



ARO-MUC5AC INHALATION SOLUTION
STUDY NO. AROMUC5AC-1001
PROTOCOL AMENDMENT 4.0
DATED 21 FEBRUARY 2024

A PHASE 1/2A STUDY EVALUATING THE EFFECTS OF
ARO-MUC5AC INHALATION SOLUTION IN HEALTHY
SUBJECTS AND PATIENTS WITH MUCO-
OBSTRUCTIVE LUNG DISEASE

Investigational Product:	ARO-MUC5AC Inhalation Solution
Study Phase:	1/2a
Indication:	Asthma and Chronic Obstructive Pulmonary Disease
EudraCT Number	2022-003467-21
Amendment 3	Version Date: 09 June 2023
South Korea-Specific Amendment 2.1	Version Date: 28 February 2023
Amendment 2	Version Date: 31 January 2023
South Korea-Specific Amendment 1.1	Version Date: 21 December 2022
Amendment 1	Version Date: 04 October 2022
Original Protocol:	Version Date: 14 March 2022
Study Sponsor:	Arrowhead Pharmaceuticals, Inc. 177 East Colorado Boulevard, Suite 700 Pasadena, CA 91105 USA

SIGNATURE PAGE

Sponsor's Approval

The protocol has been approved by Arrowhead Pharmaceuticals, Inc.

Clinical Development Medical Monitor:

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Clinical Operations Therapeutic Lead:

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Biostatistician:

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Refer to the final page of this protocol for electronic signature and date of approval.

INVESTIGATOR'S AGREEMENT

By my signature below, I attest to the following:

- I have received and read the Investigator's Brochure for ARO-MUC5AC Inhalation Solution. I have carefully read the AROMUC5AC-1001 study protocol.
- I agree to conduct the trial according to the protocol and in accordance with the principles of the International Council for Harmonisation (ICH) E6 Good Clinical Practice guidelines and all applicable laws, regulations, and requirements.
- I understand that any changes to the protocol must be approved by the Sponsor, Arrowhead Pharmaceuticals, Inc. and the Institutional Review Boards (IRBs) or Independent Ethics Committees (IECs) and, in certain cases, the US Food and Drug Administration (FDA) or other applicable regulatory agencies, before they may be implemented.
- I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Printed Name of Investigator

Signature of Investigator

Date

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Sponsor Medical Monitor Contact
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1. SYNOPSIS

Name of Sponsor/Company: Arrowhead Pharmaceuticals, Inc.		
Name of Investigational Product: ARO-MUC5AC Inhalation Solution (henceforth referred to as ARO-MUC5AC)		
Name of Active Ingredient: ADS-013		
Protocol Number: AROMUC5AC-1001 Amendment 4.0	Version Date: 21 February 2024	Phase: 1/2a
Title of Study: A Phase 1/2a Study Evaluating the Effects of ARO-MUC5AC Inhalation Solution in Healthy Subjects and Patients with Muco-Obstructive Lung Disease		
Objectives: <u>Primary</u> - To assess the safety and tolerability of ARO-MUC5AC in normal healthy volunteers (NHVs) and patients <u>Secondary</u> - To assess the pulmonary safety of ARO-MUC5AC in NHVs and patients using spirometry - To assess the pharmacokinetics (PK) of ARO-MUC5AC in NHVs and patients <u>Exploratory</u> - To assess the pharmacodynamics (PD) of ARO-MUC5AC in NHVs and patients - To assess the efficacy of ARO-MUC5AC in patients Endpoints: <u>Primary</u> - The incidence and frequency of treatment-emergent adverse events (TEAEs) over time through end of study (EOS) <u>Secondary</u> - Change from baseline over time through EOS in forced expiratory volume (FEV₁) as a safety assessment - Change from baseline over time through EOS in forced vital capacity (FVC) as a safety assessment - Plasma PK parameters of ARO-MUC5AC <u>Exploratory</u> <i>Safety:</i> - Change from baseline to Day 38 in bronchoalveolar lavage fluid (BALF) cell count and differential in NHVs - Change from baseline over time through EOS in chest x-ray findings - Incidence and titer of anti-drug antibodies over time through EOS		

Pharmacokinetics/Pharmacodynamics:

- Change from baseline over time through EOS in mucin 5AC (MUC5AC) and mucin 5B (MUC5B) protein concentrations from induced sputum samples
- Change from baseline to Day 38 in MUC5AC and MUC5B protein concentrations in bronchoscopic airway swab samples and bronchosorption samples from NHVs
- Change from baseline to Day 38 in MUC5AC and MUC5B protein concentrations in BALF samples from NHVs
- Change from baseline over time through EOS in MUC5AC and MUC5B mRNA expression from induced sputum samples
- Change from baseline to Day 38 in MUC5AC and MUC5B mRNA expression from BALF samples
- Change from baseline over time through EOS in serum total immunoglobulin E (IgE) concentrations in patients
- Change from baseline over time through EOS in plasma periostin in patients
- Change from baseline over time through EOS in fractional exhaled nitric oxide (FeNO) in patients
- Change from baseline over time through EOS in blood eosinophil or neutrophil count in patients
- Identification of ARO-MUC5AC metabolites in plasma and urine after a single dose of study drug in NHVs

Efficacy:

- Change from baseline over time through EOS in mucus symptom assessment in patients
- Change from baseline over time through EOS in prebronchodilator and postbronchodilator forced expiratory volume (FEV₁) in patients
- Change from baseline over time through EOS in prebronchodilator and postbronchodilator forced mid-expiratory flow (FEF₂₅₋₇₅) in patients
- Change from baseline over time through EOS in the chronic obstructive pulmonary disease (COPD) Assessment Test (CAT) score in patients
- Change from baseline over time through EOS in the St. George's Respiratory Questionnaire for COPD Patients (SGRQ-C) in patients

Study Design:

Methodology and Study Schema

This is a Phase 1/2a, randomized, double blind, placebo-controlled study in NHVs and patients with moderate-to-severe asthma and patients with moderate-to-severe COPD. ARO-MUC5AC is intended for use in patients with airways disease; however, because these patients may have baseline decreased lung function and are frequently on multiple concomitant medications, this study first evaluates dose levels in an NHV population to generate a baseline understanding of safety in a population with normal airway physiology and limited concomitant medication use prior to evaluating dose levels in patients. Additionally, NHV cohorts will generate an initial understanding of dose response, using sputum MUC5AC concentration as a biomarker of PD effect.

For all cohorts, participants who have signed an Institutional Review Board (IRB)/Independent Ethics Committee (IEC)-approved informed consent form (ICF) and met all the protocol eligibility criteria during Screening will be randomized to receive ARO-MUC5AC or placebo.

The single ascending dose (SAD) portion of the study includes 4 sequentially enrolled NHV cohorts treated with escalating single-dose levels. For each subject, a single dose at a fixed dose level will be administered once on Day 1, and the subject will be monitored up to Day 29. All subjects within a cohort will receive the same dose. Each cohort will contain 6 subjects (4 active:2 placebo). Each single-dose cohort will begin with administration of ARO-MUC5AC or placebo to 2 sentinel participants (1 ARO-MUC5AC, 1 placebo). Following the Day 2 evaluation in the sentinel participants, if there are no significant safety concerns based on the Principal Investigator's (PI's) judgment, the remaining participants in the cohort will be dosed. Dosing of participants in single-dose cohorts will be staggered by at least 30 minutes such that no 2 participants will be dosed simultaneously.

Based on available observations for all NHV subjects in each of the single-dose cohorts through the Day 15 visit, and experience from any other prior cohorts (ie, all cumulative available safety data), the Data Safety Committee (DSC) will meet to vote on the following (see Study Schema):

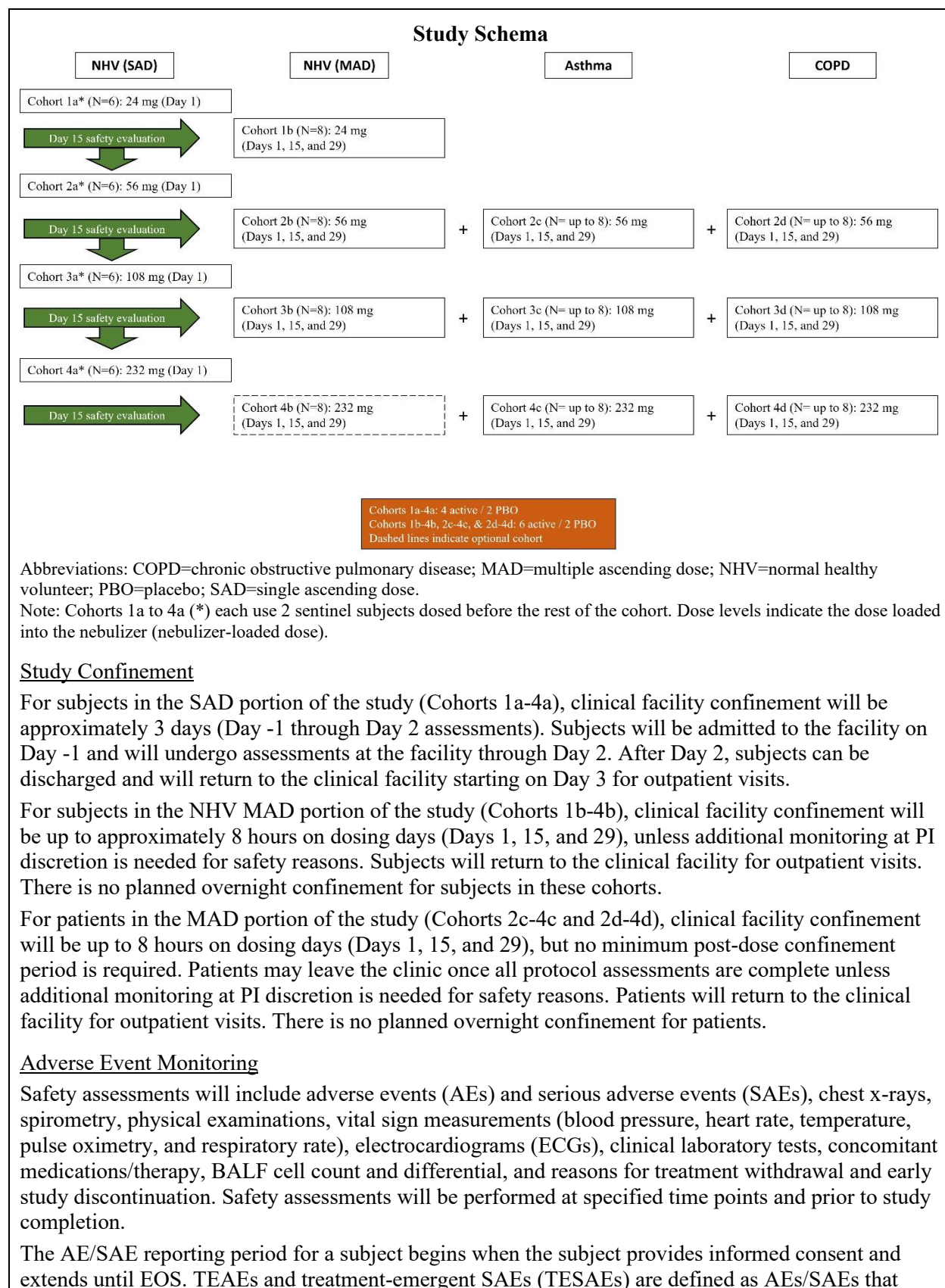
- Dose escalation to the next single-dose NHV cohort
- Initiation of multiple-dose NHV and patient cohorts when applicable per the Study Schema

The multiple ascending dose (MAD) portion of the study consists of the following cohorts: 4 NHV, 3 asthma patient, and 3 COPD patient. For both the NHV and patient cohorts, each sequential cohort will be treated with an escalating dose level. For each subject, 3 total doses at a fixed dose level will be administered, with 1 dose given on each of Days 1, 15, and 29, and the subject will be monitored up to Day 85. All subjects within a cohort will receive the same dose. Each MAD cohort will contain 8 subjects (6 active:2 placebo). No sentinel dosing will be performed in multiple-dose cohorts as all dose levels will have already been tested in single-dose cohorts. NHV MAD Cohort 4b is optional and may be enrolled at the discretion of the Sponsor following DSC approval of cohort opening.

Screening for the multiple-dose cohorts can begin prior to DSC approval of cohort opening, but dose administration may not proceed until after the DSC has opened the designated multiple-dose cohort. Multiple-dose NHV and patient cohorts will be opened for accrual once the NHV single-dose cohort at the corresponding dose level has reached Day 15 and based on a DSC vote that it is safe to proceed. Multiple-dose NHV cohorts will enroll sequentially from Cohort 1b through Cohort 4b. Multiple-dose patient cohorts may enroll in parallel with multiple-dose NHV cohorts receiving the same dose level (eg, asthma Cohort 2c may enroll in parallel with NHV Cohort 2b, see Study Schema).

Escalation to the next highest dose level will proceed until the highest planned dose level is completed or the trial is halted prematurely by the PI, DSC, or Sponsor due to safety or other concerns.

For all cohorts, the indicated dose level is the maximum allowable dose for the cohort, but the Sponsor may adjust the dose downward based on available safety and PD data.



occur following study drug administration or preexisting conditions exacerbated by study drug. The TEAE reporting period begins after the first dose and extends until the EOS visit is complete.

All SAEs that occur during the AE reporting period, in addition to reporting via electronic case report forms (eCRFs), must also be reported to the Sponsor via the SAE report form within 24 hours of being notified, regardless of the relationship of the SAE to study treatment. All AEs/SAEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. If the PI learns of an SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the PI will promptly notify the Sponsor. Laboratory or diagnostic (eg, ECG) abnormalities will be reported as AEs if considered clinically significant by the PI. Laboratory or diagnostic assessment abnormalities not reported as AEs are not to be reported as clinically significant in the study database.

Asthma exacerbation is defined in [Section 12.9.2](#). COPD exacerbation is defined in [Section 12.9.3](#).

Data Safety Committee

The DSC will consist of at least 1 Investigator, Sponsor Medical Monitor, Sponsor Pharmacovigilance Lead, and an independent physician experienced in pulmonary drug development and/or early stage clinical trials. DSC meetings will be held after all enrolled subjects finish the Day 15 study visit from each single-dose NHV cohort.

Treatment Stopping Rules

A decision to stop the trial early or discontinue the drug in an individual subject or group of subjects may be indicated based on any of the following:

- A single SAE considered related to study drug (a serious adverse reaction) in 1 subject that is confirmed to have occurred in a subject dosed with ARO-MUC5AC rather than placebo
- A severe, nonserious AE considered related to study drug (a severe adverse drug reaction) in 2 subjects in the same cohort, independent of whether they are within the same System Organ Class (SOC), that are confirmed to have occurred in subjects dosed with ARO-MUC5AC rather than placebo
- Episodes of a drop from baseline FEV₁ (which must be confirmed on repeat within 72 hours) of $\geq 20\%$ (ie, 20% absolute decline in the percent predicted FEV₁ [ppFEV₁]) in 2 separate study subjects in the same cohort that are both considered to be related to study drug, and that are confirmed to have occurred in subjects dosed with ARO-MUC5AC rather than placebo*

*FEV₁ values from spirometry sessions that fail central over-reader quality review are exempt from stopping rule evaluation. Postinduction FEV₁ values obtained as part of an induced sputum protocol are exempt from stopping rule evaluation since decreases in this setting would likely be due to sputum induction rather than study drug. Please see [Section 12.9.1](#) for suggested evaluation for clinically significant spirometry abnormalities.

If any such events occur, the DSC will meet within 7 calendar days of the Sponsor being notified of the event to review it and determine any necessary actions, as detailed below.

The following pregnancy rule will also be applied:

- A confirmed positive pregnancy test will lead to permanent discontinuation of study drug in the pregnant subject.

In the setting of a confirmed pregnancy, a mandatory DSC meeting will not be required and dosing of other subjects in the study will not cease.

The Sponsor or Investigator can discontinue any subject at any time with or without DSC consultation. If such events, as described above, occur and the subject is not discontinued from the study, the reason for not discontinuing the subject will be included in DSC meeting minutes. The DSC may pause the study and additional dosing or dose escalation to provide time to evaluate safety data and recommend the action to be taken, which may include, but is not limited to, one of the following:

- Discontinuation of a subject or group of subjects from the study
- Stopping the study immediately with no further dosing
- Continuing the study until the current cohort is completed
- Continuing the study but adjusting the next dose escalation to a level midway between the current level and the next planned level
- Continuing the study as planned

Central Labs / Local Labs / Home Health

Central labs will be utilized; however, local labs may be utilized if needed by the Investigator (including for Screening) when central labs are not readily available and for emergent situations, including time critical analytes.

In the event of logistics related disruptions, when a subject does not have direct access to the site (and only in such an event), certain site visits may be replaced by a home health visit. See [Section 6.1.8](#) for details.

Blinding

Investigators and study participants will remain blinded to treatment allocation of subjects until database lock. Selected Sponsor members will be unblinded throughout the entire study. Blinding (where applicable) will be preserved to the extent possible. However, treatment unblinding or unblinding of an individual participant may occur at the discretion of the PI or Medical Monitor, where deemed necessary, for treatment of an AE or for a decision to be made regarding trial continuation.

Number of subjects (planned):

Approximately up to 56 NHV subjects, up to 24 patients with asthma and up to 24 patients with COPD will be enrolled in the study. The study consists of 14 total cohorts. Each single-dose cohort (Cohorts 1a-4a) consists of 6 subjects randomized to ARO-MUC5AC or placebo at a 2:1 allocation (4 active:2 placebo). Each multiple-dose cohort (Cohorts 1b-4b, 2c-4c, and 2d-4d) consists of 8 subjects randomized to ARO-MUC5AC or placebo at a 3:1 allocation (6 active:2 placebo).

A subject who withdraws from the study prior to the EOS visit for reasons other than a treatment-related AE or other safety concern may be replaced.

If a subject fails to complete study assessments deemed critical to the integrity of the study (eg, subject receives at least 1 dose of study drug but fails to receive all planned doses of study drug, or subject fails to complete all planned bronchoscopies), the subject may be replaced at the discretion of the Sponsor (meaning that an additional, new subject will be added to the pertinent cohort, resulting in an increase of the cohort size).

Diagnosis and main criteria for inclusion:

Please see full inclusion/exclusion criteria in the protocol (Section 7).

NHV Cohorts: Male and nonpregnant, nonlactating healthy volunteers aged 18 to 55 years. NHV subjects must be current nonsmokers. NHVs with subnormal FEV₁, underlying pulmonary disease, or alanine transaminase (ALT) or aspartate transaminase (AST) > upper limit of normal (ULN) will be excluded. NHV subjects must be able to produce an acceptable induced sputum sample at Screening.

Asthma Cohorts: Male and nonpregnant, nonlactating subjects aged 18 to 65 years (aged 19 to 65 years where applicable according to local regulation). Subjects must have a clinical diagnosis of asthma. Asthma subjects must be treated with ≥ 500 mcg fluticasone propionate (or equivalent) daily plus 1 additional maintenance asthma controller medication. Asthma subjects must have a prebronchodilator FEV₁ between 40% to 80% predicted at Screening. Asthma subjects must be able to produce an acceptable induced sputum sample at Screening. Asthma subjects with a recent asthma exacerbation, or ALT or AST >2 $\times$ ULN, will be excluded.

COPD Cohorts: Male and nonpregnant, nonlactating subjects aged 40 to 70 years. Subjects must have a clinical diagnosis of COPD, with a smoking history (either current or former smoker). COPD subjects must have a post-bronchodilator FEV₁/FVC ratio of <0.7 and a ppFEV₁ between 40% to 80% at Screening. COPD subjects must have a clinical history of chronic bronchitis and must be able to produce an acceptable induced sputum sample at Screening. COPD subjects with a recent COPD exacerbation, or ALT or AST >2× ULN, will be excluded.

Investigational product, dosage and mode of administration:

Active (Test Formulation): ARO-MUC5AC Inhalation Solution

The active pharmaceutical ingredient (ADS-013) contained in ARO-MUC5AC is composed of a synthetic, double-stranded, small interfering RNA (siRNA) duplex conjugated to an αvβ6 integrin-targeting ligand to facilitate pulmonary epithelial delivery, formulated in normal saline to be delivered by inhalation of nebulized solution.

Duration of treatment:

Subjects in the SAD portion of the study (Cohorts 1a-4a) will receive a single dose on Day 1. Their approximate duration of study is 9 weeks from the beginning of the Screening period to the Day 29 EOS visit.

Subjects in the MAD portion of the study (Cohorts 1b-4b, 2c-4c, and 2d-4d) will receive 3 total doses, with 1 dose given on each of Days 1, 15, and 29. For subjects in the MAD portion of the study, the approximate duration of the study is 17 weeks (NHVs) or 16 weeks (patients) from the beginning of the Screening period to the Day 85 EOS visit.

Reference therapy, dosage and mode of administration:

Placebo (Reference Formulation): Normal saline (0.9%) administered by inhalation of nebulized solution; volume-matched to the corresponding ARO-MUC5AC dose volume.

Parameters for evaluation:

Pharmacokinetics

- Blood samples will be collected from each subject for PK analysis per the Schedule of Assessments (SOA)
- Blood and urine samples will be collected from each subject per the SOA for pooled metabolite analysis

Pharmacodynamics

- MUC5AC and MUC5B protein concentration in induced sputum
- MUC5AC and MUC5B protein concentration in bronchoscopic airway swab samples
- MUC5AC and MUC5B protein concentration in bronchosorption samples
- MUC5AC and MUC5B protein concentration in BALF
- MUC5AC and MUC5B mRNA expression in induced sputum
- MUC5AC and MUC5B mRNA expression in BALF
- Serum total IgE
- FeNO
- Blood eosinophil or neutrophil count
- Plasma periostin concentration

Immunogenicity

- Blood samples will be collected for potential future anti-drug antibody testing per the SOA

Efficacy (Patient Cohorts Only)

- Spirometry (prebronchodilator and postbronchodilator FEV₁ and FEF₂₅₋₇₅)
- Mucus symptom assessment
- COPD Assessment Test
- SGRQ-C

Safety

- Vital signs: Resting heart rate, semi-supine systolic/diastolic blood pressure, respiratory rate, pulse oximetry, and temperature
- Clinical laboratory measurements (eg, blood chemistry, hematology, coagulation, urinalysis)
- Resting ECG measurements (measure after participant is semi-supine for at least 3 minutes)
- Chest x-ray (posteroanterior [PA] and lateral)
- Spirometry
- BALF cell count/differential
- At each visit (or phone call), participants will be asked about concomitant medications/therapy and will be instructed to volunteer any information regarding AEs and SAEs that he/she may have experienced.
- Physical exams

Statistical Considerations:

Normal healthy volunteers, patients with asthma, and patients with COPD will be analyzed separately. For each NHV and patient population, subjects will be analyzed by cohort. In addition, subjects receiving placebo or investigational product will be pooled within each population.

Tolerability and Safety Analyses

In general, safety analyses will be performed based on the Safety Analysis Set, which includes all randomized subjects who receive at least 1 dose of study drug (placebo or active). Postdose safety assessments will be compared with measurements recorded at baseline. TEAEs will be summarized using the Medical Dictionary for Regulatory Activities (MedDRA) under Version 24.1 or later by SOC and Preferred Term (PT). The incidence and frequency of TEAEs, SAEs, related AEs, and AEs leading to withdrawal, dose modification, or treatment discontinuation will be summarized by cohort per SOC, PT, and severity. All AEs will also be presented in listings. The duration of AEs will be determined and included in listings, along with the action taken and outcome. The incidence of laboratory abnormalities will be assessed using descriptive summary statistics and shift tables. Vital sign measurements will be summarized at each scheduled time point using descriptive statistics. Abnormal physical examination findings will be summarized by time point and presented in subject listings. ECG parameters, changes from baseline, and qualitative assessments will be summarized. Pregnancy and follicle-stimulating hormone (FSH) test results will be listed separately by time point. FEV₁ and FVC values will be summarized at each scheduled time point using descriptive statistics. Physical exam and chest x-ray data will be listed.

Pharmacokinetics

Plasma concentrations of ARO-MUC5AC full-length antisense strand will be used to calculate the following PK parameters, when feasible: maximum observed plasma concentration (C_{max}), area under the plasma concentration time curve (AUC) from time 0 to 24 hours (AUC₀₋₂₄), AUC from time 0 to the last quantifiable plasma concentration (AUC_{last}), AUC from time 0 extrapolated to infinity

(AUC_{inf}), terminal elimination half-life ($t_{1/2}$), apparent systemic clearance (CL/F), and apparent terminal-phase volume of distribution (V_z/F). Additional PK parameters may be calculated as needed. PK parameters will be determined using noncompartmental methods. Descriptive statistics of PK parameters will include mean, SD coefficient of variation, median, minimum, and maximum. PK results will be analyzed for dose proportionality.

The PK population is all subjects who received at least 1 dose of study drug and who have measurable PK concentration data. A subject may be excluded from PK summary and statistical analysis if the subject has a protocol deviation(s) that is thought to impact PK analysis.

Pharmacodynamics

MUC5AC and MUC5B protein concentrations and mRNA expression, including percent change and duration of response from baseline to EOS will be analyzed and summarized by cohort. Blood eosinophil or neutrophil count, serum IgE, plasma periostin, and FeNO at each scheduled assessment and change from baseline will be summarized by patient cohort.

Efficacy

A descriptive summary of FEV₁ and FEF₂₅₋₇₅, SGRQ-C score, and CAT score at each scheduled assessment and change from baseline will be summarized by patient cohort. A listing of mucus symptom assessment results will be provided.

Immunogenicity

Shift tables for the incidence and titers of anti-drug antibodies may be displayed by cohort or by dose.

Baseline Values

Baseline for data analysis purposes is defined as the predose value occurring closest to the first dose of ARO-MUC5AC or placebo administered in the study, unless otherwise indicated.

Additional details will be provided in the Statistical Analysis Plan.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and special terms are used in this study protocol.

Table 2: Abbreviations

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
API	active pharmaceutical ingredient
AST	aspartate transaminase
ATS	American Thoracic Society
BALF	bronchoalveolar lavage fluid
BMI	body mass index
CAT	COPD assessment test
COPD	chronic obstructive pulmonary disease
COVID-19	coronavirus disease 2019
CRA	Clinical Research Associate
CRO	Contract Research Organization
CVA	cerebrovascular accident
DBP	diastolic blood pressure
DSC	Data Safety Committee
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EOS	end of study
ERS	European Respiratory Society
FDA	(US) Food and Drug Administration
FEF ₂₅₋₇₅	forced mid-expiratory flow
FeNO	fractional exhaled nitric oxide
FEV ₁	forced expiratory volume
FIH	first-in-human
FVC	forced vital capacity
GCP	Good Clinical Practice
GINA	Global Initiative for Asthma
GLP	Good Laboratory Practice

Abbreviation	Definition
Hb	hemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IgE	immunoglobulin E
IL	interleukin
IP	investigational product
IRB	Institutional Review Board
IWRS	interactive web response system
LABA	long-acting β_2 agonist
LAMA	long-acting muscarinic antagonist
LTRA	leukotriene receptor antagonist
MABEL	minimal anticipated biological effect level
MAD	multiple ascending dose
mRNA	messenger RNA
MUC5AC	mucin 5AC
MUC5B	mucin 5B
NAAT	nucleic acid amplification test
NHV	normal healthy volunteer
NLD	nebulizer loaded dose
NOAEL	no-observed-adverse-effect level
NYHA	New York Heart Association
PCP	phencyclidine
PD	pharmacodynamics
PDD	pulmonary deposited dose
PI	Principal Investigator
PK	pharmacokinetics
ppFEV ₁	percent predicted FEV ₁

Abbreviation	Definition
PT	Preferred Term
RDD	respirable delivered dose
RISC	RNA-induced silencing complex
RNAi	RNA interference
SABA	short-acting β 2 agonist
SAD	single ascending dose
SAE	serious adverse event
SBP	systolic blood pressure
SGRQ-C	St. George's Respiratory Questionnaire for COPD Patients
siRNA	small interfering RNA
SOA	Schedule of Assessments
SOC	System Organ Class
TEAE	treatment-emergent adverse event
THC	tetrahydrocannabinol
TIA	transient ischemic attack
TSLP	thymic stromal lymphopoietin
ULN	upper limit of normal

4. INTRODUCTION

4.1. Muco-obstructive Lung Disease and the Role of MUC5AC

Mucus that lines airway epithelial cells provides an important barrier function, protecting the underlying airway from inhaled pathogens and toxins. The mucociliary clearance mechanism allows for directional transport of mucus and its trapped contents out of the airway. This transport relies on a 2-gel layer resting above the airway epithelial cell: a periciliary layer surrounding the epithelial cilia and a mucus layer resting on top of the cilia ([Button 2012](#)). Impairment of any component of this transport mechanism (ciliary beating, periciliary hydration, or mucus composition) may lead to dysfunctional mucociliary clearance and resultant muco-obstructive disease ([Boucher 2019](#)).

Mucins are polymeric, highly glycosylated proteins that serve as key components of airway mucus (together with water, salt, and other proteins). In humans, 2 secreted mucins contribute to the airway mucus layer: mucin 5AC (MUC5AC) and mucin 5B (MUC5B) ([Bonser 2017](#)). MUC5B, expressed by both superficial epithelia and submucosal glands, is the dominant mucin in the normal airway ([Okuda 2019](#)). MUC5B is necessary for proper mucociliary clearance and defense against bacterial pathogens ([Roy 2014](#)). In normal airways, MUC5AC is expressed primarily in superficial epithelia of the proximal airway ([Okuda 2019](#)) and is not necessary for mucociliary clearance or airway defense ([Roy 2014](#)). In contrast to the normal airway, MUC5AC is upregulated in the inflamed airway in response to various stresses and stimuli, serving as a “response mucin” ([Okuda 2019](#)). Prolonged, pathologic airway inflammation in the setting of multiple disease states results in disruption of the normal mucin balance, with a marked increase in MUC5AC production that is out of proportion to the change in MUC5B production ([Lachowicz-Scroggins 2016](#); [Radicioni 2021](#); [Ramsey 2020](#)). Thus, altered airway mucus composition, with a skew toward MUC5AC, is a hallmark of multiple muco-obstructive diseases.

4.1.1. Asthma

Asthma is a chronic respiratory disease characterized by variable symptoms of wheezing, shortness of breath, chest tightness and/or cough, and expiratory airflow limitation ([GINA 2021](#)). Asthmatic airflow obstruction can be triggered by a range of stimuli, such as exposure to allergens and inhaled irritants, viral infections, and exercise ([GINA 2021](#)). This airflow obstruction is due to a number of factors that decrease airway diameter, including smooth muscle contraction, airway edema, and mucus production.

Clinical data have pointed to the centrality of MUC5AC-driven mucus plugging in asthmatic airflow obstruction and subsequent morbidity and mortality. Asthmatic inflammation results in upregulation of MUC5AC expression to such an extent that MUC5AC, rather than MUC5B, becomes the predominant airway mucin ([Lachowicz-Scroggins 2016](#)). A high airway MUC5AC:MUC5B ratio correlates with increased mucus plugging, as seen on chest computed tomography (CT) scans in patients with asthma ([Dunican 2018](#)). The increased extent of mucus plugging is, in turn, associated with worsened airflow obstruction, poor asthma symptom control, and increased asthma severity ([Dunican 2018](#)). Thus, upregulated MUC5AC is associated with increased mucus plugging, which narrows airways and thereby drives airflow obstruction and associated symptoms.

In addition to airflow obstruction, MUC5AC is also implicated in the onset of acute asthma exacerbations. *MUC5AC* belongs to a module of mucus-related genes that are upregulated in children who experience an asthma exacerbation following a cold (Altman 2019); subsequent studies have clarified that there is an inducible increase specifically in *MUC5AC* expression during asthma exacerbations (Altman 2021). Finally, sputum MUC5AC protein concentrations are higher during exacerbations than during stable disease (Welsh 2017).

Beyond the significant morbidity associated with dysregulated mucus production in asthma, autopsy studies have indicated that airway mucus plugging is a significant contributor to fatal asthma (Kuyper 2003) and revealed that mucostasis in the setting of fatal asthma is triggered by tethering of MUC5AC-containing mucus gels to the airway epithelium (Bonser 2016).

This clinical data implicating MUC5AC in asthma pathology is bolstered by genetic studies in both humans and animals. Multiple genome-wide association studies have demonstrated a correlation between *MUC5AC* single nucleotide polymorphism variants, which result in increased MUC5AC expression, and the development of asthma (Pividori 2019; Shrine 2019). Additionally, mice with genetic deletion of *Muc5ac* are protected from airway hyperreactivity in the setting of allergic stimuli, again supporting the concept that MUC5AC-driven mucus plugging plays a central role in allergen-induced airflow obstruction (Evans 2015).

Current therapies for asthma are focused on smooth muscle relaxation with bronchodilators as well as various anti-inflammatory approaches, from corticosteroid therapy to targeted biologics, which target key pathways contributing to bronchoconstriction. However, there is currently no therapy that directly targets muco-obstruction. In patients with asthma who have significant mucus plugging, even aggressive therapy with bronchodilators and systemic corticosteroids is insufficient to normalize airflow obstruction (Dunican 2018). This supports the concept that mucus represents a critical contributor to airflow limitation in asthma and that mucus-directed therapy remains a significant unmet need for these patients.

4.1.2. Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is defined by persistent airflow obstruction that shows limited reversibility (Celli 2019). This obstruction is a common end to multiple pathways, both genetic and environmental (such as inhalation of tobacco smoke), that affect the development and function of the lungs (Agustí 2019). The chronic inflammatory response to these inhaled noxious irritants results in tissue remodeling that is characterized by emphysematous destruction of alveolar walls, chronic bronchitis, and obstruction of small airways with mucus (Hogg 2009).

As in patients with asthma, MUC5AC expression is closely tied to airflow obstruction in patients with COPD. The concentration of MUC5AC and the MUC5AC:MUC5B ratio in sputum are markedly increased in patients with severe COPD and a history of smoking, compared with healthy controls (Kesimer 2017). Increased sputum MUC5AC concentration is associated with multiple measures of COPD morbidity, including decreased forced expiratory volume (FEV₁), increased exacerbation frequency, and higher symptom scores (Radicioni 2021). Finally, the extent of mucus plugging, quantified on chest CT imaging, is associated with the severity of airflow obstruction in COPD, as it is in asthma (Dunican 2021).

Beyond its role in chronic airflow obstruction, MUC5AC is also implicated in the pathogenesis of COPD exacerbations (Singanayagam 2022). Airway MUC5AC concentration is increased during disease exacerbations and is associated with multiple markers of exacerbation severity. In animal models of viral-induced exacerbation, MUC5AC amplifies airway inflammation, suggesting that MUC5AC silencing could ameliorate exacerbations.

Current treatments for COPD are focused on smooth muscle relaxation and reducing inflammation, with a notable lack of disease-modifying therapies. There is no current therapy that directly targets the muco-obstructive component of COPD pathology.

4.2. Overview of ARO-MUC5AC Development

4.2.1. Mechanism of ARO-MUC5AC Action and Therapeutic Rationale

The active pharmaceutical ingredient (API) (ADS-013) contained within ARO-MUC5AC Inhalation Solution is an RNA interference (RNAi) conjugate designed to specifically silence pulmonary epithelial *MUC5AC* messenger RNA (mRNA). The conjugate is composed of a synthetic, double-stranded RNAi trigger conjugated to an $\alpha\beta6$ integrin-targeting ligand for the $\alpha\beta6$ receptor, which is expressed on pulmonary epithelial cells. This targeting approach facilitates the binding of ADS-013 to pulmonary epithelial cells, internalization through receptor-mediated endocytosis, and release of the double-stranded RNAi trigger into the cytoplasm from the endosome, leading to loading of the RNAi trigger antisense strand into the machinery of the RNA-induced silencing complex (RISC). This association leads to highly sequence-specific degradation of *MUC5AC* mRNA. Active RISC is a multiple-turnover enzyme complex. Therefore, incorporation of a single RNAi trigger into RISC can result in the degradation of many mRNA molecules, and subsequently, prolonged reduction of the expression of the corresponding protein. Because a small amount of guide strand can be active in the cytoplasm of target cells for extended periods of time, the pharmacologic activity of ADS-013 persists beyond the period of plasma or local tissue exposure.

MUC5AC is implicated in the development of mucus plugging and airflow obstruction in multiple muco-obstructive diseases, including asthma and COPD. Reduction of MUC5AC expression via RNAi is designed to reduce the amount of MUC5AC protein secreted by airway epithelium into the airway lumen. Reduced airway MUC5AC concentration should lead to decreased mucus plugging and improvements in airflow obstruction and exacerbation frequency.

4.2.2. Preclinical Studies

The Sponsor is conducting a comprehensive preclinical program to support ARO-MUC5AC development. Proof-of-concept studies in animal models support the use of small interfering RNA (siRNA) against *MUC5AC* as a potential treatment for airway muco-obstructive diseases. Findings of potential clinical significance and relevance to this protocol are summarized in the Investigator's Brochure (IB). Additional details regarding preclinical pharmacology, pharmacokinetics (PK), and toxicology results are also provided in the IB.

The Sponsor has conducted Good Laboratory Practice (GLP) toxicology studies in rats and in cynomolgus monkeys to enable clinical studies in humans. The results of animal toxicology studies are presented in the IB.

4.2.3. Clinical Studies

AROMUC5AC-1001 is a first-in-human (FIH) Phase 1/2a clinical study of ARO-MUC5AC, which is designed to assess the safety, PK, pharmacodynamics (PD), and efficacy of ARO-MUC5AC, initially in normal healthy volunteers (NHVs) and subsequently in patients with asthma and COPD.

Subsequent clinical development plans will follow from the results of AROMUC5AC-1001.

4.3. Potential Risks of Study Participation

This is an FIH dose-ranging study to evaluate the effects of inhaled ARO-MUC5AC therapy in NHVs, patients with asthma, and patients with COPD. The trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements. Considerations on risks to study participants are detailed below.

4.3.1. MUC5AC-Related Risks

The Sponsor is not aware of any prior clinical trials evaluating MUC5AC inhibitors, and thus is not aware of prior clinical data to inform the risk of MUC5AC depletion. Mice with genetic deletion of *Muc5ac* survive normally without any identified physiologic abnormality. Specifically, mice with genetic deletion of *Muc5ac* do not show any dysfunction in mucociliary clearance or in airway defense against pathogens (Roy 2014).

Animals studies suggest that MUC5AC may play a role in limiting the spread of helminth infections to the lung, which suggests a theoretical risk that ARO-MUC5AC could exacerbate pulmonary manifestations of systemic helminth infection (Campbell 2019). To mitigate this risk, subjects with known active parasitic infections or recent treatment for parasitic infections are excluded from the study.

4.3.2. Pulmonary Risks

GLP-compliant animal toxicology studies have not revealed evidence of pulmonary toxicity at clinically relevant doses, and no-observed-adverse-effect levels (NOAELs) from such studies provide a wide therapeutic index. However, as this is the first administration of ARO-MUC5AC in humans, careful monitoring of pulmonary function is prudent to mitigate risk. Frequent measurement of forced expiratory volume (FEV₁) and forced vital capacity (FVC), as described in the Schedule of Assessments (SOA) (Table 3, Table 4, Table 5, and Table 6), and stopping rules for adverse changes in airflow are intended to mitigate the risk of pulmonary toxicity. Pulse oximetry, chest x-ray imaging, and profiling of bronchoalveolar lavage fluid (BALF) inflammatory cells are also included to assess for potential pulmonary toxicity.

4.3.3. Embryo-Fetal Risks

GLP-compliant embryo-fetal toxicity studies in rats and rabbits did not indicate any ADS-013-related effects on any ovarian or uterine parameters (mean number of implants, corpora lutea, percent post-implantation loss, resorptions, fetuses [live and dead], percent post-implantation loss), mean fetal body weights (combined, male and female), or fetal external, visceral, or skeletal malformations or variations at ≤ 160 mg/kg/day when administered twice during the period of development. However, as a precaution, both male and female eligible subjects

enrolled in this study (including partners) must agree to use a highly effective form of contraception, in addition to a condom, during the study or agree to abstinence (acceptable only if this method is in alignment with the normal lifestyle of the subject) (see [Appendix 2](#)).

4.3.4. Procedural Risks

4.3.4.1. Bronchoscopy Risks

This is a semi-invasive procedure involving sedation, local anesthesia, and insertion of a bronchoscope into the airways. Following insertion of the bronchoscope, bronchoalveolar lavage and airway swabs will be performed; notably, no transbronchial biopsies will be taken. Airway swabs will be taken from large airways under direct bronchoscopic visualization. Complications of bronchoscopy include sore throat, bronchospasm, hypoxemia, laryngospasm, laryngeal injury, fever, and adverse reactions to sedation. The most common adverse event (AE) is laryngospasm, which occurs in about 0.6% of flexible bronchoscopies ([Pue 1995](#)). More severe complications such as pneumothorax and pulmonary hemorrhage are rare (0.1%-0.2% for each) and occur more frequently in the setting of transbronchial biopsies, which will not be performed in this study ([Facciolongo 2009](#); [Pue 1995](#)). Subject selection has a significant impact on procedural complications, and performance of bronchoscopies in NHVs only, rather than those with underlying respiratory disease, will significantly mitigate the risk of the procedure. Post-procedural phone calls will take place per the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)) to identify any procedural complications so that they can be appropriately monitored and treated as necessary.

4.3.4.2. Induced Sputum Risks

Sputum induction with hypertonic saline is a well-tolerated procedure. Prior efforts at collecting induced sputum for diagnosis of tuberculosis, for example, indicated that there were no complications in any of the subjects; 3% of subjects developed severe cough following induction, which subsided in less than 5 minutes in all cases ([Anderson 1995](#)). The sputum induction procedure has also been shown to be safe in patients with asthma, including those with severe disease ([de la Fuente 1998](#)). A sputum induction protocol, with frequent monitoring of lung function via spirometry throughout the procedure and provisions to stop the induction procedure in the setting of significant drop in lung function will be followed to mitigate risk of complications.

4.3.5. Benefits

As this is a FIH study, clinical benefits remain undetermined. There is no expected benefit of ARO-MUC5AC therapy to NHV subjects. In patients with asthma or COPD, ARO-MUC5AC has the potential to decrease MUC5AC concentration in the airway and to thereby reduce mucus plugging, which may improve lung function and asthma symptoms, and reduce exacerbation frequency.

4.3.6. Overall Risk/Benefit Conclusion

The collective results of nonclinical pharmacology studies, nonclinical safety studies, and clinical safety assessments did not identify any effects considered to pose a significant risk to

human safety at the doses proposed in this clinical study. The totality of the data supports a favorable benefit-to-risk profile and favors the ongoing clinical development of ARO-MUC5AC.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1. Objectives

5.1.1. Primary Objective

- To assess the safety and tolerability of ARO-MUC5AC in NHVs and patients

5.1.2. Secondary Objectives

- To assess the pulmonary safety of ARO-MUC5AC in NHVs and patients using spirometry
- To assess the PK of ARO-MUC5AC in NHVs and patients

5.1.3. Exploratory Objectives

- To assess the PD of ARO-MUC5AC in NHVs and patients
- To assess the efficacy of ARO-MUC5AC in patients

5.2. Endpoints

5.2.1. Primary Endpoint

- The incidence and frequency of treatment-emergent adverse events (TEAEs) over time through end of study (EOS)

5.2.2. Secondary Endpoints

- Change from baseline over time through EOS in FEV₁ as a safety assessment
- Change from baseline over time through EOS in FVC as a safety assessment
- Plasma PK parameters of ARO-MUC5AC

5.2.3. Exploratory Endpoints

Safety:

- Change from baseline to Day 38 in BALF cell count and differential in NHVs
- Change from baseline over time through EOS in chest x-ray findings
- Incidence and titer of anti-drug antibodies over time through EOS

Pharmacokinetics/Pharmacodynamics:

- Change from baseline over time through EOS in MUC5AC and MUC5B protein concentrations from induced sputum samples
- Change from baseline to Day 38 in MUC5AC and MUC5B protein concentrations in bronchoscopic airway swab samples and bronchosorption samples from NHVs
- Change from baseline to Day 38 in MUC5AC and MUC5B protein concentrations in BALF samples from NHVs

- Change from baseline over time through EOS in MUC5AC and MUC5B mRNA expression from induced sputum samples
- Change from baseline to Day 38 in MUC5AC and MUC5B mRNA expression from BALF samples
- Change from baseline over time through EOS in serum total immunoglobulin E (IgE) concentrations in patients
- Change from baseline over time through EOS in plasma periostin in patients
- Change from baseline over time through EOS in fractional exhaled nitric oxide (FeNO) in patients
- Change from baseline over time through EOS in blood eosinophil or neutrophil count in patients
- Identification of ARO-MUC5AC metabolites in plasma and urine after a single dose of study drug in NHVs

Efficacy:

- Change from baseline over time through EOS in mucus symptom assessment in patients
- Change from baseline over time through EOS in prebronchodilator and postbronchodilator FEV₁ in patients
- Change from baseline over time through EOS in prebronchodilator and postbronchodilator forced mid-expiratory flow (FEF₂₅₋₇₅) in patients
- Change from baseline over time through EOS in CAT score in patients
- Change from baseline over time through EOS in the St. George's Respiratory Questionnaire for COPD Patients (SGRQ-C) in patients

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

6.1.1. Overview of Study Design

This is a Phase 1/2a, randomized, double-blind, placebo-controlled FIH study of ARO-MUC5AC in NHVs and patients with moderate-to-severe asthma and patients with moderate-to-severe COPD. The purpose of this study is to evaluate the safety, tolerability, PK, PD, and efficacy of ARO-MUC5AC. The study is divided into initial NHV cohorts evaluating single ascending doses (SADs), followed by NHV and patient cohorts evaluating multiple ascending doses (MADs).

ARO-MUC5AC is intended for use in patients with airways disease; however, because these patients may have baseline decreased lung function and are frequently on multiple concomitant medications, this study first evaluates dose levels in an NHV population to generate a baseline understanding of safety in a population with normal airway physiology and limited concomitant medication use prior to evaluating dose levels in patients. Additionally, NHV cohorts will generate an initial understanding of dose response using sputum MUC5AC concentration as a biomarker of PD effect.

For all cohorts, participants who have signed an Independent Ethics Committee (IEC) /Institutional Review Board (IRB)-approved informed consent form (ICF) and met all the protocol eligibility criteria during Screening will be randomized to receive ARO-MUC5AC or placebo.

The SAD portion of the study includes 4 sequentially enrolled NHV cohorts treated with escalating single-dose levels. For each subject, a single dose at a fixed dose level will be administered once on Day 1 and the subject will be monitored up to Day 29. All subjects within a cohort will receive the same dose. Each cohort will contain 6 subjects (4 active:2 placebo). Each single-dose cohort will begin with administration of ARO-MUC5AC or placebo to 2 sentinel participants (1 ARO-MUC5AC, 1 placebo). Following the Day 2 evaluation in the sentinel participants, if there are no significant safety concerns based on the Principal Investigator's (PI's) judgment, the remaining participants in the cohort will be dosed. The dosing of participants in single-dose cohorts will be staggered by at least 30 minutes such that no 2 participants will be dosed simultaneously.

Based on available observations for all NHV subjects in each of the single-dose cohorts through the Day 15 visit, and experience from other prior cohorts (ie, all cumulative available safety data), the Data Safety Committee (DSC) will meet to vote on the following (see Study Schema, [Figure 1](#)):

- Dose escalation to the next single-dose NHV cohort
- Initiation of multiple-dose NHV and patient cohorts when applicable per the Study Schema ([Figure 1](#))

The MAD portion of the study consists of the following cohorts: 4 NHV, 3 asthma patient, and 3 COPD patient. For both the NHV and patient cohorts, each sequential cohort will be treated with an escalating dose level. For each subject, 3 total doses at a fixed dose level will be

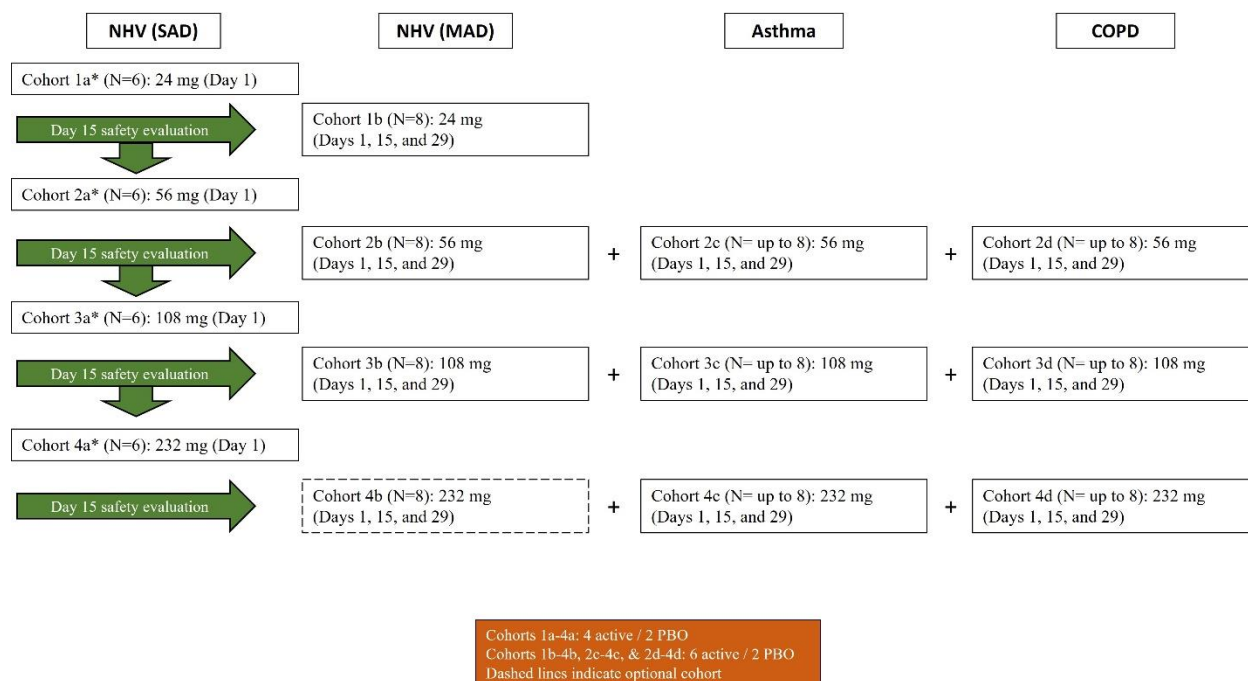
administered, with 1 dose given on each of Days 1, 15, and 29, and the subject will be monitored up to Day 85. All subjects within a cohort will receive the same dose. Each MAD cohort will contain 8 subjects (6 active:2 placebo). No sentinel dosing will be performed in multiple-dose cohorts as all dose levels will have already been tested in single-dose cohorts. NHV MAD Cohort 4b is optional and may be enrolled at the discretion of the Sponsor following DSC approval of cohort opening.

Screening for the multiple-dose cohorts can begin prior to DSC approval of cohort opening, but dose administration may not proceed until after the DSC has opened the designated multiple-dose cohort. Multiple-dose NHV and patient cohorts will be opened for accrual once the NHV single-dose cohort at the corresponding dose level has reached Day 15 and based on a DSC vote that it is safe to proceed. Multiple-dose NHV cohorts will enroll sequentially from Cohort 1b through Cohort 4b. Multiple-dose patient cohorts may enroll in parallel with multiple-dose NHV cohorts receiving the same dose level (eg, asthma Cohort 2c may enroll in parallel with NHV Cohort 2b, see Study Schema).

Escalation to the next highest dose level will proceed until the highest planned dose level is completed or the trial is halted prematurely by the PI, DSC, or Sponsor due to safety or other concerns.

For all cohorts, the indicated dose level is the maximum allowable dose for the cohort, but the Sponsor may adjust the dose downward based on available safety and PD data.

Figure 1: Study Schema



Abbreviations: COPD=chronic obstructive pulmonary disease; MAD=multiple ascending dose; NHV=normal healthy volunteer; PBO=placebo; SAD=single ascending dose.

Note: Cohorts 1a to 4a (*) each use 2 sentinel subjects dosed before rest of the cohort. Dose levels indicate the dose loaded into the nebulizer (nebulizer-loaded dose).

Table 3: Schedule of Assessments for Single-Dose Normal Healthy Volunteer Cohorts (Cohorts 1a-4a)

Assessment	Screen		Day 1	Day 2	Day 3	Day 8	Day 15	Day 22	Day 29 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9
Visit Window	Days -35 to -2	Day -1	±0 days			±2 days	±2 days	±2 days	±2 days
Admit to study site		X							
Informed Consent	X								
Eligibility Criteria	X		X						
Randomization			X						
Study Drug Dosing			X						
Demographics	X								
Medical History	X								
Drug Screen	X								
Hepatitis/HIV Serologies	X								
FSH (in women of postmenopausal age only)	X								
Urine Cotinine	X								
COVID-19 test ^a	X								
Urine qualitative pregnancy test	X	X							X
Body Mass Index/Height/Weight ^{b,c}	X		X						X
Physical Exam (may be symptom directed after Day 1) ^b	X		X	X	X	X	X	X	X
ECG ^d	X		X	X	X		X		X

Assessment	Screen		Day 1	Day 2	Day 3	Day 8	Day 15	Day 22	Day 29 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9
Visit Window	Days -35 to -2	Day -1	±0 days			±2 days	±2 days	±2 days	±2 days
Vital Signs (BP, temp, pulse ox, RR, heart rate), perform before other evaluations ^b	X		X	X	X	X	X	X	X
Clinical Labs ^{b,c}	X		X	X	X	X	X	X	X
PK ^f			X	X	X				
Plasma metabolite ID ^f			X	X	X				
Urine collection for excretion and metabolite ID ^g			X	X					
Anti-drug antibodies ^{b,c}			X				X		X
Spirometry ^{e,h,i}	X		X	X	X	X	X	X	X
Chest x-ray (PA and lateral) ^j	X								X
Induced sputum MUC5AC and MUC5B protein ^{b,e,i,k}	X		X			X	X	X	X
Induced sputum MUC5AC and MUC5B mRNA ^{b,e,i,k}	X		X			X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X
Concomitant Medications/Therapies	X	X	X	X	X	X	X	X	X

Abbreviations: BP=blood pressure; COVID-19=coronavirus disease 2019; ECG=electrocardiogram; EOS=end of study; FSH=follicle-stimulating hormone; HIV=human immunodeficiency virus; ID=identification; MUC5AC=mucin 5AC; MUC5B=mucin 5B; NHV=normal healthy volunteer; PA=posteroanterior; PI=Principal Investigator; PK=pharmacokinetic; pulse ox=pulse oximetry; RR=respiratory rate; temp=temperature; Term=termination.

^a COVID-19 antigen testing may be performed at any time during Screening window, including Day -1, with timing at the discretion of the PI.

^b If this assessment is performed on a dosing day, then it must be performed prior to study drug dosing.

^c Screening height measurement can be used for all future visits.

- ^d On dosing days, ECGs will be performed predose (-2 hours $\pm$ 1 hour) and postdose (2 hours $\pm$ 15 minutes based on start of inhalation); all ECGs will be performed in triplicate with 3- to 5-minute intervals between each measurement.
- ^e Order of assessments: Spirometry, induced sputum, blood draw. Order of other assessments is flexible with the stipulation that indicated assessments must occur prior to dosing or at other specified time points as detailed in (d), (f), (g), and (h).
- ^f Plasma samples for PK analysis and metabolite identification will both be collected at the following time points:
- Day 1: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, 6, 8, and 12 hours
 - Day 2: 24 hours
 - Day 3: 48 hours
- The recommended allowance of time windows is $\pm$ 5 minutes for the 0.5-hour time point and $\pm$ 10% of nominal time for all other time points. All times are referenced to the beginning of inhalation dosing. If a time window is missed, every effort should be made to collect the PK blood samples with documentation of actual collection times.
- ^g Urine will be collected predose and cumulatively from 0 to 6 hours and 6 to 24 hours postdose on Day 1. Subjects must have a spot urine void predose, which must be collected and used as a predose background for analysis. The 24-hour urine collection will end with a urine void prior to the 24-hour PK blood draw. Metabolite ID will be performed on collected urine samples (pooled analysis).
- ^h On dosing day (Day 1), spirometry measures will be completed predose and postdose at 15 minutes ($\pm$ 10 minutes), 60 minutes ($\pm$ 10 minutes), and 120 minutes ($\pm$ 15 minutes). On nondosing days, spirometry measures will only be completed once per visit. Additional spirometry measures will be required as safety evaluations when the induced sputum protocol is performed (see [i]). For NHVs, spirometry measurements will not involve bronchodilation.
- ⁱ The induced sputum protocol requires baseline and repeat spirometry measurements. The initial spirometry measure of the visit will serve as the baseline spirometry measure for the induced sputum protocol and must be performed within 2 hours of the induced sputum protocol.
- ^j To minimize the risk associated with radiation exposure, a chest x-ray performed within 90 days prior to first dose (even if performed prior to the Screening window) may be used for Screening purposes.
- ^k Samples for sputum MUC5AC and MUC5B will be collected on all subjects who are screened; however, MUC5AC and MUC5B measurement on these samples will only be performed for those subjects who are dosed with study drug.

Table 4: Schedule of Assessments for Multiple-Dose Normal Healthy Volunteer Cohorts (Cohorts 1b-4b)

Assessment	Screen			Day 1	Day 8	Day 15	Day 22	Day 29	Day 35	Day 38 ^a	Day 39	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Visit Window	Days -35 to -1			±0 days	±2 days	±1 day	±2 days	±1 day	±1 day	±2 days	+1 day	±1 day	±2 days	±2 days	±5 days	±5 days
Informed Consent	X															
Eligibility Criteria	X			X												
Randomization				X												
Study Drug Dosing				X		X		X								
Demographics	X															
Medical History	X															
Drug Screen	X															
Hepatitis/HIV Serologies	X															
FSH (in women of postmenopausal age only)	X															
Urine Cotinine	X															
COVID-19 test ^b	X															
Urine qualitative pregnancy test ^c	X			X		X		X								X
Body Mass Index/ Height and Weight ^{c,d}	X			X				X						X		X

Assessment	Screen			Day 1	Day 8	Day 15	Day 22	Day 29	Day 35	Day 38 ^a	Day 39	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Visit Window	Days -35 to -1			±0 days	±2 days	±1 day	±2 days	±1 day	±1 day	±2 days	+1 day	±1 day	±2 days	±2 days	±5 days	±5 days
Physical Exam (may be symptom directed after Day 1) ^{c,e}	X	X		X	X	X	X	X	X	X		X	X	X	X	X
ECG ^f	X			X		X		X				X				X
Vital Signs (BP, temp, pulse ox, RR, heart rate), perform before other evaluations ^c	X	X		X	X	X	X	X	X	X		X	X	X	X	X
Clinical Labs ^{c,g}	X			X	X	X	X	X	X			X	X	X	X	X
Serum urea, protein, and albumin		X								X						
PK ^h				X		X		X								
Anti-drug antibodies ^{c,g}				X		X		X				X			X	X
Spirometry ^{g,i,j}	X			X	X	X	X	X	X			X	X	X	X	X
Chest x-ray (PA and lateral) ^k	X								X							X
Induced sputum MUC5AC and MUC5B protein ^{c,g,j,l}	X			X	X	X	X	X	X			X	X	X	X	X

Assessment	Screen			Day 1	Day 8	Day 15	Day 22	Day 29	Day 35	Day 38 ^a	Day 39	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Visit Window	Days -35 to -1			±0 days	±2 days	±1 day	±2 days	±1 day	±1 day	±2 days	+1 day	±1 day	±2 days	±2 days	±5 days	±5 days
Induced sputum MUC5AC and MUC5B mRNA ^{c,g,j,l}	X			X	X	X	X	X	X			X	X	X	X	X
Bronchoscopy ^m		X								X						
BALF MUC5AC and MUC5B mRNA ^m		X								X						
BALF MUC5AC and MUC5B protein ^m		X								X						
Bronchosorption MUC5AC and MUC5B protein ^m		X								X						
BALF cell count/differential ^{lm}		X								X						
Bronchoscopic airway swab for MUC5AC and MUC5B protein ^m		X								X						
Phone Call ⁿ			X								X					
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications/Therapies	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BALF=bronchoalveolar lavage fluid; BP=blood pressure; COVID-19=coronavirus disease 2019; ECG=electrocardiogram; EOS=end of study; FSH=follicle-stimulating hormone; HIV=human immunodeficiency virus; MUC5AC=mucin 5AC; MUC5B=mucin 5B; NHV=normal healthy volunteer; PA=posteroanterior; PI=Principal Investigator; PK=pharmacokinetic; pulse ox=pulse oximetry; RR=respiratory rate; temp=temperature; Term=termination.

^a There must be at least a 48-hour window between sputum induction at Visit 9 and bronchoscopy at Visit 10.

^b COVID-19 antigen testing may be performed at any time during Screening window, with timing at the discretion of the PI. COVID-19 testing is the sole Screening assessment that may occur following the Screening bronchoscopy (see [I]) should the PI deem such timing appropriate.

^c If this assessment is performed on a dosing day, then it must be performed prior to study drug dosing.

^d Screening height measurement can be used for all future visits.

^e Physical exam during bronchoscopy visits (Visits #2 and #10) may be limited to exam that PI deems clinically indicated prior to bronchoscopy.

^f On dosing days, ECGs will be performed predose (-2 hours $\pm$ 1 hour) and postdose (2 hours $\pm$ 15 minutes based on start of inhalation); all ECGs will be performed in triplicate with 3- to 5-minute intervals between each measurement.

^g Order of assessments: Spirometry, induced sputum, blood draw. Order of other assessments is flexible with the stipulation that indicated assessments must occur prior to dosing or at other specified time points as detailed in (f), (h), and (i).

^h Plasma samples for PK analysis will be collected at the following time points:

- Day 1: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, and 6 hours
- Day 15: Predose only
- Day 29: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, and 6 hours

The recommended allowance of time windows is $\pm$ 5 minutes for the 0.5-hour time point and $\pm$ 10% of nominal time for all other time points. All times are referenced to the beginning of inhalation dosing. If a time window is missed, every effort should be made to collect the PK blood samples with documentation of actual collection times.

ⁱ On dosing days, spirometry measures will be completed predose and postdose at 15 minutes ($\pm$ 10 minutes), 60 minutes ($\pm$ 10 minutes), and 120 minutes ($\pm$ 15 minutes). On nondosing days, spirometry measures will be completed only once per visit. Additional spirometry measures will be required as safety evaluations when the induced sputum protocol is performed (see [j]). For NHVs, spirometry measurements will not involve bronchodilation.

^j The induced sputum protocol requires baseline and repeat spirometry measurements. The initial spirometry measure of the visit will serve as the baseline spirometry measure for the induced sputum protocol and must be performed within 2 hours of the induced sputum procedure.

^k To minimize the risk associated with radiation exposure, a chest x-ray performed within 90 days prior to first dose (even if performed prior to the Screening window) may be used for Screening purposes.

^l Samples for sputum MUC5AC and MUC5B will be collected on all subjects who are screened; however, MUC5AC and MUC5B measurement on these samples will only be performed for those subjects who are dosed with study drug.

^m The window for the Screening bronchoscopy is Day -19 to Day -5. The Screening bronchoscopy will be performed after all other Screening procedures are complete and the subject has successfully screened into the study (ie, met all inclusion and exclusion criteria); see (b) for potential exception. It will serve to provide baseline measures and will not serve to fulfill any inclusion or exclusion criteria. For any study visit involving a bronchoscopy, all other visit assessments will be performed prior to the bronchoscopy, with the exception of blood draws, which may be performed in a $\pm$ 24-hour window around the bronchoscopy visit.

ⁿ The phone call during Screening will occur within a window of +1 day following bronchoscopy.

Table 5: Schedule of Assessments for Multiple-Dose Asthma Cohorts (Cohorts 2c-4c)

Assessment	Screen	Day 1	Day 2	Day 15	Day 29	Day 30	Day 36	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12
Visit Window	Days -28 to -1	±0 days		±1 day	±1 day		±2 days	±2 days	±3 days	±3 days	±5 days	±5 days
Informed Consent	X											
Eligibility Criteria	X	X										
Randomization		X										
Study Drug Dosing		X		X	X							
Demographics	X											
Medical History	X											
Drug Screen	X											
Hepatitis/HIV Serologies	X											
FSH (in women of postmenopausal age only)	X											
Urine Cotinine	X											
COVID-19 test ^a	X											
Urine qualitative pregnancy test ^b	X	X		X	X							X
Body Mass Index/ Height and Weight ^{b,c}	X	X			X					X		X
Physical Exam (may be symptom directed after Day 1) ^b	X	X	X	X	X	X	X	X	X	X	X	X

Assessment	Screen	Day 1	Day 2	Day 15	Day 29	Day 30	Day 36	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12
Visit Window	Days -28 to -1	±0 days		±1 day	±1 day		±2 days	±2 days	±3 days	±3 days	±5 days	±5 days
ECG ^d	X	X		X	X			X				X
Vital Signs (BP, temp, pulse ox, RR, heart rate), perform before other evaluations ^b	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Labs ^{b,e}	X	X		X	X		X	X	X	X	X	X
Blood eosinophil count ^{b,e}	X	X		X	X		X	X	X	X	X	X
Serum total IgE ^{b,e}	X	X		X	X		X	X		X		X
PK ^f		X	X	X	X	X						
Anti-drug antibodies ^{b,e}		X		X	X			X			X	X
Spirometry ^{e,g,h}	X	X	X	X	X	X	X	X	X	X	X	X
Chest x-ray (PA and lateral) ⁱ	X											X
Induced sputum MUC5AC and MUC5B protein ^{b,e,h,j}	X	X		X	X		X	X		X		X
Induced sputum MUC5AC and MUC5B mRNA ^{b,e,h,j}	X	X		X	X		X	X		X		X
FeNO ^{b,e}	X	X		X	X		X	X		X		X
Plasma Periostin ^{b,e}	X	X		X	X		X	X		X		X

Assessment	Screen	Day 1	Day 2	Day 15	Day 29	Day 30	Day 36	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12
Visit Window	Days -28 to -1	±0 days		±1 day	±1 day		±2 days	±2 days	±3 days	±3 days	±5 days	±5 days
Mucus Symptom Assessment ^b		X						X		X		X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Meds/Therapies	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BP=blood pressure; COVID-19=coronavirus disease 2019; ECG=electrocardiogram; EOS=end of study; FeNO=fractional exhaled nitric oxide; FSH=follicle-stimulating hormone; HIV=human immunodeficiency virus; IgE=immunoglobulin E; MUC5AC=mucin 5AC; MUC5B=mucin 5B; PA=posteroanterior; PI=Principal Investigator; PK=pharmacokinetic; pulse ox=pulse oximetry; RR=respiratory rate; temp=temperature; Term=termination.

^a COVID-19 antigen testing maybe performed at any time during Screening window, with timing at the discretion of the PI.

^b If this assessment is performed on a dosing day, then it must be performed prior to study drug dosing.

^c Screening height measurement can be used for all future visits.

^d On dosing days, ECG will be performed predose (-2 hours ±1 hour) and postdose (2 hours ±15 minutes based on start of inhalation); all ECGs will be performed in triplicate with 3- to 5-minute intervals between each measurement.

^e Order of assessments: FeNO, spirometry, induced sputum, blood draw. Order of other assessments is flexible with the stipulation that indicated assessments must occur prior to dosing or at other specified time points as detailed in (d), (f), and (g).

^f Plasma samples for PK analysis will be collected at the following time points:

- Day 1: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, and 6 hours
- Day 2: 24 hours
- Day 15: Predose only
- Day 29: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, and 6 hours
- Day 30: 24 hours

The recommended allowance of time windows is ±5 minutes for the 0.5-hour time point and ±10% of nominal time for all other time points. All times are referenced to the beginning of inhalation dosing. If a time window is missed, every effort should be made to collect the PK blood samples with documentation of actual collection times.

^g On dosing days, spirometry measures will be completed predose and postdose at 15 minutes (±10 minutes) and 120 minutes (±15 minutes). Additional spirometry measures will be required as safety evaluations when the induced sputum protocol is performed (see [h]). For patients with asthma, the first spirometry assessment of each study visit will include bronchodilator administration; any subsequent spirometry measures on that study day will not include bronchodilator administration.

^h The induced sputum protocol requires baseline and repeat spirometry measurements. The initial spirometry measure of the visit will serve as the baseline spirometry measure for the induced sputum protocol and must be performed within 2 hours of the induced sputum procedure.

- ⁱ To minimize the risk associated with radiation exposure, a chest x-ray performed within 90 days prior to first dose (even if performed prior to the Screening window) may be used for Screening purposes.
- ^j Samples for sputum MUC5AC and MUC5B will be collected on all subjects who are screened. However, measurement on these samples will only be performed for those subjects who are dosed with study drug.

**Table 6: Schedule of Assessments for Multiple-Dose COPD Cohorts
(Cohorts 2d-4d)**

Assessment	Screen	Day 1	Day 2	Day 15	Day 29	Day 30	Day 36	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12
Visit Window	Days -28 to -1	±0 days		±1 day	±1 day		±2 days	±2 days	±3 days	±3 days	±5 days	±5 days
Informed Consent	X											
Eligibility Criteria	X	X										
Randomization		X										
Study Drug Dosing		X		X	X							
Demographics	X											
Medical History	X											
Drug Screen	X											
Hepatitis/HIV Serologies	X											
FSH (in women of postmenopausal age only)	X											
Urine Cotinine	X											
COVID-19 test ^a	X											
Urine qualitative pregnancy test ^b	X	X		X	X							X
Body Mass Index/Height and Weight ^{b,c}	X	X			X					X		X

Assessment	Screen	Day 1	Day 2	Day 15	Day 29	Day 30	Day 36	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12
Visit Window	Days - 28 to -1	±0 days		±1 day	±1 day		±2 days	±2 days	±3 days	±3 days	±5 days	±5 days
Physical Exam (may be symptom directed after Day 1) ^b	X	X	X	X	X	X	X	X	X	X	X	X
ECG ^d	X	X		X	X			X				X
Vital Signs (BP, temp, pulse ox, RR, heart rate), perform before other evaluations ^b	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Labs ^{b,e}	X	X		X	X		X	X	X	X	X	X
Blood neutrophil count ^{b,e}	X	X		X	X		X	X	X	X	X	X
PK ^f		X	X	X	X	X						
Anti-drug antibodies ^{b,e}		X		X	X			X			X	X
Spirometry ^{e,g,h}	X	X	X	X	X	X	X	X	X	X	X	X
Chest x-ray (PA and lateral) ⁱ	X											X
Induced sputum MUC5AC and MUC5B protein ^{b,e,h,j}	X	X		X	X		X	X		X		X

Assessment	Screen	Day 1	Day 2	Day 15	Day 29	Day 30	Day 36	Day 43	Day 50	Day 57	Day 71	Day 85 (EOS) or Early Term
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12
Visit Window	Days -28 to -1	±0 days		±1 day	±1 day		±2 days	±2 days	±3 days	±3 days	±5 days	±5 days
Induced sputum MUC5AC and MUC5B mRNA ^{b,e,h,j}	X	X		X	X		X	X		X		X
SGRQ-C ^b	X	X						X		X		X
COPD Assessment Test ^b	X	X						X		X		X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Meds/Therapies	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: BP=blood pressure; COPD=chronic obstructive pulmonary disease; COVID-19=coronavirus disease 2019; ECG=electrocardiogram; EOS=end of study; FSH=follicle-stimulating hormone; HIV=human immunodeficiency virus; IgE=immunoglobulin E; MUC5AC=mucin 5AC; MUC5B=mucin 5B; PA=posteroanterior; PI=Principal Investigator; PK=pharmacokinetic; pulse ox=pulse oximetry; RR=respiratory rate; temp=temperature; Term=termination.

^a COVID-19 antigen testing maybe performed at any time during Screening window, with timing at the discretion of the PI.

^b If this assessment is performed on a dosing day, then it must be performed prior to study drug dosing.

^c Screening height measurement can be used for all future visits.

^d On dosing days, ECG will be performed predose (-2 hours ±1 hour) and postdose (2 hours ±15 minutes based on start of inhalation); all ECGs will be performed in triplicate with 3- to 5-minute intervals between each measurement.

^e Order of assessments: spirometry, induced sputum, blood draw. Order of other assessments is flexible with the stipulation that indicated assessments must occur prior to dosing or at other specified time points as detailed in (d), (f), and (g).

^f Plasma samples for PK analysis will be collected at the following time points:

- Day 1: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, and 6 hours
- Day 2: 24 hours
- Day 15: Predose only
- Day 29: Predose, then postdose (based on start of inhalation) at 0.5, 1, 2, 4, and 6 hours
- Day 30: 24 hours

The recommended allowance of time windows is ±5 minutes for the 0.5-hour time point and ±10% of nominal time for all other time points. All times are referenced to the beginning of inhalation dosing. If a time window is missed, every effort should be made to collect the PK blood samples with documentation of actual collection times.

- ^g On dosing days, spirometry measures will be completed predose and postdose at 15 minutes (± 10 minutes) and 120 minutes (± 15 minutes). Additional spirometry measures will be required as safety evaluations when the induced sputum protocol is performed (see [h]). For patients with COPD, the first spirometry assessment of each study visit will include bronchodilator administration; any subsequent spirometry measures on that study day will not include bronchodilator administration.
- ^h The induced sputum protocol requires baseline and repeat spirometry measurements. The initial spirometry measure of the visit will serve as the baseline spirometry measure for the induced sputum protocol and must be performed within 2 hours of the induced sputum procedure.
- ⁱ To minimize the risk associated with radiation exposure, a chest x-ray performed within 90 days prior to first dose (even if performed prior to the Screening window) may be used for Screening purposes.
- ^j Samples for sputum MUC5AC and MUC5B will be collected on all subjects who are screened. However, measurement on these samples will only be performed for those subjects who are dosed with study drug.

6.1.2. Informed Consent

Informed consent will be obtained before the subject can participate in the study. The contents and process of obtaining informed consent will be in accordance with all applicable regulatory requirements. Study participation includes all Screening procedures as well as required washout of excluded medications.

It is the responsibility of the PI, or medically qualified designee, to obtain a written informed consent from everyone participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study. The PI, or medically qualified designee, must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for documenting a written consent will be provided by the PI or by Arrowhead Pharmaceuticals, Inc. (Arrowhead).

For this study, each eligible subject will be required to provide written informed consent before participation in the study.

All eligible subjects will have the study explained by the PI or designee. They will receive a full explanation, in lay terms, of the aims of the study, the discomforts, risks, and benefits in taking part as well as of insurance and other procedures for compensation in case of injury. It will be explained that the study is for research purposes only and is not expected to provide any therapeutic benefit to the individual. It will be pointed out that they can withdraw from the study at any time without prejudice. Each subject will acknowledge receipt of this information by giving written informed consent for participation in the study. The subject will be given a copy of the signed ICF to retain.

Additionally, subjects in this study will be given the option to sign (or decline) a consent form allowing unused portions of their study samples (eg, blood, sputum, and bronchoscopic samples) to be made available to Arrowhead for possible future research purposes. Signing, or declining, this optional consent will not impact the ability of the subject to participate in this study.

6.1.3. Screening

A screen failure occurs when a subject who consents to participate in the clinical study is not subsequently randomized in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, concomitant procedures, adverse events, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened subjects should be assigned a new subject number for every Screening/rescreening event. There is no limit on the number of rescreening attempts a subject may undergo.

In the multiple-dose NHV cohorts, a baseline bronchoscopy procedure will take place during the Screening period. This bronchoscopy serves to provide baseline measurements of PD markers and does not serve a Screening purpose. Therefore, only subjects who have successfully met all inclusion and exclusion criteria will undergo the bronchoscopy assessment during the Screening period, with the expectation that all such subjects will be randomized and treated with study drug. If a subject undergoes a Screening bronchoscopy but does not subsequently enroll in the cohort they had screened for, then the subject may repeat Screening for a subsequent cohort. In such a scenario, repeat Screening would not involve a repeat Screening bronchoscopy; instead, bronchoscopy results from the subject's initial Screening attempt would be used as baseline measures.

To minimize the risk associated with radiation exposure, a chest x-ray performed within 90 days prior to first dose (even if performed prior to the Screening window) may be used for Screening purposes.

6.1.4. Treatment Period

Subjects in the SAD portion of the study (Cohorts 1a-4a) will receive a single dose on Day 1. Their approximate duration of study is 9 weeks from the beginning of the Screening period to the Day 29 EOS visit.

Subjects in the MAD portion of the study (Cohorts 1b-4b, 2c-4c, and 2d-4d) will receive 3 total doses, with 1 dose given on each of Days 1, 15, and 29. For subjects in the MAD portion of the study, the approximate duration of the study is 17 weeks (NHVs) or 16 weeks (patients) from the beginning of the Screening period to the Day 85 EOS visit.

6.1.5. Adverse Event Monitoring

Safety assessments will include AEs and SAEs, chest x-rays, spirometry, physical examinations, vital sign measurements (blood pressure, heart rate, temperature, pulse oximetry, and respiratory rate), electrocardiograms (ECGs), clinical laboratory tests, BALF cell count and differential, concomitant medications/therapy, and reasons for treatment withdrawal and study discontinuation. Safety assessments will be performed at specified time points and prior to study completion.

The AE/SAE reporting period for subjects begins when the subject provides informed consent and extends until EOS. TEAEs and treatment-emergent SAEs are defined as AEs/SAEs that occur following study drug administration, or exacerbation of a preexisting condition by the study drug. The TEAE reporting period begins after the first dose and extends until the EOS visit is complete.

All SAEs that occur during the AE reporting period, in addition to reporting via electronic case report forms (eCRFs), must also be reported to the Sponsor via the SAE report form within 24 hours of being notified, regardless of the relationship of the SAE to study treatment. All AEs/SAEs will be followed until resolution, until the condition stabilizes, until the event is

otherwise explained, or until the subject is lost to follow-up. If the PI learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product (IP), the PI will promptly notify the Sponsor. Laboratory or diagnostic (eg, ECG) abnormalities will be reported as AEs if considered clinically significant by the PI. Laboratory or diagnostic assessment abnormalities not reported as AEs are not to be reported as clinically significant in the study database.

Asthma exacerbation is defined in [Section 12.9.2](#). COPD exacerbation is defined in [Section 12.9.3](#).

6.1.6. Early Termination

The reason for early termination will be documented in source documents and eCRFs. Procedures outlined in the SOA will be completed. In all cohorts, subjects who terminate the study early for reasons not related to safety or a treatment-related AE may be replaced at the discretion of the Sponsor.

If a subject fails to complete study assessments deemed critical to the integrity of the study (eg, subject receives at least 1 dose of study drug but fails to receive all planned doses of study drug, or subject fails to complete all planned bronchoscopies), the subject may remain in the study for further safety evaluation if deemed necessary by Sponsor. In such cases, subsequent study visits will focus on safety, which may involve cessation of certain study assessments (eg, PK sample collection). If a subject fails to complete study assessments deemed critical to the integrity of the study, the subject may be replaced at the discretion of the Sponsor (meaning that an additional, new subject will be added to the pertinent cohort, resulting in an increase of the cohort size).

6.1.7. Data Safety Committee

Scheduled DSC meetings that occur during each single-dose cohort will review available aggregate safety data through the Day 15 study visit of that cohort. The DSC will vote to escalate to single-dose cohorts at higher dose levels as well as to open multiple-dose cohorts as per [Figure 1](#). The DSC may also meet as needed to review and discuss any safety issues that may arise.

The DSC will consist of at least 1 Investigator, Sponsor Medical Monitor, Sponsor Pharmacovigilance Lead, and an independent physician experienced in pulmonary drug development and/or early-stage clinical trials.

6.1.8. Local Labs and Home Health

Central labs will be utilized. However, local labs may be utilized (including Screening visit) only when central labs are not readily available and for emergent situations, including time critical analytes.

In the event of logistics related disruptions when a subject does not have direct access to the site (and only in such an event), certain site visits may be replaced by a home health visit.

Use of home health care services is permissible contingent upon prior approval from the Sponsor, compliance with local laws and regulations, and the capability of the PI to adequately monitor subject safety. In cases where home health care services are to be used, this approach, and any specific risks associated with it, must be clearly outlined in the IEC/IRB-approved ICF.

The following study visits may not be replaced by a home health visit under any circumstances: initial Screening visit (Visit #1), any visit involving study drug administration, or any visit involving bronchoscopy.

If a home health visit is deemed necessary, this visit may incorporate the following assessments: blood and/or urine-based laboratory collections, physical exam, body weight, vital signs, ECG, mucus symptoms assessment, and review of AEs and concomitant medications. A home health visit will not incorporate the following assessments: study drug dosing, bronchoscopy, sputum induction, spirometry, FeNO, or chest x-ray.

Labs may be obtained at a home health visit using either a local lab kit with local analysis or a central lab kit with central analysis, as determined on a case-by-case basis.

6.2. Number of Subjects

The study will be conducted in approximately up to 56 NHV subjects (6-8 per cohort) and up to 24 patients with asthma (8 per cohort) as well as 24 patients with COPD (8 per cohort). A subject who withdraws from the study prior to the scheduled EOS visit, for reasons not related to safety or a treatment-related AE, may be replaced at the discretion of the Sponsor.

If a subject fails to complete study assessments deemed critical to the integrity of the study (eg, subject fails to complete all planned bronchoscopies, or subject receives at least 1 dose of study drug but fails to receive all planned doses of study drug), the subject may be replaced at the discretion of the Sponsor (an additional, new subject will be added to the pertinent cohort, resulting in an increase of the cohort size).

6.3. Treatment Assignment

All potential subjects who sign an ICF at Screening will receive a unique 9-digit identifier. The first 3 digits will represent the country code and the next 3 digits will represent the assigned site number and will be the same for each subject that screens at an individual site. The last 3 digits will be assigned sequentially at each site (starting with 001). For subjects who are deemed eligible, this 9-digit identifier will become the subject's permanent study ID number.

6.4. Dose Adjustment Criteria

There will be no dose escalation or dose reduction within a cohort (ie, the same study drug dose will be administered to each subject within the cohort). However, specified dose levels represent the highest dose that may be used in each cohort. The Sponsor or the DSC may adjust the dose level for any cohort downward based on available safety and PD data.

6.4.1. Safety Criteria for Adjustment or Stopping Doses

A decision to stop the trial early or discontinue the drug in an individual subject or group of subjects may be indicated based on any of the following:

- A single SAE considered related to study drug (a serious adverse reaction) in 1 subject that is confirmed to have occurred in a subject dosed with ARO-MUC5AC rather than placebo

- A severe, nonserious AE considered related to study drug (a severe adverse drug reaction) in 2 subjects in the same cohort, independent of whether they are within the same System Organ Class (SOC), that are confirmed to have occurred in subjects dosed with ARO-MUC5AC rather than placebo
- Episodes of a drop from baseline FEV₁ (which must be confirmed on repeat within 72 hours) of $\geq 20\%$ (ie, 20% absolute decline in the percent predicted FEV₁ [ppFEV₁]) in 2 separate study subjects in the same cohort that are both considered to be related to study drug, and that are confirmed to have occurred in subjects dosed with ARO-MUC5AC rather than placebo*

*FEV₁ values from spirometry sessions that fail central over-reader quality review are exempt from stopping rule evaluation. Postinduction FEV₁ values obtained as part of an induced sputum protocol are exempt from stopping rule evaluation since decreases in this setting would likely be due to sputum induction rather than study drug. Please see [Section 12.9.1](#) for suggested evaluation for clinically significant spirometry abnormalities.

If any such events occur, the DSC will meet within 7 calendar days of the Sponsor being notified of the event to review it and determine any necessary actions, as detailed below.

The following pregnancy rule will also be applied:

- A confirmed positive pregnancy test will lead to permanent discontinuation of study drug in the pregnant subject.

In the setting of a confirmed pregnancy, a mandatory DSC meeting will not be required and dosing of other subjects in the study will not cease.

The Sponsor or Investigator can discontinue any subject at any time with or without DSC consultation. If such events, as described above, occur and the subject is not discontinued from the study, the reason for not discontinuing the subject will be included in DSC meeting minutes. The DSC may pause the study and additional dosing or dose escalation to provide time to evaluate safety data and recommend the action to be taken, which may include, but is not limited to, one of the following:

- Discontinuation of a subject or group of subjects from the study
- Stopping the study immediately with no further dosing
- Continuing the study until the current cohort is completed
- Continuing the study but adjusting the next dose escalation to a dose level midway between the current level and the next planned level
- Continuing the study as planned

6.5. Criteria for Study Termination

The Sponsor reserves the right to discontinue the trial at any time. Reasons will be provided in the event of this happening. The PI reserves the right to discontinue the study at his/her site for safety reasons at any time in collaboration with the Sponsor.

6.6. Rationale for Study Design, Including Choice of Control Group

This FIH study is designed to investigate ARO-MUC5AC to evaluate the drug's safety, tolerability, PK profile, PD effects, and efficacy following a single-inhaled dose and multiple-inhaled doses.

In all cohorts, a placebo control will be used for comparison of safety, PK, PD, and efficacy results between those treated with ARO-MUC5AC and those treated with placebo. The placebo group will inhale normal saline via nebulization. Randomization and blinding will be used in all cohorts to minimize bias in both the assignment of subjects to study treatment and the assessment of study endpoints.

This FIH study will first evaluate NHVs to assess the safety and tolerability of escalating single doses of ARO-MUC5AC in a population with normal airway physiology and with minimal concomitant medication use. Toxicology studies suggest a low safety risk to NHVs at the planned dose levels. MUC5AC protein is quantifiable in sputum from NHVs and represents a measurable biomarker of PD effect. Thus, escalating doses in NHVs will allow for PD dose-response assessment utilizing sputum MUC5AC concentrations. Of note, ARO-MUC5AC is not expected to have an effect on MUC5B concentrations; MUC5B concentrations will be measured to verify that the drug has a specific effect on MUC5AC.

A DSC meeting will occur following Study Day 15 of each NHV single-dose cohort to discuss safety data and to make decisions regarding the appropriateness of dose escalation and of opening multiple-dose cohorts. Sheep pharmacology data suggest that maximal PD effect should occur within 2 weeks following initial dosage; thus, any toxicity related to MUC5AC depletion would be most likely to manifest within a 15-day timeframe. Following a favorable interim assessment by the DSC of the study drug's safety profile during each single-dose NHV cohort, the study will subsequently enroll both NHVs and patients into cohorts that will evaluate multiple doses of study drug. The NHV multiple-dose cohorts are designed to interrogate whether subsequent doses of study drug alter its safety, PK, or PD effect profile.

The patient multiple-dose cohorts are designed to provide initial insights into safety, PK, PD, and efficacy in a population with obstructive lung disease. This study will evaluate patients with moderate-to-severe asthma because sputum MUC5AC concentration is known to be increased in this population. The COPD patient population is added to this study to further assess safety in a population characterized by older age and more comorbidities, and to further assess target engagement in airways characterized by irreversible airflow obstruction and high mucus production. Asthma and COPD each represent potential populations for later phase development, contingent upon results of this first-in-human study.

Bronchoscopic collections are included in the NHV MAD cohorts to provide an additional approach to measurement of MUC5AC concentration, in case sputum samples prove difficult to obtain in some NHV subjects. Bronchoscopies are limited to the MAD cohorts because nonclinical animal pharmacology data suggest that a single dose of ARO-MUC5AC may have limited effect on MUC5AC protein concentrations.

The highest-dose NHV MAD cohort, Cohort 4b, is made optional in the setting of both review of initial PD data from prior NHV cohorts (including NHV Cohorts 1b-3b), along with the addition of further patient cohorts (ie, the COPD cohorts) to the study. For safety, PK, and PD endpoints, patient data following multiple doses are expected to provide more pertinent information than

data from healthy volunteers, as subsequent development of ARO-MUC5AC is anticipated to occur in patients with muco-obstructive lung disease. With the enlargement of the patient component of the study, this NHV MAD cohort may not prove necessary to fulfill the objectives of the study.

6.7. Rationale for Dose and Schedule of Administration

A starting dose of 24 mg will be administered in Cohort 1a, with planned escalation to a maximum dose of 232 mg in Cohort 4a. Multiple-dose cohorts will assess a similar range of dose levels, with repeat dosing at 2-week intervals for a total of 3 doses.

The doses of ARO-MUC5AC to be evaluated in this clinical trial were chosen based on pharmacology data from sheep and toxicology findings from rats and monkeys. The dose of drug that is loaded into an inhalational device is not the same as the dose that deposits in the lung. In animals, pulmonary deposited dose (PDD) refers to the amount of drug reaching the lung tissue. In humans, respirable delivered dose (RDD) refers to the amount of nebulized drug contained in aerosol droplets capable of entering the lung. Respirable delivered dose serves as an estimate of how much drug is deposited in the human lung. Human doses (RDD) for the clinical trial are thus selected based on animal doses (PDD) that are pharmacologically efficacious in nonclinical studies and that are associated with acceptable safety margins based on nonclinical toxicology studies. Using data from nebulizer performance testing, the amount of ARO-MUC5AC that must be loaded into the nebulizer to achieve the target RDD is then calculated. Detailed dosing calculations are provided in the IB.

Pharmacologic data in sheep have indicated that doses (PDD) of 0.25 mg/kg body weight to 1.0 mg/kg body weight attenuate allergen-induced increases in airway resistance, with escalating doses resulting in greater response (please see IB for details). A dose of 0.25 mg/kg body weight represents an estimate of the minimal anticipated biological effect level (MABEL) in sheep. Appropriate dose scaling for inhaled drugs between sheep and humans would suggest that doses, expressed as mg drug per kg body weight, can be converted on a roughly 1:1 scale between these organisms (Phillips 2017). This clinical study will initiate at a dose (RDD) below the estimated MABEL of 0.2 mg/kg body weight and will initially plan to explore a 5-fold dose range up to a dose of 1.0 mg/kg body weight. Based on nebulizer performance data, this range of target RDDs (between 0.2 and 1.0 mg/kg body weight) translates to nebulizer loaded doses (NLDs) between 24 and 108 mg. Efficacy in sheep pharmacology studies was observed when dosing was given at 2-week intervals, which informs the dosing frequency in this clinical study. Loss of pharmacologic effect is seen at 4 weeks following a single dose, suggesting that longer dosing intervals may be less efficacious.

Additional cohorts (Cohorts 4a-4d), dosed at a NLD of 232 mg (which results in a RDD of 2.0 mg/kg body weight), were introduced in response to review of initial safety and PK data from the ongoing clinical Study AROMUC5AC-1001. Nonclinical pharmacology data suggest that exploration of a higher dose level is necessary to fully define the dose-response to ARO-MUC5AC. Available safety data from lower-dose NHV cohorts in the ongoing Study AROMUC5AC-1001 have not identified any safety concerns around further dose escalation. Updated safety margins, utilizing clinical PK data rather than weight-based comparisons for systemic effects, indicate appropriate margins for the higher dose level.

GLP-compliant 4-week toxicology studies in rats indicated a NOAEL at a dose (PDD) of 11 mg/kg body weight, while 4-week GLP studies in monkeys indicated a NOAEL at a dose (PDD) of 10.2 mg/kg. For systemic effects, safety margin calculations are made comparing the animal plasma AUC at the NOAEL with the human plasma AUC at a given dose level (human AUC data are modeled for the planned highest dose level). For lung effects, safety margin calculations are made using the dose (PDD at the NOAEL in animals and RDD in humans) divided by lung weight. [Table 7](#) shows safety margin calculations for systemic effects for all doses utilized in this clinical trial, while [Table 8](#) shows safety margin calculations for lung effects. All doses in this clinical trial are associated with safety margins exceeding those considered adequate by the US Food and Drug Administration (FDA) (for lung weight-based margins: 10-fold safety margin in rats and 5-fold safety margin in monkeys; for systemic AUC-based margins: 1-fold safety margins in rats and monkeys) ([Tepper 2016](#)).

Table 7: Safety Margins for Systemic Effects for All Dose Levels Utilized in AROMUC5AC-1001

Human Nebulizer Loaded Dose (mg)	Human Respirable Delivered Dose (mg)	Human RDD/kg Body Weight (mg/kg)	Human Plasma AUC _{0-inf} (ng·h/mL)	Rat PDD/kg Body Weight at NOAEL (mg/kg)	Rat Plasma AUC ₀₋₉₆ at NOAEL (ng·h/mL)	Rat Safety Margin	Monkey PDD/kg Body Weight at NOAEL (mg/kg)	Monkey Plasma AUC ₀₋₁₂₀ at NOAEL (ng·h/mL)	Monkey Safety Margin
24	14	0.2	51.0	11	35,300	692-fold	10.2	89,800	1761-fold
56	35	0.5	299	11	35,300	118-fold	10.2	89,800	300-fold
108	70	1.0	1,910	11	35,300	18-fold	10.2	89,800	47-fold
232	139	2.0	8,570 ^a	11	35,300	4-fold	10.2	89,800	10-fold

Abbreviations: AUC=area under the plasma concentration time curve; AUC₀₋₉₆=area under the plasma concentration time curve from time 0 to 96 hours; AUC₀₋₁₂₀=area under the plasma concentration time curve from time 0 to 120 hours; AUC_{0-inf}=area under the plasma concentration time curve from time 0 extrapolated to infinity; NOAEL=no-observed-adverse-effect level; PDD=pulmonary deposited dose; RDD=respirable delivered dose.

^a Human AUC exposure data at this dose level were extrapolated using a dose-exposure regression during dose-proportionality analysis.

Note: Assumes human body weight of 70 kg (Davies 1993).

Table 8: Safety Margins for Lung Effects for All Dose Levels Utilized in AROMUC5AC-1001

Human Nebulizer Loaded Dose (mg)	Human Respirable Delivered Dose (mg)	Human RDD/g Lung Weight (mg/g)	Rat PDD/g Lung Weight at NOAEL (mg/g)	Rat Safety Margin	Monkey PDD/g Lung Weight at NOAEL (mg/g)	Monkey Safety Margin
24	14	0.014	2.29	164-fold	1.67	119-fold
56	35	0.035	2.29	65-fold	1.67	48-fold
108	70	0.070	2.29	33-fold	1.67	24-fold
232	139	0.139	2.29	16-fold	1.67	12-fold

Abbreviations: NOAEL=no-observed-adverse-effect level; PDD=pulmonary deposited dose; RDD=respirable delivered dose.

Note: Assumes human lung weight of 1000 g (Davies 1993).

7. SELECTION OF STUDY POPULATION AND WITHDRAWAL OF SUBJECTS

Inclusion and exclusion criteria for NHV cohorts and patient cohorts are outlined below. Of note, any assessment may be repeated once during Screening, with the repeat result used for inclusion/exclusion purposes. If, based upon the participant's medical history or Screening results, a PI raises concern that a participant's safety may be at risk and/or that the safety data may confound or conflict with the study results, then the PI has the option to exclude the enrollment of the participant.

7.1. Inclusion Criteria for NHV Cohorts

To be eligible for enrollment, NHV subjects must meet all the following inclusion criteria:

1. Male or nonpregnant, nonlactating female volunteers
2. Age 18 to 55 years old at Screening
3. Normal ppFEV₁ (>80%) at Screening, prior to sputum induction
4. Able and willing to provide written informed consent prior to the performance of any study-specific procedures
5. Body mass index (BMI) between 18.0 and 35.0 kg/m² at Screening
6. A 12-lead ECG at Screening with no abnormalities that may compromise participant's safety in this study
7. Nonsmoker (defined as someone who has not smoked a cigarette for at least 6 months) with current nonsmoking status confirmed by urine cotinine at Screening AND previous smoking history prior to 6 months must be <15 pack-years. Subjects may be on nicotine replacement (patch or gum). Nicotine e-cigarettes (vapor) are not permitted. A positive urine cotinine result due to nicotine replacement is acceptable for enrollment at the discretion of the PI.
8. Participants of childbearing potential must agree to use a highly effective form of contraception in addition to a condom, during the study and for at least 90 days following the end of the study or last dose of study drug, whichever is later. Subjects must not donate sperm or eggs during the study and for at least 90 days following the end of the study or last dose of study drug, whichever is later. See [Appendix 2](#) for details.
9. Able and willing to comply with all study assessments and adhere to the protocol schedule
10. Able to produce an induced sputum sample at Screening that meets criteria of acceptable quality (See [Section 10.2.1.2.1](#) for quality criteria)

7.2. Exclusion Criteria for NHV Cohorts

Normal healthy volunteers who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Acute lower respiratory infection within 30 days prior to first dose
2. Acute upper respiratory infection within 7 days prior to first dose
 - a. In the case of an upper respiratory infection within 7 days of the first dose, the PI may elect to extend the Screening period to >35 days such that the first dose is given >7 days following clinical resolution of the infection. In no case will the Screening period be extended to >45 days.
3. Positive COVID-19 test during Screening window. Any record of positive test during the Screening window, whether a protocol-mandated antigen test or any nucleic acid amplification test (NAAT) or antigen test obtained outside of the study, serves as a potential exclusion criterion (see below).
 - a. In the event of a positive COVID-19 test during Screening, and if the PI deems the subject clinically appropriate to proceed to study drug dosing and no other exclusion criteria are met (eg, #1), the PI may elect to proceed to randomize the subject. In such a case, the first dose may not be given until >7 days after both clinical resolution of infection and conversion of antigen test from positive to negative, whichever is later. If necessary, the Screening period may be extended to >35 days, but in no case will the Screening period be extended to >45 days.
4. Any history of chronic pulmonary disease (including, but not limited to: asthma, COPD, or interstitial lung disease). Childhood asthma, which has subsequently resolved, will not exclude a subject from this study.
5. Known unresolved parasitic infection or prior parasitic infection that has required antiparasitic therapy within 30 days prior to first dose
6. Any history of anaphylaxis
7. Human immunodeficiency virus (HIV) infection, as shown by presence of anti-HIV antibodies (seropositive)
8. Seropositive for hepatitis B virus (HBV) or hepatitis C virus (HCV) (positive result for anti-HCV antibodies must be confirmed with positive HCV RNA test for exclusion)
9. Uncontrolled hypertension (systolic blood pressure [SBP] >150 mmHg or diastolic blood pressure [DBP] >100 mmHg) at Screening
10. A history of Torsades de Pointes, ventricular rhythm disturbances (eg, ventricular tachycardia or fibrillation), pathologic sinus bradycardia (<50 bpm with symptoms), heart block (excluding first-degree block, being PR prolongation only), congenital long QT syndrome, new ST segment elevation or depression, or new Q wave on ECG. Participants with a history of atrial arrhythmias may be enrolled if the PI judges the safety risk to be low. Participants with a history of cardiac rhythm abnormality that has been treated with a device (eg, pacemaker) may be enrolled if the PI judges the safety risk to be low.
11. A family history of congenital long QT syndrome or unexplained sudden cardiac death
12. Use of medications known to prolong the QTc interval within 30 days prior to first dose
13. Symptomatic heart failure (per New York Heart Association [NYHA] guidelines), unstable angina, myocardial infarction, severe cardiovascular disease (ejection fraction

<20%), transient ischemic attack (TIA), or cerebrovascular accident (CVA) within 24 weeks prior to first dose

14. History of malignancy within the past 5 years except for adequately treated basal cell carcinoma, squamous cell skin cancer, superficial bladder tumors, or in situ cervical cancer. Participants with other curatively treated malignancies who have no evidence of metastatic disease and >5-year disease-free interval may be enrolled if the PI judges the risk of recurrence to be low.
15. History of major surgery within 12 weeks prior to first dose
16. Unwilling to limit alcohol consumption to within moderate limits for the duration of the study, as follows: not more than 14 units per week for women and 21 units per week for men (1 unit=150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol)
17. Use of illicit drugs (such as cocaine, phencyclidine [PCP]) within 1 year prior to Screening or positive urine drug screen at Screening (a urine drug screen deemed positive due to prescription medications or for benzodiazepines, opioids, or marijuana is acceptable and inclusion is at the discretion of the PI). Subjects who smoke or vape prescription tetrahydrocannabinol (THC)/marijuana may be enrolled if the PI judges the safety risk to be low.
18. Use of an investigational agent or device within 30 days or 5 half-lives (whichever is longer) prior to first dose or current participation in an investigational study
19. Donation or loss of whole blood (excluding the volume of blood that will be drawn during the Screening procedures of this study) prior to first dose as follows: 50 mL to 499 mL of whole blood within 30 days, or more than 499 mL of whole blood within 56 days prior to first dose.
20. Any finding at Screening or any concomitant medical or psychiatric condition or social situation that would make it difficult to comply with protocol requirements or to complete the study, or would put the participant at additional safety risk
21. Participants who are unable to return for all scheduled study visits
22. Any of the following laboratory values at Screening:
 - a. Alanine transaminase (ALT) or aspartate transaminase (AST) > upper limit of normal (ULN)
 - b. Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m²
23. Known allergy or possible allergy to either ARO-MUC5AC or to its excipients

7.3. Inclusion Criteria for Asthma Cohorts

To be eligible for enrollment, patients with asthma must meet all the following inclusion criteria:

1. Male or nonpregnant, nonlactating female volunteers
2. Age 18 to 65 years at Screening. Age 19 to 65 years at Screening, where applicable according to local regulation.

3. Diagnosis of asthma which must have been confirmed and documented (based on source verifiable medical record) for at least 12 months prior to Screening
4. Documented treatment with a total daily dose of inhaled corticosteroids ≥ 500 mcg fluticasone propionate dry powder formulation (or equipotent inhaled corticosteroid) for at least 3 months prior to Screening (see [Appendix 1](#) for equipotent doses)
5. Documented treatment with at least 1 additional maintenance asthma controller medication (eg, a long-acting β_2 -agonist [LABA], a leukotriene receptor antagonist [LTRA], or a long-acting muscarinic antagonist [LAMA]) for at least 3 months prior to Screening
6. Prebronchodilator ppFEV₁ between 40% and 80% inclusive at Screening, prior to sputum induction
7. Stable dose of asthma controller medications for at least 28 days prior to Screening
8. If on allergen-specific immunotherapy, patients must be on a stable maintenance dose for at least 90 days prior to first dose
9. If on biologic therapy for asthma (eg, anti-IgE, anti-IL5/IL5R, anti-IL4R, or anti-thymic stromal lymphopoietin [TSLP]), patients must be on a stable maintenance dose for at least 16 weeks prior to first dose
10. Chest x-ray taken at Screening that, according to the Investigator, excludes significant alternative respiratory disease
11. Able and willing to provide written informed consent prior to the performance of any study-specific procedures
12. BMI between 18.0 and 35.0 kg/m² at Screening
13. A 12-lead ECG at Screening with no abnormalities that may compromise participant's safety in this study
14. Nonsmoker (defined as someone who has not smoked a cigarette for at least 6 months) with current nonsmoking status confirmed by urine cotinine at Screening AND previous smoking history prior to 6 months must be <10 pack-years. Subjects may be on nicotine replacement (patch or gum). Nicotine e-cigarettes (vapor) are not permitted. A positive urine cotinine result due to nicotine replacement is acceptable for enrollment at the discretion of the PI.
15. Participants of childbearing potential must agree to use a highly effective form of contraception, in addition to a condom, during the study and for at least 90 days following the end of the study or last dose of study drug, whichever is later. Subjects must not donate sperm or eggs during the study and for at least 90 days following the end of the study or last dose of study drug, whichever is later. See [Appendix 2](#) for details.
16. Able and willing to comply with all study assessments and adhere to the protocol schedule
17. Able to produce an induced sputum sample at Screening that meets criteria of acceptable quality (see [Section 10.2.1.2.1](#) for details)

7.4. Exclusion Criteria for Asthma Cohorts

Patients with asthma who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Acute lower respiratory infection or asthma exacerbation within 30 days prior to first dose
2. Acute upper respiratory infection within 7 days prior to first dose
 - a. In the case of an upper respiratory infection within 7 days of the first dose, the PI may elect to extend the Screening period to >28 days such that the first dose is given >7 days following clinical resolution of the infection. In no case will the Screening period be extended to >45 days.
3. Positive COVID-19 test during Screening window. Any record of positive test during the Screening window, whether a protocol-mandated antigen test or any NAAT or antigen test obtained outside of this study, serves as a potential exclusion criterion (see below).
 - a. In the event of a positive COVID-19 test during Screening, and if the PI deems the subject clinically appropriate to proceed to study drug dosing and no other exclusion criteria are met (eg, #1), the PI may elect to proceed to randomize the subject. In such a case, the first dose may not be given until >7 days after both clinical resolution of infection and conversion of antigen test from positive to negative, whichever is later. If necessary, the Screening period may be extended to >28 days, but in no case will the Screening period be extended to >45 days.
4. Prior history of bronchial thermoplasty treatment
5. Diagnosis of vocal cord dysfunction, reactive airways dysfunction syndrome, panic attacks with hyperventilation, or other mimics of asthma
6. Any concomitant pulmonary disease that, in the opinion of the Investigator, will interfere with the evaluation of the study drug or interpretation of patient safety or study results (including, but not limited to: interstitial lung disease, cystic fibrosis, lung cancer, tuberculosis, and bronchiectasis). Note that patients with only radiographic findings of bronchiectasis may be included.
7. Known unresolved parasitic infection or prior parasitic infection that has required antiparasitic therapy within 30 days prior to first dose
8. Any history of organ transplant
9. HIV infection, as shown by presence of anti-HIV antibodies (seropositive)
10. Seropositive for HBV or HCV (positive result for anti-HCV antibody must be confirmed with positive HCV RNA test for exclusion)
11. Uncontrolled hypertension (SBP >150 mmHg or DBP >100 mmHg) at Screening
12. A history of Torsades de Pointes, ventricular rhythm disturbances (eg, ventricular tachycardia or fibrillation), pathologic sinus bradycardia (<50 bpm with symptoms), heart block (excluding first-degree block, being PR prolongation only), congenital long QT syndrome, new ST segment elevation or depression, or new Q wave on ECG.

Participants with a history of atrial arrhythmias may be enrolled if the PI judges the safety risk to be low. Participants with a history of cardiac rhythm abnormality that has been treated with a device (eg, pacemaker) may be enrolled if the PI judges the safety risk to be low.

13. A family history of congenital long QT syndrome or unexplained sudden cardiac death
14. Use of medications known to prolong the QTc interval within 30 days prior to first dose (eg, azithromycin)
15. Use of theophylline within 30 days prior to first dose
16. Symptomatic heart failure (per NYHA guidelines), unstable angina, myocardial infarction, severe cardiovascular disease (ejection fraction <20%), TIA, or CVA within 24 weeks prior to first dose
17. History of malignancy within the past 5 years except for adequately treated basal cell carcinoma, squamous cell skin cancer, superficial bladder tumors, or in situ cervical cancer. Participants with other curatively treated malignancies who have no evidence of metastatic disease and >5-year disease-free interval may be enrolled if the PI judges the risk of recurrence to be low.
18. History of major surgery within 12 weeks prior to first dose
19. Unwilling to limit alcohol consumption to within moderate limits for the duration of the study, as follows: not more than 14 units per week for women and 21 units per week for men (1 unit=150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol)
20. Use of illicit drugs (such as cocaine, PCP) within 1 year prior to Screening or positive urine drug screen at Screening (a urine drug screen deemed positive due to prescription medications or for benzodiazepines, opioids, or marijuana is acceptable and inclusion is at the discretion of the PI). Subjects who smoke or vape prescription THC/marijuana may be enrolled if the PI judges the safety risk to be low.
21. Use of an investigational agent or device within 30 days or 5 half-lives (whichever is longer) prior to first dose or current participation in an investigational study
22. Donation or loss of whole blood (excluding the volume of blood that will be drawn during the Screening procedures of this study) prior to first dose as follows: 50 mL to 499 mL of whole blood within 30 days, or more than 499 mL of whole blood within 56 days prior to first dose.
23. Any finding at Screening or any concomitant medical or psychiatric condition or social situation that would make it difficult to comply with protocol requirements or to complete the study, or would put the participant at additional safety risk
24. Participants who are unable to return for all scheduled study visits
25. Any of the following laboratory values at Screening:
 - a. ALT or AST >2× ULN
 - b. eGFR <60 mL/min/1.73m²
26. Known allergy or possible allergy to either ARO-MUC5AC or to its excipients

7.5. Inclusion Criteria for COPD Cohorts

To be eligible for enrollment, patients with COPD must meet all the following inclusion criteria:

1. Male or nonpregnant, nonlactating female volunteers
2. Age 40 to 70 years at Screening
3. Diagnosis of COPD for at least 12 months prior to Screening, based on source verifiable medical record, confirmed with a post-bronchodilator ratio of FEV₁ to FVC < 0.7 at Screening
4. History of chronic bronchitis (defined as both cough and phlegm “most days a week” or “several days a week”) elicited on the SGRQ-C at Screening
5. Current smoker or ex-smoker (meaning >1 year of smoking cessation) with a smoking history of ≥ 10 pack-years
6. Post-bronchodilator ppFEV₁ between 40% and 80% inclusive at Screening, prior to sputum induction
7. Subjects treated with either single, double, or triple therapy for COPD, as described below:
 - a. Single therapy consisting of: long-acting beta agonist (LABA) or long-acting muscarinic antagonist (LAMA)
 - b. Double therapy consisting of: LABA + LAMA, or LABA + inhaled corticosteroid (ICS), or LAMA + ICS
 - c. Triple therapy consisting of: LABA + LAMA + ICS
 - d. Please note that additional use of other agents, such as azithromycin or roflumilast, will not exclude the subject.
8. All treatments for COPD have been stable for at least 1 month prior to Screening (meaning no new medications or changes in dose quantity or dose frequency) and subject is willing to continue this treatment regimen without change for study duration.
9. Able and willing to provide written informed consent prior to the performance of any study-specific procedures
10. BMI between 18.0 and 35.0 kg/m² at Screening
11. A 12-lead ECG at Screening with no abnormalities that may compromise participant’s safety in this study
12. Participants of childbearing potential must agree to use a highly effective form of contraception in addition to a condom, during the study and for at least 90 days following the end of the study or last dose of study drug, whichever is later. Subjects must not donate sperm or eggs during the study and for at least 90 days following the end of the study or last dose of study drug, whichever is later. See [Appendix 2](#) for details.
13. Able and willing to comply with all study assessments and adhere to the protocol schedule
14. Able to produce an induced sputum sample at Screening that meets criteria of acceptable quality (see [Section 10.2.1.2.1](#) for details).

7.6. Exclusion Criteria for COPD Cohorts

Patients with COPD who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Acute lower respiratory infection or COPD exacerbation within 30 days prior to first dose
2. Acute upper respiratory infection within 7 days prior to first dose
 - a. In the case of an upper respiratory infection within 7 days of the first dose, the PI may elect to extend the Screening period to >28 days such that the first dose is given >7 days following clinical resolution of the infection. In no case will the Screening period be extended to >45 days.
3. Positive COVID-19 test during Screening window. Any record of positive test during the Screening window, whether a protocol-mandated antigen test or any NAAT or antigen test obtained outside of this study, serves as a potential exclusion criterion (see below).
 - a. In the event of a positive COVID-19 test during Screening, and if the PI deems the subject clinically appropriate to proceed to study drug dosing and no other exclusion criteria are met (eg, #1), the PI may elect to proceed to randomize the subject. In such a case, the first dose may not be given until >7 days after both clinical resolution of infection and conversion of antigen test from positive to negative, whichever is later. If necessary, the Screening period may be extended to >28 days, but in no case will the Screening period be extended to >45 days.
4. Any significant, concomitant pulmonary disease that, in the opinion of the Investigator, will interfere with the evaluation of the study drug or interpretation of patient safety or study results (including, but not limited to: interstitial lung disease, cystic fibrosis, lung cancer, and tuberculosis).
5. History of lung volume reduction surgery (either surgical or bronchoscopic) or pneumonectomy
6. Need for chronic (>15 hours/day) oxygen support at Screening
7. Prior history of bronchial thermoplasty treatment
8. Participation in the acute phase of pulmonary rehabilitation (ie started < 4 weeks prior to Screening)
9. Known unresolved parasitic infection or prior parasitic infection that has required antiparasitic therapy within 30 days prior to first dose
10. Any history of organ transplant or active listing for a solid organ transplant at Screening
11. HIV infection, as shown by presence of anti-HIV antibodies (seropositive)
12. Seropositive for HBV or HCV (positive result for anti-HCV antibody must be confirmed with positive HCV RNA test for exclusion)
13. Uncontrolled hypertension (SBP >150 mmHg or DBP >100 mmHg) at Screening
14. A history of Torsades de Pointes, ventricular rhythm disturbances (eg, ventricular tachycardia or fibrillation), pathologic sinus bradycardia (<50 bpm with symptoms), heart block (excluding first-degree block, being PR prolongation only), congenital long QT

syndrome, new ST segment elevation or depression, or new Q wave on ECG. Participants with a history of atrial arrhythmias may be enrolled if the PI judges the safety risk to be low. Participants with a history of cardiac rhythm abnormality that has been treated with a device (eg, pacemaker) may be enrolled if the PI judges the safety risk to be low.

15. A family history of congenital long QT syndrome or unexplained sudden cardiac death
16. Symptomatic heart failure (per NYHA guidelines), unstable angina, myocardial infarction, severe cardiovascular disease (ejection fraction <20%), TIA, or CVA within 24 weeks prior to first dose
17. History of malignancy within the past 5 years except for adequately treated basal cell carcinoma, squamous cell skin cancer, superficial bladder tumors, or in situ cervical cancer. Participants with other curatively treated malignancies who have no evidence of metastatic disease and >5-year disease-free interval may be enrolled if the PI judges the risk of recurrence to be low.
18. History of major surgery within 12 weeks prior to first dose
19. Unwilling to limit alcohol consumption to within moderate limits for the duration of the study, as follows: not more than 14 units per week for women and 21 units per week for men (1 unit=150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol)
20. Use of illicit drugs (such as cocaine, PCP) within 1 year prior to Screening or positive urine drug screen at Screening (a urine drug screen deemed positive due to prescription medications or for benzodiazepines, opioids, or marijuana is acceptable and inclusion is at the discretion of the PI). Subjects who smoke or vape prescription THC/marijuana may be enrolled if the PI judges the safety risk to be low.
21. Use of an investigational agent or device within 30 days or 5 half-lives (whichever is longer) prior to first dose or current participation in an investigational study
22. Donation or loss of whole blood (excluding the volume of blood that will be drawn during the Screening procedures of this study) prior to first dose as follows: 50 mL to 499 mL of whole blood within 30 days, or more than 499 mL of whole blood within 56 days prior to first dose.
23. Any finding at Screening or any concomitant medical or psychiatric condition or social situation that would make it difficult to comply with protocol requirements or to complete the study, or would put the participant at additional safety risk
24. Participants who are unable to return for all scheduled study visits
25. Any of the following laboratory values at Screening:
 - a. ALT or AST >2× ULN
 - b. eGFR <50 mL/min/1.73m²
26. Known allergy or possible allergy to either ARO-MUC5AC or to its excipients

7.7. Subject Withdrawal Criteria

Subjects will be advised that they are free to withdraw from the study at any time for any reason or, if necessary, the PI, medically-trained designee, or Sponsor may withdraw a subject from the study to protect the subject's health or the integrity of the study data, per the following criteria:

- The need to take medication that may interfere with study measurements or is incompatible with the IP
- Intolerable/unacceptable adverse events
- Major violation of or deviation from study protocol procedures
- Noncompliance of subject with protocol
- Subject is unwilling to proceed, or consent is withdrawn
- Withdrawal from the study if, in the Investigator's judgment, it is in the subject's best interest

The date and reasons for withdrawal, along with any AEs and any necessary medical treatment, will be recorded on the eCRF.

If a subject is withdrawn from the study due to a significant AE or SAE, the Investigator or medically-trained designee will evaluate the urgency of the event. If the situation warrants, the Investigator or medically-trained designee will take appropriate diagnostic and therapeutic measures. If the situation is not an immediate emergency, the Investigator or medically-trained designee at the clinical study facility will attempt to contact the Medical Monitor or medically-qualified designee for consultation. No medical help, diagnosis, or advice will be withheld from the subject due to an inability to contact the Medical Monitor. The subject will be encouraged to remain available for follow-up medical monitoring. The Sponsor will be notified as soon as possible of any subject withdrawals.

If a subject fails to complete study assessments deemed critical to the integrity of the study (eg, subject receives at least 1 dose of study drug but fails to receive all planned doses of study drug, or subject fails to complete all planned bronchoscopies), the subject may remain in the study for further safety evaluation if deemed necessary by Sponsor. In such cases, subsequent study visits will focus on safety, which may involve cessation of certain study assessments (eg, PK sample collection). If a subject fails to complete study assessments deemed critical to the integrity of the study, the subject may be replaced at the discretion of the Sponsor (meaning that an additional, new subject will be added to the pertinent cohort, resulting in an increase of the cohort size).

8. TREATMENT OF SUBJECTS

8.1. Description of Study Drug

The study drug will be either active treatment or placebo treatment administered by inhalation using a nebulizer.

8.1.1. Active Treatment

The active treatment is ARO-MUC5AC Inhalation Solution. The API (ADS-013) contained in ARO-MUC5AC is a synthetic, double-stranded, siRNA duplex conjugated to an $\alpha v\beta 6$ integrin-targeting ligand to facilitate pulmonary epithelial delivery, formulated in normal saline to be delivered by inhalation of nebulized solution.

8.1.2. Placebo

The placebo is normal saline (0.9%) administered by inhalation of nebulized solution, volume-matched to the corresponding ARO-MUC5AC dose volume.

8.1.3. Doses and Number of Doses Per Treatment

Part 1: NHV SAD cohorts (Cohorts 1a-4a)

Normal healthy volunteer cohorts, Cohorts 1a to 4a, will receive escalating single doses of study drug on Day 1, administered by inhalation. The planned doses start at 24 mg (Cohort 1a) and escalate up to 232 mg (Cohort 4a):

- Cohort 1a: 24 mg
- Cohort 2a: 56 mg
- Cohort 3a: 108 mg
- Cohort 4a: 232 mg

Part 2: NHV MAD cohorts (Cohorts 1b-4b)

Normal healthy volunteer cohorts, Cohorts 1b to 4b, will receive escalating multiple doses of study drug on Days 1, 15, and 29, administered by inhalation. The planned doses start at 24 mg (Cohort 1b) and escalate up to 232 mg (Cohort 4b). Cohort 4b is optional and will be enrolled at the discretion of the Sponsor.

- Cohort 1b: 24 mg
- Cohort 2b: 56 mg
- Cohort 3b: 108 mg
- Cohort 4b: 232 mg

Part 3: Asthma MAD cohorts (Cohorts 2c-4c)

Asthma cohorts, Cohorts 2c to 4c, will receive escalating multiple doses of study drug on Days 1, 15, and 29, administered by inhalation. The planned doses start at 56 mg (Cohort 2c) and escalate up to 232 mg (Cohort 4c).

- Cohort 2c: 56 mg
- Cohort 3c: 108 mg
- Cohort 4c: 232 mg

Part 4: COPD MAD cohorts (Cohorts 2d-4d)

COPD cohorts, Cohorts 2d to 4d, will receive escalating multiple doses of study drug on Days 1, 15, and 29, administered by inhalation. The planned doses start at 56 mg (Cohort 2d) and escalate up to 232 mg (Cohort 4d).

- Cohort 2d: 56 mg
- Cohort 3d: 108 mg
- Cohort 4d: 232 mg

For all cohorts, the indicated dose level is the maximum allowable dose for the cohort, but the Sponsor may adjust the dose downward based on available safety and PD data. Dose levels will not be adjusted above a maximum dose of 232 mg.

8.1.4. Sputum Induction Procedure

Sterile 0.9%, 3%, 4%, and 5% saline solution may be used for sputum induction. Sterile saline inhalation solution is supplied by the Sponsor for use in sputum induction as 0.9% saline solution, 3% saline solution, and 6% saline solution. If locally supplied saline is to be used for sputum induction, it must be approved by the Sponsor. The drug products for sputum induction are saline administered by inhalation of nebulized solution.

8.2. Restrictions and Concomitant Medications

8.2.1. Confinement and Study Visits

For subjects in the SAD portion of the study (Cohorts 1a-4a), clinical facility confinement will be approximately 3 days (Day -1 through Day 2 assessments). Subjects will be admitted to the facility on Day -1 and will undergo assessments at the facility through Day 2. After Day 2, subjects can be discharged and will return to the clinical facility starting on Day 3 for outpatient visits.

For subjects in the NHV MAD portion of the study (Cohorts 1b-4b), clinical facility confinement will be up to approximately 8 hours on dosing days (Days 1, 15, and 29), unless additional monitoring at PI discretion is needed for safety reasons. Subjects will return to the clinical facility for outpatient visits. There is no planned overnight confinement for subjects in these cohorts.

For patients in the patient MAD portion of the study (Cohorts 2c-4c and 2d-4d), clinical facility confinement will be up to 8 hours on dosing days (Days 1, 15, and 29), but no minimum post-dose confinement period is required. Patients may leave the clinic once all protocol assessments are complete unless additional monitoring at PI discretion is needed for safety reasons. Patients will return to the clinical facility for outpatient visits. There is no planned overnight confinement for patients.

8.2.2. Fasting

Fasting from food and drink will be required for at least 6 hours prior to a scheduled bronchoscopy.

Fasting from food and caffeinated drinks will be required for 2 hours prior to all induced sputum procedures.

Subjects are required to fast from food and drink for at least 1 hour prior to a FeNO measurement. Additionally, there are specific foods that should be avoided on study days involving FeNO measurement; please see [Section 10.2.2](#) for details.

No fasting will be required for other study visits.

8.2.3. Recreational Drugs & Alcohol

Subjects will be instructed to abstain from consuming alcohol for at least 48 hours prior to their clinic visit on dosing days and during the clinic visit. In addition, subjects will be instructed to refrain from excessive use of alcohol (ie, more than 14 units per week for women and 21 units per week for men [1 unit=150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol]) for the study duration. Subjects must abstain from the use of recreational drugs throughout the study. Subjects must not smoke tobacco or use e-cigarettes (vaping) during the study.

8.2.4. Blood Donation

Subjects will be instructed to abstain from donating any blood or blood products following first dose of study drug through EOS.

8.2.5. Concomitant Medications

A prior medication is any drug or substance taken prior to the time the subject enters Screening (ie, up until the time at which they provide informed consent). All prior medications will be recorded on the eCRF, noting the name, dose, duration, and indication.

Concomitant medication is defined as any medication, including prescription and over-the-counter medicines other than the study medication, that is taken during the study from the date of signature of informed consent until the EOS visit or last treatment visit (ie, the last visit for the subject). All concomitant medications taken by the subject during the study from the date of signature of informed consent are to be recorded in the appropriate section of the eCRF, noting the name, dose, duration, and indication. Nondrug interventions and any changes to a concomitant medication or other intervention should also be recorded in the eCRF.

8.2.5.1. Asthma Controller Medications

For asthma cohorts (Cohorts 2c to 4c), all subjects will have been treated with inhaled corticosteroids (at a total daily dose of ≥ 500 μ g fluticasone propionate dry powder formulation, or equivalent) for at least 3 months prior to Screening. All subjects will also have been treated with 1 additional asthma controller medication (eg, LABA, LTRA, or LAMA) for at least 3 months prior to Screening.

Concomitant use of oral corticosteroids, biologic therapy, and/or allergen-specific immunotherapy as controller medication is allowed. Biologic therapy dose regimen must be

stable for at least 16 weeks prior to first dose. Immunotherapy dosing must be stable for at least 90 days prior to first dose.

Aside from biologics and immunotherapy, all other asthma controller medication dosing regimens must be stable for at least 4 weeks prior to Screening.

The dose of all asthma controller medications should remain stable throughout the study, unless changes are required due to disease worsening (eg, asthma exacerbation).

8.2.5.2. COPD Controller Medications

For COPD cohorts (Cohorts 2d to 4d), all subjects will have been treated with LABA, LAMA, and/or ICS. These may be used as either single therapy (ie, LABA or LAMA alone) or in combination as double or triple therapy.

Use of other therapies for COPD, such as roflumilast, azithromycin, or oral corticosteroids, is allowed, in addition to the above therapies.

All COPD treatments must have been stable for at least 4 weeks prior to Screening.

8.2.5.3. QT-Prolonging and Arrhythmogenic Medications

No cardiac safety signal has emerged from nonclinical or clinical experience to date with ARO-MUC5AC, and interference with cardiac conduction has not been associated with other siRNA therapeutics. However, rigorous cardiac eligibility criteria and frequent monitoring have been implemented to mitigate unanticipated cardiac risk in this FIH trial.

8.2.5.3.1. NHV and Asthma Cohorts

Standard asthma controller medications are not commonly associated with increased risk of QT prolongation or arrhythmias. However, some patients with asthma may be on chronic azithromycin and/or theophylline therapy as controller medication. Azithromycin therapy is associated with increased risk of QT prolongation, and thus, concomitant chronic azithromycin therapy is an exclusion criterion. Theophylline use is associated with increased risk of arrhythmias, and thus, concomitant theophylline therapy is also an exclusion criterion.

Subjects should not be initiated on QT-prolonging or arrhythmogenic medications during the study unless there is a medical need for the introduction of one of these agents and an alternative, non-arrhythmogenic medication is not available. The initiation of any of QT-prolonging or arrhythmogenic medications must be approved by the Sponsor in advance and only occur if required for treatment of subjects. If such a medication is initiated, subjects should be carefully monitored for the development of QTc prolongation or other ECG abnormalities as indicated.

8.2.5.3.2. COPD Cohorts

In the ongoing AROMUC5AC-1001 clinical trial, there have been no clinically significant ECG findings to date and no adverse events due to ECG changes. In light of this, and in recognition of the fact that patients with COPD frequently have multiple comorbidities requiring administration of multiple medications (including medications that may prolong the QT interval), there are no restrictions placed on use of QT-prolonging or potentially arrhythmogenic medications for subjects in the COPD Cohorts of the AROMUC5AC-1001 study.

8.2.5.4. All Other Medications

Any medications (other than those excluded by the protocol) that are considered necessary for any subject's welfare and will not interfere with the study medication may be given at the Investigator's discretion. Any additional concomitant therapy that becomes necessary during the study and any change to concomitant drugs must be recorded in the corresponding section of the eCRF, noting the name, dose, duration, and indication.

8.2.5.5. Prohibited Medications

The following medications will be prohibited:

- Any medication known to be associated with increased risk of QTc prolongation (NHV and asthma cohorts only)
- Theophylline (NHV and asthma cohorts only)

8.2.5.6. Medications To Be Withheld Prior to Study Visits

Inhaled bronchodilators (short-acting β_2 agonists [SABAs], LABAs, and LAMAs) and LTRAs are to be withheld for a specified time period prior to any study visit involving spirometry or FeNO measurement. See [Section 11.1.2](#) and [Section 10.2.2](#) for details.

8.3. Notification of General Practitioner

It is the responsibility of the PI or designee to notify, where applicable, with the consent of the subject, the General Practitioner of the subject's participation in the trial, by sending a letter stating the nature of the trial, treatments, expected benefits or AEs, and concomitant drugs to be avoided.

8.4. Treatment Compliance

All study drug will be administered at the study site. The date and time (start and stop times) of inhaled administration of study drug will be recorded on the eCRF on dosing days.

8.5. Randomization and Blinding

8.5.1. Randomization

All potential study subjects who sign an ICF at Screening will receive a unique identifier (ie, a Screening number). For study subjects who are deemed eligible, this unique Screening number will become the subject's permanent study ID number.

Eligible subjects will be allocated a unique randomization number, in accordance with the randomization schedule. Treatments will be administered per the randomized sequence generated by interactive web response system (IWRS). The randomization numbers will be grouped in blocks. In NHV Cohorts 1a to 4a, the first 2 healthy volunteers (sentinels) will be randomized separately to 1 active and 1 placebo.

The randomization schedules and randomization number assignments will be maintained electronically in the IWRS. The personnel involved in the dispensing of IPs will be accountable for ensuring compliance to randomization schedules. The nonblinded Clinical Research

Associate (CRA) will have IWRS access to verify the subject's treatment arm (ie, ARO-MUC5AC or placebo) and will compare it against the dispensing log to verify the correct study drug assignment.

8.5.2. Blinding

Investigators and study participants will remain blinded to treatment allocation of subjects until database lock. Selected Sponsor members will be unblinded to treatment allocation of subjects throughout the entire study.

Blinding of active treatment/placebo assignment is essential to the integrity of this clinical study. It is expected that in most cases, AEs can be properly managed without the need for unblinding. However, in the event of a medical emergency in which knowledge of an individual subject's assignment is considered critical to the subject's wellbeing and management, the PI, documented designated treating physician, or the Sponsor Medical Monitor can unblind the treatment assignment. If the situation is not an immediate emergency, the PI should contact the responsible Medical Monitor to discuss the subject and circumstances requiring the unblinding. The blind will be broken only for the specific subject under discussion. The Study Medical Monitor should be informed promptly. In addition, disclosure of treatment allocation information to DSC members may occur, if necessary, to determine whether a stopping rule has been met and, if so, for subsequent DSC discussion regarding the conduct of the trial.

If the PI considers an AE to be of such severity as to require immediate specific knowledge of the identity and dose of the relevant product, unblinding will be completed via IWRS. The "Medical Emergency Unblinding" transaction in the IWRS is only accessible to the designated unblinded Pharmacist, PI, and Subinvestigator. The Medical Monitor should be informed promptly.

If a subject requires emergency unblinding (with or without a discussion between the PI and the Medical Monitor preceding the unblinding), the PI may also be required to complete a "Drug Safety Unblinding Request/Notification Form" to document the medical rationale necessitating the unblinding. This form is then forwarded to the local Medical Monitor.

8.6. Emergency Contact With Principal Investigator

Suitable arrangements will be made for subjects to contact the PI or medically-trained designee in the event of an emergency.

9. STUDY DRUG MATERIALS AND MANAGEMENT

9.1. Study Drug

ARO-MUC5AC Inhalation Solution is manufactured by dissolution of the lyophilized API (ADS-013) into aqueous normal saline solution, which is then aseptically filled into glass vials. The Sponsor is responsible for the supply of ARO-MUC5AC together with detailed instructions (in the Pharmacy Manual) describing the preparation of ARO-MUC5AC. The placebo (sterile 0.9% sodium chloride for inhalation) will be supplied by the clinical site or may be provided by the Sponsor upon request. ARO-MUC5AC Inhalation Solution will be supplied as sterile solution for inhalation via nebulization, containing 40 mg/mL of ADS-013 salt-free form.

9.2. Study Drug Packaging and Labeling

ARO-MUC5AC Inhalation Solution will be supplied as sterile solution for inhalation containing 40 mg/mL of ADS-013 salt-free form packaged in a 10-mL Type I glass vial and closed with a fluorocarbon-lined butyl stopper and an orange flip-off seal. Each vial contains a 1.8-mL nominal volume to provide a 1.6-mL withdrawable volume.

Table 9: Study Drug Description

Strength	40 mg/mL ADS-013 (salt-free basis)
Volume	1.8 mL
Appearance	Clear, colorless to yellow solution
Inactive ingredients	0.9% saline (0.9% sodium chloride in water)
Shipment and storage	Refrigerated, 2 °C-8 °C

The investigational product (IP) will be labeled per current Good Manufacturing Practice (cGMP) and GCP. Investigational product supplies will be stored at clinical sites securely under the appropriate conditions.

Placebo (sterile 0.9% sodium chloride for inhalation, USP, preservative-free) will be supplied by the clinical site or the Sponsor and the volume administered (mL) will match the volume of corresponding ARO-MUC5AC dose based on the respective cohort for which the subject has been assigned.

9.3. Study Drug Storage

ARO-MUC5AC will be supplied by the Sponsor and labeled with the drug name, batch number, expiration date (as applicable), and storage conditions. The Sponsor will provide the PI with sufficient IP. The PI must ensure that IP deliveries from the Sponsor are correctly received by a responsible person, that all receipts of IP shipments are recorded on the appropriate drug accountability forms prepared by the pharmacy at the clinical site, and that the IP is stored in a secure area under the recommended storage conditions. The PI must also ensure that the integrity of packaged IP is not jeopardized prior to dispensing.

Only subjects enrolled in the study may receive IP, in accordance with all applicable regulatory requirements. Only authorized site staff may supply or administer IP. The IP must be stored in a

secure area with access limited to the PI and authorized staff and under the physical conditions that are consistent with the IP-specific requirements.

Placebo (0.9% saline) will be supplied by the clinical site or by the Sponsor and stored as directed on the label.

9.4. Study Drug Preparation

A Pharmacy Manual will be prepared to define the procedures for dispensing. ARO-MUC5AC will be prepared, per the Pharmacy Manual, by a staff Pharmacist or other designated, qualified staff at the clinical site. The time of preparation for active treatment must be documented and tracked to demonstrate administration within prepared drug stability boundaries. The dispensing will be verified by a second member of clinical site staff whenever possible. Individual doses will be dispensed by clinical study site staff members on the day of dosing and recorded in the drug accountability records. Please refer to the Pharmacy Manual for more detailed instructions.

9.5. Study Drug Administration

Each dose of ARO-MUC5AC or placebo will be administered by inhalation of nebulized solution by the PI or by appropriately trained and qualified clinical staff designated by the PI.

The dose specified to be administered per protocol is the NLD. [Table 10](#) shows the NLD, dose concentration, nebulizer loaded volume, approximate nebulization time, and corresponding estimated RDD for each cohort. See the Pharmacy Manual and Study Procedure Manual for more information.

Table 10: Dose Administration Per Cohort

Cohort	Nebulizer Loaded Dose (mg)	Dose Concentration (mg/mL)	Nebulizer Loaded Volume (mL)	Approximate Nebulization Time (min)	Estimated Respirable Delivered Dose (mg)
1a	24	10	2.4	6	14
2a	56	40	1.4	4	35
3a	108	40	2.7	7	70
4a ^a	232	40	5.8	14	139
1b	24	10	2.4	6	14
2b	56	40	1.4	4	35
3b	108	40	2.7	7	70
4b ^a	232	40	5.8	14	139
2c	56	40	1.4	4	35
3c	108	40	2.7	7	70
4c ^a	232	40	5.8	14	139
2d	56	40	1.4	4	35

Cohort	Nebulizer Loaded Dose (mg)	Dose Concentration (mg/mL)	Nebulizer Loaded Volume (mL)	Approximate Nebulization Time (min)	Estimated Respirable Delivered Dose (mg)
3d	108	40	2.7	7	70
4d ^a	232	40	5.8	14	139

^a Dosage for Cohorts 4a, 4b, 4c, and 4d will be given utilizing the device with an 8 mL nebulizer reservoir. Dosage for all other cohorts will be given using the device with a 4 mL reservoir.

9.6. Study Drug Accountability

All material supplied is for use only in this clinical study and should not be used for any other purpose. The PI is responsible for the IP accountability, reconciliation, and record maintenance at the investigational site. In accordance with all applicable regulatory requirements, the PI or designated clinical site staff must maintain IP accountability records throughout the course of the study. This person will document the amount of IP received from the Sponsor and the amount administered to subjects. An unblinded CRA will perform initial and ongoing IP and placebo accountability. The unblinded CRA will protect the integrity of the assignment blind and will not participate in data review for study subjects. Used vials of study drug will be retained sequestered per subject (where allowable by local policy) and made available to the unblinded CRA during IP and placebo reconciliation.

A drug dispensing log must be kept current and will contain the following information:

- The identification of the subject to whom the drug was dispensed
- The date(s), quantity, lot number(s), and expiration date(s) of the drug dispensed to the subject

The date and time of dose preparation and release will be maintained to support administration of IP. The authorized Pharmacist or qualified staff will be unblinded to treatment assignment (ie, active or placebo). The pharmacy will dispense the study medication and the study center will administer the study medication only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the IP as assigned by the IWRS. The IP administration will be documented and recorded on the eCRFs. The inventory must be available for inspection by the unblinded CRA during the study. Drug supplies will either be collected at the end of the study by the CRA or returned by the PI or designee to the Sponsor or the designated Sponsor-approved depot.

9.7. Study Drug Handling and Disposal

The Sponsor will provide the Investigator with a sufficient quantity of clinical drug supplies. The Investigator must ensure that deliveries of IP from the Sponsor are correctly received by a responsible person, that all receipts of drug shipments are recorded on the appropriate Drug Accountability Forms prepared by the pharmacy at the clinical site, and that the products are stored in a secure area under the recommended storage conditions. It is also the responsibility of the Investigator to ensure that the integrity of packaged IP not be jeopardized prior to dispensing.

Only subjects enrolled in the study may receive IP, in accordance with all applicable regulatory requirements. Only authorized site staff may supply or administer IP. The IP must be stored in a secure area, with access limited to the Investigator and authorized staff, and under the physical conditions that are consistent with the IP-specific requirements.

An authorized and trained staff member at each clinical study site will dispense the IP per predefined drug dispensing requirements. The dispensing will be verified by a second member of site staff whenever possible.

ARO-MUC5AC will be supplied by the Sponsor and labeled with the drug name, batch number, expiration date (as applicable), and storage conditions. The IP will be dispensed by clinical study site staff members on the day of dosing and recorded in the Drug Accountability Records. A Pharmacy Manual will be provided to define the procedures for dispensing.

10. PHARMACOKINETIC AND PHARMACODYNAMIC ASSESSMENTS

10.1. Pharmacokinetic Assessments

10.1.1. Sample Collection

Blood and/or urine samples for PK analysis of circulating and excreted ARO-MUC5AC and its metabolites will be obtained from subjects at various time points before and after study drug administration, as outlined in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)). Whole blood will be collected and processed per the Laboratory Manual. Unused portions of collected blood and urine samples may be retained and stored for future research if the subject consents (see [Section 6.1.2](#)).

10.1.2. Sample Analysis

Plasma samples will be assayed by a validated hybridization-based peptide nucleic acid method. The criteria for repeat analysis, as defined in the respective in-house procedure, will be followed. The validation study conducted by the appointed bioanalytical laboratory to establish validity including accuracy, precision, reproducibility, specificity, recovery, and frozen stability of the analytical method will be appended to the final report.

10.2. Pharmacodynamic Assessments

10.2.1. Target Engagement Biomarkers

10.2.1.1. Sample Collection for Mucin Measurements

MUC5AC and MUC5B protein concentrations will be assessed in induced sputum samples in all cohorts, as well as in bronchoscopic airway swab samples, bronchosorption samples, and BALF samples in NHV MAD cohorts. MUC5AC and MUC5B mRNA expression will be assessed in induced sputum samples in all cohorts, as well as in BALF samples in NHV MAD cohorts. Change in MUC5AC expression, but not MUC5B expression, is expected following treatment with ARO-MUC5AC. MUC5B will be measured to verify that the drug has a specific effect on MUC5AC. Induced sputum collections and bronchoscopies for airway swab, bronchosorption, and BALF samples will occur as noted in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)) and as described below. Unused portions of collected sputum and bronchoscopic samples may be retained and stored for future research if the subject consents (see [Section 6.1.2](#)).

10.2.1.2. Induced Sputum Collection

Sputum samples will be obtained from NHVs and patients via sputum induction at all time points noted in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)). A sputum induction protocol will be provided in the Study Procedure Manual.

Per the SOAs, the sputum induction procedure will always follow the spirometry assessment on a given study visit. This initial spirometry assessment will serve as a baseline measure of lung function prior to sputum induction and must be performed within 2 hours of sputum induction. Prior to the sputum induction, subjects will be asked to provide a noninduced, expectorated

sputum sample, if possible. Sputum induction will still be performed whether a subject is able to produce a spontaneous expectorated sputum or not. During the sputum induction protocol, subjects will be monitored both symptomatically and via spirometry, and the procedure will be discontinued in the setting of concerning symptoms or a significant ($>20\%$) fall in FEV₁ compared to baseline (as detailed in the Study Procedure Manual). All subjects will undergo three 7-minute rounds of sputum induction unless the procedure is terminated for safety reasons. Sputum samples generated during each round of induction will be pooled. A note should be made in the eCRF if the sputum sample contains any blood.

When the induced sputum procedure is performed on a dosing day, dosing of study drug will not occur until the subject has recovered from the sputum induction. Recovery is defined as return of FEV₁ to within 10% of the preinduction prebronchodilator FEV₁ (eg, if prebronchodilator FEV₁ is 3.0 L prior to sputum induction, then the subject must recover to FEV₁ ≥ 2.7 L prior to dosing of study drug). If the subject is unable to recover FEV₁ to within 10% of the FEV₁ obtained prior to sputum induction but is otherwise clinically well (eg, if the preinduction FEV₁ was abnormally high for the subject), the site Investigator may elect to proceed with study drug dosing at their clinical discretion.

Postinduction spirometry measurements made during the induced sputum procedure will be recorded on a Procedure Log (see Study Procedure Manual) but will not be entered into the eCRF or sent for review to the central spirometry vendor.

Induced sputum samples will be kept on ice until processing. Sputum processing details are provided in the Central Laboratory Manual and Study Procedure Manual. Sputum samples will be used for measurement of MUC5AC and MUC5B protein concentrations and mRNA expression.

10.2.1.2.1. Induced Sputum Quality Criterion for Screening

Both NHV subjects and patients must produce an induced sputum sample of acceptable quality at Screening to successfully meet inclusion criteria ([Section 7.1](#) and [Section 7.3](#)). A sputum sample of acceptable quality at Screening is one that meets the following criterion:

- Weight of sputum sample after removal of saliva ≥ 100 mg

10.2.1.3. Bronchoscopies

Bronchoscopic samples (BALF, bronchosorption, and airway swabs) will be obtained from NHVs in the MAD cohorts (Cohorts 1b-4b) at all time points noted in the SOA ([Table 4](#)). A bronchoscopy protocol will be provided in the Central Laboratory Manual.

Subjects will be required to fast for at least 6 hours prior to the procedure. Following sedation with fentanyl and midazolam (or similar agents as deemed appropriate by the bronchoscopist), the bronchoscope will be introduced into the airway and topical anesthesia with lidocaine will be applied to the airway. Subsequently, bronchosorption, airway swab and BALF samples will be obtained, in that order.

10.2.1.3.1. Bronchosorption Sample Collection

A bronchosorption catheter is a catheter containing a filter, or swab, that absorbs airway mucosal lining fluid. A bronchosorption catheter will be passed down the working channel of the

bronchoscope into the bronchus intermedius of the right lung. The bronchosorption filter will then be extruded and placed on the bronchial mucosa of the bronchus intermedius for 20 to 30 seconds under direct vision. The bronchosorption filter will then be retracted back into its catheter and withdrawn from the airway. Please see the Study Procedures Manual for further details, including the number of bronchosorption collections. Processing details for the bronchosorption filter are provided in the Study Procedures Manual. Airway epithelial lining fluid derived from the bronchosorption filter will be used for quantification of MUC5AC and MUC5B protein concentration.

10.2.1.3.2. Airway Swab Sample Collection

Two large airway swab samples will be obtained utilizing a brush. A sheathed cytology brush will be passed down the working channel of the bronchoscope into the right upper lobe. The brush will be unsheathed and samples collected in one segment of the right upper lobe. Under direct vision, the brush will be applied firmly to the airway wall at this site for 25 seconds. The brush will be retracted back into the sheath and withdrawn from the airway. The mucus material will then be washed off the brush into a sample container (see Central Laboratory Manual for details). This process will be repeated with a new brush, this time inserted into another segment of the right upper lobe. Mucus material from this brush will be washed off into the same sample container and pooled with the first sample. Brushing samples will be kept on ice until processing. Further processing details are provided in the Central Laboratory Manual. Brushing samples will be used for quantification of MUC5AC and MUC5B protein concentrations.

10.2.1.3.3. Bronchoalveolar Lavage Fluid Sample Collection

The bronchoscope will be wedged into a subsegment of the right middle lobe and three 50-mL aliquots of normal saline will be instilled; thus, a total of 150 mL of saline will be instilled into the lung. Suctioning will be performed following instillation of each aliquot (see Central Laboratory Manual for details). BALF will be kept on ice until processing. Processing details are provided in the Central Laboratory Manual. BALF samples will be used for measurement of MUC5AC and MUC5B protein concentration and mRNA expression, as well as cell count and differential. For cell count and differential measurements, BALF will be processed to generate Cytospin slides, which will be sent to a central reading facility for staining and reading (see Central Laboratory Manual and Study Procedure Manual for additional information).

10.2.1.4. Sample Analysis

Following processing of induced sputum, BALF, bronchosorption, and airway swab samples, MUC5AC and MUC5B protein concentrations will be measured by liquid chromatography-mass spectrometry.

Following processing of induced sputum and BALF samples to generate a cell pellet, MUC5AC and MUC5B mRNA expression will be measured in the cell pellet.

If measurement of both protein concentration and mRNA expression is not feasible for any given sample, then either measurement from that sample may be omitted, as indicated.

Sputum MUC5AC and MUC5B protein concentrations from only those sputum samples that meet the acceptability criterion indicated in [Section 10.2.1.2.1](#) will be used for analysis purposes. If an induced sputum sample does not meet the acceptable weight threshold, the induced sputum

sample may be combined with the subject's spontaneously expectorated sputum sample (if available), with acceptability then reassessed based on the combined sample.

Of note, while sputum samples will be collected from all subjects who undergo Screening, sputum MUC5AC and MUC5B concentration measurements will only be performed on Screening sputum samples from subjects who are subsequently dosed with study drug. MUC5AC and MUC5B concentration measurements will be performed on all collected bronchoscopic airway swab samples.

The baseline MUC5AC and MUC5B measurement will be derived using all available predose measurements.

10.2.2. FeNO

FeNO will be utilized as an assessment of airway inflammation and will be performed on patients with asthma in accordance with ATS clinical practice guidelines ([American Thoracic Society \(ATS\)/European Respiratory Society \(ERS\) 2005](#)).

All subsequent FeNO measurements should be performed within ± 1.5 hours of the time that the initial FeNO measurement (ie Screening measurement) was performed. At all study visits, FeNO must be measured prior to any spirometry measurements since pulmonary function measurements can impact FeNO results. Additionally, for all dosing days, FeNO must be measured prior to study drug dosing. Subjects should not use SABA medications (eg, albuterol/salbutamol) within 6 hours prior to the FeNO measurement and should withhold other inhaled medications (eg, LABA, LAMA, and LTRA) prior to FeNO for the period specified in the spirometry section ([Section 11.1.2](#)).

Subjects should not eat or drink for at least 1 hour prior to a FeNO measurement. On study days involving FeNO measurement, subjects should not consume the following foods prior to the FeNO measurement: spinach, lettuce, beetroot, celery, chervil, radish, turnip tops, strawberries, currants, raspberries, cherries, gooseberries, ham, bacon, or salami. If a subject has consumed any of these foods, the FeNO measurement should still be performed but a note should be made of the food consumed and the time it was consumed.

The subject should be sitting during the FeNO measurement. If the subject is unable to sit, standing is acceptable. Subject positioning (whether sitting or standing) must be consistent at every study visit and should be recorded in the source document. FeNO measurements will be performed with the NIOX VERO (Circassia) device. After closing lips on the mouthpiece, the subject will inhale to total lung capacity. The subject will then exhale for 10 seconds at a flow rate of 50 mL/second, assisted by cues on the NIOX monitor.

For each FeNO assessment, repeat exhalations (up to a maximum of 6 exhalations) will be performed until 2 measurements that agree within 10% of each other are achieved. At least 2 minutes of relaxed tidal breathing off the FeNO device should elapse between exhalations. All exhalation values will be recorded in the source document. The average of the 2 reproducible measurements will be used for analysis purposes.

10.2.3. Serum Total IgE

Blood samples will be collected at the time points indicated in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)) and will be sent to the central laboratory for measurement of serum total

IgE concentrations. Serum total IgE concentrations will only be measured on blood samples from patients with asthma, as indicated in the SOA ([Table 5](#)) and will not be measured on blood samples from NHVs. Unused portions of collected blood samples may be retained and stored for future research if the subject consents (see [Section 6.1.2](#)).

10.2.4. Blood Eosinophil or Neutrophil Count

Blood samples will be collected at the time points indicated in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)) and will be sent to the central laboratory for measurement of blood eosinophil or neutrophil count. Blood eosinophil or neutrophil count will only be measured as a PD assessment on blood samples from patients, as indicated in the SOA ([Table 5](#)). Unused portions of collected blood samples may be retained and stored for future research if the subject consents (see [Section 6.1.2](#)).

10.2.5. Plasma Periostin

Blood samples will be collected at the time points indicated in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)) and will be sent to the central laboratory for measurement of plasma periostin concentration. Plasma periostin concentration will only be measured on blood samples from patients with asthma, as indicated in the SOA ([Table 5](#)) and will not be measured on blood samples from NHVs. Unused portions of collected blood samples may be retained and stored for future research if the subject consents (see [Section 6.1.2](#)).

11. ASSESSMENT OF EFFICACY

11.1. Spirometry

11.1.1. Spirometry Assessments

Spirometry will be evaluated in NHVs and patients per the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)). In NHVs, spirometry serves as a safety assessment only. In patients, spirometry serves dual purposes as an assessment of both safety and efficacy. In patients, efficacy analyses will be performed on the first spirometry assessment of a study visit (via comparison with baseline spirometry results). Any subsequent spirometry measures obtained during the study visit (ie, after dosing with study drug or after sputum induction) serve primarily safety purposes.

Subjects should refrain from vigorous exercise for 1 hour prior to spirometry measurements.

Spirometry will be conducted in accordance with American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines ([Graham 2019](#)) using equipment provided by a central vendor. The central vendor is responsible for assuring that the spirometer meets ATS and ERS recommendations and that the study center personnel who will be performing the testing are properly certified. The required spirometer reference range to use is the Global Lung Function Initiative range. In the event that the central spirometer is not available or functional at a given study visit, another spirometer at the clinical site may be used.

While spirometry must always be conducted according to ATS/ERS guidelines, Screening spirometry results need not meet ATS/ERS criteria for acceptability or repeatability in order to be used for inclusion/exclusion purposes. It is at the discretion of the PI to judge whether spirometry results are of sufficient quality to inform inclusion/exclusion criteria.

Spirometry assessments will be performed either with or without bronchodilator administration during the assessment, as indicated in [Table 11](#). For spirometry assessments where bronchodilator administration is required, the prebronchodilator maneuvers will be completed, the bronchodilator will be administered, and then the postbronchodilator maneuvers will be completed. Bronchodilation will be performed with albuterol (90 µg metered dose) or salbutamol (100 µg metered dose), preferably with a spacer device, repeated for a maximum total of 4 inhaled doses. Less than 4 inhaled doses may be used if there is concern about a subject's heart rate, tremor, or safety; in this case, the reason and number of doses administered should be recorded. Postbronchodilator maneuvers will be performed 15 minutes after bronchodilator dosing (with a +10-minute window).

Table 11: Bronchodilator Challenge as Part of the Spirometry Assessment

Spirometry Assessment	With or Without Bronchodilation
NHVs	
All spirometry assessments	Without bronchodilation
Patients With Asthma	
First spirometry assessment of each study visit	With bronchodilation
All other spirometry assessments	Without bronchodilation

Spirometry Assessment	With or Without Bronchodilation
Patients With COPD	
First spirometry assessment of each study visit	With bronchodilation
All other spirometry assessments	Without bronchodilation

Abbreviations: COPD=chronic obstructive pulmonary disease; NHV=normal healthy volunteer.

When spirometry is performed without bronchodilation, maneuvers will be repeated to achieve 3 acceptable FEV₁ and FVC measurements. An upper limit of 8 maneuvers should be performed to attempt to produce 3 acceptable measurements. Spirometry data will be transmitted to a central vendor for quality review.

When spirometry is performed with bronchodilation, prebronchodilator maneuvers will be repeated to achieve 3 acceptable FEV₁ and FVC measurements. An upper limit of 8 prebronchodilator maneuvers should be performed to attempt to produce 3 acceptable measurements. Following bronchodilation, postbronchodilator maneuvers will be repeated to achieve 3 acceptable FEV₁ and FVC measurements. An upper limit of 8 postbronchodilator maneuvers should be performed to attempt to produce 3 acceptable measurements. Spirometry data will be transmitted to a central vendor for quality review.

The best prebronchodilator and postbronchodilator FEV₁ and FVC from a given spirometry assessment will be used for analysis purposes.

11.1.2. Medication Withholding Prior to Spirometry Assessments

Subjects, either NHVs or patients, who use the following concomitant medications should withhold the medication(s) prior to a study visit involving spirometry, as specified below:

- SABA: Withhold for at least 6 hours prior to first spirometry assessment of study visit
- Twice daily LABA- or LAMA-containing therapies: Withhold for at least 12 hours prior to first spirometry assessment of study visit
- Once daily LABA- or LAMA-containing therapies: Withhold for at least 24 hours prior to first spirometry assessment of study visit
- LTRA: Withhold for at least 24 hours prior to first spirometry assessment of study visit

If a subject has failed to withhold any of the above medications as specified, spirometry may be performed with a notation indicating that the subject failed to withhold medication appropriately, including note of the relevant medication and when it was last used relative to the first spirometry assessment of the visit.

Any concomitant medications that a subject has withheld should be taken as prescribed immediately after the first spirometry assessment of the visit is completed (ie, after prebronchodilator and postbronchodilator maneuvers are completed). Thus, withheld concomitant medications should be taken prior to initiation of the sputum induction protocol and prior to dosing with study drug on the relevant study days.

11.2. Mucus Symptoms Assessment

At the time points indicated in the SOA ([Table 5](#)), asthma patients will be asked the following 2 questions:

1. Over the last 2 weeks, how often have you coughed?
2. Over the last 2 weeks, how often have you brought up phlegm (sputum)?

For each question, the subjects will be asked to choose an answer from one of the following options:

- Almost every day
- Several days a week
- A few days a month
- Only with lung/respiratory infections
- Not at all

Based on responses to these questions, an asthma subject will be classified as experiencing chronic bronchitis for analysis purposes if they indicate that they have both cough and phlegm almost every day or several days a week.

11.3. COPD Assessment Test

The CAT is a validated, 8-item, patient-completed questionnaire developed to measure the health status of patients with COPD ([Jones 2009](#); [Jones 2012](#)). Subjects are asked to rate their experiences on a 6-point scale, ranging from 0-5, thus resulting in a total score ranging between 0 to 40. Higher scores reflect greater disease impact on health.

11.4. SGRQ-C

The SGRQ-C is a revised version of the SGRQ intended for patients with COPD. It is a slightly shorter version of the SGRQ that produces scores directly analogous to those from the SGRQ. It is a self-administered questionnaire that measures health status in 3 domains: symptoms, activity, and impact ([Meguro 2007](#)). Scores may be calculated for each domain, as well as a total score. A lower score indicates better quality of life.

12. ASSESSMENT OF SAFETY

12.1. Safety Parameters

The following safety assessments will be obtained at time points outlined in the SOA ([Table 3](#), [Table 4](#), [Table 5](#), and [Table 6](#)).

12.1.1. Demographic/Medical History

Subject demographics (eg, age, sex, ethnicity) and medical history will be collected during the Screening period. Medical history will include medication use over the previous 1 month prior to Screening, including vitamins, over-the-counter medications, prescription drugs, recreational drugs or supplements, and history of alcohol and tobacco use. Medical history will also include lifetime history of cigarette use.

Patients with asthma will be assessed for a diagnosis of asthma based on the inclusion criteria described in [Section 7.3](#).

For patients with asthma, the following medical history information will be collected: age at asthma onset, presence of atopy, number of total asthma exacerbations in the previous year, number of asthma exacerbations in the previous year that resulted in hospitalization, and whether FEV₁ reversibility has been documented in the past (ie, postbronchodilator FEV₁ reversibility of at least 12% and at least 200 mL).

For patients with COPD, the following medical history information will be collected: age at diagnosis of COPD, number of total COPD exacerbations in the previous year, number of moderate or severe COPD exacerbations in the previous year, and smoking history.

12.1.2. Vital Signs

Vital signs (body temperature, respiration rate, resting heart rate, semi-supine systolic and diastolic blood pressure measurements, and oxygen saturation) will be evaluated at the visits indicated in the SOA. All vital signs will be measured after the subject has been resting in a semi-supine position for at least 3 minutes. Blood pressure measurements are to be taken in the same arm from Day 1 through EOS. Unless otherwise noted, vital signs should be obtained before venipuncture and other invasive procedures during a study visit, whenever possible.

12.1.3. Physical Examination

A complete physical examination will be performed at Screening and Day 1 (head, eyes, ears, nose, and throat; heart; lungs; abdomen; skin; axillary lymph nodes; and neurological and musculoskeletal systems). At Screening, height (without shoes) and weight (without shoes) will be measured. Body mass index (BMI) will be measured at other time points as indicated in the SOA; Screening height can be used for post-Screening BMI measures. At all other time points, a symptom-directed physical examination will be performed if indicated.

12.1.4. Electrocardiogram

Twelve-lead ECG measurements will be obtained in triplicate at time points outlined in the SOA after the subject has been semi-supine for at least 3 minutes. ECG measures will occur in

3- to 5-minute intervals. All ECGs will be performed prior to venipuncture and any other invasive procedures.

12.1.5. Clinical Laboratory Assessments

Blood and urine samples will be collected and assessed by a central laboratory. Samples from local laboratories will be used if central laboratory testing is not available or will not meet the required turnaround time, or in the event of emergent situations, including time critical analytes.

At the Screening visit, a blood and urine sample will be collected for the laboratory tests detailed in the SOA to establish baseline data and eligibility for enrollment. Any laboratory assessment may be repeated once during Screening if indicated, with the repeat result used to establish eligibility. The results will be assessed by the PI or medically qualified designee before study enrollment. Any abnormality in laboratory values at Screening (that is confirmed on repeat), deemed clinically significant by the Investigator or medically qualified designee, that would jeopardize the safety of the subject or impact on the validity of the study results will result in exclusion of that subject. Following Screening, laboratory tests will also be performed on subjects' blood and urine at specified time points listed in the SOA. Unused portions of collected blood and urine samples may be retained and stored for future research if the subject consents (see [Section 6.1.2](#)).

Clinical laboratory tests, or “clinical labs” as specified in the SOA, consist of the following analyses: hematology, blood chemistry, coagulation, and urinalysis ([Section 12.1.5.1](#) through [Section 12.1.5.4](#)). In addition to “clinical labs”, the SOA includes additional laboratory assessments as detailed below. Refer to the Laboratory Manual for additional details on laboratory tests.

12.1.5.1. Hematology

The following will be assessed: complete blood count with differential, which includes hemoglobin (Hb), red blood cell count, hematocrit, mean cell volume, mean cell Hb, mean cell Hb concentration, platelets, white cell count, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

12.1.5.2. Blood Chemistry

The following will be assessed: sodium, potassium, chloride, bicarbonate, glucose, urea, creatinine (including eGFR), creatine kinase, uric acid, phosphate, total calcium, anion gap, albumin, protein, total bilirubin, amylase, lipase, conjugated bilirubin, gamma glutamyl transferase, alkaline phosphatase, ALT, AST, lactate dehydrogenase, and C-reactive protein.

12.1.5.3. Coagulation

The following will be assessed: partial thromboplastin time and prothrombin time with international normalized ratio.

12.1.5.4. Urinalysis

The following will be assessed: leukocytes, nitrites, urobilinogen, protein, pH, blood, specific gravity, ketone, bilirubin, and glucose. Additionally, microscopic urinalysis of white blood cells, red blood cells, epithelial cells, and bacteria will be performed as a reflex test if indicated.

12.1.5.5. Virus Serology

The following will be assessed: HBV surface antigen, HCV antibody (reflex HCV RNA for positive antibody test), and HIV antibody screen. If necessary, subjects will be counseled by the PI, or medically trained designee, concerning the blood tests for HBV surface antigen, HCV and HIV antibodies, and their subsequent results.

12.1.5.6. COVID-19 Testing

COVID-19 testing will be conducted by antigen testing using a nasopharyngeal swab or nasal swab.

12.1.5.7. Drug Screen

A urine sample will be screened for use of drugs such as benzodiazepines, amphetamines, barbiturates, methamphetamines, methadone, opiates, phencyclidine, cannabinoids, 3,4-methylenedioxymethamphetamine (MDMA), and cocaine. A urine drug screen deemed positive due to prescription medications or for benzodiazepines, opioids, or THC/marijuana may be acceptable and inclusion is at the discretion of the PI. Subjects who smoke or vape prescription THC/marijuana may be enrolled at the discretion of the PI.

12.1.5.8. Urine Cotinine

Urine will be tested for urine cotinine to evaluate for smoking of tobacco. A positive urine cotinine result due to nicotine replacement (eg, patch or gum) is acceptable for enrollment at the discretion of the PI.

12.1.5.9. Pregnancy Screen

Postmenopausal status will be confirmed by a follicle-stimulating hormone (FSH) level consistent with the postmenopausal state. Urine qualitative pregnancy tests will be obtained for women of childbearing potential.

12.1.5.10. Anti-Drug Antibodies

Serum samples will be collected and stored for potential future assessment for anti-drug antibodies.

12.1.6. Chest X-ray

Posteroanterior and lateral chest radiographs will be performed at time points outlined in the SOA.

12.1.7. Spirometry

Spirometry serves as a safety assessment in NHVs and patients as well as an efficacy assessment in patients. Spirometry will be performed at least once at each study visit indicated in the SOA. Additional spirometry measures during the study visit will also be performed following study drug dosing, as detailed in the SOA, and as part of the sputum induction procedure, as detailed in the Study Procedure Manual. Please see [Section 11.1](#) for further details on spirometry measurements.

12.1.8. Phone Call

A phone call to the subject will take place at time points listed in the SOA. The purpose of this phone call is to assess for any complications following bronchoscopy. The subject will be questioned about the presence of any AEs and any change in concomitant medications. If any complications of bronchoscopy are identified during the phone call, further appropriate evaluations will occur at the judgment of the PI.

12.1.9. BALF Cell Count and Differential

Change from baseline in BALF cell count and differential will be used to assess for evidence of study drug-induced pulmonary inflammation. BALF will be collected from all bronchoscopies performed in NHV MAD cohorts, as detailed in [Section 10.2.1.3.3](#). BALF samples will be processed to generate Cytospin slides, as detailed in the Central Laboratory Manual, and slides will be sent to a central reading facility for measurement of cell counts and differentials.

12.2. Adverse Events

The PI and clinical facility staff are responsible for detection, recording, and reporting of events that meet the criteria and definition of AEs as listed below. Adverse events will be recorded from the time of signed consent through the EOS visit; only AEs that occur postdose will be considered treatment emergent. Refer to [Section 12.8.2](#) for pregnancy reporting details.

12.2.1. Definition of Adverse Events

12.2.1.1. Adverse Event

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding or diagnostic test), symptom, or disease temporally associated with the use of a medicinal (investigational/experimental) product, whether related to this product or not (refer to International Council for Harmonisation [ICH] Guideline E2a: *Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, 27 October 1994).

TEAEs will be defined as AEs with onset after administration of the study drug, or when a preexisting medical condition increases in severity or frequency after study drug administration.

Adverse events will not include:

- A medical procedure such as surgery, endoscopy, tooth extraction, or transfusion (although the condition that leads to the procedure may be an AE)
- A preexisting disease or condition present at the start of the study that does not worsen during the study
- Any situation where an untoward medical occurrence has not occurred (for example, hospitalizations for cosmetic elective surgery or “social” admissions)
 - Please note that hospitalization for preplanned procedures or surgery (eg, knee replacement) should not be reported as an AE or SAE

- An overdose of either the IP or a concurrent medication without any resulting signs or symptoms

12.2.1.2. Serious Adverse Event

An SAE is an AE occurring during any study phase (ie, baseline, treatment, washout, or follow-up), and at any dose of the IP, comparator, or placebo that fulfills one or more of the following:

- Results in death
- Is immediately life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but might jeopardize the subject or might require medical or surgical intervention, to prevent one of the other serious outcomes listed in the above definition. These should also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, development of drug dependency, or drug abuse.

12.3. Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events

Abnormal assessments (eg, ECGs and vital signs) that are judged by the PI as clinically significant or result in clinical sequelae will be recorded as AEs. Laboratory abnormalities will be reported by the PI as AEs if the abnormality is considered clinically significant or results in clinical sequelae.

Clinically significant abnormal laboratory findings or other clinically significant abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs.

The PI (or medically qualified designee) will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding, or other abnormal assessment is clinically significant.

12.4. Timing, Frequency, and Method of Detecting Adverse Events

Any preexisting conditions or signs and/or symptoms present in a subject prior to the start of the study (ie, before informed consent) should be recorded as Medical/Surgical History.

All AEs occurring after informed consent and on or before the final visit must be reported as AEs; only AEs that occur postdose will be considered treatment emergent. All AEs must be

recorded irrespective of whether they are considered related to study drug. Adverse events will be collected through the EOS visit or through 28 days after the last dose, whichever is longer ([Section 12.8.5](#)). Monkey studies indicate that the plasma half-life of the API contained in ARO-MUC5AC is up to 16 hours (depending on dose); thus 28 days is greater than 5 elimination half-lives.

At each visit/assessment in the period defined above, AEs will be evaluated by the PI (or medically qualified designee) and recorded.

12.5. Recording Adverse Events

When an AE occurs, it is the responsibility of the PI or medically qualified designee to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) relative to the event. The PI or medically qualified designee will then record the AE on the AE eCRF. Additional reporting requirements for an AE that meets serious criteria are discussed in [Section 12.2.1.2](#).

The PI or medically qualified designee will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In all cases, when available, the diagnosis should be reported as the event and not the individual signs/symptoms. It is not acceptable for the PI to send photocopies of the subject's medical records to the Sponsor in lieu of completion of the appropriate AE eCRF pages.

12.6. Evaluating Adverse Events

12.6.1. Assessment of Severity

The PI or medically qualified designee will assess severity for each AE reported during the study. The assessment will be based on the PI's (or medically qualified designee's) clinical judgment. The severity of all AEs should be assigned to one of the following categories:

- **Mild:** An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities. Intervention not indicated.
- **Moderate:** An event that is sufficiently discomforting to interfere with normal everyday activities. Minimal, local, or noninvasive intervention indicated.
- **Severe:** An event that prevents normal everyday activities and requires emergent invasive intervention which may include hospitalization.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilized for rating the severity of an event; both AEs and SAEs can be assessed as severe. An event is defined as "serious" when it meets one of the predefined outcomes as described in [Section 12.2.1.2](#).

12.6.2. Assessment of Causality

The PI, or medically qualified designee, is obligated to assess the relationship between IP and the occurrence of each AE. The PI, or medically qualified designee, will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the IP will

be considered and investigated. The PI, or medically qualified designee, will also consult the IB in the determination of his/her assessment.

There may be situations when an SAE has occurred and the PI has minimal information to include in the initial SAE report. However, it is very important that the PI, or medically qualified designee, always assess causality for every event prior to transmission of the SAE report form. The PI, or medically qualified designee, may change his/her opinion of causality considering follow-up information, amending the SAE report form accordingly. The causality assessment is one of the criteria used when determining global regulatory reporting requirements.

The PI or medically qualified designee will provide the assessment of causality utilizing 2 possible categories: Related or Not Related.

An AE will be considered “Not Related” to the use of the product if any of the following conditions are met:

- An unreasonable temporal relationship between administration of the product and the onset of the AE (eg, the event occurred either before or too long after administration of the product for it to be considered product-related)
- A causal relationship between the product and the AE is biologically implausible (eg, death as a passenger in an automobile accident)
- A clear more likely alternative explanation for the AE is present (eg, typical adverse reaction to a concomitant drug and/or typical disease-related event)

12.7. Follow-up of Adverse Events

After the initial AE, the PI is required to proactively follow each subject and provide further information on the subject’s condition as deemed appropriate.

All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject is lost to follow-up. Once resolved, the appropriate AE eCRF page and SAE report form (if event is serious) will be updated. The PI or medically qualified designee will ensure that follow-up includes any supplemental investigations that may be indicated to elucidate the nature and/or causality of the AE or SAE. These may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. In the event of a fatal outcome in an SAE, the PI or medically qualified designee will attempt to obtain postmortem findings, including histopathology, and provide all additional information in a follow-up SAE report.

New or updated information regarding an SAE will be recorded on a new SAE report form marked as follow-up with the appropriate follow-up number added to the report. The follow-up report will be signed and dated by the PI.

12.8. Prompt Reporting of Serious Adverse Events

Any AE meeting serious criteria MUST be reported promptly to the Sponsor’s designated Pharmacovigilance Contract Research Organization (CRO), and the IRB and/or IEC in accordance with applicable local/institutional requirements.

12.8.1. Completion and Transmission of the Serious Adverse Event Reports

Once a PI becomes aware that an SAE has occurred in a study subject, she/he will report the information on an SAE report form to the designated Pharmacovigilance CRO within 24 hours. The SAE report form will always be completed as thoroughly as possible with all available details of the event and signed by the PI or medically qualified designee. If the PI does not have all information regarding an SAE, she/he will not wait to receive additional information before reporting the event. The SAE report form will be updated when additional information is received.

The PI, or medically qualified designee, will always provide an assessment of causality at the time of the initial report as described in [Section 12.6.2](#). However, as new information becomes available, causality may be modified.

Email transmissions of the SAE report forms are the preferred methods to transmit this information to the designated Pharmacovigilance CRO. Facsimile is acceptable if email is unavailable. In rare circumstances, notification by telephone is acceptable, with a copy of the SAE report sent by overnight mail. Initial notification via the telephone does not replace the need for the PI, or medically qualified designee, to complete and sign the SAE report form within the outlined time frames.

The Sponsor will provide a list of project contacts for SAE receipt, fax numbers, telephone numbers, and mailing addresses. Any event that in the opinion of the PI may be of immediate or potential concern for the subject's health or wellbeing will be reported to the Sponsor emergency contact listed in [Table 1](#).

12.8.2. Pregnancy Reporting

The PI and clinical facility staff are responsible for detection, recording, and reporting of pregnancy and appropriate follow up. Any known pregnancy that occurs during the study and until 90 days following the EOS visit, or 90 days from the last dose, should be reported by the subject to the PI. Monkey studies indicate that the plasma half-life of the API contained in ARO-MUC5AC is up to 16 hours (depending on dose); thus 90 days is greater than 5 elimination half-lives. In the event of a pregnancy, the Sponsor would request to collect follow-up information on the pregnancy over the course of the pregnancy, outcome of the pregnancy, and approximately 1 year after birth.

Pregnancy occurring in a subject or in the female partner of a male subject during the study must be reported on the pregnancy notification form or on an SAE form (if pregnancy details are not immediately available) and sent to the designated Pharmacovigilance CRO within 24 hours of initially becoming aware of the pregnancy by the PI. While pregnancy may be reported on an SAE form, a pregnancy is not an SAE.

Contingent on the consent of the subject or the subject's partner, as appropriate, pregnancy data will be collected at the end of pregnancy (ie, birth or termination of the pregnancy). Additionally, contingent on consent, should the pregnancy result in the birth of a child, data will be collected on the health of the child at 1 year following birth.

Should there be any SAEs that occur with the pregnancy, fetus, or during the first year of child's life (eg, serious maternal complications, therapeutic or spontaneous abortion, ectopic pregnancy, stillbirth, significant illnesses, congenital deformities, or other medical abnormalities etc.), these

events must be reported in accordance with the procedure for reporting SAEs. As such, the event will be followed until resolution or stabilization which may extend beyond the end of pregnancy or the first year of child's life.

12.8.3. Serious Adverse Event Reports to the IRB/IEC

The PI, or responsible person per local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the appropriate IRB and/or IEC.

12.8.4. Regulatory Requirements for Reporting of Serious Adverse Events

The PI, or medically qualified designee, will promptly report all SAEs in accordance with the procedures detailed in [Section 12.8.1](#). Prompt notification of SAEs by the PI is essential so that the Sponsor may comply with its regulatory obligations.

Any SAEs requiring expedited reporting will be reported by the Sponsor to relevant regulatory authorities, PIs, and IRBs and/or IECs in accordance with the Sponsor's procedures and local regulatory requirements.

12.8.5. Poststudy Adverse Events

A poststudy AE is defined as any event that occurs outside of the AE detection period defined in [Section 12.4](#). The PI is not obligated to actively seek AEs in former study subjects. However, if the PI learns of any SAE at any time after a subject has been discharged from the study, and she/he considers the event reasonably related to the IP, the PI will promptly notify the Sponsor.

12.8.6. Serious Adverse Events Related to Study Participation

An SAE considered related to study participation (eg, procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported promptly ([Section 12.8.1](#)).

12.9. Respiratory Adverse Events

Respiratory AEs are of particular interest for an inhaled therapy and include the following AEs:

- Asthma exacerbation
- COPD exacerbation
- Lower respiratory infection
- Upper respiratory infection
- Dyspnea
- Cough
- A clinically significant spirometry abnormality
- A clinically significant chest x-ray abnormality
- A clinically significant abnormality in oxygen saturation

12.9.1. Suggested Evaluation for Respiratory Adverse Events

The occurrence of a respiratory AE should trigger further evaluation of the study subject to assess the underlying etiology. This evaluation should be guided by the site Investigator's clinical judgment and is thus not strictly protocolized. However, the following work-up should be considered:

- History: Presence of infectious symptoms (eg, fever), recent sick contacts, recent allergic or environmental exposures, recent medication changes (especially respiratory medications)
- Physical exam: Pulmonary and cardiovascular exams and vital signs, including pulse oximetry
- Nasopharyngeal swab for respiratory virus panel, including COVID-19 and other seasonal respiratory viruses as appropriate
- Sputum bacterial culture, if productive cough is present and differential includes bacterial infection
- Spirometry
- Chest x-ray, if concern for alveolar infiltrate

12.9.2. Asthma Exacerbation

Of note, an AE of asthma exacerbation should be indicated for those events that meet the following definition of asthma exacerbation. Asthma exacerbation is defined as a worsening of asthma that leads to any of the following:

- A temporary burst of systemic corticosteroids (or a temporary increase in stable oral corticosteroid background dose) for at least 3 consecutive days to treat symptoms of asthma worsening; a single depo-injectable dose of corticosteroids will be considered equivalent to a 3-day burst of systemic corticosteroids.
- An emergency room or urgent care visit (defined as evaluation and treatment for <24 hours in an emergency department or urgent care center) due to asthma that required systemic corticosteroids (as per the above)
- An inpatient hospitalization (defined as admission to an inpatient facility and/or evaluation and treatment in a healthcare facility for ≥ 24 hours) due to asthma

12.9.3. COPD Exacerbation

Of note, an AE of COPD exacerbation should be indicated for those events that meet the following definition of COPD exacerbation. COPD exacerbation is defined as an acute worsening of respiratory symptoms that lead to any of the following:

- Use of systemic corticosteroids for at least 3 days; a single depot injectable dose of corticosteroids will be considered equivalent to a 3-day course of systemic corticosteroids
- New initiation of antibiotic therapy or change in antibiotic therapy if patient is on chronic antibiotics

- An inpatient hospitalization or a visit to an emergency room due to COPD

13. STATISTICS

13.1. Statistical Hypotheses

No hypothesis is considered for this FIH exploratory study.

13.2. Analysis Populations

For the purposes of analysis, the following analysis sets are defined:

- Safety Analysis Set: All subjects who receive at least 1 dose of IP. The Safety Analysis Set will be used for safety analyses and efficacy analyses. Subjects will be included in the analyses according to the treatment they received.
- PK Analysis Set: All subjects who receive at least 1 dose of IP and have sufficient plasma concentration data to facilitate determination of PK parameters. Subjects with major protocol violations will be assessed on a subject-by-subject basis for inclusion in the PK Analysis Set. The PK Analysis Set will be used for PK analyses.

13.3. Sample Size Considerations

No formal sample size calculations were performed to determine cohort sizes in this Phase 1/2a study aimed primarily at evaluating the safety and tolerability of ARO-MUC5AC. When the probability of observing a TEAE from a single subject who received active drug is 30%, the chance of observing at least 1 TEAE is higher than 75% among 4 dosed subjects (Cohorts 1a-4a) and 88% among 6 dosed subjects (Cohorts 1b-4b, 2c-4c, 2d-4d).

13.4. Analysis Methods

13.4.1. General Considerations

Descriptive statistics will be presented for all analyses unless otherwise specified. For continuous variables, data will be presented as number (n), mean, median, SD, minimum, and maximum. Discrete variables will be presented as frequencies and proportions or percent. Data will be analyzed by population (NHV subjects, patients with asthma, and patients with COPD), cohort, and treatment groups. Data summarized in tables and figures will also be presented in subject listings. For each population, subjects who receive placebo will be pooled and subjects who receive IP will be pooled.

A detailed Statistical Analysis Plan will be provided.

13.4.2. Safety Analyses

In general, safety analyses will be performed based on the Safety Analysis Set, which will include all enrolled subjects who receive at least 1 dose of study drug (placebo or active). Results will be summarized by population (NHV subjects, patients with asthma, and patients with COPD) and cohort. Placebo subjects from each cohort will be pooled by population. Subjects who receive active treatment will also be pooled by population.

TEAEs will be summarized by SOC and Preferred Term (PT), classified from verbatim terms, with Medical Dictionary for Regulatory Activities (MedDRA) Version 24.1 or later. The

incidence and percentage of participants with at least 1 occurrence of a PT will be included per the most severe grade using a 3-point scale (mild, moderate, severe). The number of events per PT will also be summarized. Causality (relationship to study treatment) will be summarized separately. TEAEs will be summarized by PT in descending order of frequency.

The incidence and frequency of TEAEs, SAEs, related AEs, related SAEs, and AEs leading to withdrawal, dose modification, or treatment discontinuation will be summarized by population and cohort by SOC and PTs. All AEs and SAEs will also be summarized in listings. The duration of AEs will be determined and included in listings along with the action taken and the outcome.

Laboratory parameters will be summarized at each scheduled time point using descriptive statistics. The incidence of laboratory abnormalities will be summarized. Results for variables that are not coded will be presented in the listings as “below, within, and above” the normal limits of the laboratory. Pregnancy test results will be listed.

Vital sign measurements will be summarized at each scheduled time point using descriptive statistics. Physical examination findings will be summarized by time point and presented in subject listings.

ECG parameters at each scheduled study visit, changes from baseline, and qualitative assessments will be summarized.

Pregnancy and FSH test results will be listed. BALF cell count and differential in NHVs will be summarized at each scheduled time point using descriptive statistics. Physical exam and chest x-ray data will be listed.

Descriptive statistics of FEV₁ and FVC by scheduled visit will be provided for NHV cohorts and the derived change from baseline in FEV₁ and FVC will be summarized.

13.4.3. Pharmacodynamics/Efficacy Analyses

Descriptive statistics will be performed by scheduled study visit and the derived and percent change from baseline will be provided for the following measures of pharmacodynamic activity and efficacy:

- MUC5AC and MUC5B protein concentrations from induced sputum samples, BALF samples, bronchosorption samples, and bronchoscopic airway swab samples
- MUC5AC and MUC5B mRNA expression from induced sputum samples and BALF samples
- Serum total IgE concentration in patients
- Plasma periostin in patients
- Prebronchodilator and postbronchodilator FEV₁ in patients (efficacy)
- Prebronchodilator and postbronchodilator FEF₂₅₋₇₅ in patients (efficacy)
- FeNO in patients
- Blood eosinophil or neutrophil count in patients
- CAT score in patients with COPD (efficacy)

- SGRQ-C score in patients with COPD (efficacy)

A listing of mucus symptom assessment results will be provided.

Efficacy data for patients will be analyzed by cohort. Placebo subjects and subjects receiving active drug will be pooled separately. Efficacy analyses will be completed based on the Safety Analysis Set.

13.4.4. Pharmacokinetics Analyses

Plasma and urine ARO-MUC5AC concentrations from NHVs will be analyzed using noncompartmental analysis. The following plasma PK parameters will be calculated as applicable:

- Maximum observed plasma concentration ($C_{\max}$)
- AUC from time 0 to 24 hours (AUC_{0-24})
- AUC from time 0 to the last quantifiable plasma concentration (AUC_{last})
- AUC from time 0 extrapolated to infinity (AUC_{inf})
- Elimination half-life ($t_{1/2}$)
- Apparent systemic clearance (CL/F)
- Apparent terminal-phase volume of distribution (V_z/F)

The following urine PK parameters will be calculated as applicable:

- Recovery of unchanged drug in urine over 0 to 24 hours (amount excreted; A_e)
- Percentage of the administered drug recovered in urine over 0 to 24 hours postdose
- Renal clearance (CL_R)

Additional PK parameters may be estimated as appropriate. Urine and plasma ARO-MUC5AC concentrations will be listed, summarized, and plotted as appropriate. Urine and plasma ARO-MUC5AC PK parameters will be listed and summarized descriptively. The plasma PK parameters ($C_{\max}$ and AUC) will be analyzed for dose proportionality and sex differences, if supported by data. Urine metabolite concentrations will be analyzed as appropriate.

The sparse PK concentration data from subjects will be listed as appropriate.

A population PK analysis of ARO-MUC5AC plasma concentration data will be performed using nonlinear mixed effect methods and appropriate software. If there is sufficient diversity in demographics and other baseline characteristics in the study population, an attempt will be made to evaluate these baseline characteristics as potential covariates on ARO-MUC5AC population PK. The PK data collected in this study may be combined with those from other clinical studies to develop an integrated population PK model.

13.4.5. Immunogenicity Analyses

The number and percentage of subjects who are positive for anti-drug antibodies at baseline and at any postbaseline visit may be summarized. A listing of subjects with positive anti-drug antibody assay results may be provided.

13.5. Interim Analysis

For all NHV cohorts, interim analyses may occur after completion of each NHV cohort (ie, after all subjects within a cohort complete the study or discontinue early). For patient cohorts, interim analyses may occur when all subjects within each cohort complete their Day 85 visit (or discontinue early). Additional exploratory analyses may be done prior to completion of each cohort. Unblinded tables, listings, and figures from interim analyses will be kept in a secure location. Only designated unblinded personnel, documented in the Data Access Plan, will have access to individual treatment assignment before database lock.

14. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

14.1. Study Monitoring

Arrowhead is responsible for assuring the proper conduct of the study about protocol adherence and validity of the data recorded on the eCRFs. Subject confidentiality will be maintained.

In accordance with applicable regulations, GCP, and Arrowhead procedures, Arrowhead will be responsible for assigning a Study Monitor (CRA) who will contact the site to organize a visit prior to subject enrolment to review the protocol and data collection procedures with site staff. In addition, the assigned Study Monitor will periodically contact the site and conduct onsite visits. The extent, nature, and frequency of onsite visits will be based on such considerations as the study objective or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these site visits, the Study Monitor will:

- Check the progress of the study
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution
- Check IP accountability
- Review blood and urine samples and ensure they are labeled and stored correctly

This will be done to verify that the:

- Data are authentic, accurate, and complete
- Safety and rights of subjects are being protected
- Study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements

The PI agrees to allow the Monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the Monitor to discuss findings and any relevant issues.

At study closure, a Study Monitor will conduct the following activities in conjunction with the PI or site staff as appropriate:

- Return of all study data to Arrowhead
- Data queries
- Accountability, reconciliation, and arrangements for unused IP(s)
- Inventory and final disposition (eg, destruction, shipping to repository)
- Review of site study records for completeness

14.2. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. The PI will conduct the study in compliance with the approved protocol and will not implement any deviation from or changes to the protocol without prior agreement by the Sponsor and review and documented approval from the IRB and/or IEC of an amendment, except where necessary to eliminate an immediate hazard to study subjects.

Deviations may result from the action or inaction of the subject, PI, or site staff. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications, food, drink, herbal remedies, or supplements that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements (for vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc.), such that tests are not done, incorrect tests are done, or tests are not done within the time frame specified in the protocol
- Procedural deviations such as incorrect storage of study drug, failure to update the ICF when new risks become known, failure to obtain IRB and/or IEC approvals for the protocol, and ICF revisions

Protocol deviations impacting subject safety or eligibility will be reported to the Sponsor or CRO within 2 business days of occurrence and to the IRB and/or IEC-competent regulatory authority per local regulatory requirements.

The PI is responsible for ensuring that any known protocol deviations are recorded and reported as agreed. The nature and reasons for protocol deviations will be recorded.

14.3. Clinical Laboratory Certification and Reference Ranges

Before the initiation of this study, the PI or designee will obtain a copy of the certification form, with certification number and expiration date for all clinical laboratories (excluding central laboratories) used in the study. Reference ranges for each clinical laboratory test used in this study will be obtained from the appropriate central laboratory, which will perform the test for the study. In the event that central labs are not readily available or in the event of emergent situations, local labs with associated local lab reference ranges may be utilized with prior Sponsor approval.

14.4. Audits and Inspections

To ensure compliance with GCP and all applicable regulatory requirements, Arrowhead may conduct a quality assurance audit of the study site. Regulatory agencies may also conduct a

regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the PI and clinical site agree to notify Sponsor as soon as possible following awareness of an impending regulatory inspection. The PI and clinical site agree to allow the auditor/inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

14.5. Institutional Review Board / Independent Ethics Committee

Prior to initiation of the study, written IRB and/or IEC approval of the protocol and ICFs, based on the principles of ICH GCP procedures, will be received. A copy of the signed and dated letter of approval will be provided to the clinical site and Arrowhead prior to study commencement. Any written information or advertisements to be used for subject recruitment will be approved by the IRB and/or IEC prior to use. A list of the IRB and/or IEC voting members, their titles or occupations, Federal Wide Assurance number (where applicable) and their institutional affiliations will be requested before study initiation.

Protocol modifications that may impact subject safety or the validity of the study will be approved by the IRB and/or IEC, following written agreement from the Sponsor.

15. ETHICS

15.1. Ethics Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB and/or IEC as appropriate. The PI must submit such written approval to the Sponsor before he or she can enroll any subject into the study.

The PI is responsible for informing the IRB and/or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB and/or IEC must approve all advertising used to recruit subjects for the study. The protocol must be reapproved by the IRB and/or IEC upon receipt of amendments and annually, as local regulations require.

The PI is also responsible for providing the IRB and/or IEC with reports of any reportable serious adverse drug reactions from any other study conducted with the IP. The Sponsor will provide this information to the PI.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB and/or IEC according to local regulations and guidelines.

15.2. Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirements. The protocol will be submitted for approval to the IRB and/or IEC, and written approval obtained before subjects are enrolled. The composition of the IRB and/or IEC will also be provided to the Sponsor. If approval is suspended or terminated by the IRB and/or IEC, the PI will notify the Sponsor immediately.

Where applicable, the clinical site and Arrowhead agree to abide by the local compensation guidelines for injury resulting from participating in a company-sponsored research project. Compensation will only be provided on the understanding that the provision of compensation does not amount to an admission of legal liability.

15.3. Written Informed Consent

Informed consent will be obtained before the subject can participate in the study. The contents and process of obtaining informed consent will be in accordance with all applicable regulatory requirements. Study participation includes all Screening procedures as well as any washout of excluded medications.

It is the responsibility of the PI or medically qualified designee to obtain written informed consent from everyone participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study. The PI or medically qualified designee must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for documenting a written consent will be provided to the PI by Arrowhead or its designee.

For this study, each eligible subject will be required to provide written informed consent before participation in the study.

All eligible subjects will have the study explained by the PI or medically qualified designee. They will receive a full explanation, in lay terms, of the aims of the study, the discomforts, risks and benefits in taking part as well as of insurance and other procedures for compensation in case of injury. It will be explained that the study is for research purposes only and is not expected to provide any therapeutic benefit to the individual. It will be pointed out that they can withdraw from the study at any time without prejudice. Each subject will acknowledge receipt of this information by giving written informed consent for participation in the study. The subject will be given a copy of the signed ICF to retain.

16. DATA HANDLING AND RECORDKEEPING

16.1. Inspection of Records

The Sponsor or its designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The PI agrees to allow the Monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relevant to the conduct of the study.

16.2. Retention of Records

Following closure of the study, the PI must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval when needed (eg, audit or inspection) and to allow for any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. When permitted by local laws/regulations or institutional policy, some of these records can be maintained in a format other than hard copy (eg, microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The PI must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including regenerating a hard copy, if required. Furthermore, the PI must ensure there is an acceptable backup of these reproductions and that an acceptable quality control process exists for making these reproductions.

Arrowhead will inform the PI of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or Arrowhead standards/procedures; otherwise, the retention period will default to 15 years, whichever is the longer time period.

The material to be stored shall include, but is not limited to, the following:

- Signed and dated copy of the final study protocol and any amendments
- Signed and dated letter of IRB and/or IEC approval, letter of constitution of the IRB and/or IEC, and copies of any other correspondence relevant to the study with the IRB and/or IEC or regulatory authorities
- The IRB- and/or IEC-approved ICF
- Current curriculum vitae (signed and dated) of the PI and coworkers with major responsibilities in the trial
- Site Signature and Delegation of Responsibility Log
- Food and Drug Administration Form 1572 (where applicable)
- Financial Disclosure Form(s)
- Signed subject ICFs
- Laboratory reference ranges (signed and dated)
- The completed Clinical Trial Notification Application Form (where applicable)

- Clinical raw data including the source data forms, all clinical laboratory report forms, subject case report forms, drug accountability forms, and dispensing records, etc.

16.3. Data Protection

Subjects will be assigned a unique identifier by the site. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only, not the subject name.

The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject who will be required to give consent for their data to be used as described in the informed consent.

The subject must be informed that his/her medical records may be examined by the Sponsor, Arrowhead Pharmaceuticals, Inc., and its designated representatives, by the IRB/IEC responsible for reviewing the study, by regulatory authorities such as the FDA, the European Medicines Agency, and other health agencies that regulate this research, and by the study doctor, study staff, and other authorized healthcare providers at the study site.

16.4. Source Documents

Source documents must be maintained for each subject in the study, consisting of all demographic and medical information, including clinical laboratory data, and entered into the electronic data capture system within 5 days of the subject visit. A copy of the signed ICF must be retained. All information in the eCRFs must be traceable to these source documents in the subject's file.

Data recorded in all subjects' eCRFs will be subject to a quality control review.

17. PUBLICATION POLICY

[REDACTED]

[REDACTED]

[REDACTED]

17.1. Ownership

[REDACTED]

17.2. Confidentiality

[REDACTED]

18. LIST OF REFERENCES

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APPENDIX 1. INHALED CORTICOSTEROID DOSE DEFINITIONS

Table 12: Definitions of Low, Medium, and High Doses of Inhaled Corticosteroids Based on Global Initiative for Asthma (GINA) 2021 Guidelines

ICS	Total Daily ICS Dose (mcg)		
	Low	Medium	High
Beclometasone dipropionate (pMDI, standard particle, HFA)	200-500	>500-1000	>1000
Beclometasone dipropionate (DPI or pMDI, extrafine particle, HFA)	100-200	>200-400	>400
Budesonide (DPI, or pMDI, standard particle, HFA)	200-400	>400-800	>800
Ciclesonide, (pMDI, extrafine particle, HFA)	80-160	>160-320	>320
Fluticasone furoate (DPI)	100		200
Fluticasone propionate (DPI)	100-250	>250-500	>500
Fluticasone propionate (pMDI, standard particle, HFA)	100-250	>250-500	>500
Mometasone furoate (DPI)	200		400
Mometasone furoate (pMDI, standard particle, HFA)	200-400		>400

Abbreviations: DPI=dry powder inhaler; HFA=hydrofluoroalkane; ICS=inhaled corticosteroid; pMDI=pressurized metered-dose inhaler.

Source: [GINA 2021](#)

APPENDIX 2. CONTRACEPTION

Women not of childbearing potential must be either permanently sterile following surgery, congenitally sterile, or postmenopausal (defined as cessation of regular menstrual periods for at least 12 months without an alternative medical cause) with supportive follicle-stimulating hormone (FSH) test consistent with postmenopausal state based on laboratory reference ranges.

Female subjects who are permanently sterile following surgery are subjects who have undergone hysterectomy, bilateral oophorectomy, or bilateral salpingectomy.

Male subjects considered permanently sterile following surgery, and thus not able to inseminate, are subjects who have undergone bilateral orchidectomy.

Subjects who have the ability to conceive who are partnered with individuals who have the ability to inseminate, and subjects who have the ability to inseminate who are partnered with individuals who have the ability to conceive must consent to use a method of highly effective contraception in addition to a condom during the study and for at least 90 days following the end of study (EOS) or last dose of study treatment, whichever is later.

Each of the following constitute an acceptable method of highly effective contraception:

- Birth control pills (the pill [combined estrogen and progestogen containing] or the mini-pill [progestogen only])
- Injectable contraceptives
- Birth control patch (estrogen and progestogen containing)
- Hormonal implants associated with inhibition of ovulation
- Contraceptive devices: Intrauterine device (IUD) / Intravaginal delivery systems (estrogen and progesterone containing)
- Hormonal intrauterine systems (IUSs)
- Bilateral tubal occlusion
- Vasectomy in a male partner

Subjects (or partners) who have the ability to conceive and who are on hormonal contraceptives must be stable on the medication for ≥ 1 menstrual cycle prior to Day 1. Otherwise, the method of highly effective contraception must be initiated at least 28 days prior to first dose of study medication. Use of a condom must start at the time of signing of Informed Consent. The subject should not change their method of highly effective contraception during the course of their participation in the study.

True sexual abstinence for the duration of the study and for at least 90 days following the EOS visit or after the last dose of IP, whichever is later, is an acceptable method of contraception only when in line with the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea methods are not considered “true” abstinence and are not acceptable methods of contraception.

As detailed in the Investigator's Brochure (IB), in vitro studies of ARO-MUC5AC showed no inhibition of tested drug metabolizing enzymes, efflux transporters or uptake transporters. Thus, clinical drug-drug interactions with hormonal contraceptives due to inhibition of commonly encountered drug transporters are considered unlikely. Further, ARO-MUC5AC showed no induction of tested human CYP enzymes. Therefore, ARO-MUC5AC is expected to have low potential to cause or be subjected to clinical drug-drug interactions with hormonal contraceptives that are mediated by the common drug metabolizing enzymes and transporters. These results indicate minimal risk of ARO-MUC5AC involvement in drug-drug interactions for contraceptives or other concomitant medications.

If a subject's urine pregnancy test is positive and pregnancy is confirmed by serum human chorionic gonadotropin (HCG), the subject will be referred to their primary care provider for follow-up. Female subjects with a positive pregnancy test at Screening or on Day 1 predose will not be enrolled in the study. Female subjects who become pregnant after Day 1 and before subsequent planned doses of investigational product (IP) will not receive subsequent administration of IP but may otherwise continue in the study, with potential modification of subsequent study assessments as deemed clinically appropriate.

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AROMUC5AC-1001 Clinical Study Protocol Amendment 4.0

Approval	John Huetsch Medical 21-Feb-2024 22:07:15 GMT+0000
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