genetic mutations leading to loss of ENaC function result in pseudohypoaldosteronism in which these patients have difficulty managing sodium balance in the body. This may result in renal sodium wasting, hyponatremia, and hyperkalemia. These were not seen in the Phase 1 study of ION-827359 but subjects will be monitored for these in this trial.

Due to the current COVID-19 pandemic, there may be risks to the patients by traveling to research sites. Sites should follow their specific regional guidance (i.e., institutional, local, state, federal, country-level, as applicable) with regard to receiving patients for clinical trials. Visits should continue as long as it is deemed safe to do so. Additional mitigation steps and a study pause may be necessary as conditions warrant. Post-treatment follow-up visits may be done remotely, if necessary. If a study patient becomes infected with COVID-19 or develop COVID-19-related symptoms, the patient should notify the study staff/Investigator and notify their treating Physician that they are participating in a clinical trial with ION-827359. To our knowledge, ION-827359 has no immunosuppressive effects.

The known potential risks to study participants associated with ION-827359 are elaborated on in the Guidance to Investigator section of the Investigator's Brochure.

2.5.1. Overall Assessment of Benefit:Risk

Taking into account the measures taken to minimize risk to patients participating in this study, the potential risks identified in association with ION-827359 are justified by the anticipated benefits that may be afforded to patients with CB.

3. EXPERIMENTAL PLAN

3.1. Study Design

This will be a Phase 2a, double blind, randomized, placebo-controlled, dose-ranging study. Patients with CB associated with COPD will be randomized to ION-827359 or matching placebo administered by oral inhalation once weekly for 13 weeks.

3.2. Number of Study Centers

This study will be conducted at multiple centers in Europe.

3.3. Number of Patients

Approximately 180 patients are planned to be enrolled in this study. Patients will be randomized to ION-827359 at a dose of 37.5 mg, ION-827359 at a dose of 75 mg, or placebo.

3.4. Overall Study Duration and Follow-up

The study will consist of Screening, Treatment, and Post-Treatment Periods. Please refer to the Schedule of Procedures in Appendix A.

The study for an individual patient will generally consist of the following Periods:

- A ≤ 4-week Screening Period. The last week of which is the Run-in Period
- A 13-week Treatment Period during which study medication (ION-827359 or placebo) will be administered by nebulization once per week (both in-clinic and at home dosing)
- A 10-week Post-Treatment Period

Patients may be required to attend additional visits for monitoring of AEs or abnormal investigation results. The frequency of additional monitoring will be determined by the Study Medical Monitor in consultation with the Investigator.

3.4.1. Screening

Subject eligibility for the study will be determined within 4 weeks prior to study entry.

3.4.2. Treatment

Eligible patients will report to the Study Center for study treatment as per the Schedule of Procedures in Appendix A. Study medication (ION-827359 or placebo) will be administered once per week, for 13 weeks with dosing both in-clinic and at home.

3.4.3. Post-Treatment

Patients are to return to the Study Center for follow-up visits on Study Days 92 and 120. The final study visit will be Study Day 162.

3.5. End-of-Study

The End-of-Study is defined as last patient, last visit when all patients have completed the last visit of the Post-Treatment Period.

4. PATIENT ENROLLMENT

4.1. Screening

Before patients may be enrolled into the study, the Sponsor or designee requires a copy of the Study Center's written Independent Ethics Committee/Institutional Review Board (IEC/IRB) approval of the protocol, informed consent form, and all other subject information and/or recruitment material.

Patients or their legally acceptable representatives must sign the consent form before any screening tests or assessments, including fasting prior to screening blood draws, are performed. At the time of consent, the subject will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including screening procedures, are performed. At the time of randomization, patients will be assigned a unique subject identification number. This number will be used to identify the subject throughout the trial and must be used on all study documentation related to that subject. The screening number and subject identification number must remain constant throughout the entire trial. In the event the subject is re-consented and re-screened the subject must be given a new screening number. Screening numbers and subject identification numbers, once assigned, will not be re-used.

4.2. Randomization/Registration

Patients will be randomized, after all Baseline and Screening assessments have been completed and after the Investigator has verified that they are eligible per criteria in Sections 5.1 and 5.2. No subject may begin treatment prior to randomization and assignment of a unique subject identification number.

Using an Interactive Voice/Web-Response System (IXRS), eligible patients will be randomized in a 2:2:1:1 ratio to receive ION-827359 37.5 mg (1.5 mL), ION-827359 75 mg (3 mL), Placebo 1.5 mL, and Placebo 3 mL, respectively. Cohort A will be defined as those subjects receiving 1.5 mL of Study Drug or placebo, and Cohort B as those receiving 3 mL of Study Drug or placebo. These cohorts will run concurrently.

There will be 4 separate and independent randomizations:

- PK subgroup: approximately 24 patients
- HRCT subgroup: approximately 60 patients
- Bronchoscopy subgroup: approximately 18 patients
- Other: patients who are not in the above 3 subgroups, approximately 78

The randomization lists will be prepared by an independent external vendor. Sites will be designated as to which subgroups they will be participating in. All subjects at those sites will contribute to the specific subgroup, unless the subject refuses to consent to the procedure in which case they may enroll into the Other subgroup.

4.3. Replacement of Patients

Patients who withdraw from the study will not be replaced.

4.4. Unblinding of Treatment Assignment

The Sponsor and all patients, monitors, and Study Center personnel related to the study, will be blinded throughout the study. However, if a subject has suffered a SAE (as defined in Section 9.3.3), and/or when knowledge of the treatment assignment will impact the clinical management of the subject, the Investigator will have the ability to unblind the treatment assignment for that subject using the automated interactive response technology (IRT) system. The Sponsor or designee will be informed of the unblinding of a subject within 24 hours. An unblinded randomization schema will be maintained securely at the Sponsor's designated vendor. In addition, all suspected unexpected serious adverse reaction (SUSAR) will be unblinded by the Sponsor or designee for the purpose of regulatory reporting (see Section 9.2).

Every reasonable attempt should be made to complete the early termination study procedures and observations (see Appendix A and Appendix B) prior to unblinding, as knowledge of the treatment arm could influence subject assessment.

An unblinded interim analysis may be performed and the results summarized by treatment group at the end of the Treatment Period of the study.

5. SUBJECT ELIGIBILITY

To be eligible to participate in this study candidates must meet the following eligibility criteria at Screening and randomization.

5.1. Inclusion Criteria

1. Must have given written informed consent (signed and dated) and any authorizations required by local law and be able to comply with all study requirements
2. Males or females. Aged 40–70 inclusive at the time of informed consent
3. Females must be non-pregnant and non-lactating, and either surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy) or post-menopausal (defined as 12 months of spontaneous amenorrhea without an alternative medical cause and follicle stimulating hormone (FSH) levels in the post-menopausal range for the laboratory involved)

   Males must be surgically sterile or abstinent*, if engaged in sexual relations with a female of child-bearing potential, the subject must be using a condom and a highly effective contraceptive method by the partner from the time of signing the informed consent form until at least 10 weeks after the last dose of Study Drug (ION-827359 or placebo).

* Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.

4. BMI < 35.0 kg/m^2
5. Patients with a diagnosis of COPD as defined by the American Thoracic Society (ATS)/European Respiratory Society (ERS)
   - Ability to perform acceptable and reproducible spirometry
   - Post-bronchodilator (4 puffs of albuterol) spirometry at Screening demonstrating the following:
     - FEV_1/ forced vital capacity (FVC) ratio of < 0.70
     - $FEV_1 \geq 50\%$ and $\leq 90\%$ of predicted normal
6. Clinically stable COPD in the 4 weeks prior to Screening (Visit 1)
7. Current and former smokers with smoking history of ≥ 20 pack years
8. Meet SGRQ definition of CB (See Section 6.1.1)
9. CAT score ≥ 10

5.2. Exclusion Criteria

1. Clinically significant abnormalities in medical history (e.g., previous acute coronary syndrome within 6 months of screening, congestive heart failure, major surgery within 3 months of Screening) or physical examination
2. Screening laboratory results as follows, or any other clinically significant abnormalities in screening laboratory values that would render a subject unsuitable for inclusion
   - Urine protein/creatinine (P/C) ratio ≥ 0.3 mg/mg. In the event of P/C ratio above this threshold eligibility may be confirmed by a quantitative total urine protein measurement of < 300 mg/24 hr
   - Positive test (including trace) for blood on urinalysis. In the event of a positive test eligibility may be confirmed with urine microscopy showing ≤ 5 red blood cells per high power field
   - alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, alkaline phosphatase (ALP), serum creatinine, blood urea nitrogen (BUN) > 1.5 × upper limit of normal (ULN)
   - Platelet count < LLN
   - Serum potassium > 5.2 mmol/L
   - Estimated GFR < 60 mL/min (as determined by the Cockcroft-Gault Equation for creatinine clearance)
3. Any active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to first day Study Drug product is administered to the patient (Study Day 1)

4. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator
5. Active infection with human immunodeficiency virus (HIV), hepatitis C or hepatitis B
6. Uncontrolled hypertension (blood pressure (BP) > 160/100 mm Hg)
7. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix that has been successfully treated. Patients with a history of other malignancies that have been treated with curative intent and which have no recurrence within 5 years may also be eligible if approved by the PI and reviewed by the Sponsor Medical Monitor
8. Treatment with another investigational drug, biological agent, or device within 1 month of Screening, or 5 half-lives of investigational agent, whichever is longer
9. Previous treatment with an oligonucleotide (including small interfering ribonucleic acid [siRNA]) within 4 months of screening if single dose received, or within 12 months of screening if multiple doses received
10. Clinically important pulmonary disease other than COPD
11. Asthma as a primary or main diagnosis according to the Global Initiative for Asthma (GINA) guidelines (GINA 2011) or other accepted guidelines. Patients with a past medical history of asthma (e.g. childhood or adolescence) may be included
12. Treatment with systemic corticosteroids and/or antibiotics, and/or hospitalization for a COPD exacerbation within 4 weeks prior to enrolment (Visit 1)
13. Acute upper or lower respiratory infection requiring antibiotics or antiviral medication within 4 weeks prior to enrolment (Visit 1)
14. Long term oxygen therapy (LTOT)
15. Patients participating in, or scheduled for, an intensive (active) COPD rehabilitation program (patients who are in the maintenance phase of a rehabilitation program are eligible to take part)
16. Recent history of, or current drug or alcohol abuse
17. Concomitant medication restrictions: Oral anticoagulants, oral steroids (e.g., prednisone or Medrol), theophylline, chronic azithromycin, or roflumilast
18. Have any other conditions, which, in the opinion of the Investigator would make the subject unsuitable for inclusion, or could interfere with the subject participating in or completing the Study
19. A positive PCR test for SARS-CoV-2 at any time prior to randomization

6. STUDY PROCEDURES

6.1. Study Schedule

All required study procedures are outlined in Appendix A, Appendix B and Appendix C.

6.1.1. Screening

Written informed consent for the study will be obtained prior to the performance of any study-related procedures including screening procedures. A 4-week period is provided for completing screening assessments and determining subject eligibility for the study. Safety labs may be re-tested up to 2 additional times for determination of subject eligibility. Patients will also receive a SARS-CoV-2 PCR test. The study site will record basic personal information including name, contact details, gender, height, weight, date of birth, age, ethnicity, and racial origin (to be used only for clinical purposes).

During the Screening Period, subjects will undergo a medical history and physical examination including vital signs, 12-lead ECG, and have blood and urine samples taken for clinical laboratory testing. Chronic obstructive pulmonary disease specific questionnaires (SGRQ, CAT) will be administered and scoring performed on the SGRQ to assess the diagnosis of CB. Spirometry will be performed at Screening including efforts before and 20-30 minutes after inhalation of 4 puffs of albuterol. Plethysmography for determination of lung volumes will also be performed during the Screening Period, as per Section 8.3.4.

The definition of CB by the SGRQ is based on the following 2 questions:

1. Over the last 4 weeks, I have coughed:
    - Almost every day
    - Several days a week
    - A few days a month
    - Only with lung/respiratory infections
    - Not at all
2. Over the last 4 weeks, I have brought up phlegm (sputum):
    - Almost every day
    - Several days a week
    - A few days a month
    - Only with lung/respiratory infections
    - Not at all

In order to be diagnosed as CB the patients must answer that they have cough AND phlegm almost every day or several times a week.

A subset of up to about 60 patients will undergo low dose, HRCT scanning of the lung, with scanning to occur at 2 different lung volumes – FRC and at total lung capacity (TLC). See Section 8.3.5.

A separate subset of up to 18 patients will undergo bronchoscopy, as per Section 8.3.3.

Training for self-administration (by patient or caregiver) of Study Drug will be initiated at Week-1 and will include appropriate use and cleaning of the eFlow® nebulizer. Training will continue at randomization and throughout the Treatment Period.

6.1.2. Baseline Period

One (1) week (7 ± 1 days) prior to randomization the patient will return for the start of the Run-in Period. At this visit the patient will be dispenses an electronic diary and be instructed in its use. The diary is to be used once daily during the Run-in and Treatment Periods.
A minimum of 4 days of diary entry in the 7 days prior to Day 1 is required for the patient to be randomized. The patient will also receive education on the use of a nebulizer and it's cleaning. The patient will be instructed to return in 1 week for the randomization visit (Day 1).

6.1.3. Treatment Period

Eligible patients will be administered Study Drug (ION-827359 or placebo) on a weekly basis for a total of 13 doses. These doses will be administered in the clinic on Days 1, 15, 29, 43, 57, and 85. Doses on Days 8, 22, 36, 50, 64, 71, and 78 will be self-administered by the patient at home. Safety and clinical laboratory evaluations will be performed periodically throughout the Treatment Period (Appendix A, Appendix B and Appendix C). A subset of about 24 patients will undergo additional blood sampling for PK analysis after dosing on Day 1 and Day 85. The subset of patients undergoing HRCT scans will have a follow-up scan performed on Day 92.

Any AEs and concomitant medications will be recorded. All safety data including AEs, BP, and concomitant medications will be reviewed by the Sponsor's Medical Monitor or designee on an ongoing basis throughout the trial.

Prior to each visit (after Screening) the patient's bronchodilators should be withheld as follows:

Table 1: Withhold Time for Bronchodilators Prior to Study Visit:

Bronchodilator	**Withhold Time**
SABA (e.g., albuterol or salbutamol)	6 hours
SAMA (e.g., ipratropium bromide)	12 hours

Patients should be contacted prior to their visits to remind them to withhold medications as above. Patients should be queried at each visit about their concomitant medications, and visits should be rescheduled if these withhold times are not met. The usual dose of bronchodilators should not be taken the morning of the visit, until after spirometry is performed and prior to dosing (if applicable) with the study medication. Albuterol (salbutamol) 2 puffs, should be dosed prior to study drug both for in-clinic and at-home dosing.

Visits should occur at the same time of day as on Day 1 (± 2 hours).

6.1.4. Post-Treatment Period

After the last dose (Study Day 85), or last dose for early termination subjects, subjects will return to the clinic on Study Day 92 for final efficacy assessments. The efficacy assessments on Day 92 include COPD questionnaires (SGRQ and CAT), spirometry, and plethysmography (to be performed in that order). Safety assessments will also be performed at this visit. There will be additional post-treatment study visits on Days 120 and 162, which can occur remotely if necessary. Patients who discontinue from the study during this period should return for a final visit with the procedures as per the schedule for the Day 162 visit. All safety data including AEs, and concomitant medications will be reviewed by the Sponsor's Medical Monitor or designee on an ongoing basis throughout the trial.

6.2. Study/Laboratory Assessments

Laboratory analyte samples will be collected throughout the study. A list of these analytes is contained in Appendix B.

If the platelet value, serum creatinine or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient or missing), a repeat blood specimen should be re-drawn as soon as possible (ideally within 7 days).

6.3. Restriction on the Lifestyle of Subjects

6.3.1. Contraception Requirements

Male subjects must refrain from sperm donation and either be abstinent† or, if engaged in sexual relations with a woman of child-bearing potential (WOCBP), the subject must use a condom and the subject's non-pregnant female partner must use a highly effective contraception method from the time of signing the informed consent form until at least 10 weeks after their last dose of Study Drug. Highly effective contraception for the male subject comprises a vasectomy with negative semen analysis at Follow-up. Highly effective contraception for WOCBP partners of male subjects comprises surgical sterilization (e.g., bilateral tubal occlusion), hormonal contraception associated with inhibition of ovulation (combined estrogen and progestogen containing, or progestogen-only), intrauterine contraception device or intrauterine hormone-releasing system (IUS). Male subjects with partners that are pregnant must use condoms to ensure that the fetus is not exposed to the Study Drug.

For subjects who are exclusively in same sex relationships contraceptive requirements do not apply. If a subject who is in a same sex relationship at the time of signing the ICF becomes engaged in a heterosexual relationship, they must agree to use contraception as described and as outlined in the protocol and ICF.

†**Note:** Abstinence (i.e., refraining from heterosexual intercourse throughout the duration of study participation) is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial and withdrawal are not acceptable methods of contraception.

For the purposes of this study, WOCBP are defined as any female who has experienced menarche, and who does not meet 1 of the following conditions:

- Post-menopausal: 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the post-menopausal range for the laboratory involved
- 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Posthysterectomy

Note: A female condom and a male condom should not be used together as friction between the 2 can result in either or both products failing.

6.3.2. Other Requirements

Patients must refrain from taking strenuous exercise/activity (for example heavy lifting, weight training, intense aerobics classes etc.) for at least 72 hours prior to study visits.

All patients will be required to fast for at least 2 hours before and 1 hour after dosing. Additionally, patients must not smoke 2 hours before and 1 hour after dosing with Study Drug. Furthermore, patients should refrain from smoking within an hour of each spirometry.

7. STUDY DRUG

7.1. Study Drug Description

Study Drug (ION-827359 or Placebo) characteristics are listed in Table 2.

7.1.1. ION-827359

A solution of ION-827359 (25 mg/mL) contained in stoppered glass vials and its storage and preparation instructions will be provided by the Sponsor. ION-827359 must be stored securely at 2-8 °Celsius (C)and be protected from light.

7.1.2. Placebo

Phosphate-buffered saline (PBS) Solution for Inhalation contained in stoppered glass vials will be used as the placebo for this study. Storage and preparation instructions will be provided by the Sponsor. PBS Solution for Inhalation must be stored at 2-8 °C and be protected from light.

Table 2: Study Drug Characteristics

Study Drug	**ION-827359**	**Placebo**
Strength	25 mg/mL	not applicable
Route of Administration	Inhaled via PARI nebulizer	Inhaled via PARI nebulizer

7.2. Packaging and Labeling

The Sponsor will provide the Investigator with packaged Study Drug (ION-827359 or placebo) labeled in accordance with specific country regulatory requirements.

7.3. Study Drug Accountability

The study staff is required to document the receipt, dispensing, and return/destruction of Study Drug (ION-827359 or placebo) supplies provided by the Sponsor. After accountability is complete, the Study Center must either destroy used Study Drug or return all used and unused Study Drug (ION-827359 or placebo) to the Sponsor or designee.

8. TREATMENT OF PATIENTS

8.1. Study Drug Administration

Please refer to the Study Drug Manual provided by the Sponsor for more detailed instructions for Study Drug (ION-827359 or placebo) preparation and administration.

Table 3: Study Drug Dosing Information

Cohort	Volume to Administer	Total Dose
A	1.5 mL	37.5 mg or placebo
B	3.0 mL	75 mg or placebo

8.2. Other Protocol-Required Drugs

Per eligibility criteria, patients must be on a stable regimen of medications to treat their COPD and will continue on these medications throughout the trial. The study nebulizer (Pari eFlow®) is only for use with the study medication and no other medications should be administered via this nebulizer.

8.3. Protocol-Required Treatment Procedures

8.3.1. Spirometry

Spirometry assessments will be made in accordance with ATS/ERS guidelines (Graham et al. 2019). At all time points, 3 technically acceptable measurements should be made and recorded. Spirometry assessments may be performed up to 8 times to obtain 3 acceptable readings according to ATS guidelines (Graham et al. 2019). The highest FEV_1 and FVC readings from each assessment will be used for analysis even if the FEV_1 and FVC values come from 2 different forced exhalations. The baseline values are those performed immediately (within 30 minutes) prior to inhalation of study medication. The reference values will be that of GLI 2012. Spirometry will be performed by a central vendor who will perform quality control on spirometry efforts.

8.3.2. Diffusing Capacity (DLCO)

Diffusing capacity will be performed during the Screening Period. Assessments will be made in accordance with ATS/ERS guidelines, including the use of appropriate quality control measures to minimize data variability (Macintyre et al. 2005). The average of at least 2 acceptable tests will be reported, with a maximum of 5 attempts. There should be a minimum of 4 minutes between tests. The report will also include the percent of predicted DLCO (Crapo and Morris 1981), and the DLCO corrected for alveolar volume (V_A). A DLCO corrected for haemoglobin will be calculated using the standard equation: DLCO, predicted for Hb = DLCO, predicted x. (1.7Hb/(10.22 + Hb)).

[redacted]

8.3.4. Plethysmography

Lung volumes will be determined by plethysmography during the Screening Period and again at Day 92. The technique for determination of lung volumes will be made in accordance with ATS/ERS guidance (Wanger et al. 2005). The lung volumes will include the FRC, or the amount of gas present in the lung at end-expiration during tidal breathing, and the RV, or the volume of gas remaining in the lung after maximal exhalation. For quality control, the mouth pressure transducer should be physically calibrated daily prior to use.

8.3.5. HRCT

A subset of 60 patients will undergo HRCT of their lungs during Screening and on Day 92. Each site performing these scans will be individually trained in the scanning protocol, sending images to the central vendor, and monitoring patients breathing during the scan. The computed tomography (CT) scans will be performed at both TLC and FRC. The following parameters will be calculated for each patient:

- Lung and Lobar Volume at TLC and FRC
- Specific Airway Volume at TLC and FRC
- Specific Airway Resistance at TLC and FRC
- Blood vessel % and density on lobar level at TLC
- Internal Airflow Distribution based on lobar expansion from FRC to TLC
- Image based ventilation/perfusion (V/Q)
- Airway Wall Volume at TLC
- Emphysema score at TLC
- Air Trapping at FRC
- Aerosol deposition from FRC to TLC

8.3.6. Sputum Collection

Patients will be encouraged to produce spontaneous sputum which will be collected from patients during the Screening Period, and in the mornings prior to study visits on Days 1, 29, 43, 85, 92, and 162. The sputum will be frozen at the study site and shipped on dry ice to a central laboratory. The laboratory manual will provide further instructions.

8.3.7. SGRQ

The SGRQ is a patient completed, disease-specific instrument designed to measure impact on overall health, daily life, and perceived well-being in patients with obstructive airways disease. This study will utilize the shorter 40-item version (SGRQ-C) which does not specify a Recall Period and has been validated specifically for COPD patients. Scores of the SGRQ-C range from 0 to 100, with higher scores indicating more limitations.

8.3.8. COPD Assessment Test (CAT)

The CAT is an eight-item patient completed questionnaire designed to quantify the impact of COPD symptoms on the health status of patients. The CAT is easy for patients to complete and provides a score of 0–40 to indicate the impact of disease. In clinically stable COPD patients, the CAT was found to be closely related to the St George's Respiratory Questionnaire. The CAT score can also provide a reliable measure of exacerbation severity in COPD patients (Jones et al. 2011).

8.3.9. EXACT – Respiratory Symptoms (E-RS)

The EXACT –E-RS scale is a patient-reported outcome (PRO) measure designed to measure the symptoms of patients with COPD. The E-RS utilizes 11 respiratory symptom items from the existing and validated 14-item EXACT, which measures symptoms of exacerbation. The E-RS total score quantifies respiratory symptom severity, and 3 domains assess breathlessness, cough and sputum, and chest symptoms (Leidy et al. 2014). The E-RS will be collected on the daily e-diary, which will include all 14 items from the EXACT questionnaire.

8.4. Treatment Precautions

Patients should be dosed in a different room from any other patient participating in the study.

8.5. Safety Monitoring Rules

Please refer also to the 'Guidance for Investigator' section of the Investigator's Brochure.

For the purposes of Safety Monitoring Baseline is defined as:

- Monitoring Rules for Liver Chemistry Tests – Baseline is defined as the average of Screening and Day 1

In addition to the standard monitoring of clinical safety parameters, the following guidelines are provided for the monitoring of selected parameters chosen based on preclinical and clinical observations.

Confirmation Guidance: At any time during the study (Treatment or Post-Treatment Periods), the initial clinical laboratory results meeting the safety monitoring criteria presented below **must be confirmed** by performing measurements (ideally in the same laboratory that performed the initial measurement) on new specimens. All new specimen collections should take place as soon as possible (ideally within 3 days of the initial collection). For stopping rules, if the initial laboratory result is observed during the Treatment Period, the results from the retest **must be available** prior to administering the next dose of Study Drug (ION-827359 or placebo).

Re-dosing Guidance: Patients with initial laboratory test values that reach a stopping rule must not be re-dosed until the re-test results are available. In general, patients who do not meet the stopping rules based upon retest may continue dosing. However, the Investigator and the Sponsor Medical Monitor (or appropriately qualified designee) should confer as to whether additional close monitoring of the subject is appropriate. If any of the stopping criteria described below (refer to Sections 8.6.1 to 8.6.4 are met, the subject will be permanently discontinued from further treatment with Study Drug (ION-827359 or placebo), evaluated fully as outlined below and in consultation with the Sponsor Medical Monitor or appropriately qualified designee, and will be followed up in accordance with Section 8.8 of the protocol.

8.5.1. Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," issued by the U.S. Department of Health and Human Services, Food and Drug Administration, July 2009. For a definition of Baseline, please refer to guidance in Section 8.5 above.

In the event of an ALT or AST measurement that is > 3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was > ULN) at any time during the study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed as described above. Additional, confirmatory measurements should also be performed if ALT or AST levels increase to 5 × ULN.

Frequency of Repeat Measurements: Patients with confirmed ALT or AST levels > 3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was > ULN) should have their liver chemistry tests (ALT, AST, ALP, international normalized ratio [INR] and total bilirubin) retested at least once-weekly until ALT and AST levels become ≤ 1.2 × ULN or 1.2 × baseline value if the baseline value was > ULN.

Further Investigation into Liver Chemistry Elevations: For patients with confirmed ALT or AST levels > 3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was > ULN), the following evaluations should be performed:

- Obtain a more detailed history of symptoms and prior and concurrent diseases
- Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Obtain a history for exposure to environmental chemical agents and travel
- Serology for viral hepatitis (hepatitis A virus (HAV) IgM, HBsAg, hepatitis C virus (HCV) antibody, Cytomegalovirus (CMV) immunoglobulin M (IgM), and EBV antibody panel)
- Serology for autoimmune hepatitis (e.g., antinuclear antibody [ANA])

Additional liver evaluations, including gastroenterology/hepatology consultations, hepatic CT or magnetic resonance imaging (MRI) scans, may be performed at the discretion of the Investigator, in consultation with the Sponsor Medical Monitor. Repetition of the above evaluations should be considered if a subject's ALT and/or AST levels reach 5 × ULN.

For a definition of Baseline, please refer to guidance in Section 8.5.

8.5.2. Safety Monitoring Rules for Platelet Count Results

Platelet count will be monitored at least every 4 weeks during the Treatment Period. The Investigator should review all platelet count results within 48 hours of receipt. If a patient's platelet count falls to 100,000/mm^3 or less, then the patient's platelet counts should be monitored weekly. In case of platelet reduction to below 75,000/mm^3, the platelet monitoring rule defined in Stopping rules (Section 8.6.3) should be followed.

In the event of a platelet count < 75,000/mm^3, additional laboratory investigations should be conducted (Table 4).

Table 4: Additional Labs to be Performed in the Event of a Platelet Count < 75,000/mm^3

Peripheral smear (should be performed locally, fixed and sent to central lab for review)
Fibrinogen split products or D-dimer on fresh blood
Citrated sample for platelets
Coagulation panel (prothrombin time [PT]/INR, activated partial thromboplastin time [aPTT])
CBC with reticulocytes and mean platelet volume (MPV)
Serum B12 and folate
Fibrinogen
von Willebrand factor
Total globulins, total IgA, IgG and IgM
Complement: total C3, total C4, Bb, C5a
CRP measured by high sensitivity assay (hsCRP)
Serology for:
hepatitis B virus (HBV), HCV, HIV (if not done for screening)
Rubella
CMV
EBV
Parvo B19
Helicobacter pylori (IgG serum test)
Auto-antibody screen:
Antiphospholipid
Rheumatoid factor
Anti-dsDNA
Anti-thyroid
To Be Performed at Specialty Lab(s):
Antiplatelet antibodies and Anti-PF4 assay
Anti-ASO antibody

Note: The above labs may change as additional data is assessed, and sites will be updated regarding any changes.

8.6. Stopping Rules for Liver Chemistry Elevations

For the purposes of the stopping rules, Baseline is defined as:

- Insert a description of what will constitute the Baseline for each of the tests that will be used in the stopping rules. This does not need to be the same definition as will be used in the statistical testing, but it is anticipated that in the same definition will be used wherever possible to avoid confusion
- Monitoring rules for liver chemistry tests – Baseline is defined as average of Screening and Day 1
- Temporary stopping rules for renal function tests – Baseline is defined as average of Screening and Day 1
- Stopping rules for platelets – Baseline is defined as the last non-missing value prior to the first dose

8.6.1. Stopping Rules for Liver Chemistry Elevations

In the event of laboratory results meeting the following criteria, **and the event is without an alternative explanation as discussed with the Sponsor Medical Monitor,** dosing of a subject with Study Drug (ION-827359 or placebo) will be stopped permanently; values that are not confirmed due to failure to retest or missing lab values will be presumed confirmed:

1. ALT or AST > 8 × ULN, which is confirmed
2. ALT or AST > 5 × ULN, which is confirmed and persists for ≥ 2 weeks
3. ALT or AST > 3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was > ULN), which is confirmed **and** total bilirubin > 2 × ULN or INR > 1.5
4. ALT or AST > 3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was > ULN), which is confirmed, **and** the new appearance (i.e., onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (> ULN)

8.6.2. Stopping Rules for Renal Function Test Results / Temporary Stopping Rules for Renal Function Test Results

In the event of laboratory results for either of the following criteria, dosing of a subject with Study Drug (ION-827359 or placebo) will be stopped permanently:

1. Confirmed serum creatinine increase that is both ≥ 0.5 mg/dL (26.5 µmol/L) and ≥ 40% above Baseline creatinine values (refer to definition of Baseline in Section 8.6)
2. Proteinuria, dipstick 2 + (confirmed by dipstick retest and then further confirmed by a quantitative total urine protein measurement of > 1.0 g/24 hour)

The follow-up schedule for any events meeting either of these stopping criteria will be determined by the Investigator in consultation with the Sponsor Medical Monitor or designee.

In the event of a persistent elevation that is observed over 2 consecutive weeks, for either of the 2 criteria below, dosing of a patient with Study Drug (ION-827359 or placebo) may be stopped temporarily:

1. Serum creatinine increase that fulfills all of the following criteria: ≥ 0.5 mg/dL (26.5 µmol/L) and ≥ 40% above Baseline creatinine values and > ULN (refer to definition of Baseline in Section 8.6)
2. Proteinuria, dipstick 2 + (confirmed by dipstick retest and then further confirmed by a quantitative total urine protein measurement of > 1.0 g/24 hour)

The possible dosing re-initiation or follow-up schedule for any events meeting either of these criteria will be determined by the Investigator **in consultation with** the Sponsor Medical Monitor or designee.

8.6.3. Stopping Rule for Platelet Count Results

In the event of a confirmed platelet count less than 75,000/mm^3, dosing of a subject with Study Drug (ION-827359 or placebo) will be stopped permanently. The platelet count should be tested weekly until it is above 100,000/mm^3 then subsequent monitoring should be per the schedule of procedures.

8.6.4. Stopping Rule for Serum Potassium

In the event of a laboratory result demonstrating a severe elevation of serum potassium (> 5.6 mmol/L) at any time point, which was confirmed on reanalysis, dosing of the subject will be stopped permanently unless there is a clear etiology explaining the observed increase.

8.7. Adjustment of Dose and/or Treatment Schedule

There is no adjustment of dose and/or treatment schedule.

8.8. Discontinuation of Study Drug/Treatment

A subject must permanently discontinue study treatment for any of the following:

- The subject becomes pregnant. Report the pregnancy according to instructions in Section 9.5.4
- The subject withdraws consent
- The subject experiences an AE that necessitates permanent discontinuation of Study Drug
- The subject develops laboratory test abnormalities that meet any of the stopping rules listed in Sections 8.6.1 to 8.6.3
- The subject experiences an AE that necessitates unblinding of the Investigator to the subject's treatment assignment
- Subject has PCR test positive for SARS-CoV-2

The reason for discontinuation of Study Drug must be recorded in the electronic Case Report Form (eCRF) and source documentation.

Patients who discontinue Study Drug will remain in the study and continue protocol required tests and assessments will enter the Post-Treatment Period unless consent is withdrawn. For patients withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination of study procedures and observations at the time of withdrawal (see Appendix A) and ideally within 2 weeks from the last dose of Study Drug. If the patient declines or is unable to participate in the above, the Investigator should clarify what type of follow-up the subject is agreeable to: in person, by phone/mail, through family/friends, via correspondence/communication with other physicians, and/or from review of the medical records. Wherever possible these patients should continue to be followed up via the agreed means to collect information on AEs, concomitant medications and survival status. At the very least, the patient's status at the end of the protocol defined Study Period should be ascertained and documented wherever possible. The agreed means of follow-up will be documented in the patient records and notified to the Sponsor.

8.9. Withdrawal of Patients from the Study Procedures

Patients must be withdrawn from study procedures for any of the following:

- Withdrawal of consent
- The subject is unwilling or unable to comply with the protocol

Other reasons for withdrawal of patients from study procedures might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from study, must be recorded in the eCRF as appropriate.

Any subject who withdraws consent to participate in the study will be removed from further treatment and study observation immediately upon the date of request. These patients should be encouraged to complete the early termination study procedures and observations at the time of withdrawal (Appendix A).

8.10. Concomitant Therapy and Procedures

The use of concomitant therapies or procedures defined below must be recorded on the subject's eCRF. Adverse events related to administration of these therapies or procedures must also be documented on the appropriate eCRF.

8.10.1. Concomitant Therapy

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications and vitamin supplements) administered between Screening and the last visit (Visit 11).

Allowed Concomitant Therapy

Any other medications deemed necessary by the Investigator are allowed except those listed in the disallowed concomitant therapy.

Disallowed Concomitant Therapy

The following are disallowed concomitant therapies:

- Oral corticosteroids
- Theophylline
- Roflumilast
- See Table 1, Section 6.1.3 for bronchodilator use prior to visits

8.10.2. Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between Screening and last study visit (Visit 11).

8.11. Treatment Compliance

Compliance with treatment dosing is to be monitored and recorded in the eCRF by Study Center staff.

Patients will record treatment in a dosing diary that will be reviewed periodically by Study Center staff and the Clinical Monitor.

9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING

9.1. Sponsor Review of Safety Information

Safety information will be collected, reviewed, and evaluated by the Sponsor or designee in accordance with the applicable Ionis and/or designee SOPs throughout the conduct of the clinical trial.

9.2. Regulatory Requirements

The Sponsor or designee is responsible for regulatory submissions and reporting to the Investigators of SAEs including SUSARs per the International Conference on Harmonization (ICH) guidelines E2A and ICH Good Clinical Practice (GCP). Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

Institutional Review Boards (IRB)/Independent Ethics Committees (IEC) will be notified of any SAE according to applicable regulations.

In addition to the Investigator's assessment of relatedness, the Sponsor or designee will evaluate the available information and perform an independent assessment of all reported SAEs and determine if there is a reasonable possibility that the Study Drug (ION-827359 or placebo) is causally related to a reported SAE. While the Sponsor may upgrade an Investigator's decision it

is not permissible to downgrade the Investigator's opinion for the purposes of determining whether the SAE meets the definition of a SUSAR.

The Sponsor or designee will evaluate the available information for all reported SAEs and decide if there is a reasonable possibility that the Study Drug (ION-827359 or placebo) caused the AE and, therefore, meets the definition of a SUSAR.

Appropriate personnel at the Sponsor or designee will unblind SUSARs for the purpose of regulatory reporting. The Sponsor or designee will submit SUSARs to Regulatory Agencies in blinded or unblinded fashion according to local law. The Sponsor or designee will submit SUSARs to Investigators in a blinded fashion.

For the purpose of regulatory reporting of SUSARs, there are no "expected" AEs in this study population. For Study Drug (ION-827359 or placebo) "expected" AEs, refer to the Investigator's Brochure.

The Sponsor or designee will monitor these protocol-specified SAEs using the incidence rate of the event compared to the rate expected in a non-Study Drug exposed population. If the aggregate analysis indicates that an event is occurring more frequently or at a greater severity than expected, then the event will be reported.

SAEs will be evaluated on a case by case basis to determine whether there is a reasonable possibility that the drug caused the event. If the event is considered by the Sponsor or designee to meet the definition of a SUSAR, the event will be reported.

9.3. Definitions

9.3.1. Adverse Event

An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of medicinal (investigational) product, whether or not the AE is considered related to the medicinal (investigational) product.

An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (e.g., headache) not present at Baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from Study Drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

9.3.2. Adverse Drug Reaction and Suspected Unexpected Adverse Drug Reaction

Adverse Drug Reaction (ADR)

In the *pre-approval* clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not have been established, ADR is defined as follows:

> All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.

The phrase "responses to a medicinal product" means that a causal relationship between the medicinal product and the AE has been determined by the Sponsor as at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Suspected Unexpected Adverse Drug Reaction

A suspected unexpected ADR is any ADR, the nature or severity of which is not consistent with the applicable product information, e.g., Investigator's Brochure for an unapproved medicinal (investigational) product.

A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

9.3.3. Serious Adverse Event (SAE)

A SAE is any AE that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in a congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE]; OR Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials; the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

9.4. Monitoring and Recording Adverse Events

Any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible. Before a diagnosis is confirmed, all symptoms should be reported as separate AEs.

9.4.1. Serious Adverse Events

In the interest of subject safety, and in order to fulfill regulatory requirements**,** all SAEs (regardless of their relationship to Study Drug) should be reported to the Sponsor or designee within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the subject signs the informed consent form and stop at the end of the subject's Follow-up Period which is defined as the last study visit (Visit 11). When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message. SAEs should be reported using an electronic SAE submission form whenever possible. In situations where the electronic SAE submission is unavailable, an Initial SAE Form should be completed and a copy should be faxed or emailed to the Sponsor or designee. The SAE reporting instruction, including the fax number and email address can be found in the Investigator site file for the study.

Detailed information should be actively sought and included as Follow-Up as soon as additional information becomes available. All SAEs will be followed until resolution. Serious Adverse Events that remain ongoing past the subject's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and Sponsor agree the subject's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

9.4.2. Non-Serious Adverse Events

The recording of non-serious AEs will begin after the subject signs the informed consent form and will stop at the end of the subject's Follow-up Period, which is defined as Visit 11. The Investigator will monitor each subject closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

9.4.3. Evaluation of Adverse Events (Serious and Non-Serious)

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form.

9.4.3.1. Relationship to the Study Drug

The event's relationship to the Study Drug (ION-827359 or placebo) is characterized by 1 of the following:

- **Related:** There is clear evidence that the event is related to the use of Study Drug, e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug (ION-827359 or placebo) administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug (ION-827359 or placebo) administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)
- **Not Related:** The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

9.4.3.2. Severity

The severity of AEs and SAEs relating to laboratory tests and AEs at the injection site will be graded based on criteria from the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Sept 2007 (refer to Appendix D). Any AE not listed in Appendix D will be graded as follows:

- **Mild:** The event is easily tolerated by the subject and does not affect the subject's usual daily activities
- **Moderate:** The event causes the subject more discomfort and interrupts the subject's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the subject's usual daily activities

The severity of AEs and SAEs relating to laboratory tests and AEs at the injection site will be graded based on criteria from the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017 (refer to Appendix D). Any AE not listed in Appendix D will be graded as follows:

- **Mild:** The event is easily tolerated by the subject and does not affect the subject's usual daily activities
- **Moderate:** The event causes the subject more discomfort and interrupts the subject's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the subject's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in Section 9.3.3).

9.4.3.3. Action Taken with Study Drug

Action taken with Study Drug (ION-827359 or placebo) due to the event is characterized by 1 of the following:

- **None:** No changes were made to Study Drug (ION-827359 or placebo) administration and dose
- **Not Applicable:** SAE/AE was reported during Screening Period prior to Study Drug administration
- **Permanently Discontinued:** Study Drug was discontinued and not restarted
- **Temporarily Interrupted, Restarted – Same Dose:** Dosing and/or dosing frequency was temporarily interrupted/changed or delayed due to the AE and restarted at the same dose
- **Reduced Dose:** Dosing was reduced, temporarily interrupted or delayed due to the AE and restarted at the next lower dose or reduced dosing frequency

9.4.3.4. Treatment Given for Adverse Event

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form. Treatment should also be recorded on the concomitant treatment or ancillary procedures eCRF, as appropriate.

9.4.3.5. Outcome of the Adverse Event

If the event is a non-serious AE, then the event's outcome is characterized by 1 of the following:

- **AE Persists:** Patient terminates from the trial and the AE continues
- **Recovered:** Patient recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity (if applicable):** AE severity changed

If the event is an SAE, then the event's outcome is characterized by 1 of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Patient has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE eCRF (the SAE resolution date should be entered as the date of onset of that AE)
- **Recovered:** Patient recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Recovered with Sequelae:** The signs/symptoms of the reported SAE have improved but not completely resolved, and a new baseline for the subject is established since full recovery is not expected
- **Fatal:** Patient died (the date of death should be entered as the SAE resolution date)
- **Unknown:** The outcome of the reported SAE is not available, e.g., patient is lost to follow-up

9.4.3.6. Follow-up of Adverse Event

Investigator Follow-Up

During the Study Period, the Investigator should follow each AE until the event has resolved to baseline grade or better, the event is assessed as stable, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to Study Drug or related to study procedures until a final outcome can be reported.

Resolution of AE (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification.

Investigator should follow-up, or support the Sponsor's effort to follow up with all pregnancies reported during the study from either the study subject or the female partner of male study subject until pregnancy outcome is available.

Sponsor Follow-Up

For SAEs, adverse event of special interest (AESI) and pregnancy cases in patients who have completed or terminated study, the Sponsor or a designee should follow -up by telephone, fax, email, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

9.5. Procedures for Handling Special Situations

9.5.1. Abnormalities of Laboratory Tests

Clinically significant (CS) abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. CS abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Sponsor Medical Monitor. Laboratory abnormalities deemed not clinically significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet. If the platelet value, serum creatinine or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing a repeat blood specimen should be re-drawn as soon as possible (ideally within 7 days)

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents and should include the Investigator's assessment of clinical significance of out of range/abnormal laboratory values.

9.5.2. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

9.5.3. Dosing Errors

Study Drug (ION-827359 or placebo) errors (including overdose, underdose, and administration error) should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional.

Dosing details should be captured on the Dosing Case Report Form. If the subject takes a dose of Study Drug (ION-827359 or placebo) that exceeds protocol specifications and the subject is symptomatic, then the symptom(s) should be documented as an AE and be reported per Section 9.4.

An overdose is the accidental or intentional use of a drug in an amount higher than the dose being studied. An overdose or incorrect administration of study treatment is not itself an AE, but it may result in an AE. All AE associated with an overdose or incorrect administration of Study Drug should be recorded on the AE eCRF. If the associated AE fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event).

Should an overdose occur, the Investigator or designee should refer to the Guidance to Investigator's section of the Investigator's Brochure and contact the Sponsor or designee within 24 hours.

9.5.4. Contraception and Pregnancy

Patients must continue to use appropriate contraception with their partners, or refrain from sexual activity, as described in Section 6.3.1.

If a subject becomes pregnant or a pregnancy is suspected, or if a male subject makes or believes that he has made someone pregnant during the study, then the Study Center staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported by designating as 'Follow-up' on the Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the subject's responsibility.

Female patients: If a suspected pregnancy occurs while on the study (including follow-up), a pregnancy test will be performed. The subject with a confirmed pregnancy will be immediately withdrawn from treatment with Study Drug. However, the subject will be encouraged to complete the post-treatment follow-up portion of the study to the extent that study procedures do not interfere with the pregnancy. Regardless of continued study participation, the study physician will assist the subject in getting obstetrical care and the progress of the pregnancy will be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, the Study Center and Sponsor may require access to the mother and infant's medical records to obtain additional information relevant to the pregnancy progress and outcome**.** A longer follow-up may be required if a newborn child experiences a medical condition. Follow-up will be performed to the extent permitted by the applicable regulations and privacy considerations; e.g., pregnancy ICF may be required.

Male patients: The progress of the pregnancy of a male subject's partner should be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, **the Study Center and Sponsor may follow-up with the mother and may request access to the mother and infant's medical records** to obtain additional information relevant to the pregnancy progress and outcome. A longer follow-up may be required if a newborn child experiences a medical condition. Follow-up will be performed to the extent permitted by the applicable regulations and privacy considerations; e.g., partner ICF may be required.

10. STATISTICAL CONSIDERATIONS

10.1. Stratification, Subsets, and Covariates

There are 3 subgroups: PK, HRCT and Bronchoscopy subgroups. Details of the prespecified subgroup analyses will be provided in the Statistical Analysis Plan (SAP).

In general, the covariates will include baseline measurements (where applicable). The details will be provided in Section 10.6.3.

10.2. Sample Size Considerations

The sample size assumptions for the primary endpoint include:

- The increase in FEV_1 from Baseline to the primary time point in the ION-827359 treatment group is 100 mL; no change in the placebo group
- Standard deviation of change in FEV_1 is 220 mL
- Significance level (alpha) of 0.05 (2-sided test)

With the above assumptions, a sample size of 174 patients (58 patients in each ION-827359 treatment groups and 29 patients in each placebo group) will provide a power of at least 80% for the primary comparison (pooled ION-827359 treatment groups vs. pooled placebo groups). Approximately 180 patients will be enrolled in this trial to account for a 3% dropout rate.

10.3. Populations

Full Analysis Set (FAS): all randomized patients who received at least 1 dose of Study Drug (ION-827359 or placebo) and who have at least 1 post-Baseline efficacy assessment (i.e., post-Baseline FEV_1 assessment, E-RS score, CAT score, SGRQ-C score, or post-bronchodilator FEV_1 assessment).

Per Protocol Set (PPS): a subset of FAS who have received at least 9 doses of Study Drug and have no significant protocol deviations that would be expected to affect efficacy assessments.

Safety Set: All patients who are randomized and receive at least 1 dose of Study Drug.

PK Population: All patients who are randomized and receive at least 1 dose of Study Drug.

10.4. Definition of Baseline

For all assessments, baseline will be defined as the last non-missing measurement prior to the first Study Drug administration, except for liver chemistry which is an average of the values at Screening and Day 1.

10.5. Interim Analysis and Multiplicity

No interim analyses are planned.

10.6. Planned Methods of Analysis

All eCRF data, lab data transfers, and any outcomes derived from the data will be provided in the patient data listings. Patient data listings will be presented for all patients enrolled into the study. Descriptive summary statistics including n, mean, median, standard deviation, standard error, interquartile range (25^{th} percentile, 75^{th} percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data by treatment group. Where appropriate, p-values will be reported. All primary and secondary efficacy endpoints will be assessed on the FAS and PPS, with the former being the basis for the primary efficacy analysis. All safety assessments will be performed on the Safety Set. The PK analyses will be conducted in the PK population.

The 2 placebo groups will be pooled and analyzed as a single placebo group.

10.6.1. Demographic and Baseline Characteristics

Demographic and Baseline characteristics will be summarized using descriptive statistics by treatment group. Patient randomization will be summarized by treatment group. The patient disposition will be summarized. All patients enrolled will be included in a summary of patient disposition.

10.6.2. Safety Analysis

Treatment duration and amount of Study Drug (ION-827359 or placebo) received will be summarized by treatment group.

All treatment emergent AEs, all treatment emergent AEs potentially related to Study Drug, all treatment emergent serious AEs, and all treatment emergent serious AEs potentially related to

Study Drug (ION-827359 or placebo) will be summarized for each treatment group using the Medical Dictionary for Regulatory Activities (MedDRA™) coding system, by system organ class, preferred term, relationship to Study Drug, and severity. Narratives of deaths, SAEs, including early withdrawals from treatment and from study due to AE, will also be provided.

Laboratory tests to ensure subject safety including chemistry panel, complete blood count with differential, coagulation panel, etc., will be summarized by study visits for each treatment group. These safety variables will also be presented as change and percent change from Baseline over time after Study Drug administration, as appropriate.

Vital sign and ECG measures will be tabulated by treatment group. In addition, the number of patients who experience abnormalities in clinical laboratory evaluations will be summarized by treatment group.

10.6.3. Efficacy Analysis

10.6.3.1. Analysis of Primary Endpoint

The primary analysis of the primary endpoint is to compare the change from Baseline to the primary time point (defined as the average of Weeks 13 and 14) in FEV_1 between the pooled ION-827359 treatment group (ION-827359 37.5 mg and ION-827359 75 mg) and the pooled placebo group using the Mixed Effects Model with Repeated Measures (MMRM) model. The response variable is the change from Baseline at post-Baseline visit up to the primary time point. The MMRM model will include effects of treatment (ION-827359 or placebo), time (categorical), treatment-by-time interaction, and Baseline value. The primary analysis will be conducted in the FAS. The null hypothesis of the primary analysis is that the change from Baseline to the primary time point in FEV_1 is the same between the pooled ION-827359 and pooled placebo treatment groups. The alternative hypothesis is that the change from Baseline to the primary time point in FEV_1 is different between the pooled ION-827359 and pooled placebo treatment groups.

The primary analysis described above will be repeated in the PPS as a sensitivity analysis. The secondary analyses of the primary endpoint will include the comparison of each individual ION-827359 treatment group to the pooled placebo group in both FAS and PPS. The data will be analyzed using the same method as the primary analysis.

The exploratory analyses of the primary measurement will include the change from Baseline to each individual time point other than the primary time point.

10.6.3.2. Analysis of Secondary Endpoints

The secondary endpoints include change from Baseline to the primary time point in the E-RS total and subscale scores, change from Baseline to Week 14 in CAT score, SGRQ-C total and component scores, change from Baseline to the primary time point in post-bronchodilator FEV_1. The primary analysis of each secondary endpoint is the comparison between the pooled ION-827359 treatment group (ION-827359 37.5 mg and ION-827359 75 mg) and the pooled placebo group in the FAS. The analyses will be repeated in PPS as sensitivity analyses.

The secondary analyses of each secondary endpoint will include the comparison of each individual ION-827359 treatment group to the pooled placebo group in both FAS and PPS.The data will be analyzed using a similar method to the primary endpoint.

The exploratory analyses of the secondary measurements will include the change from Baseline to each individual time point other than the primary time point or Week 14.

10.6.4. Pharmacokinetic Analysis

The plasma PK of ION-827359 will be assessed in subjects from the PK subgroup following inhalational administration by nebulization. Non-compartmental PK analysis of ION-827359 will be carried out on each individual subject data set. The maximum observed drug concentration (C_{max}) and the time taken to reach maximal concentration (T_{max}) will be obtained directly from the concentration-time data. Partial areas under the plasma concentration-time curve from zero time (pre-dose) to selected times (t) after the inhalational administration (AUC_t) will be calculated using linear-up log-down trapezoid method. Plasma clearance (CL/F) will be calculated by dose/AUC_t, where appropriate. The plasma half-life ($t_{½}\lambda_z$) associated with the apparent terminal elimination will be calculated if data permits, from the equation, $t_{½}\lambda_z = 0.693/\lambda_z$, where λ_z is the rate constant associated with the apparent terminal elimination phase.

Other PK parameters, as appropriate, may be determined or calculated at the discretion of the PK scientist.

Plasma PK parameters will be summarized using descriptive statistics. Additional details regarding the PK analysis will be described in the SAP.

Potential relationships between selected PD and PK measures may also be explored, where deemed appropriate.

10.6.5. Additional Analyses

The exploratory and PD analyses include comparison of change from Baseline in total sputum mucin, MUC5AC, MUC5B levels, FRC and RV, regional lung volumes as measured by HRCT, and ENaC mRNA analysis from bronchial brushings between ION-827359 and placebo groups. Cytokines will also be analyzed from the BAL as in Appendix B. Details of these analyses will be provided in the SAP.

11. INVESTIGATOR'S REGULATORY OBLIGATIONS

11.1. Informed Consent

The written informed consent document should be prepared in the language(s) of the potential patient population, based on an English version provided by the Sponsor or designee.

Before a subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the subject after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific Screening procedures or any Study Drug (ION-827359 or placebo) are administered. The subject must be given sufficient time to consider whether to participate in the study.

The acquisition of informed consent and the subject's agreement or refusal to notify his/her primary care physician should be documented in the subject's medical records and the informed consent form should be signed and personally dated by the subject or a legally acceptable representative and by the person who conducted the informed consent discussion (not necessarily an Investigator). The original signed informed consent form should be retained in the Study Master File and in any other locations required by institutional policy, and a copy of the signed consent form should be provided to the subject or legally acceptable representative.

If a potential subject is illiterate or visually impaired and does not have a legally acceptable representative, the Investigator must provide an impartial witness to read the informed consent form to the subject and must allow for questions. Thereafter, both the subject or legally acceptable representative and the witness must sign the informed consent form to attest that informed consent was freely given and understood.

11.2. Ethical Conduct of the Study

All applicable regulations and guidelines of current GCP as well as the demands of national drug and data protection laws and other applicable regulatory requirements must be followed.

11.3. Independent Ethics Committee/Institutional Review Board

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material must be submitted to the IEC/IRB for written approval. A copy of the written approval of the protocol and informed consent form must be received by the Sponsor or designee before recruitment of patients into the study and shipment of Study Drug.

A copy of the written approval of any other items/materials that must be approved by the Study Center or IEC/IRB must also be received by the Sponsor or designee before recruitment of patients into the study and shipment of Study Drug. The Investigator's Brochure must be submitted to the IEC/IRB for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the IEC/IRB for all subsequent protocol amendments and changes to the informed consent document. The Investigator should notify the IEC/IRB of deviations from the protocol in accordance with ICH GCP. The Investigator should also notify the IEC/IRB of SAEs occurring at the Study Center and other AE reports received from the Sponsor or designee, in accordance with local procedures.

The Investigator will be responsible for obtaining annual IEC/IRB approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all IEC/IRB submissions and the IEC/IRB continuance of approval must be sent to the Sponsor or designee.

11.4. Subject Confidentiality

The Investigator must ensure that the subject's confidentiality is maintained. On the case report forms or other documents submitted to the Sponsor or designee, patients should be identified by initials (if permitted by local law) and a subject identification number only. Documents that are

not for submission to the Sponsor or designee (e.g., signed informed consent forms) should be kept in strict confidence by the Investigator.

In compliance with Federal and local regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IEC/IRB direct access to review the subject's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The Investigator is obligated to inform and obtain the consent of the subject to permit named representatives to have access to his/her study-related records without violating the confidentiality of the subject.

12. ADMINISTRATIVE AND LEGAL OBLIGATIONS

12.1. Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor or designee. Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent document. The regulatory authority and IEC/IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the patients or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the IEC/IRB to the Sponsor or designee.

12.2. Study Termination

The Sponsor or designee reserves the right to terminate the study. The Investigator reserves the right to terminate their participation in the study, according to the terms of the site contract. The Investigator/Sponsor or designee should notify the IEC/IRB in writing of the trial's completion or early termination and send a copy of the notification to the Sponsor or designee.

12.3. Study Documentation and Storage

An eCRF utilizing an Electronic Data Capture (EDC) application will be used for this study.

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the subject's case report form data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, imaging, and correspondence.

The Investigator and Study Center staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with ICH GCP, suitable for inspection at any time by representatives from the Sponsor or designee and/or applicable regulatory authorities. Elements should include:

- Subject files containing completed case report forms, informed consents, and supporting copies of source documentation

- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the IEC/IRB and the Sponsor or designee
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction, final Study Drug product reconciliation, and all drug-related correspondence

In addition, all original source documents supporting entries in the case report forms must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor or designee and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor or designee.

12.4. Study Monitoring

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., case report forms and other pertinent data) provided that subject confidentiality is respected.

The Sponsor monitor or designee is responsible for inspecting the case report forms at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the case report forms.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing case report forms, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department (or designees). Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data a clinical data management review will be performed on subject data received by the Sponsor or designee. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor or designee.

The Principal Investigator will sign and date the indicated places on the case report form. These signatures will indicate that the Principal Investigator inspected or reviewed the data on the case report form, the data queries, and the Study Center notifications, and agrees with the content.

12.5. Language

Case report forms must be completed in English. Generic names and trade names are acceptable for concomitant medications. Combination medications should be recorded using their trade name.

All written information and other material to be used by patients and investigative staff must use vocabulary and language that are clearly understood.

12.6. Compensation for Injury

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Patients will be treated and/or compensated for any study related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

13. REFERENCES

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14. APPENDICES

APPENDIX A. SCHEDULE OF PROCEDURES

Appendix A. Schedule of Procedures

	Screen	**Run-in Period**	**Treatment Period (13 Weeks)**							**Post-Treatment Period (10 Weeks)+**		
Study Week/Day	**S-28 to S-7**	**Day -7**	**W1 D1**	**W3 D15**	**W5 D29**	**W7 D43**	**W9 D57**	**W13 D85**	**W14 D92 (and Early Term)**	**W15 D99[15]**	**W18 D120**	**W24 D162**
Visit Window (Days)	**NA**	**± 1**	**NA**	**± 2**	**± 3**	**± 3**	**± 3**	**± 2**	**± 5**	**± 5**	**± 5**	**± 5**
Scheduled Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**9[a]**	**10**	**11**
Informed Consent	X											
Inclusion/Exclusion	X											
Medical History	X											
Body Weight and Height[10]	X								X			X
Physical Exam[1]	X								X			X
Vital Signs[2]	X	X	X[a, c]	X[a]	X[a]	X[a]	X[a]	X[a, c]	X		X	X
HIV, Hepatitis B & C	X											
FSH/ βhCG[3]	X											
SARS-CoV-2 PCR test	X											
Chemistry Panel[5, 6]	X		X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X		X	X
Hematology[6]	X		X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X		X	X
Urinalysis[7]	X		X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X		X	X
Drug/Alcohol Screen[9]	X		X									
PT, INR, aPTT[4]	X							X				X
Spirometry	X[b]		X[g]	X[a]	X[a]	X[a]	X[d]	X[b]	X		X	X
Collect sputum for mucin	X		X[f]		X[f]	X[f]		X[f]	X[f]			X[f]
Plethysmography	X								X			

Appendix A. Schedule of Procedures (Continued)

	Screen	**Run-in Period**	**Treatment Period (13 Weeks)**							**Post-Treatment Period (10 Weeks)+**		
Study Week/Day	**S-28 to S-7**	**Day -7**	**W1 D1**	**W3 D15**	**W5 D29**	**W7 D43**	**W9 D57**	**W13 D85**	**W14 D92 (and Early Term)**	**W15 D99[15]**	**W18 D120**	**W24 D162**
Visit Window (Days)	**NA**	**± 1**	**NA**	**± 2**	**± 3**	**± 3**	**± 3**	**± 2**	**± 5**	**± 5**	**± 5**	**± 5**
Scheduled Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**9a**	**10**	**11**
Diffusing Capacity	X											
Administer SGRQ and CAT questionnaires	X		X[a]		X[a]		X[a]		X			X[a]
Inflammatory biomarkers[8]			X[a]						X			X
Dispense electronic diary and instruct in its use		X										
Collect electronic diary									X			
ECG (12-Lead) in Triplicate	X		X[d]	X[a]	X[a]		X[d]	X[a]	X			X
Study Drug (ION-827359 or placebo) Administration[12]			X	X	X	X	X	X				
Dispense Study Drug for home administration			X[16]			X[17]						
Educate on use of nebulizer and dispense			X									
Review use of nebulizer		X		X	X	X	X					
Collect nebulizer								X				

Appendix A. Schedule of Procedures (Continued)

	Screen	**Run-in Period**	**Treatment Period (13 Weeks)**							**Post-Treatment Period (10 Weeks)+**		
Study Week/Day	**S-28 to S-7**	**Day -7**	**W1 D1**	**W3 D15**	**W5 D29**	**W7 D43**	**W9 D57**	**W13 D85**	**W14 D92 (and Early Term)**	**W15 D99[15]**	**W18 D120**	**W24 D162**
Visit Window (Days)	**NA**	**± 1**	**NA**	**± 2**	**± 3**	**± 3**	**± 3**	**± 2**	**± 5**	**± 5**	**± 5**	**± 5**
Scheduled Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**9a**	**10**	**11**
HRCT scans[13]	X								X			
[redacted]												
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
PK Blood Sampling[11]			X[a, e]	X[a]	X[a]		X[a]	X[a, e]	X	X	X	X

Note: If not specifically labeled, "X" means anytime. S, Screening Day, D, Day, W, Week

* Subjects that terminate early during the Post-Treatment Period should complete all procedures for Study Day 162

[+] Post-Treatment Period visits can occur remotely, if necessary.

[1] Full physical exam to be given at Screening and abbreviated physical exam to be given during Treatment and Follow-up Period as indicated to assess changes from Screening

[2] BP, heart rate (HR), respiratory rate (RR), temperature, and pulse oximetry

[3] Women who are not surgically sterile

[4] For subjects in the bronchoscopy subgroup

[5] Fasting only for the screening lab. During this time the patient can drink water and they should ensure that they consume sufficient water in order to not become dehydrated

[6] If the platelet value, serum creatinine or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing a repeat blood specimen should be re-drawn as soon as possible (ideally within 7 days)

[7] If hematuria or 2 + proteinuria is observed, see confirmation guidance in Section 8.5

[8] Stored at -80 °C. Can be used for follow-up exploration of laboratory findings and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc.) in this or subsequent clinical studies of ION-827359

[9] Urine test at Screening. Day 1 can be done at the site using established methods, e.g., a breathalyzer or urine test

[10] Height at Visit 1 only

[11] If time is not specified PK draw can be done anytime

[12] At-home dosing to occur on Days 8, 22, 36, 50, 64, 71, and 78 (± 1 day)

Appendix A. Schedule of Procedures (Continued)

Legend (Continued)

[13] For subjects in the HRCT subgroup

■ [redacted]

[15] Visit is only for subjects in the PK subgroup
[16] Dispense 1 box of Study Drug for use at-home. Additional drug may be dispensed as necessary (e.g., lost, broken, or spilled vials)
[17] Dispense 1 box of Study Drug for patients in Dose Cohort B.

Time (time is in reference to Study Drug (ION-827359 or placebo) administration):

[a] Pre-dose
[b] Pre- and 20-30 minutes post-albuterol dose
[c] Pre-dose, and 2 hour
[d] Pre-dose, 1, and 2 hour
[e] For the subset of patients with full PK sampling; Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8,12, 24 hours post-inhalation (window of ± 10% of the timepoint in minutes)
[f] In the morning, prior to study visit
[g] Perform spirometry (pre-Study Drug) pre and 20-30 minutes post albuterol dose (2 puffs) and then perform spirometry 1 and 2 hours post-Study Drug dosing.

APPENDIX B. LIST OF LABORATORY ANALYTES

Appendix B. List of Laboratory Analytes

Based on emerging data from this or future studies, additional tests not listed below may be performed on stored samples to better characterize the profile of ION-827359 or other similar oligonucleotides.

Clinical Chemistry Panel
- Sodium
- Potassium
- Chloride
- Bicarbonate
- Total protein
- Albumin
- Calcium
- Magnesium
- Phosphorus
- Glucose
- BUN
- Creatinine
- Cholesterol
- Uric Acid
- Total bilirubin
- Direct (conjugated) bilirubin
- Indirect (unconjugated) bilirubin
- ALT
- AST
- Alkaline phosphatase
- Creatinine kinase
- GGT
- eGFR (calculation)

Screening Tests
- HBsAg
- Hepatitis C antibody
- HIV antibody
- FSH (women only)
- Serum βhCG
- Drug/Alcohol screen

Sputum
- Total Mucin
- MUC5AC
- MUC5B

Coagulation[4]
- aPTT (sec)
- PT (sec)
- INR

Hematology
- Red blood cells
- Hemoglobin
- Hematocrit
- MCV, MCH, MCHC
- Platelets
- White blood cells
- WBC Differential (% and absolute)
- Neutrophils
- Eosinophils
- Basophils
- Lymphocytes
- Monocytes

Pharmacokinetics[1]
- ION-827359 concentrations in plasma

Inflammatory
- Hs-CRP

BAL Biomarkers[3]
- Cell count and diff
- IL-8
- IL-5
- IP-10
- TGF-β

Urinalysis
- Color
- Appearance
- Specific gravity
- pH
- P/C Ratio
- Protein
- Blood
- Ketones
- Urobilinogen
- Glucose
- Bilirubin
- Leukocyte esterase
- Nitrate
- Microscopic examination[2]

[1] Plasma and urine PK samples may also be used for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity testing (or possibly for purposes of immunogenicity assay development and/or validation), or to assess other actions of ION-827359 with plasma constituents
[2] Will be performed on abnormal findings unless otherwise specified
[3] Additional cytokines and mediators may be measured as needed
[4] For subjects in the bronchoscopy subgroup

APPENDIX C. PK SAMPLING SCHEDULE

Appendix C. PK Sampling Schedule

	W1 D1	**W3 D15**	**W5 D29**	**W9 D57**	**W13 D85**	**W14 D92 (and Early Term)**	**W15 D99**	**W18 D120**	**W24 D162**
Non-PK Subgroup	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Anytime	n/a	Anytime	Anytime
PK Subgroup	Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8,12, and 24 hours Post-inhalation	Pre-dose	Pre-dose	Pre-dose	Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8,12, and 24 hours Post-inhalation	Anytime	Anytime	Anytime	Anytime

APPENDIX D. GRADING SCALE FOR ADVERSE EVENTS RELATING TO LABORATORY ABNORMALITIES

Appendix D. Grading Scale for Adverse Events Relating to Laboratory Abnormalities

The following grading recommendations for AEs relating to lab test abnormalities and AEs at the injection site are based upon the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017

Adverse Event	Mild	Moderate	Severe
Hematology			
aPTT prolonged	1.0 – 1.2 x ULN	>1.2 – 1.4 x ULN	> 1.4 x ULN
Eosinophils increased	650 – 1,500 cell/mm^3	1,501 - 5,000 cell/mm^3	>5,000 cell/mm^3
Fibrinogen decreased	150 – 200 mg/dL	125 – 149 mg/dL	< 125 mg/dL
Fibrinogen increased	400 – 500 mg/dL	501 – 600 mg/dL	> 600 mg/dL
Hemoglobin decreased Male Female	 12.5 – 13.5 g/dL 11.0 – 12.0 g/dL	 10.5 – 12.4 g/dL 9.5 – 10.9 g/dL	 < 10.5 g/dL < 9.5 g/dL
INR increased[†]	>1.2 - 1.5; >1 - 1.5 times above baseline if on anticoagulation	>1.5 - 2.5; >1.5 - 2.5 x baseline if on anticoagulation; monitoring only indicated	>2.5; >2.5 x baseline if on anticoagulation; dose adjustment indicated
Lymphocyte count decreased	750 – 1,000 cell/mm^3	500 – 749 cell/mm^3	< 500 cell/mm^3
Neutrophil count decreased	1,500 – 2,000 cell/mm^3	1,000 – 1,499 cell/mm^3	< 1,000 cell/mm^3
Platelet count decreased	125,000 – 140,000 cell/mm^3	100,000 – 124,000 cell/mm^3	< 100,000 cell/mm^3
Prothrombin time (PT)	1.0 – 1.1 x ULN	>1.1 – 1.2 x ULN	> 1.2 x ULN
White blood cell decreased	2,500 – 3,500 cell/mm^3	1,500 – 2,499 cell/mm^3	< 1,500 cell/mm^3
White blood cell increased	10,800 – 15,000 cell/mm^3	15,001 – 20,000 cell/mm^3	>20,000 cell/mm^3
Chemistry			
Alanine aminotransferase increased[†]	>ULN - 3.0 x ULN if baseline normal 1.5 - 3.0 x baseline if baseline abnormal	>3.0 - 5.0 x ULN if baseline normal >3.0 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Alkaline phosphatase increased	1.1 – 2.0 x ULN	>2.0 – 3.0 x ULN	> 3 x ULN

Appendix D. Grading Scale for Adverse Events Relating to Laboratory Abnormalities (Continued)

Adverse Event	Mild	Moderate	Severe
Aspartate aminotransferase increased†	>ULN - 3.0 x ULN if baseline normal 1.5 - 3.0 x baseline if baseline abnormal	>3.0 - 5.0 x ULN if baseline normal >3.0 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Blood bilirubin increased			
When accompanied by any increase in liver function test	1.1 – 1.25 x ULN	>1.25 – 1.5 x ULN	> 1.5 x ULN
When liver function test is normal	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	> 2 x ULN
Blood urea nitrogen	23 – 26 mg/dL	27 – 31 mg/dL	>31 mg/dL
CPK increased*	>ULN - <6 ULN	6 - 10 x ULN	>10 x ULN
Creatinine increased	1.5 – 1.7 mg/dL	1.8 – 2.0 mg/dL	≥ 2.1 mg/dL
GGT increased†	>ULN - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 x ULN
Hypercalcemia	10.5 – 11.0 mg/dL	11.1 – 11.5 mg/dL	≥ 11.6 mg/dL
Hyperglycemia††	Fasting glucose value ≥126 mg/dL (7.0 mmol/L)	Change in daily management to maintain fasting blood glucose <126 mg/dL (7.0 mmol/L); e.g. addition of oral antiglycemic agent; workup for diabetes	Insulin therapy initiated; hospitalization indicated
Hyperkalemia	5.1 – 5.2 mmol/L	5.3 – 5.4 mmol/L	≥5.5 mmol/L
Hypernatremia	144 – 145 mmol/L	146 – 147 mmol/L	≥148 mmol/L
Hypoalbuminemia	2.8 – 3.1 g/dL	2.5 – 2.7 g/dL	< 2.5 g/dL
Hypocalcemia	8.0 – 8.4 mg/dL	7.5 – 7.9 mg/dL	< 7.5 mg/dL
Hypoglycemia	65 – 69 mg/dL	< 64 mg/dL	Requires assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions‡
Hypokalemia	3.5 – 3.6 mmol/L	3.3 – 3.4 mmol/L	< 3.3 mg/dL
Hypomagnesemia	1.3 – 1.5 mg/dL	1.1 – 1.2 mg/dL	< 1.1 mg/dL
Hyponatremia	132 – 134 mmol/L	130 – 131 mmol/L	<130 mg/dL
Hypophosphatemia	2.3 – 2.5 mg/dL	2.0 – 2.2 mg/dL	< 2.0 mg/dL
Hypoproteinemia	5.5 – 6.0 g/dL	5.0 – 5.4 g/dL	< 5.0 g/dL
Lipase increased	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	> 2 x ULN
Serum amylase increased	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	> 2 x ULN

Appendix D. Grading Scale for Adverse Events Relating to Laboratory Abnormalities (Continued)

Adverse Event	Mild	Moderate	Severe
Urine			
Proteinuria	Trace	1+	≥ 2+
Hematuria	1 - 10 cells per high power field	11 – 50 cells per high power field	> 50 cells per high power field
Adverse Events at the Injection Site			
Adverse events at the injection site**	An event at the injection site (e.g. erythema, tenderness, itching) that is easily tolerated by the subject and does not affect the subject's usual daily activities	- Persistent (>24 hours) pain, phlebitis or edema; OR - Lipodystrophy, hair growth or alopecia, OR - Prolonged (>1 month) hypo/hyperpigmentation	- Ulceration or necrosis; severe tissue damage; operative intervention indicated, OR - Any event at the injection site that is incapacitating

*Grading for this parameter is derived from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2.0, Nov 2014

†Grading for this parameter is derived from the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 27, 2017

††Modified for consistency with ADA "Standards of Medical Care in Diabetes - 2018" Diabetes Care 2018;41(Suppl. 1):S13–S27. https://doi.org/10.2337/dc18-S002

‡Modified for consistency with ADA "Glycemic Targets: Standards of Medical Care in Diabetes - 2018", Diabetes Care 2018;41(Suppl. 1):S55–S64. https://doi.org/10.2337/dc18-S006

**Grading for this parameter is adapted from the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 27, 2017

Protocol

Version:	1
Version Date:	02 Feb 2021
Title:	ION-827359-CS2 Protocol Amendment 3: A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis

APPROVALS:

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Official Title:	**A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis**
NCT Number:	NCT04441788
Document Date:	SAP Version 1.0 : 26 August 2021

Statistical Analysis Plan

ION-827359 CS2

A Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Safety, Tolerability, and Efficacy of ION-827359 in Patients with Mild to Moderate COPD with Chronic Bronchitis

Date: August 24, 2021

Version: 1.0

Statistical Analysis Plan Signature Page

Approved by: See electronic signature and date attached at end of document

[redacted]

Ionis Pharmaceuticals, Inc.

See electronic signature and date attached at end of document

[redacted]

Ionis Pharmaceuticals, Inc.

See electronic signature and date attached at end of document

[redacted]

Ionis Pharmaceuticals, Inc.

Table of Contents

1 INTRODUCTION

This document provides a description of the study organization, study procedures, and the plan for the statistical analysis of the study data. Section 1 discusses study design, objectives, and endpoints; Section 2 provides the study procedures; Section 3 provides the detailed plan for the statistical analyses.

As with any statistical analysis plan (SAP), the proposed methods and approaches to the data analysis should be viewed as flexible. The statistical analysis to some degree is iterative since so much of the planning is based on statistical and other assumptions, which require verification.

1.1 Study Overview

This is a Phase 2a, double blind, randomized, placebo-controlled, dose-ranging study, will be conducted at multiple centers in Europe. Patients with CB associated with COPD will be randomized to ION-827359 or matching placebo administered by oral inhalation once weekly for 13 weeks.

Approximately 180 patients are planned to be enrolled in this study. Patients will be randomized in a 2:2:1:1 ratio to the following 4 treatment groups respectively:

- ION-827359 37.5 mg (1.5 mL) once per week
- ION-827359 75mg (3 mL) once per week
- Placebo (1.5 mL) once per week
- Placebo (3 mL) once per week

There are 3 subset studies:

1. A subset of up to about 24 patients will have PK profile sampling performed after the first and last dose
2. A subset of up to about 60 patients will have low dose inspiratory and expiratory HRCT scans to assess regional lung ventilation performed before and post-treatment
3. A subset of up to about 18 patients will undergo bronchoscopy and BAL for target engagement and inflammatory biomarkers before and post-treatment

A patient may be enrolled in a maximum of 1 subset study.

The study will consist of Screening, Treatment, and Post-Treatment Periods. Please refer to the Schedule of Procedures in protocol Appendix A. The study for an individual patient will generally consist of the following Periods:

- A ≤ 4-week Screening Period. The last week of which is the Run-in Period
- A 13-week Treatment Period during which study medication (ION‑827359 or placebo) will be administered by nebulization once per week (both in-clinic and at home dosing)
- A 10-week Post-Treatment Period

Patients may be required to attend additional visits for monitoring of AEs or abno1mal investigation results. The frequency of additional monitoring will be determined by the Sponsor Medical Monitor in consultation with the Investigator.

Due to a combination of findings in a recently completed chronic toxicology study in non-human primates and the totality of the data collected to date in this and other studies ofENaC inhibition, the study treatment has been te1minated on April 29th, 2021, after 60 patients have been randoinized and 59 patients have been dosed. All patients who were on treatment at the time of study treatment tennination will stop the treatment, continue in the study to complete the early tennination visit (Week 14 Day 92) and the 10-week Post-Treatment period.

1.2 Objectives

1.2.1 Primary Objective

To evaluate the effect ofION-827359 on forced expirato1y volume in 1 second (FEVl) in patients with mild to moderate chronic obstructive pulmonaiy disease (COPD) with chronic bronchitis (CB).

1.2.2 Secondary Objectives

- To evaluate the effect ofION-827359 on symptoms of CB
- To evaluate the effect ofION-827359 on quality of life (QoL) in patients of CB
- To evaluate the phaimacokinetics (PK) ofION-827359 in patients with CB
- To evaluate the safety and tolerability of ION-827359 compai·ed to placebo

1.2.3 Exploratory Objectives

- Assess changes in PD markers
- Assess changes in lung volumes
- Assess changes in lung ventilation
- Assess tai·get engagement

1.3 Endpoints

1.3.1 Primary Efficacy Endpoints

Change from Baseline to the primary time point (defined as the average of Weeks 13 and 14) in FEVl compared to placebo.

1.3.2 Secondary Efficacy Endpoints

- Change from Baseline in the EXACT respiratory symptoms (E-RS) (evaluating respiratory symptoms) daily symptom diaiy to the primaiy time point
- Change from Baseline in the COPD assessment test (CAT) to the Week 14 time point

- Change from Baseline in St. George's Respiratory Questionnaire (SGRQ) to the Week 14 time point
- Change from Baseline in post-bronchodilator FEV1
- Pharmacokinetics

1.3.3 Safety Endpoints

- Incidence and severity of treatment-emergent adverse events (TEAE)
- Abnormal findings in laboratory assessments, electrocardiogram (ECGs), and vital signs

1.3.4 Exploratory Endpoints

The tertiary and PD endpoints include:

- Change from Baseline in sputum mucins (total, MUC5AC, and MUC5B)
- Change from Baseline in epithelial sodium channel (ENaC) messenger ribonucleic acid (mRNA) levels from bronchial brushings
- Change from Baseline in inflammatory biomarkers from bronchoalveolar lavage (BAL)
- Change from Baseline in functional respiratory capacity (FRC) and residual volume (RV)
- Change from Baseline in regional lung volumes as measured by high resolution CT scanning (HRCT)

1.3.5 Exploratory Endpoints

Plasma exposure over time will be summarized using data from PK profile samples collected following the first and last dose in a subset of patients, as well as trough samples collected in all patients throughout the study. In addition, potential PK/PD correlation on relevant biomarkers may be evaluated.

2 PROCEDURES

2.1 General Overview of Procedures

Ionis Pharmaceuticals, Inc. will review all study data including source documents, CRFs, laboratory reports, and other external data. The study site will enter subject source data into the case report form. Laboratory data will be transferred electronically to Ionis Pharmaceuticals, Inc.

2.2 Randomization & Treatment Allocation

Patients will be randomized, after all Baseline and Screening assessments have been completed and after the Investigator has verified that they are eligible per criteria in protocol Sections 5.1 and 5.2.

No subject may begin treatment prior to randomization and assignment of a unique subject identification number.

Eligible patients will be randomized using an Interactive Voice/Web-Response System (IXRS) in a 2:2:1:1 ratio to receive ION-827359 37.5 mg (1.5 mL), ION-827359 75 mg (3 mL), Placebo 1. 5 mL, and Placebo 3 mL, respectively. Cohort A will be defined as those subjects receiving 1.5 mL of Study Drug or placebo, and Cohort B as those receiving 3 mL of Study Drug or placebo. These cohorts will run concurrently.

There will be 4 separate and independent randomizations:

- PK subgroup: approximately 24 patients
- HRCT subgroup: approximately 60 patients
- Bronchoscopy subgroup: approximately 18 patients
- Other: patients who are not in the above 3 subgroups, approximately 78

Randomization information will be concealed from the Investigators and patients until the end of the study, with the exception of an emergency situation involving a patient that requires unblinding of the treatment assignment.

2.3 Conduct

The study will be conducted in accordance with current Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) guidelines, the World Medical Association Declaration of Helsinki guidelines, the Food and Drug Administration (FDA) Code of Federal Regulations, and all other local regulatory requirements.

2.4 Data Monitoring

2.4.1 Safety Data Monitoring

Ionis Pharmaceuticals, Inc. (Ionis) (or designee) is responsible for processing all reported adverse events (AEs). All serious adverse events (SAEs), reported to Ionis (or designee), are reviewed according to standard operating procedures. The medical monitor will review all AEs and SAEs on an ongoing basis throughout the study. Ionis (or designee) will prepare and submit safety reports to the health authorities worldwide in accordance with local requirements. If it becomes necessary to communicate new safety information, Ionis (or designee) will also prepare a safety notification letter and transmit it to study site.

2.5 Data Management

2.5.1 Case Report Form Data

BioClinica (or designee) is responsible for creating the Electronic Data Capture (EDC) data entry screens, database and edit checks using definitions developed by Ionis. Ionis is responsible for the review, data management querying and locking of the database.

Data are single-entered into the EDC system by the investigator site staff. Programmed edit checks (computer logic that checks the validity of the data entered and also prompts for missing data that is expected to be entered) are run and automatic queries are generated. Ionis reviews all data for accuracy and validity and generates additional queries in the EDC system when necessary. The data is corrected or an explanation concerning the query is provided in the EDC system. After all data are entered, reviewed (by Data Management and Clinical Development) and queried, and all queries resolved, the database is locked.

2.5.2 Laboratory Data

2.5.2.1 Bal/Bronchial Brushing

Bal cell counts and differentials will be analyzed by local labs and entered in the EDC system by the investigator site staff.

2.5.2.2 Sputum Mucins (Total, MUC5AC, and MUC5B)

The sputum samples will be analyzed by PPD Development (PPD) for sputum mucins. Additionally, sputum from baseline and end of study will be sent to Spirovation for determination of cytokines and complement levels. Ionis, PPD, and Spirovation are responsible for the format of the safety laboratory electronic data transfers and the transfer schedule. The data results will not be stored in the EDC system. PPD, Spirovation, and Ionis are responsible for the review of the data. This process involves reviewing the patient and visit identifiers in the PPD data against the visit identifiers collected in the EDC system.

2.5.2.3 Central Laboratory Data

The sites will collect the blood and urine samples and send to the central vendor, Medpace. Ionis and Medpace are responsible for the format of the safety laboratory electronic data transfers and the transfer schedule. Central laboratory data results will not be stored in the EDC system. Medpace and Ionis are responsible for the review of the clinical laboratory data. This process involves reviewing the patient and visit identifiers in the central laboratory data results data against the central lab data identifiers collected in the EDC system. Investigator sites have access to the data via lab reports sent directly from the laboratory or through the laboratory's web portal (in which case Investigators only have access to data from their site).

2.5.3 ECG Data

Ionis and ERT are responsible for the format of the cardiac safety and ECG data transfers and the transfer schedule. Cardiac safety and ECG data will not be stored in the EDC system.

Ionis and ERT are also responsible for the data reconciliation, which involves reviewing the patient and visit identifiers in the cardiac safety and ECG data against the visit identifiers collected in the EDC system.

2.5.4 Spirometry, DLCO, and Plethysmography Data

Ionis and ERT are responsible for the format of the spirometry, DLCO and plethysmography data transfers and the transfer schedule. The spirometry, DLCO and plethysmography data will not be stored in the EDC system. Ionis and ERT are also responsible for the data reconciliation, which involves reviewing the patient and visit identifiers in the spirometry, DLCO and plethysmography data against the visit identifiers collected in the EDC system.

2.5.5 HRCT Data

The sites will perform the computed tomography (CT) scans at both TLC and FRC and send the images to a central vendor, FLUIDDA. The outcome data will be generated by FLUIDDA. Ionis and FLUIDDA are responsible for the format of the data transfers and the transfer schedule. The data will not be stored in the EDC system. Ionis and ERT are also responsible for the data reconciliation, which involves reviewing the patient and visit identifiers in the cardiac safety and ECG data against the visit identifiers collected in the EDC system.

Since no patients have been enrolled in the HRCT subgroup at the time of treatment termination, the HRCT data are not collected.

2.5.6 Diary and Questionnaire Data (E-RS, CAT, SGRQ)

The diary and questionnaire data will be captured by ERT eCOA System. Patients will enter their assessments into the system using a tablet or other handheld devices. Ionis and ERT are responsible for the format of the electronic data transfers and the transfer schedule. The data will not be stored in the EDC system. Ionis and ERT are also responsible for the review of the data. This process involves reviewing the patient and visit identifiers in the questionnaire data against the data identifiers collected in the EDC system.

2.5.7 Pharmacokinetics Data

Ionis Pharmaceuticals, Inc. is responsible for the management and review of the plasma drug concentration data. Final data, which has been approved by Quality Assurance, will be stored in version-controlled repository

3 ANALYTICAL PLAN

3.1 General Overview of Analyses

3.1.1 Statistical Methods

Descriptive summary statistics including number of subjects, mean, median, standard deviation, standard error, interquartile range (25th percentile, 75th percentile), and range

(minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data. All statistical tests will be conducted using 2-sided tests with 5% Type I error rate unless otherwise stated. As a Phase 2a study, adjustments for multiplicity of testing will generally not be used.

PK parameters will be summarized using number of Subjects, mean, standard deviation, coefficient of variation (CV), geometric mean, geometric %CV, median, minimum, and maximum.

General Presentation Considerations

The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean, median, lower quartile and upper quartile will be reported to one more decimal place than the raw data recorded in the database. The standard deviation will be reported to two more decimal places than the raw data recorded in the database. In general, the maximum number of decimal places reported shall be four for any summary statistic.

Categorical data will be summarized in terms of the number of subjects providing data at the relevant time point (n), frequency counts and percentages. Any planned collapsing of categories will be detailed in the text and the data displays.

Percentages will be presented to one decimal place and not be presented for zero counts. Percentages will be calculated using n as the denominator.

P-values greater than or equal to 0.0001, in general, will be presented to four decimal places. P-values less than 0.0001 will be presented as "<0.0001".

Confidence intervals will be presented to one more decimal place than the raw data.

Baseline definition

Baseline E-RS total and subscale scores are defined as the average of daily scores collected on Day 1 and within 6 days prior to Day 1.

Baseline liver chemistry parameters (ALT, AST, and total, direct and indirect bilirubin, alkaline phosphatase) are defined as the average of the non-missing values at Screening and Day 1 pre-dose, and any values in between.

Baseline ECG assessments are defined as the last triplicate prior to the first Study Drug administration. For numeric results, the baseline is the average of the triplicate. For character results, baseline is the worst result of the triplicate.

Baseline for other assessments is defined as the last non-missing measurement prior to the first Study Drug administration.

Primary time point definition

The primary time point is defined as the average of Weeks 13 and 14. If the assessment is missing in one of the two time points, then non-missing assessment will be used.

End of treatment period assessment definition

The end of treatment period assessments are defined as the last available assessments within 14 days after the last dose.

Analytical visits:

Efficacy/PD data including spirometry, CAT, SGRQ, sputum mucins, ENaC mRNA, inflammatory biomarkers, during the treatment period will be mapped to analysis visit as specified in the table (

Efficacy/PD Measure Visit Windows) below. The intent of these visit windows is not to align with those prescribed for visit scheduling in the clinical study protocol but, rather, based on the protocol-defined target study day, to delineate mutually exclusive windows so that all assessments proximal to a particular study week can be integrated to best represent the patient's status during that period of the study.

If a patient discontinued early from the treatment period but attended applicable landmark visits to collect assessments, then those assessments collected within 14 days after the last dose of Study Drug will be included as long as they are within the defined analysis windows.

The unscheduled visits will also be included if they are collected within 14 days after the last dose of Study Drug and within the defined analysis windows.

If there are multiple assessments within a visit window, the visit nearest the scheduled date will be used unless 2 visits are equally near, in which case the average will be used for continuous results, and the worst will be used for categorical results.

Efficacy/PD Measure Visit Windows

Measure	Mapped Visit (Week)	Target Day	Study Day Window
Spirometry assessments	1 – 1 hour post dose	1	1
	1 – 2 hours post dose	1	1
	3	15	2-22
	5	29	23 to 36
	7	43	37 to 50
	9	57	51 to 71
	9 – 1 hour post dose	57	51 to 71
	9 – 2 hours post dose	57	51 to 71
	13	85	72 to 88
	14	92	89 to 99
CAT and SGRQ	5	29	2 to 43
	9	57	44 to 74
	14	92	75 to 99
EXACT	2	8	2-8
	3-14	15-99	(Week-1)*7-5 to (Week-1)*7+1
Sputum mucins	5	29	23 to 36

Measure	Mapped Visit (Week)	Target Day	Study Day Window
	7	43	37 to 64
	13	85	65 to 88
	14	92	89 to 99
Plethysmography assessments,	14	92	75 to 99

Data collected under visit labels containing Weeks 18, 24, Days 120 or 162 will not be mapped to analysis week.

Visits after Week 14 are part of the post-treatment assessment period. The efficacy and PD data during the post-treatment period will be summarized using the visit labels provided in the data.

Other data will be summarized using the visit labels provided in the data. Multiple results with the same visit label will be averaged. Results with visit labels as "Unscheduled" will not be included in the by-visit summary tables and figures, but will be included in determining baseline, and in the laboratory abnormality summaries and shift analyses.

All data will be presented in data listings.

Laboratory data:

For potassium all central and local labs will be included in the data summary; for other lab assessments, only the central lab assessments will be included in the data summary.

All central and local labs will be included in the data listings.

3.1.2 Subject Population Analyzed

Full Analysis Set (FAS): all randomized patients who received at least 1 dose of Study Drug (ION-827359 or placebo) and who have at least 1 post-Baseline efficacy assessment (i.e., post-Baseline FEV1 assessment, E-RS score, CAT score, or SGRQ-C score).

Safety Set: All patients who are randomized and receive at least 1 dose of Study Drug.

PK Population: All subjects who are randomized and receive at least 1 dose of active Study Drug (ION-827359) and have at least 1 evaluable PK sample collected and analyzed with reportable result. This population will be used for PK analyses.

In addition to the above analysis populations, it is recognized that some data displays will be provided for "All Screened", "Screening Failures" and "All Randomized" subjects but no data analysis will be executed in these populations except for the disposition table that includes all screened subjects.

The Per-Protocol Set (PPS), FAS - Bronchoscopy Subgroup, PPS - Bronchoscopy Subgroup, FAS-HRCT and PPS-HRCT subgroups are defined in the protocol but have been removed from this SAP.

3.1.3 Sample Size Consideration

The sample size assumptions for the primary endpoint include:

- The increase in FEV_1 from Baseline to the primary time point in the ION‑827359 treatment group is 100 mL; no change in the placebo group
- Standard deviation of change in FEV_1 is 220 mL
- Significance level (alpha) of 0.05 (2-sided test)

With the above assumptions, a sample size of 174 patients (58 patients in each ION-827359 treatment groups and 29 patients in each placebo group) will provide a power of at least 80% for the primary comparison (pooled ION-827359 treatment groups vs. pooled placebo groups).

Approximately 180 patients will be enrolled in this trial to account for a 3% dropout rate.

3.1.4 Planned Interim Analysis

No interim analysis is planned for this study.

3.1.5 Incomplete or Missing Data

For EXACT daily diary, if no more than three items are missing, and no more than two of items 9–11 are missing, the missing items will be imputed using the mean of the remaining answered items, rounded to the nearest integer and capped at the maximum score available for that item. Otherwise, the E-RS score will be considered missing for the day.

For CAT questionnaire, if the score is missing for only one item, the missing value will be imputed by the mean value of the remaining seven items. If two or more items are missing, then the CAT score will be considered missing for the visit.

For SGRQ-C questionnaire Part 1, missed items are treated as if the answer was in the negative. A maximum of one missed item is permitted for this section.

For SGRQ-C questionnaire Part 2, items in questions 9, 10, 11, 12, 13 all require a response of either ‘True’ or ‘False’. If neither box is ticked, the item should be coded as missing. The weight for that item should then be removed from the total possible for that component (and the total score). A maximum of 3 missed items for the Activity component (items in questions 9 and 12) and a maximum of 5 items for the Impacts component (items in questions 8, 10, 11, 13, 14) are permitted.

3.2 Demographic and Baseline Characteristics and Patient Disposition

Baseline and demographic variables will be summarized descriptively by treatment group and for each analysis population.

Demographic and baseline characteristics to be presented include age, age category (<18, 18 to 64, ≥65 years), gender, race, ethnicity, height, weight, BMI, , percent predicted DLCO, , baseline spirometry assessments, E-RS scores, CAT scores, SGRQ scores, sputum mucins, FRC and RV.

For race summary, if multiple races are recorded in database, 'Multiple Race' will be used in the summary table but details records in the listing.

A separate table will be created for the targeted medical history and smoking history, and will include duration since diagnosis of COPD, COPD exacerbations that required hospitalization in the last year (yes/no), number of hospitalizations for COPD exacerbations in the last year, COPD exacerbations that required oral corticosteroids and/or antibiotic in the last year (yes/no), number of COPD exacerbations in the last year that required oral corticosteroids and/or antibiotics, diagnosis with asthma (yes/no), diagnosis with coronary artery disease (yes/no), diagnosis of congestive heart failure (yes/no), ever used tobacco cigarettes (yes/no), number of packs tobacco per day and number of years smoking.

The duration since diagnosis of COPD will be calculated as the difference between informed consent date and COPD diagnosis date plus 1 day then divided by 365.25. The partial COPD dates will be imputed using the following rules:

- If both month and day are missing then assign July 1st
- If only day is missing then assign 15th

Other medical history data will be provided in the data listing and summarized using MedDRA SOC and preferred term.

Subject enrollment and disposition will be summarized by treatment group. The summary will include: the number of subjects screened, the number of screen failures, the reason for screen failure, the number of subjects randomized, the number and percentage of subjects dosed, the number and percentage of subjects in each analysis population, the number and percentage of subjects in each subgroup, the number and percentage of subjects completing treatment, the primary reason for terminating treatment, the number of subjects completing post-treatment follow-up, and the primary reason for terminating post-treatment follow-up. The percentages will be calculated based on the number of patients randomized.

3.3 Efficacy Analyses

All efficacy analyses will be performed on the FAS. Due to the fact that study treatment terminated after only 60 patients have been randomized, 59 patients have been dosed, and 10 patients have completed the Week 14, all analyses will be descriptive. The model-based analyses described in the protocol will not be conducted.

3.3.1 Change from Baseline in FEV_1 at the Primary Time Point

The FEV1 and other spirometry assessments, as well as change and percent change from baseline to the primary time point, end of treatment period, and other study visits will be summarized by treatment group using descriptive statistics.

At each time point, 3 technically acceptable measurements will be made and recorded. The best readings from each time point for each assessment will be used for analysis.

In addition to the descriptive summary, the proportion of patients who have a greater than 20% reduction in FEV1 post treatment and the proportion of doses that resulted in a greater than 20% reduction in FEV1 will be tabulated by treatment group.

3.3.2 Analysis of Secondary Endpoints

3.3.2.1 Change from Baseline in E-RS Total and Subscale Scores at the Primary Time Point

The E-RS assesses respiratory symptoms using the following 11 respiratory symptom items from the EXACT.

The response for each item will be scored according to the E-RS User Manual Version 3.0 as below:

E-RS™ Individual Items	**Response**	**Score**
1. Chest congested	Not at all	0
	Slightly	1
	Moderately	2
	Severely	3
	Extremely	4
2. Cough	Not at all	0
	Rarely	1
	Occasionally	2
	Frequently	3
	Almost constantly	4
3. Mucus/phlegm	Not at all	0
	A little	1
	Some	1
	A great deal	2
	A very great deal	3

E-RS™ Individual Items	**Response**	**Score**
4. Difficulty bring up mucus	Not at all	0
	Slightly	1
	Moderately	2
	Quite a bit	3
	Extremely	4
5. Chest discomfort	Not at all	0
	Slight	1
	Moderate	2
	Severe	3
	Extreme	4
6. Tight chest	Not at all	0
	Slightly	1
	Moderately	2
	Severely	3
	Extremely	4
7. Breathlessness	Not at all	0
	Slightly	1
	Moderately	2
	Severely	3
	Extremely	4
8. Describe breathlessness	Unaware of breathlessness	0
	Breathlessness during strenuous activity	1
	Breathlessness during light activity	2
	Breathlessness when washing or dressing	3
	Present when resting	3
9. Short of breath personal activities	Not at all	0
	Slightly	1
	Moderately	2
	Severely	3
	Extremely	3
	Too breathlessness to do these	4

E-RS™ Individual Items	Response	Score
10. Short of breath indoor activities	Not at all	0
	Slightly	1
	Moderately	2
	Severely	3
	Extremely	3
	Too breathlessness to do these	3
11. Short of breath outdoor activities	Not at all	0
	Slightly	1
	Moderately	2
	Severely	3
	Extremely	3
	Too breathlessness to do these	3

Patients' daily E-RS total scores will be computed by summing across responses to the 11 items, the score ranges from 0 to 40. The same summation procedure will be used for obtaining the three subscale scores: E-RS Breathlessness is the sum of items 7, 8, 9, 10 and 11, the score ranges from 0 to 17; E-RS Cough & Sputum is the sum of items 2, 3, 4, the score ranges from 0 to 11; and E-RS Chest is the sum of items 1, 5, 6, and score ranges from 0 to 12. The higher scores indicate more severe respiratory symptoms.

Missing EXACT items will be imputed using the rules described in Section 3.1.5 in this SAP.

The data will be mapped to study week based on the visit window specified in section 3.1.1. The data in the visit window will be averaged. If patients complete less than 4 daily assessments in a week, then the E-RS scores will be considered as missing for the week.

The change from baseline in the E-RS total and the 3 subscale scores to primary time point and over time as well as change from baseline to the end of treatment period will be summarized by treatment group using descriptive statistics.

3.3.2.2 Change from Baseline in CAT at Week 14

The CAT contains eight items: cough, phlegm, chest tightness, breathlessness, limited activities, confidence leaving home, sleeplessness and energy. Each item is presented as a semantic 6-point differential scale from 0 to 5. The total CAT score ranges from 0–40. Higher scores indicate a more severe impact of COPD on a patient's life.

If the score is missing for only one item, the missing value will be imputed by the mean value of the remaining seven items. If two or more items are missing, then the CAT score will be considered missing for the visit.

The change from baseline in the CAT score at Week 14 and other study visits as well as change from baseline to the end of treatment period will be summarized by treatment group using descriptive statistics.

3.3.2.3 Change from Baseline in SGRQ at Week 14

The SGRQ is a patient completed, disease-specific instrument designed to measure impact onoverall health, daily life, and perceived well-being in patients with obstructive airways disease and includes 50 items addressing three domains of HRQL (symptoms, activity limitations, and impact of the disease on daily life). Recall periods in the questionnaire include the past 4 weeks and the present.

This study will utilize the shorter 40-item version (SGRQ-C) which does not specify a Recall Period. A five-point scale is used for rating the symptoms, and a true/false binary scale is used for activity limitations. Items are assigned a weight according to the degree of distress in the calculation of the overall score as below:

Part 1:
Question 1: I cough:
Most days 80.6
Several days 46.3
With chest infections 28.1
Not at all 0.0
Question 2: I bring up phlegm (sputum):
Most days 76.8
Several days 47.0
With chest infections 30.2
Not at all 0.0
Question 3: I have shortness of breath:
Most days 87.2
Several days 50.3
Not at all 0.0
Question 4: I have attacks of wheezing:
Most days 86.2
Several days 71.0
A few days 45.6
With chest infection 36.4
Not at all 0.0
Question 5: How many attacks of chest trouble have you had
3 or more 80.1
1 or 2 attacks 52.3
None 0.0
Question 6: How often do you have good days (with little chest trouble)?
None 93.3
A few 76.6
Most are good 38.5
Every day 0.0
Question 7: If you have a wheeze, is it worse in the morning?
No 0.0
Yes 62.0

Part 2

Question 8: How would you describe your chest condition?

The most important problem I have 82.9
Causes me a few problems 34.6
Causes no problem 0.0

Question 9: Questions about what activities usually make you feel breathless.

Getting washed or dressed 82.8
Walking around the home 80.2
Walking outside on the level 81.4
Walking up a flight of stairs 76.1
Walking up hills 75.1

Question 10: More questions about your cough and breathlessness.

My cough hurts 81.1
My cough makes me tired 79.1
I get breathless when I talk 84.5
I get breathless when I bend over 76.8
My cough or breathing disturbs my sleep 87.9
I get exhausted easily 84.0

Question 11: Questions about other effects your chest trouble may have on you.

My cough or breathing is embarrassing in public 74.1
My chest trouble is a nuisance to my family, friends or neighbors 79.1
I get afraid or panic when I cannot get my breath 87.7
I feel that I am not in control of my chest problem 90.1
I have become frail or an invalid because of my chest 89.9
Exercise is not safe for me 75.7
Everything seems too much of an effort 84.5

Question 12: Questions about how activities may be affected by your breathing.

I take a long time to get washed or dressed 74.2
I cannot take a bath or shower, or I take a long time 81.0
I walk more slowly than other people, or I stop for rests 71.7
Jobs such as housework take a long time, or I have to stop for rests 70.6
If I walk up one flight of stairs, I have to go slowly or stop 71.6
If I hurry or walk fast, I have to stop or slow down 72.3
My breathing makes it difficult to do things such as walk up hills, carry things up stairs, light gardening such as weeding, dance, play bowls or play golf 74.5
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim 71.4

Question 13: We would like to know how your chest trouble usually affects your daily life.

I cannot play sports or games 64.8
I cannot go out for entertainment or recreation 79.8
I cannot go out of the house to do the shopping 81.0
I cannot do housework 79.1
I cannot move far from my bed or chair 94.0

Question 14: Tick the statement which you think best describes how your chest affects you.
It does not stop me doing anything I would like to do 0.0
It stops me doing one or two things I would like to do 42.0
It stops me doing most of the things I would like to do 84.2
It stops me doing everything I would like to do 96.7

The symptoms component consists of all the questions in Part 1. The activity component consists of questions 9 and 12 in Part 2 of the questionnaire. The impact component consists of questions 8, 10, 11, 13, 14 in Part 2 of the questionnaire

The score for each component is calculated separately by dividing the summed weights by the maximum possible weight for that component and expressing the result as a percentage:

$$\text{Score} = 100 \times \frac{\text{Summed weights from all positive items in that component}}{\text{Sum of weights for all items in that component}}$$

The total score is calculated in similar way:

$$\text{Score} = 100 \times \frac{\text{Summed weights from all positive items in the questionnaire}}{\text{Sum of weights for all items in the questionnaire}}$$

The 0% and 100% represent the best and worst possible health status, respectively.

Missing items will be imputed using the rules described in Section 3.1.5 in this SAP.

The SGRQ-C total score and component scores, as well as the absolute and percent change from baseline at Week 14, end of treatment period and other study visits will be summarized by treatment group using descriptive statistics.

3.3.2.4 Change from Baseline in Post-Bronchodilator FEV_1 at Week 13

The post-bronchodilator FEV1, as well as the absolute and percent changes from baseline will be summarized by treatment group and study visit using descriptive statistics. The absolute and percent changes from baseline to the end of treatment period will also be summarized.

3.3.3 Analysis of Exploratory Endpoints

3.3.3.1 Change from Baseline in Sputum Mucins (Total, MUC5AC, and MUC5B)

The sputum mucins results, absolute change and percent change from baseline will be summarized by treatment group and study visit using descriptive statistics.

3.3.3.2 Change from Baseline in ENaC (mRNA) Levels from Bronchial Brushings at Week 13

Due to the limited post-treatment bronchial brushing samples, the data will only be provided in the data listing.

3.3.3.3 Change from Baseline in Inflammatory Biomarkers from BAL at Week 13

Due to the limited post-treatment BAL samples, the data will only be provided in the data listing.

3.3.3.4 Change from Baseline in Inflammatory Biomarkers from Sputum

The results, absolute change and percent change from baseline in the inflammatory biomarkers will be summarized by treatment group and study visit using descriptive statistics.

3.3.3.5 Change from Baseline in FRC and RV at Week 14

The FRC and RV, as well as the absolute change and percent change from baseline will be summarized by treatment group and study visit using descriptive statistics.

3.3.3.6 Change from Baseline in Regional Lung Volumes as measured by HRCT at Week 14

Since no patients have been enrolled in the HRCT subgroup at the time of treatment termination and the HRCT data are not collected. The data analysis will not be conducted.

3.4 Safety Analyses

Safety analyses will be performed on the safety population.

3.4.1 Exposure

Treatment duration, number of doses, amount of Study Drug and number of patients with dose interruption will be summarized by Part and treatment group.

The treatment duration (days) for each patient is defined as last dose date - first dose date +1.

3.4.2 Adverse Events

An adverse event will be regarded as treatment emergent if it is present prior to receiving the first dose of study drug and subsequently worsens, or is not present prior to receiving the first dose of study drug but subsequently appears.

In addition, if the severity of an AE changes during the study, a separate AE will be recorded for each severity on the AE CRF. The “first” and “second” AE records will be identified based on the AE start date. AE start date of the second record will be the AE stop date of first record. These linked events should be compared pairwise. Consider the following two cases,

where the AE severity (mild/moderate/severe) and seriousness (Yes/No) between the two records in a pair are compared.

Case 1: The first AE record in the pair occurs before the first dosing, and the second AE record occurs after the first dosing.

If the AE severity or seriousness of the second record is worse than that of the first record, then only the second AE is deemed as a TEAE. Otherwise, neither record is considered as TEAE.

Case 2: Both AE records in the pair occur after first dosing.

The worst AE is considered as a TEAE.

The most conservative approach will be used to determine if the event occurs after the treatment. For example, if the onset date or resolution date of an AE is prior to the first study treatment date, it will be considered to have occurred prior to the study period. If the onset or resolution date of an AE is a partial date with only month or year available or complete missing, then the event is assumed to be within the study period unless the year is prior to the year of the first study treatment date, or if in the same year, the month is prior to the month of the first study treatment date.

The incidence of AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ class for:

- Any treatment emergent adverse events
- Related treatment emergent adverse events. Related is defined as "Related", "Possible", or missing relationship to study drug
- Any treatment emergent adverse events by severity. At each level of patient summarization, a patient is classified according to the highest severity if the patient reported one or more events. Adverse events with missing severity will categorized as "Missing" for this summary
- Related treatment emergent adverse events by severity
- Serious treatment emergent adverse events
- Serious and related treatment emergent adverse events

AEs that lead to investigational drug discontinuation will be listed. Non-treatment emergent adverse event will be flagged in the data listing.

3.4.3 Laboratory Measurements

The list of safety lab analytes collected throughout the study is provided in protocol Appendix B.

All lab data collected will be presented in subject listings.

Results from chemistry, hematology, complements, and coagulation panels as well as the change and percent change from baseline will be summarized by treatment group and study visit using descriptive statistics.

For ALT and AST, the number and percent of subjects falling in each of the following categories based on the confirmed results will be tabulated by treatment group

- ALT/AST > 3 x ULN, which is confirmed
- ALT/AST > 5 x ULN, which is confirmed

For platelet, the number and percentage of subjects falling in each of the following categories based on the confirmed results will be tabulated by treatment group for 100,000/mm3 to <140,000/mm3 and <100,000/mm3.

A confirmed value is based on a consecutive lab value within 7 days. If that value is in the same or worse category the initial value is confirmed. If the consecutive value is in a better category, then the initial value is confirmed using the consecutive value category. If there is no retest within 7 days, then the initial value is presumed confirmed. If there are multiple results on the same day, then the worst value will be utilized in the analysis.

3.4.4 Vital Signs, Weight, and BMI

Vital signs will include heart rate, respiratory rate, body temperature, systolic and diastolic blood pressure and pulse pressure. Summary tables will be created to present the descriptive statistics for vital sign values, weight, and BMI as well as the change and percent change from baseline at each study visits.

3.4.5 Physical Examinations

Adverse changes in physical examinations that are deemed clinically significant by the Investigator will be classified as adverse events. All physical examination data will be provided in a data listing.

3.4.6 12-Lead Electrocardiograms (ECG)

The ECG data will include heart rate, RR interval, PR interval, QRS Duration, QT interval, QTcF, QTcB, and overall interpretation.

For the continuous variables above, descriptive statistics of the results at each study visit, as well as the changes and percent changes from baseline to each study visit, will be summarized by treatment group. The average of triplicates will be used in the summary. For the categorical responses to overall interpretation, the counts and percentages will be tabulated by treatment group. The worst case result of triplicates will be used in the summary.

In addition to the descriptive summary, a shift analysis from baseline to the worst (highest) post-baseline QTcF and QTcB by treatment group will be conducted. The categories for the shift analysis will be: ≤450 msec, >450 msec to ≤480 msec, >480 msec to ≤500 msec, and >500 msec. The average of triplicates will be used to categorize the data.

The number and percent of patients experiencing an increase from baseline in QTcF and QTcB of greater than 30 msec or 60 msec at any time post-baseline will be summarized by treatment group. The average of triplicates will be used to determine the increase.

Only data collected from the central reader and provided by ERT will be included in the summary table. The local ECG data will only be listed if collected.

The ECG data will be presented in a subject listing.

3.4.7 Prior and Concomitant Medications

Prior medications include medications started prior to the first dose of study medication regardless whether continued while on treatment or not. Concomitant medications include medications that patients are exposed to on or after the first dose of study medication. Partial or missing medication start date or end date will be imputed by the following imputation rules:

Start date:

- If year, month and day are all missing then assign the date of first dose of Study Drug
- If month and day are missing and year is:
  - earlier than the year of the first dose of Study Drug then assign December 31
  - otherwise, assign January 1
- If only day is missing and month-year is:
  - earlier than the month-year of the first dose of Study Drug then assign the last day of the month
  - otherwise, assign the first day of the month

End date: imputation will be performed for the end date only if the day or month is missing (i.e., year is present):

- If month and day are missing, then assign December 31
- If only day is missing, then assign the last day of the month

If the imputed start date is later than the imputed end date, then set the imputed start date to the imputed end date.

Prior and concomitant medications will be coded using WHO Drug dictionary and summarized by ATC class, preferred name and by treatment group and Part.

3.5 Pharmacokinetic Analysis

For pharmacokinetic (PK) assessment, plasma trough PK samples during the 13-week treatment period and post-treatment samples during the 10-week post-treatment follow up period will be collected from all patients in the study. Additionally, extensive plasma PK

samples will be collected in the PK subgroup following the first (Day 1) and last (Day 85) inhalation administration.

PK analysis will be conducted for the PK Population only.

3.5.1 Plasma Concentration Data

Plasma concentrations of ION-827359 over time, along with the scheduled (nominal) and actual samples times (i.e., time from the end of inhalation) will be listed (when applicable) for each subject, by treatment, group/subgroup, cohort, nominal dose, and day. In addition, percent differences between scheduled and actual sampling times will be listed for all subjects. Percent differences between actual administered dose and nominal dose will also be listed.

Plasma concentrations below the lower limit of quantification (LLOQ) will be indicated by "BLQ". For the purpose of calculating typical descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for plasma concentrations, all BLQ values will be set to zero. Mean plasma concentrations that are BLQ will be presented as BLQ, and the corresponding SD, %CV, geometric mean, geometric %CV will be reported as not applicable. Summary statistics of the ION-827359 plasma concentrations will be tabulated by treatment, group/subgroup, cohort, nominal dose, day, and scheduled time point. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

ION-827359 plasma concentration versus time (actual) profiles (up to 24 hours post dose and/or full profiles) following the dose on Days 1 and 85 for each subject in the PK subgroup and received ION-827359 active treatment, as well as the median plasma concentrations versus time (scheduled) profiles, will be presented graphically on linear and semilogarithmic scales. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from the median plots if there are large deviations between scheduled and actual sampling times.

3.5.2 Plasma Pharmacokinetic Parameters

Non-compartmental pharmacokinetic analysis of ION-827359 will be carried out on each individual subject data set where full PK sampling profiles are collected (PK subgroup) using Phoenix WinNonlin version 8.0 or higher (Pharsight Corporation, Mountain View, CA). Plasma pharmacokinetic parameters in each subject (when applicable) will be determined. For calculation of PK parameters, all BLQ values will be set to zero. The following plasma PK parameters for ION-827359 will be calculated (when applicable and not necessarily limited to) and based on actual sampling times:

- C_{max}: the maximum observed ION-827359 concentration in plasma
- T_{max}: the time at which C_{max} occurs

- $AUC_{0\text{-}24h}$: areas under the plasma concentration-time curve from time zero (pre-dose) to 24 hours will be calculated using the linear up-log down trapezoidal rule following the dose on Day 1 and Day 85
- $AUC_{0\text{-}168h}$ (AUC_{τ}): partial area under the plasma concentration-time curve (AUC) from time zero to 168 hours will be calculated using the linear up-log down trapezoidal rule following the dose on Day 85
- CL_{ss}/F: Apparent plasma clearance at steady-state after inhalation administration. This parameter will be calculated as CL_{ss}/F = Actual Dose/AUC_{τ} following the dose on Day 85
- $t_{1/2\lambda z}$: apparent terminal elimination half-life will be calculated from the equation, $t_{1/2\lambda z} = 0.693/\lambda_z$, where λ_z is the rate constant associated with the apparent terminal elimination phase. This parameter will be calculated following the dose on Day 85.
- V_z/F (L): Apparent volume of distribution in the terminal phase will be calculated from V_z/F = Actual Dose/(AUC_{τ} x λz) following the dose on Day 85.

λz shall be determined over a span equal to at least 1.5 x t1/2λz. A minimum of three data points in the elimination phase will be used to calculate lz using log-linear regression analysis and the adjusted correlation of determination values (r2_adj) shall be at or greater than 0.8 for the estimate to be accepted. If at least one of these three conditions are not fulfilled, the PK parameters depending on lz (i.e., t1/2λz and Vz/F) shall be flagged as not reliable if calculated and listed. They will generally be excluded from descriptive statistics and statistical testing procedures, or at the discretion of the pharmacokineticist, who is in charge of the PK analysis.

Plasma pharmacokinetic parameters will be summarized using descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) by cohort, nominal dose, and day.

Exposure-response relationships will not be conducted due to the early termination of the study.

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