PROTOCOL NUMBER:	AROENAC1001
STUDY TITLE:	A Phase 1/2a Dose-Escalating Study to Evaluate the Safety, Tolerability and Pharmacokinetic Effects of ARO-ENaC in Normal Healthy Volunteers and Safety, Tolerability and Efficacy in Patients with Cystic Fibrosis
STUDY TREATMENT (Active):	ARO-ENaC
ROUTE:	Nebulized Solution (Inhalation)
SPONSOR'S RESPONSIBLE MEDICAL MONITOR:	[redacted] Clinical Development
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IND NUMBER:	IND152991
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1. PROTOCOL SYNOPSIS

Study Title: A Phase 1/2a Dose-Escalating Study to Evaluate the Safety, Tolerability and Pharmacokinetic Effects of ARO-ENaC in Normal Healthy Volunteers and Safety, Tolerability and Efficacy in Patients with Cystic Fibrosis

Study Number: AROENAC1001

Phase: Phase 1 First-in-Human, Phase 2a in CF patients

Number of Sites: Normal Healthy Volunteers (NHVs): Single site in New Zealand

Cystic Fibrosis (CF) patients: Multiple sites in New Zealand, Australia, and the United States

Study Treatments:

There will be two study treatments; one active (Test Formulation) and one placebo (Reference Formulation) both administered by inhalation of nebulized solution.

Test Formulation:

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

Reference Formulation:

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

Study Objective: The objective of the study is to assess the safety, tolerability, pharmacokinetics of ARO-ENaC in normal healthy volunteers (NHVs) and patients with Cystic Fibrosis (CF).

Primary Endpoint:

- The incidence and frequency of adverse events over time through end of study using escalating multiple doses in NHVs and in CF patients.

Secondary Endpoints:

- Changes from pre-dose baseline in serum electrolytes in NHVs and CF patients as a safety measure.
- Changes from pre-dose baseline in Forced Expiratory Volume (FEV1) in NHVs as a measure of safety.
- Pharmacokinetics of ARO-ENaC in NHVs and in CF patients

Exploratory Endpoints:

- Changes from pre-dose baseline in FEV1 in CF patients
- Changes from pre-dose baseline in lung clearance index (LCI) in CF patients
- Changes from pre-dose baseline in the revised cystic fibrosis questionnaire (CFQ-R) in CF patients
- Rate of pulmonary exacerbations in CF patients receiving ARO-ENaC versus PBO
- Changes from pre-dose baseline in body mass index (BMI) in CF patients
- Plasma metabolite identification in NHVs (reported in a separate report outside of this study)
- Determination of urinary excretion and metabolite identification in NHVs (reported in a separate report outside of this study).
- Changes from pre-dose baseline to Day 18 in expression of αENaC using bronchoscopic brush biopsy samples in NHVs
- Changes from pre-dose baseline to Day 18 in expression of αENaC using bronchoalveolar lavage (BAL) samples in NHVs
- Changes from pre-dose baseline to Day 18 in cytokine levels as well as cell count and differential in BAL fluid in NHVs

Study Population/Patient Number:

Summary of Participant Profile by Cohort:

- Cohorts 1-4: 6 (randomized 4 active: 2 PBO) NHVs per cohort, adult healthy volunteers
- Cohort 5: 12 adult NHVs (randomized 8 active: 4 PBO); only this cohort will undergo bronchoscopies
- Cohorts 2b, 3b: up to 6 (randomized 4 active:2 PBO) CF adult patients per cohort
- Cohort 4b: up to 12 (randomized 9 active: 3 PBO) CF adult patients with baseline ppFEV1 of > 70%.
- Cohort 6: up to 24 (randomized 18 active: 6 PBO) CF adult patients

NHV subjects will enroll sequentially into a total of 5 cohorts (with 6 subjects in each of cohorts 1-4 and 12 subjects in cohort 5), randomized to receive ARO-ENaC or PBO (4 active: 2 PBO in cohorts 1-4 and 8 active: 4 PBO in cohort 5) in a double blinded fashion. Dose escalation will occur by Data Safety Committee (DSC) vote after review of cumulative safety once all healthy volunteers in the current cohort have completed their Day 21 study visit. There will be no DSC review during cohort 5. Cohort 5 is added to the study for the purpose of obtaining bronchoscopic samples in NHVs.

CF patient cohorts 2b and 3b will each enroll up to 6 patients to receive escalating multiple doses of ARO-ENaC or PBO. CF patient Cohort 4b will enroll up to 12 patients to receive multiple doses of ARO-ENaC or PBO. CF cohort 6 will enroll up to 24 patients to receive multiple doses of ARO-ENaC or PBO. Cohort 6 is included in the study to assess the safety and efficacy of dosing ARO-ENaC on days 1, 15, and 29 (as opposed to all other CF cohorts which dose on days 1/2/3 and 22/23/24).
CF patients not indicated or not treated with CFTR modulators/correctors at baseline or CF patients on a stable regimen (last 2 months) of CFTR modulators/correctors are eligible. CF patients are required to continue their established treatment regimen for the duration of the study without any changes. Sub-group analysis of CF patients will be conducted according to CFTR modulator/corrector use at baseline.

**Any CF patient already in Screening when a CF patient cohort has reached capacity (defined as six patients who have completed Day 1 in Cohorts 2b and 3b, twelve participants who have completed Day 1 in Cohort 4b, and twenty-four patients who have completed Day 1 in Cohort 6), may participate in the study up to an additional two patients per cohort.*

NHV subjects who withdraw from the study prior to collection of the final pharmacokinetic blood sample, for reasons other than an adverse event or otherwise due to safety, may be replaced. CF patients who are withdrawn or discontinue prior to EOS visit for reasons other than an adverse event, may be replaced.

A maximum total of approximately 36 NHVs and up to 56 CF participants (including up to 2 extra patients per cohort already in Screening and not including potential replacements) may be enrolled in the study.

Number of Doses: Healthy Volunteer Cohorts (Cohorts 1 through 5) will receive a single cycle of three doses on Days 1, 2 and 3.
CF cohorts 2b, 3b, 4b will receive two cycles of three doses on Days 1, 2, 3 and Days 22, 23, 24.
CF cohort 6 will receive three total doses, with one dose given on Days 1, 15, and 29.

Study Duration: For each NHV subject in cohorts 1-5, the approximate duration of the study clinic visits is a maximum of 8 weeks from the beginning of the Screening period to the Day 29 End-of-Study (EOS) examination.

For each CF patient, the duration of the study is approximately 20 weeks from the beginning of the Screening period to the Day 113 EOS examination.

Study Confinement & Study Visits: For NHVs, clinical facility confinement will be approximately 4 days for single dose cycle administration (Day -1 through Day 4 assessments for cohorts 1-4 and Day 0 to Day 4 for cohort 5). Subjects will be admitted to the facility on Day -1 (or Day 0 in cohort 5) and will undergo assessments at the facility through Day 4. After Day 4 assessments NHV participants can be discharged and will return to the clinical facility starting on Day 5 for outpatient visits.
For CF patients, clinical facility confinement will be approximately 6 hours on dosing days unless additional monitoring at Principal Investigator (PI) discretion is needed for safety reasons. Patients will return to the clinical facility for outpatient visits. There is no planned overnight confinement for CF patients.

Study Design/Methods:

Normal Healthy Volunteers:

While ARO-ENaC is intended for use in CF patients, these patients may have baseline decreased lung function and may suffer from frequent disease exacerbations and are frequently on multiple concomitant medications. For this reason, this study initially evaluates each dose level in a healthy volunteer population to generate a baseline understanding of safety in a population with normal physiology and without the confounding factor of concomitant medication use.

For NHVs, 4 cohorts of 6 eligible subjects (4 active: 2 placebo) will be evaluated at each dose level starting with Cohort 1. Cohort 5 will include 12 eligible patients (8 active: 4 placebo) who will be treated with a dose equal to or less than that given to Cohort 4, with the purpose of collecting bronchoscopic samples to evaluate for αENaC mRNA knockdown. Participants who have signed an IRB/EC approved informed consent form and have met all the protocol eligibility criteria during screening, will be randomized to receive ARO-ENaC or PBO. NHV cohorts will receive a single cycle of three doses all at a fixed dose level administered daily on Days 1, 2, 3.

NHV cohorts 1-4 will begin with administration of ARO-ENaC or PBO to two sentinel participants (one ARO-ENaC, one PBO). Following the Day 4 evaluation in these participants, if there are no significant safety concerns based on PI's judgement, the remaining participants in the cohort will be dosed. Cohort 5 will not utilize sentinel participants as the dose evaluated in cohort 5 will be equal to or lower than doses used in cohorts 1-4. Dosing of participants will be staggered by at least 30 minutes such that no two participants will be dosed simultaneously. Blood samples will be drawn pre-dose on Day 1 for baseline measurements.

Based on observations for all NHV subjects in each of cohorts 1-4 through Day 21, and experience from any other prior cohorts (e.g. all cumulative available safety data), the DSC will meet to vote on the following (See Fig. 1):

1. Dose escalation to next NHV cohort
2. Initiation of CF cohort (Cohorts 2b, 3b, 4b) when applicable per Figure 1.

Escalation to the next highest dose level in NHVs will proceed in cohorts of 6 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.

Following an affirmative vote from the DSC meeting held during NHV cohort 4 allowing the opening of CF Cohort 4b, NHV cohort 5 may also be subsequently opened for accrual. The primary purpose of cohort 5 will be to assess the effect of ARO-ENaC on αENaC mRNA expression, with samples obtained via bronchoscopy. The dose in cohort 5 will be equivalent to or less than the dose utilized in cohort 4. Based on available safety data reviewed during prior DSC meetings, the Sponsor or DSC may adjust the dose in cohort 5 downward.

The DSC will consist of at least one study Investigator, Sponsor Medical Monitor, Sponsor pharmacovigilance lead, and an independent physician experienced in pulmonary drug development and/or early stage clinical trials.

Cystic Fibrosis Patients:

Cohorts 2b, 3b, 4b will enroll eligible CF patients who have signed an IRB/EC approved informed consent form to receive two cycles of ARO-ENaC or PBO administered daily on Days 1, 2 and 3, then again on Days 22, 23 and 24. Screening for the CF cohorts can begin once Cohort 2 dosing has commenced but dose administration may not proceed until after the DSC has opened the designated CF patient cohort. CF patient cohorts 2b, 3b, and 4b will be opened for accrual once the corresponding NHV cohort receiving the same dose level has reached Day 21 and based on DSC vote that it is safe to proceed. CF patient cohorts 2b, 3b, and 4b will enroll in sequence with 2b enrolling first, followed by 3b and then Cohort 4b.

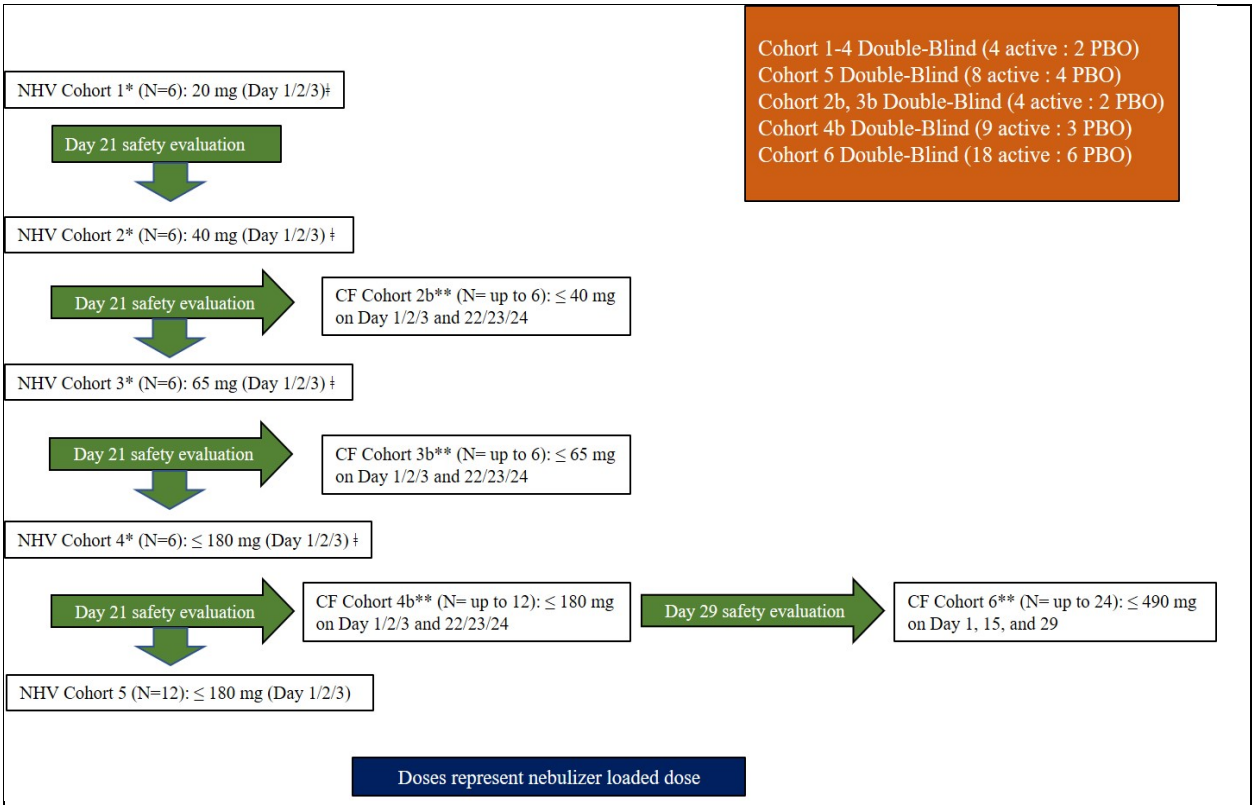
Once all subjects in CF cohort 4b have completed the Day 29 study visit, the DSC will meet to review all cumulative safety data to date and to vote on the initiation of CF cohort 6. Following an affirmative vote from this DSC meeting, CF cohort 6 may be opened for accrual. The purpose of cohort 6 is to assess the safety and efficacy of an alternate dosing regimen. In CF cohort 6, the 3-day dosing cycle used in cohort 4b is compressed to a single dose, which is given at 2 week intervals, such that subjects will be dosed on Days 1, 15, and 29. The dose given on each dosing day to a subject in CF cohort 6 (in respirable delivered dose [RDD] terms, which represents the amount of drug that reaches the lung, as opposed to the amount of drug loaded into the nebulizer) will be ≤ 3x the dose given on each dosing day to a subject in CF cohort 4b. Thus, the dose (in RDD terms) given on Day 1 to a subject in cohort 6 will be ≤ the total cumulative dosage given over Days 1, 2, and 3 to a subject in cohort 4b. Of note, the RDD does not scale identically to the nebulizer loaded dose (i.e. tripling the RDD does not necessarily result in tripling the nebulizer loaded dose).

The dose escalation schedule is outlined in **Figure 1.**

Blinding (where applicable) will be preserved to the extent possible. However, treatment un-blinding or un-blinding 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. After all subjects in a cohort (except cohort 6) have completed an EOS visit, Sponsor may be unblinded to that cohort. PI and study participants will remain blinded.

Primary analysis is planned once both all subjects in cohort 6 have completed the Day 43 study visit and all subjects from other cohorts have completed EOS. PIs and study participants will remain blinded through EOS. Sponsor will be unblinded to analyze both safety and efficacy endpoints for all NHV and CF cohorts. Final Analysis is planned when all subjects have ended study and database is locked. Safety and efficacy results will be updated based on complete database in final analysis.

Figure 1: Dose Escalation Schedule



* Cohorts 1-4 each use 2 sentinel subjects dosed before the rest of the cohort. Where specified, dose represents the highest dose that may be used in the cohort. The DSC or Sponsor may adjust the dose downward if indicated based on available safety data.

ǂ specified dose given once on Day 1, Day 2 and Day 3

** Cohorts 2b, 3b, 4b, and 6 enroll in sequence per Figure 1 and after associated DSC approval.

Adverse event monitoring:

Safety assessments will include AE/SAEs, chest x-rays, FEV1, physical examinations, vital sign measurements (blood pressure, heart rate, temperature, pulse oximetry and respiratory rate), ECGs, clinical laboratory tests including serum and urine electrolytes (spot urine electrolytes only analyzed in setting of hyperkalemia), concomitant medications/therapy, and reasons for treatment discontinuation. Safety assessments will be performed at specified time points and prior to study completion.

The AE/SAE reporting period for an enrolled participant begins when the participant provides informed consent. Treatment-emergent AEs (TEAEs) and treatment-emergent SAEs are defined as those that occur following study drug administration or are a pre-existing condition exacerbated by study drug. The TEAE reporting period begins after the first dose and extends until the End-of-Study 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 AE to study treatment. All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the participant is lost to follow-up. If the PI learns of any SAE, including a death, at any time after a patient 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 (e.g. 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.

*Any AE consistent with lower respiratory infection, dyspnea, bronchospasm, or CF exacerbation will trigger a chest X- ray and PFT measurement.

Treatment Stopping Rules & Data Safety Committee:
A decision to stop the trial early or discontinue drug in an individual subject or group of subjects **may** be indicated based on any of the following:

1. A single Serious Adverse Events (SAE, defined in Section 11.1) considered at least possibly related to study drug.
2. Two cases of a drop from baseline FEV1 (which must be confirmed on repeat within 48 hours) **in healthy volunteer cohorts** of ≥ 20% (absolute decline in % of predicted) considered to be at least possibly related to study drug by study investigator
3. One case of a drop from baseline FEV1 (which must be confirmed on repeat within 48 hours) **in cystic fibrosis cohorts** of ≥ 20% (absolute decline in % of predicted) considered to be probably related to study drug by study investigator
4. A post-dose increase in serum potassium to > 5.7 milliequivalents/Liter which must be from a non-hemolyzed sample and confirmed by repeat blood draw within 48 hours of initial results.

If any such events occur, within 5 days of Sponsor being notified of the event, the DSC will meet to review the event and determine any necessary actions as detailed below.

Central labs will be utilized. However local labs may be utilized as necessary if needed by the investigator (including for Screening) or for emergent situations, including time critical analytes such as serum potassium and repeat clinical chemistries.

Sponsor or PI 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. Including, but not limited to the events listed above, the DSC **may** pause the study to 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
- The study is stopped immediately with no further dosing
- The study will continue until the current cohort is completed
- The study will continue, but the next dose escalation will be to a level midway between the current level and the next planned level
- The study will continue as planned

The DSC will consist of at least the Sponsor Medical Monitor, Sponsor pharmacovigilance lead, one study Investigator and an independent physician experienced in Phase 1 clinical trials.

Study Assessments:

Safety Assessments:

Safety assessments will be performed at specified time points per the SOA and will include the following:

- Vital signs: Resting heart rate, semi-supine systolic/diastolic blood pressure, respiratory rate, pulse oximetry and temperature
- Clinical laboratory measurements (e.g., biochemistry, hematology, coagulation, urinalysis), urine electrolytes including potassium, sodium and urine FeNa (spot urine electrolytes will only be analyzed in the setting of hyperkalemia)
- Resting ECG measurements (measured after participant is semi-supine for at least 3 minutes).
- Chest X-ray (upright and lateral)
  At each visit, 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. Any known untoward event that occurs beyond the AE reporting period that the PI considers an SAE possibly related to study treatment will be reported to Arrowhead.
- Spirometry conducted as per ATS/ERS guidelines

Assessment of Pharmacodynamic Effect in NHVs:

- αENaC expression from bronchial brushing and BAL samples (only assessed in NHV cohort 5)

Assessments of Efficacy in CF patients:

Results including absolute change, percent change from baseline to EOS will be analyzed and summarized by dose cohort and treatment group for efficacy parameters.

Efficacy assessments will be performed at specified time points per the SOA and will include the following:

- Spirometry
- LCI
- CFQ-R
- Rate of pulmonary exacerbations
- BMI

Immunogenicity:

Blood samples for the anti-drug antibodies test will be collected per the SOA.

Genotype:

Genotype will be obtained for CFTR and ENaC (where available) mutations.

Pharmacokinetics:

Blood samples will be collected from each subject for pharmacokinetic analysis per the SOA.

Excretion and Metabolism:

Blood samples will be collected from each subject for metabolic analysis per the SOA. Samples will be frozen at -80ºC for analysis outside of this study.

Urine collections will be performed after dosing per the SOA.

Data Analysis:
Healthy volunteers and CF patients will be analyzed separately.

For each healthy volunteers and CF population, subjects will be analyzed by cohorts. Placebo subjects from each cohort will be pooled. In addition, CF patients will also be analyzed in aggregate and separately based on CFTR mutation status and based on use or no use at baseline of a stable regimen (last 2 months) of CFTR modulators/correctors as well as use or no use at baseline of inhaled hypertonic saline.

Screening, Compliance, Tolerability and Safety Data:
In general, safety analyses will be performed based on Safety Analysis Set, which includes all enrolled subjects who receive at least one dose of study drug (placebo or active). Post-dose safety assessments will be compared with measurements recorded at baseline. Treatment-emergent AEs will be summarized using the Medical Dictionary for Regulatory Activities (MedDRA) under version 23.0 or later by System Organ Class (SOC) and Preferred Term (PT). The incidence and frequency of treatment-emergent AEs, SAEs, related AEs, related SAEs, and AEs leading to discontinuation, will be summarized by cohort per SOC, PT, and severity. All AEs, SAEs 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 FSH test results will be listed separately.

Pharmacokinetics:
Plasma concentrations of ARO-ENaC product constituents will be used to calculate the following PK parameters:

maximum observed plasma concentration (C_{max}), area under the plasma concentration time curve (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}), and terminal elimination half-life ($t_{½}$). Additional PK parameters may be calculated as needed. Pharmacokinetic parameters will be determined using non-compartmental methods. Descriptive statistics of PK parameters will include mean, standard deviation (SD), coefficient of variation, median, minimum, and maximum. PK results will be analyzed for dose proportionality, and sex differences.

PK population: All NHVs and CF patients that received at least one dose of study drug and that 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.

NHV Pharmacodynamic (PD) Effect Assessment:
Results, including percent change and duration of response from baseline to EOS will be analyzed and summarized by cohort. The NHV PD population includes subjects in NHV cohort 5.

CF Patient Efficacy Assessments:
Descriptive summary of ppFEV1, LCI, BMI and CFQ-R respiratory domain score at each scheduled study visit and change from baseline will be summarized. Events rate of pulmonary exacerbation will be summarized by cohort.

Immunogenicity (Anti-Drug Antibodies):
Shift tables will be displayed by cohort or by dose in both healthy volunteers and CF patients.

Baseline for data analysis purposes is defined as the pre-dose value occurring closest to the first dose of ARO-ENaC or PBO administered in the study.

Additional details will be provided in the statistical analysis plan.

Table 1.1: Cohorts 1-4:

Assessment	Screen	Day -1 Confine	R	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 15	Day 29 EOS	Early Term.
Visit Windows	Days -28 to -1			±5 min for 30 min post dose PK draw and ±10 min for all other post dose PK timepoints only					± 2d			
Informed Consent	X											
Eligibility Criteria	X	X										
Body Mass Index/Weight/Height	X			X					X	X	X	X
Demographics	X											
Medical History	X	X										
Drug Screen	X											
Hepatitis/HIV Serology Screen	X											
Physical Exam (may be symptom directed after Day 1)	X			X (pre-dose)	X	X	X	X	X	X	X	X
FSH (in women of post-menopausal age only)	X											
Urine qualitative pregnancy test	X			X (pre-dose)							X	X
ECG[4]	X	X		X	X	X	X	X	X	X	X	X
Vital Signs (BP, temp, pulse ox., RR, heart rate) perform before other evaluations	X	X		X	X	X	X	X	X	X	X	X
Clinical Labs (heme, coag, chem, UA, Urine electrolytes)	X	X		X (pre-dose)	X (pre-dose)	X (pre-dose)	X		X	X	X	X
PK[2]				X	X	X	X	X				
Plasma metabolite ID				X	X	X	X		X	X		
Urine collection for excretion and metabolite ID[3]						X	X					
Chest X-ray (PA and Lateral)[5]	X									X	X	X
Spirometry[1]	X	X		X	X	X	X		X	X	X	X
Concomitant Meds/Therapies	X	X		X	X	X	X	X	X	X	X	X
Adverse Events	X	X		X	X	X	X	X	X	X	X	X
Dose Administration (PBO or Active)				X	X	X						
Urine Cotinine	X											
Anti-drug antibodies		X									X	

1. On dosing days (Days 1,2,3), completed at immediate pre-dose, 15 min (± 10 min), 60 min (± 10 min), 120 min (± 10 min). On non-dosing days, only completed once per visit.
2. Plasma PK collected:
   a. Day 1: Pre-dose, then based on start of inhalation 30, 60, 120 min, 4, 6, 8, 16 hours.
   b. Day 2: Pre-dose (Day 1, 24 hr timepoint), then based on start of inhalation, 1, 2, 4 hours
   c. Day 3: Pre-dose (Day 2, 24 hr timepoint), then based on start of inhalation, 30, 60, 120 min, 4, 6, 8, 16, 24 (on Day 4), 48 hours (on Day 5).
3. Urine collected cumulatively from 0-6 hours and 6-24 hours post dose on Day 3 through Day 4. Subject should void pre-dose on Day 3. Metabolite ID will be performed on urine samples collected (pooled analysis).
4. On dosing days, ECG performed pre-dose (-4hrs) and 2 hours (± 10 min) post-dose; any abnormal ECG's will be repeated in triplicate with each measurement approximately 1 minute apart.
5. Chest x-ray to be performed at screening, day 15, and day 29 (or early termination). At all other times, any AE consistent with lower respiratory infection, dyspnea, or bronchospasm will trigger a chest X-ray and PFT measurement.

Note: R = randomize.

Table 1.2: Cohort 5:

Assessment	Screen	Day 0 Confine	R	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 18	Day 19	Day 29 EOS	Early Term.
Visit Windows	Days -28 to -1			±5 min for 30 min post dose PK draw and ±10 min for all other post dose PK timepoints only					± 2d	± 3d	+ 2d	± 2d	
Informed Consent	X												
Eligibility Criteria	X												
Body Mass Index/Weight/Height	X			X					X	X		X	X
Demographics	X												
Medical History	X												
Drug Screen	X												
Hepatitis/HIV Serology Screen	X												
Physical Exam (may be symptom directed after Day 1)	X	X		X (pre-dose)	X	X	X	X	X	X		X	X
FSH (in women of post-menopausal age only)	X												
Urine qualitative pregnancy test	X			X (pre-dose)								X	X
ECG[3]	X			X	X	X	X	X	X	X		X	X
Vital Signs (BP, temp, pulse ox., RR, heart rate) perform before other evaluations	X	X		X	X	X	X	X	X	X		X	X
Clinical Labs (heme, coag, chem, UA, Urine electrolytes)	X			X (pre-dose)	X (pre-dose)	X (pre-dose)	X		X	X		X	X
PK[2]				X	X	X	X	X					
24 hour urine labs[6]		X								X		X	
Chest X-ray (PA and Lateral)[4]	X									X		X	X
Spirometry[1]	X			X	X	X	X		X	X		X	X
Concomitant Meds/Therapies	X	X		X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X		X	X	X	X	X	X	X	X	X	X
Dose Administration (PBO or Active)				X	X	X							
Urine Cotinine	X												
Anti-drug antibodies		X										X	
Bronchoscopy with bronchoalveolar lavage and bronchial brushing[5]		X								X			
Phone Call											X		

1. On dosing days (Days 1,2,3), completed at immediate pre-dose, 15 min (± 10 min), 60 min (± 10 min), 120 min (± 10 min). On non-dosing days, only completed once per visit.
2. Plasma PK collected:
   a. Day 1: Pre-dose, then based on start of inhalation 30, 60, 120 min, 4, 6, 8, 16 hours.
   b. Day 2: Pre-dose (Day 1, 24 hr timepoint), then based on start of inhalation, 1, 2, 4 hours
   c. Day 3: Pre-dose (Day 2, 24 hr timepoint), then based on start of inhalation, 30, 60, 120 min, 4, 6, 8, 16, 24 (on Day 4), 48 hours (on Day 5).
   d. Plasma metabolite identification may be performed on PK samples at the Sponsor's discretion
3. On dosing days, ECG performed pre-dose (-4hrs) and 2 hours (± 10 min) post-dose; any abnormal ECG's will be repeated in triplicate with each measurement approximately 1 minute apart.
4. Chest x-ray to be performed at screening, day 18, and day 29 (or early termination). At all other times, any AE consistent with lower respiratory infection, dyspnea, or bronchospasm will trigger a chest X-ray and PFT measurement. Day 18 chest X-ray is to be completed within the associated visit window and prior to bronchoscopy.
5. All eligibility requirements (inclusion and exclusion criteria) must be met prior to a subject undergoing bronchoscopy. On days when bronchoscopy is performed, all other assessments will be performed prior to bronchoscopy. Vital signs will be repeated during recovery from sedation post-bronchoscopy at the frequency deemed appropriate by the site.
6. 24 hour urine collection to be completed prior to dose administration on day 1. For visits not associated with confinement, 24 hour urine is to be collected by the subject at home.

Note: R = randomize.

Table 1.3: CF patient multi-dose cohorts (Cohorts 2b, 3b, 4b)

Assessment	Screen	R	Day 1	Day 2	Day 3	Day 15	Day 22	Day 23	Day 24	Day 29	Day 37	Day 57	Day 71	Day 85	Day 113 (EOS) or Early Term.
Visit Windows	Days -28 to -1		±5 min for 30 min post dose PK draw and ±10 min for all other post dose PK timepoints only			± 2d	±5 min for 30 min post dose PK draw and ±10 min for all other post dose PK timepoints only			± 2d	± 5d	± 5d	± 5d	± 5d	± 5d
Informed Consent	X														
Eligibility Criteria	X														
Body Mass Index/Weight/Height	X		X			X	X			X	X	X	X	X	X
Demographics	X														
Medical History	X														
Drug Screen	X														
Hepatitis/HIV Serology Screen	X														
Physical Exam (may be symptom directed after Day 1)	X		X	X	X	X	X	X	X	X	X	X	X	X	X
FSH (in women of post-menopausal age only)	X														
Urine qualitative pregnancy test (pre-dose to begin each cycle)	X		X				X								X
ECG[1]	X		X	X	X		X	X	X	X	X	X			X
Vital Signs (BP, temp, pulse ox., RR, heart rate) perform before other evaluations	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Labs (heme, coag, chem, UA, Urine electrolytes) Completed pre-dose on dosing days[4]	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Chest X-ray (PA and Lateral)[5]			X (pre-dose)							X					X
PK[2]			X	X	X		X	X	X						
Lung Clearance Index[6]			X (pre-dose)[4]			X [4]	X (pre-dose)[4]				X[4]	X[4]		X[4]	X[4]
CF Genotyping (See protocol for specifications)	X														
CF Questionnaire			X (pre-dose)				X (pre-dose)				X	X		X	X
Spirometry	X		X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]		X[3]	X[3]		X[3]	X[3]
Concomitant Meds/Therapies	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X		X	X	X	X	X	X	X	X	X	X	X	X	X

Dose Administration (PBO or Active)[7]			X	X	X		X	X	X						
Urine Cotinine	X														
Anti-drug antibodies			X				X								X
Evaluate and Record Occurrence of Pulmonary Exacerbation							X				X	X	X	X	X
Sweat Chloride[8]			X (pre-dose)												

1. ECG completed pre-dose (-4hrs ± 30 min) and 2 hours (± 30 min) post-dose on dosing days; any abnormal ECGs will be repeated in triplicate with each measurement approximately 1 minute apart.
2. Plasma PK collected Cycle 1:
    a. Day 1: pre-dose, then based on start of inhalation 30, 60, 120 min, 4 hour
    b. Day 2: pre-dose (Day 1, 24 hour time point), then based on start of inhalation 60 min, 120 min, 4 hour.
    c. Day 3: pre-dose (Day 2, 24 hour time point), then based on start of inhalation, 30, 60, 120 min, 4 hour

    Cycle 2:
    a. Day 22: pre-dose, then based on start of inhalation at 30 min, 4 hours post-dose.
    b. Day 23: pre-dose, then based on start of inhalation at 30 min, 4 hours post-dose.
    c. Day 24: pre-dose, then based on start of inhalation at 30, 60, 120 min, 4 hours.
3. Spirometry completed pre-dose and post-dose 60 min (± 20 min), 120 min (± 20 min) on dosing days. Spirometry is only completed once on days when dose is not administered.
4. Order of assessments: 1. Administration of any Standard of Care medications (e.g. hypertonic saline) or airway clearance treatments (e.g. percussion) 2. Lung Clearance Index testing 3. CF Questionnaire, 4. Spirometry evaluation, 5. Draw Blood, 6. Dose administration (on dosing days). Order of other assessments is not protocolized and may be done at discretion of site in concordance with specified ECG/PK windows as well as pre-dose requirements for specified assessments.
5. Chest x-ray to be performed at days 1, 29, and 113 (or early termination). At all other times, any AE consistent with lower respiratory infection, dyspnea, bronchospasm, or CF exacerbation will trigger a chest X-ray and PFT measurement.
6. At every study visit where LCI is measured per SOA, sites are asked to provide **three** acceptable LCI tests. The final value for data analysis is the mean value of all acceptable washout results.
   LCI in cohorts 2b, 3b, and 4b is only to be measured in CF patients with Day 1 ppFEV1 >70%. LCI will be performed at all sites when it is available.
7. Each treatment is three daily doses beginning on either Day 1 (Cycle 1) or Day 22 (Cycle 2).
8. Sweat chloride to be performed at all sites when available. Note: R = randomize.

Table 1.4: CF patient multi-dose cohorts (Cohort 6)

Assessment	Screen	R	Day 1	Day 2	Day 8	Day 15	Day 22	Day 29	Day 30	Day 36	Day 43	Day 57	Day 71	Day 85	Day 113 (EOS) or Early Term.
Visit Windows	Days -28 to -1		±5 min for 30 min post dose PK draw and ±10 min for all other post dose PK timepoints only		± 2d	± 2d	± 2d	± 2d ±5 min for 30 min post dose PK draw and ±10 min for all other post dose PK timepoints only		± 2d	± 2d	± 2d	± 5d	± 5d	± 5d
Informed Consent	X														
Eligibility Criteria	X														
Body Mass Index/Weight/Height	X		X		X	X	X	X		X	X	X	X	X	X
Demographics	X														
Medical History	X														
Drug Screen	X														
Hepatitis/HIV Serology Screen	X														
Physical Exam (may be symptom directed after Day 1)	X		X	X	X	X	X	X	X	X	X	X	X	X	X
FSH (in women of post-menopausal age only)	X														
Urine qualitative pregnancy test (pre-dose to begin each cycle)	X		X			X		X							X
ECG[1]	X		X	X	X	X	X	X	X	X	X	X			X
Vital Signs (BP, temp, pulse ox., RR, heart rate) perform before other evaluations	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Labs (heme, coag, chem, UA, Urine electrolytes) Completed pre-dose on dosing days[4]	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Chest X-ray (PA and Lateral)[5]			X (pre-dose)				X								X
PK[2]			X	X				X	X						
Lung Clearance Index[6]			X (pre-dose)[4]					X (pre-dose)[4]			X[4]				X[4]
CF Genotyping (See protocol for specifications)	X														
CF Questionnaire			X (pre-dose)					X (pre-dose)			X		X		X
Spirometry	X		X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]
Concomitant Meds/Therapies	X		X	X	X	X	X	X	X	X	X	X	X	X	X

Dose Administration (PBO or Active)			X			X		X							
Urine Cotinine	X														
Anti-drug antibodies			X (pre-dose)			X (pre-dose)		X (pre-dose)							X
Evaluate and Record Occurrence of Pulmonary Exacerbation			X	X	X	X	X	X	X	X	X	X	X	X	X
Sweat chloride[7]			X (pre-dose)												
Adverse Events	X		X	X	X	X	X	X	X	X	X	X	X	X	X

1. ECG completed pre-dose (-4hrs ± 30 min) and 2 hours (± 30 min) post-dose on dosing days; any abnormal ECGs will be repeated in triplicate with each measurement approximately 1 minute apart.
2. Plasma PK collected
    a. Day 1: pre-dose, then based on start of inhalation 30, 60, 120 min, 4 hours, and 24 hours (day 2) post-dose (measured from beginning of drug administration)
    b. Day 29: pre-dose, then based on start of inhalation 30, 60, 120 min, 4 hours, and 24 hours (day 30) post-dose (measured from beginning of drug administration)
3. Spirometry completed pre-dose and post-dose 60 min (± 20 min), 120 min (± 20 min) on dosing days. Spirometry is only completed once on days when dose is not administered.
4. Order of assessments: 1. Administration of any Standard of Care medications (e.g. hypertonic saline) or airway clearance treatments (e.g. percussion) 2. Lung Clearance Index testing 3. CF Questionnaire, 4. Spirometry evaluation, 5. Draw Blood, 6. Dose administration (on dosing days). Order of other assessments is not protocolized and may be done at discretion of site in concordance with specified ECG/PK windows as well as pre-dose requirements for specified assessments.
5. Chest x-ray to be obtained at days 1, 22, and 113 (or early termination). At all other times, any AE consistent with lower respiratory infection, dyspnea, bronchospasm, or CF exacerbation will trigger a chest X-ray and PFT measurement.
6. At every study visit where LCI is measured per SOA, sites are asked to provide **two** acceptable LCI tests. The final value for data analysis is the mean value of all acceptable washout results.
   LCI will be measured in all cohort 6 patients, irrespective of ppFEV1. LCI will be performed at all sites when it is available.
7. Sweat chloride to be performed at all sites when available Note: R = randomize.

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3. STUDY INFORMATION AND SIGNATURES

Investigator's Statement:

I have read and understood the information in this protocol and agree to conduct the trial according to the protocol (subject to any amendments) and in accordance with the principles of ICH E6 Good Clinical Practice guidelines. I have read and agree to comply with the Investigator obligations stated in this protocol, as well as with any and all applicable federal, state, or local laws and regulations. Any changes in procedure will only be made if necessary to protect the safety, rights or welfare of participants.

I agree to conduct or to supervise the trial in person.

I agree to ensure that all that assist me in the conduct of the study are aware of their obligations.

Principal Investigator:

_____ _____

Signature Date

_____

Printed Name

4. LIST OF ABBREVIATIONS AND TERMS

AE	Adverse event
ALT	Alanine aminotransferase
API	Active Pharmaceutical Ingredient
AST	Aspartate transaminase
ARO	Arrowhead Pharmaceuticals, Inc.
AUC	Area under the curve
AUC_{inf}	Area under the curve from time 0 to infinity
BAL	Bronchoalveolar lavage
BP	Blood pressure
cGCP	current Good Clinical Practice
cGMP	current Good Manufacturing Practice
C_{max}	Concentration maximum (peak)
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organization
CTN	Clinical Trial Notification
CVA	Cerebrovascular accident
dL	deciliter
DSC	Data Safety Committee
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EOS	End of Study
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in the first second
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBV	Hepatitis B virus
HIV	Human Immunodeficiency Virus
HR	Heart Rate
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
IWRS	Interactive web response system
Kg	Kilogram
L	Liter
LLN	Lower limit of normal
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MedDRA	Medical Dictionary for Regulatory Activities
Mg	milligram
mmHg	millimeters of mercury

NHV	Normal healthy volunteers
NOAEL	No observed adverse event level
NYHA	New York Heart Association
PBO	Placebo
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
ppFEV1	Percent predicted FEV1
PT	Prothrombin Time or Preferred Term
PTT	Partial thromboplastin time
QRS	QRS duration (complex) – a structure on the ECG that corresponds to the depolarization of the ventricles
QT	QT interval – a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
RDD	Respirable delivered dose
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious Adverse Event
SD	Standard Deviation
siRNA	Short interfering RNA oligonucleotides
SOA	Schedule of Assessments
SOC	System Organ Class
$t_{1/2}$	terminal elimination half-life
TIA	Transient Ischemic Attack
ULN	Upper Limit of Normal

5. INTRODUCTION

5.1. Background Information

Cystic fibrosis (CF), is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, which encodes a chloride and bicarbonate ion channel expressed at the apical surface of epithelial cells (Choi 2001; Stoltz 2015). In its wild-type form, the CFTR protein is localized on the cell surface and acts as a cyclic adenosine monophosphate (cAMP)-regulated ion channel (Alton 2016). The transport of anions (such as chloride and bicarbonate) through the apical membrane of epithelial cells creates an osmotic gradient for fluid secretion (Quon 2016). In the lungs, the balance of chloride ion secretion through functional CFTR protein and sodium absorption through the epithelial sodium (Na+) channel (ENaC) maintains an optimum volume, electrolyte composition, and pH of the airway surface liquid (a thin fluid layer protecting the epithelium from inspired air) (Hobbs 2013; Stoltz 2015; Vallières 2014). The CFTR protein also plays an absorptive role in some epithelial structures, particularly exocrine glands, such as the sweat gland, pancreas, gastrointestinal tract, and in the male and female reproductive tract. The absence or dysfunction of the CFTR therefore results in dehydrated, thickened secretions that obstruct these epithelium lined ducts of exocrine tissues, resulting in predisposition to infection, inflammation and tissue damage (Quon 2016).

As ENaC is negatively regulated by CFTR, reduced functional levels of or the absence of CFTR in patients with CF results in reduced chloride/bicarbonate ion and water secretion. Reduced CFTR function leads to ENaC hyperactivity, resulting in increased sodium ion and water absorption (Bangel-Ruland 2015; Hobbs 2013; Stoltz 2015) which subsequently contributes to dehydrated, thickened mucus. The combined effects of reduced chloride secretion (CFTR dysfunction) and hyperabsorption of sodium and water (ENaC hyperactivity) are dehydration of the cell surface, a reduction in the water content of the airway surface liquid, and thick, obstructive mucus that is resistant to removal (Stoltz 2015; Amaral 2015). Homeostasis of the airway surface liquid with adequate mucus hydration is essential for efficient mucociliary clearance of aerial pathogens, which is needed to maintain the sterility of the lung (Hobbs 2013; Stoltz 2015; Vallières 2014). Overly viscous and dehydrated mucus leads to impaired mucociliary clearance, resulting in infective/inflammatory sequelae, including airway mucus obstruction and disseminated bronchiectasis, recurrent bacterial infections, chronic inflammation, and lung damage/scarring (CFF Patient Registry Annual Report 2015; Maselli 2017). Finally, this cascade leads to end-stage lung disease, which can only be resolved by lung transplantation (Amaral 2015). Even in the setting of CFTR modulator/corrector therapy, there remains an unmet medical need for additional therapeutic options, particularly in certain sub-populations (e.g. those with Class I mutations). Additionally, ENaC inhibition may work synergistically with CFTR modulation (See Section 5.2.3).

5.2. Mechanism of Action of ARO-ENaC & Therapeutic Rationale

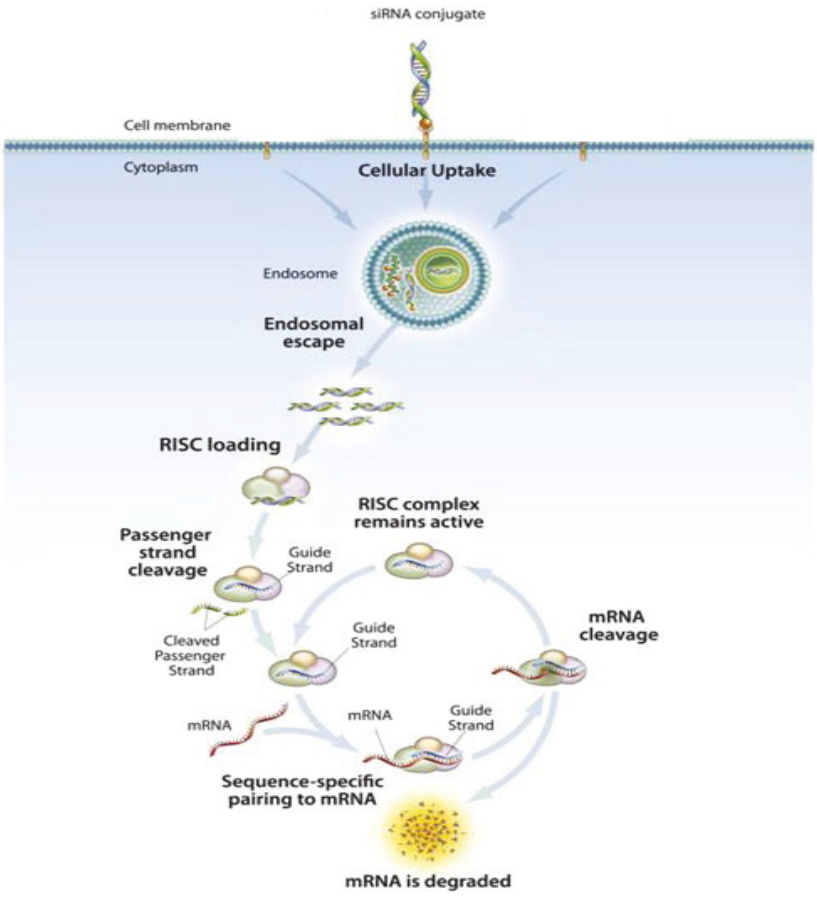
5.2.1. siRNA Mechanism of Action

RNAi-based therapeutics have the potential to silence the expression of any gene. RNAi is a naturally-occurring phenomenon by which short interfering RNA oligonucleotides (siRNAs) trigger a sequence-specific down-modulation of gene expression. RNAi triggers refer to synthetic siRNAs designed to target specific gene expression (Fire 1998).

ARO-ENaC is a synthetic, double-stranded, pulmonary epithelium-targeted RNAi trigger designed to specifically silence mRNA transcripts encoded by the αENaC gene using an RNA interference mechanism. The RNAi trigger is a short, double-stranded RNA conjugated to an αvβ6 integrin targeting ligand for the αvβ6 receptor known to be present on the pulmonary epithelial cells. This targeting approach induces epithelial cell receptor-mediated endocytosis and introduction of the

double stranded RNAi trigger into the cytoplasm of epithelial cells which results in its association with the protein components of the RNA induced silencing complex (RISC), resulting in "on target" highly sequence-specific degradation of messenger RNA complementary to the antisense strand of the RNAi trigger. ARO-ENaC targets the αENaC 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. The persistence of pharmacologic activity significantly beyond the period of plasma or local tissue exposure is due to this unique RNAi mechanism in which a small amount of guide strand can persist and be active within RISC in the cytoplasm of target cells for extended periods of time.

siRNA mechanism of action



5.2.2. ENaC Inhibition

By delivering RNAi triggers targeting the αENaC sequences to the pulmonary epithelium, it is possible to knock down expression of αENaC mRNAs in pulmonary epithelial cells and thus reduce the number of active ENaC channels on the epithelial cell.

Reduced ENaC channel activity, should lead to improved hydration and mucus clearance in patients with CF. Since mucus dehydration and impaired mucociliary clearance results in infective/inflammatory sequelae, including airway mucus obstruction and disseminated bronchiectasis, recurrent bacterial infections, chronic inflammation, and lung damage/scarring in

patients with CF, improved mucus hydration and clearance is expected to prevent further pulmonary injury and allow for a reduction in CF induced sequelae. This therapeutic rationale is supported by human genetics where CF patients who are deficient in CFTR function (F508del mutations) but also harbor an ENaC loss-of-function mutation have milder CF symptoms compared to those with wild type ENaC and are reported to live into their 5th or 6th decade. (Agrawal 2017). Patients with normal CFTR function but with an ENaC activating mutation have a CF like phenotype (Rauh 2010). Additionally, patients homozygous for ENaC loss-of-function mutations have been shown to have enhanced mucociliary clearance relative to non-carriers (Kerem 1999). Such genetic target validation has inspired development of inhaled small molecule ENaC inhibitors which have been investigated in clinical trials. However, systemic absorption via the lung has caused discontinuation of small molecule studies secondary to renal uptake of small molecules by the distal tubule leading to dose limiting hyperkalemia in some studies (O'Riordan 2014).

The RNAi trigger in ARO-ENaC was designed to specifically target only the αENaC mRNA. siRNA is poorly absorbed by most cell types without an appropriate surface receptor targeting ligand to induce endosomal uptake. Thus, uptake by non-pulmonary epithelial cells, such as cells in the renal distal tubule or collecting duct is not likely. In contrast to small molecule ENaC inhibitors, localized inhaled nebulized delivery of siRNA has shown very minimal systemic uptake with the majority of delivered drug remaining in the lung. Additionally, intravenous delivery of ARO-ENaC also does not induce significant ENaC gene silencing in the kidney and with both nebulized and intravenous delivery, no hyperkalemia has been seen in non-clinical studies (See Investigator's Brochure).

5.2.3. Combining ENaC Inhibition with CFTR Modulators

Even in the setting of broadly used CFTR modulators, there remains an unmet medical need in CF patients. CF related exacerbation rates, which are important clinical events associated with disease progression, remain a significant issue even in patients receiving CFTR modulator therapy (Middleton 2019). Patients with high unmet need include those for whom no CFTR modulator therapies are available, those who are unresponsive to CFTR modulators due to their underlying mutations (approximately 10% of the CF population), those who require frequent hospitalization due to CF-related exacerbations, and those who do not tolerate inhaled antibiotics. There is a strong rationale for the potential of synergistic effects of ENaC inhibition and CFTR modulators in the 90 % that respond to elexacaftor/tezacaftor/ivacaftor. The rescue of CFTR by modulators is not perfect (~50% at functional level) and based on physiology, inhibition of ENaC is predicted to hyperpolarize the cell membrane potential and thus increase the driving force for chloride secretion via modulator-rescued mutant CFTR. Therefore, even patients for which CFTR modulator therapy is available are expected to have additional benefit from the development of new "add-on" therapies (Mall 2019).

ENaC inhibition may act synergistically with CFTR modulators. CFTR can secrete or absorb Cl^- across epithelial surfaces depending on the driving force that is determined by i) the intra- and extracellular Cl^- concentrations that are tightly regulated and result in a Nernst potential for Cl- in the range of –30 mV in airway epithelial cells, and ii) the membrane potential of the cell that is set by the relative conductances of Cl^-, Na^+ and K^+ channels and respective intra- and extracellular concentrations of these ions (Boucher 1994; Boucher 1994; Willumsen 1989). It is therefore predicted that if ENaC is inactive or not expressed in the same cell, cAMP-mediated stimulation will lead to a concomitant activation of apical CFTR and basolateral K^+ channels that drives the membrane potential more negative than the Nernst potential of Cl^-, and thus generate a driving force for CFTR-mediated Cl^- secretion. However, if ENaC is active in the same cell, this will depolarize the membrane potential, reduce the driving force for Cl^- secretion, and may evenresult in CFTR-

mediated Cl^- absorption as observed in the sweat duct (Boucher 1994; Boucher 1994; Willumsen 1991; Willumsen 1989; Greger 1996; Quinton 1983). The implications of these physiological findings on the combination of CFTR modulators and ENaC inhibitors in patients with CF are that inhibition of hyperactive ENaC in the apical membrane of airway epithelial cells may not only block ENaC-mediated Na^+/fluid absorption, but will also hyperpolarize the apical cell membrane and thus increase the driving force for Cl^-/fluid secretion via mutant CFTR channels that have been rescued and inserted into the apical plasma membrane in the setting of CFTR modulator use. Whether this results in synergistic effects on airway surface hydration, MCC and pulmonary outcomes in patients with CF and other muco-obstructive lung diseases needs to be assessed in clinical trials. However, there is a plausible scientific rationale for synergistic effect when using CFTR modulators with ENaC inhibition.

5.3. ARO-ENaC Pre-Clinical Pharmacology Studies

Further information on the pre-clinical pharmacology studies in monkey, rodent and sheep models is provided in the Investigator's Brochure.

5.4. ARO-ENaC Pre-Clinical Pharmacokinetic and Product Metabolism Studies

PK parameters for ARO-ENaC have been evaluated in multiple species. Results of these studies can be found in the Investigator's Brochure.

5.5. ARO-ENaC Pre-Clinical Toxicology Studies

ARO-ENaC has been clinically well tolerated in rats and in non-human primate toxicology studies. Details regarding GLP and non-GLP toxicology results are provided in the Investigator's Brochure.

5.6. Rationale for the Study

AROENAC1001 is a first in human dose-ranging study to assess the safety and tolerability of ARO-ENaC in healthy volunteers and in patients with CF. Arrowhead follows general principles of EMA and FDA guidance which indicate first-in-human (FIH) studies should initiate in healthy volunteers unless the drug is known or suspected to be unavoidably toxic or associated with a high degree of toxicity (e.g. chemotherapy or other oncology compounds). This is not the case with ARO-ENaC based on GLP toxicology results (See Investigator's Brochure).

Arrowhead believes valuable scientific information will be gained from the initial NHV component of the AROENAC1001 study. For example, healthy volunteers provide valuable tolerability and PK information in the absence of disease and can provide information that can be used for comparative purposes to patient groups. Additionally, results of a FIH study in CF patients may be difficult to interpret for the following reasons:

1. CF patients as part of their underlying disease may have fluctuations in labs (e.g. ALT and AST) and pulmonary status. These factors make initial safety data interpretation difficult and support initial first in man evaluation in an NHV population with minimal co-morbidities, normal lung function and normal laboratory values at baseline. Based on the GLP toxicology results, ARO-ENaC should be appropriately safe for healthy volunteers.

2. CF patients are commonly on concomitant medications for the treatment of CF and other co-morbid conditions. It would be beneficial to initially study ARO-ENaC in a healthy volunteer population not confounded by additional medications.

For these reasons, the study initiates in healthy volunteers and pending safety evaluation in NHVs, also enrolls CF patients. Treatment with ARO-ENaC is expected to reduce pulmonary epithelial ENaC channel density via RNAi. The magnitude of the reduction and duration of effect will depend on the dose. Since there has not been human clinical exposure to ARO-ENaC, an effective therapeutic dose to administer to patients with CF in later stage efficacy studies is unknown. Thus, the study plans to dose escalate in NHVs and will also evaluate multiple dose levels in CF patients.

The study begins with single dose cycle administration to healthy volunteers to evaluate ARO-ENaC for the purpose of understanding safety profile. There are no known serum biomarkers of ENaC; thus to gauge pharmacodynamic response in NHVs bronchoscopy with bronchial brushing and bronchoalveolar lavage (BAL) is planned to obtain airway epithelial cells (from brushing) and airway exosomes (from BAL) for analysis of αENaC expression. Serum electrolytes will be monitored carefully with particular attention to potassium levels.

CF patients will require multiple doses of ARO-ENaC to sustain gene target silencing. Accordingly, this study uses a multiple-ascending dose (MAD) design to determine the dose required to reach and sustain maximal knockdown in pulmonary epithelial ENaC levels, and the dose-response relationship in patients with CF. There is no readily measurable serum assay for ENaC gene target silencing in humans. However, FEV1 and Lung Clearance Index have been used and well validated in multiple CF studies as potential markers of drug efficacy. LCI is time consuming in subjects with ppFEV1 < 70%; for feasibility reasons in CF cohorts 2b-4b, LCI will only be performed in subjects with ppFEV1 >70% and CF cohort 4b will be restricted to subjects with ppFEV1 >70% to allow for LCI assessments of all subjects in that cohort. CF cohort 6 will trial a different approach, with LCI assessments in all subjects, even in those with ppFEV1 <70%.

CF cohort 6 is included in the study in order to assess the safety and efficacy of an alternate dosing regimen in CF patients. In this cohort, patients will be dosed on Days 1, 15, and 29, as opposed to Days 1, 2, 3 and 22, 23, 24 as in other CF cohorts. The respirable delivered dose (RDD, which is the amount of drug that reaches the lung, as opposed to the amount of drug loaded in the nebulizer) given on Day 1 to subjects in cohort 6 will be equal to or less than the total cumulative RDD given over Days 1, 2, and 3 to subjects in CF cohort 4b (which is the cohort receiving the highest dose level among CF cohorts 2b, 3b, 4b). The rationale for testing this dosing regimen is that sheep pharmacology studies indicate similar efficacy of ARO-ENaC when given via one single, larger dose as when given via three smaller daily doses. Additionally, sheep studies indicate a waning of drug effect at 3 weeks post-dose, providing the rationale for testing a 2 week dosing interval.

5.7. Risk Assessment for Participants

- **Embryo-Fetal:** Limited GLP toxicology studies have been conducted. Accordingly, eligible participants enrolled in this study, both male and female (including partners), must agree to use two highly effective forms of contraception during the study and for 3 months post-dose, or agree to abstinence (acceptable only if this method is in alignment with the normal lifestyle of the patient).

- **Electrolyte Disturbances:** Small molecule inhibitors of ENaC have been associated with mild hyperkalemia (O'Riordan 2014). This was due to systemic absorption of the small molecule inhibitors with ENaC inhibition in the kidney distal tubule leading to potassium reabsorption. ARO-ENaC has not been shown to produce hyperkalemia in various GLP toxicology studies when administered via inhaled nebulized solution and even when administered intravenously.

Avoidance of renal ENaC inhibition and associated hyperkalemia is facilitated by local nebulized delivery and the poor uptake of siRNA molecules by cells lacking the appropriate receptor. Hyperkalemia is not expected in this study. However, frequent monitoring, avoidance of medications known to cause hyperkalemia, stopping rules for hyperkalemia and a hyperkalemia treatment guideline (Section 16.2) are utilized to mitigate risk.

- **Pulmonary:** GLP animal toxicology studies have not revealed evidence of pulmonary toxicity at clinically relevant doses and NOAELs from such studies provide a wide therapeutic index. However, as this is the first administration of ARO-ENaC in humans, careful monitoring of pulmonary function is prudent. Very frequent measurement and tracking of FEV1 as described in the SOA and stopping rules for adverse changes in lung function are intended to mitigate the risk of pulmonary toxicity.

- **Bronchoscopy:** 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 bronchial brushings will be performed; notably, no transbronchial biopsies will be taken. Bronchial brushings will be taken from the lung periphery under fluoroscopic visualization of brush location. Complications of bronchoscopy include sore throat, bronchospasm, hypoxemia, laryngospasm or laryngeal injury, fever, and adverse reactions to sedation. The most common adverse event is laryngospasm which occurs in 0.6% of flexible bronchoscopies (Pue 1995). More severe complications such as pneumothorax and pulmonary hemorrhage are rare (0.1 to 0.2% for each) and occur more frequently in the setting of transbronchial biopsies, which will not be performed in this study (Pue 1995; Facciolongo 2009). Patient selection has a significant impact on procedural complications and the risks of bronchoscopy in normal healthy volunteers will be lower than in patients with underlying respiratory disease.

5.8. Justification for Doses in Humans

Detailed calculations related to pulmonary deposited dose are provided in the **Investigator's Brochure Section 8.2.2**. In animals, pulmonary deposited dose (PDD) refers to the amount of drug reaching the lung tissue. Assuming a 100% deposition fraction, the equivalent measure in humans is respirable delivered dose (RDD). Pharmacologic activity of inhaled ARO-ENaC was determined in sheep, where dose dependent increases in mucociliary clearance were observed at doses of 0.04 to 0.5 mg of pulmonary deposited dose (PDD) per kg body weight dosed on each of days 1, 2, 3. This translates to human respirable delivered doses of approximately 3.0 mg to 30 mg in a 60 kg patient (0.05 to 0.5 mg respirable delivered dose per kg of body weight).
Similar pharmacologic activity was seen in sheep when a single, larger dose of 1.7 mg PDD per kg was given on day 1, which translates to a human RDD of 102 mg in a 60 kg person.

Therefore, all dose levels evaluated in the AROENAC1001 study are expected to result in dose dependent pulmonary ENaC channel suppression and increasing pharmacologic activity using the proposed dosing schedule. Sheep pharmacology studies show efficacious acceleration of mucociliary clearance at 2 weeks post-dose, which wanes at 3 weeks post-dose and returns to baseline by 4 weeks post-dose. Thus, for patient cohorts receiving multiple cycles of ARO-ENaC, dosing frequencies of either 2 weeks or 3 weeks will be tested in this study.

In assessing the human risk associated with inhalation of ARO-ENaC, consideration must be given to both the potential to elicit systemic toxicity as well as pulmonary toxicity. The initial clinical evaluation of ARO-ENaC in the AROENAC1001 study evaluates two different dosing regimens: a 3-day dosing cycle (Days 1, 2, and 3) in healthy volunteer cohorts 1-5 which is repeated after a 3-week

interval (Days 22, 23, and 24) in CF cohorts 2b-4b; as well as one, single dose repeated at 2-week intervals (Days 1, 15, and 29) in CF cohort 6. Safety margin considerations are provided for the highest dose regimen (cohort 6) utilizing exposure (AUC) and NOAEL data generated from the 24-day toxicity studies where ARO-ENaC was dosed on Days 1, 2, 3, 22, 23, and 24. While these dosing schedules differ, the total number of doses given in clinical Cohort 6 is fewer and at less frequent intervals than the doses given in the nonclinical toxicology studies.

The maximum human dose in this initial clinical study is 1.5 mg respirable delivered dose (RDD)/kg body weight given as a daily dose on Days 1, 15, and 29, to subjects in cohort 6. Based on a 60 kg human, the total human therapeutic dose of ARO-ENaC would be 90 mg RDD. Assuming a standard human lung weight of 1 kg or 1000 g (Tepper 2016) results in a lung dose of 0.09 mg RDD/g lung weight. The 0.09 mg RDD/g lung weight value can then be compared to values from the animal study to derive safety margins for lung effects. In rats, the NOAEL was considered to be 10.1 mg pulmonary deposited dose (PDD)/kg body weight and based on an average terminal body weight of 0.323 kg, the total administered dose of ARO-ENaC was 3.26 mg PDD. Assuming a standard rat lung weight of 1.5 g (Tepper 2016) results in an ARO-ENaC dose of 2.17 mg PDD/g lung weight. In monkeys, the NOAEL was considered to be 9.9 mg PDD/kg body weight and based on an average terminal body weight of 2.3 kg, the total administered dose of ARO-ENaC was 22.77 mg PDD. Assuming a standard monkey lung weight of 22 g (Tepper 2016) results in an ARO-ENaC dose of 1.04 mg PDD/g lung weight. Comparing the anticipated maximum human dose, 0.09 mg RDD/g lung weight, to the NOAEL dose in rats, 2.17 mg PDD/g lung weight, and the NOAEL dose in monkeys, 1.04 mg PDD/g lung weight, yields safety margins for lung effects of approximately 24X in rats and 12X in monkeys **(Table 1)**. These safety margins for lung effects exceed those considered adequate by the FDA, which are 10X in rats and 5X in monkeys (Tepper 2016).

A more conservative estimate of the human nominal inhaled dose than RDD is the delivered dose (DD). DD is the amount of drug aerosolized by the nebulizer, while RDD is the amount of aerosolized drug contained in droplets of a size suitable for penetration into the lungs. The maximum cohort 6 dose of 1.5 mg RDD/kg body weight results in 0.09 mg RDD/g lung weight and 0.164 mg DD/g lung weight. Comparison of the maximum human delivered dose, 0.164 mg DD/g lung weight, to the PDD at the NOAEL in rats and monkeys, results in the more conservative safety margin estimates for lung effects of 13X in rats and 6X in monkeys **(Table 1)**. This more conservative estimate of safety margins for lung effects still produces margins considered adequate by the FDA (Tepper 2016).

Safety margins for systemic effects were calculated utilizing interim clinical PK data from the ongoing AROENAC1001 study, by comparing the estimated plasma AUC_{inf} following the highest planned human dose of approximately 90 mg RDD (3730 h*ng/mL) with the plasma AUC_{last} at the NOAEL in rats (61900 h*ng/mL), and at the NOAEL in female monkeys (38900 h*ng/mL) as well as male monkeys (58400 h*ng/mL). This results in a safety margin for systemic effects of at least 16.6X in rats (61900 h*ng/mL ÷ 3730 h*ng/mL), 10.4X in female monkeys (38900 h*ng/mL ÷ 3730 h*ng/mL), and 15.7X in male monkeys (58400 h*ng/mL ÷ 3730 h*ng/mL) **(Table 2)**. These safety margins for systemic effects to support the proposed clinical dose when calculated by AUC comparisons exceed those considered adequate by the FDA, which are 1X in both rats and monkeys (Tepper 2016).

Collectively, the results of the in vitro and in vivo nonclinical safety studies, as well as the estimated safety margins for both systemic and lung effects, support the continued clinical development of ARO-ENaC at the dose levels planned in this study.

Table 1. Safety margins for lung effects using the maximum planned clinical dose of approximately 90 mg respirable delivered dose (RDD) in cohort 6

Human RDD[a] (mg/g)	Human DD[a] (mg/g)	Rat PDD at NOAEL[b] (mg/g)	Rat Safety Margin using RDD	Rat Safety Margin using DD	Monkey PDD at NOAEL[b] (mg/g)	Monkey Safety Margin using RDD	Monkey Safety Margin using DD
0.09	0.164	2.17	24	13	1.04	12	6

[a] Clinical doses given on Days 1, 15, 29. RDD and DD reflect average daily dose.
[b] GLP toxicology study doses given on Days 1, 2, 3, 22, 23, 24. PDD reflects average daily dose.

Table 2. Safety margins for systemic effects using the maximum planned clinical dose of approximately 90 mg respirable delivered dose (RDD) in cohort 6

Human RDD[a] (mg)	Estimated Human AUC_{inf} (h*ng/mL)	Rat AUC_{last} at NOAEL[b] (h*ng/mL)	Rat Safety Margin	Male Monkey AUC_{last} at NOAEL[b] (h*ng/mL)	Male Monkey Safety Margin	Female Monkey AUC_{last} at NOAEL[b] (h*ng/mL)	Female Monkey Safety Margin
92.1	3730	61,900	16.6	58,400	15.7	38,900	10.4

[a] Clinical doses given on Days 1, 15, 29.
[b] GLP toxicology study doses given on Days 1, 2, 3, 22, 23, 24.

It is important to note that while weight-based safety margins are calculated based on animal PDD compared to human RDD or DD, the RDD and DD are **not** the dose loaded into the nebulizer device. The nebulizer loaded dose required to achieve a certain RDD depends on the efficiency of the nebulizer. A table converting RDD into actual nebulizer loading dose is provided **(Table 3).**

Table 3: Nebulizer loaded dose for each cohort

Cohort	Nebulizer Loaded Dose	Concentration	Loaded Volume for Nebulization	Approximate Nebulization Time	Calculated Respirable Delivered Dose (RDD)
1	20 mg	10 mg/mL	2.0 mL	3-5 min	2.9 mg
2	40 mg	10 mg/mL	4.0 mL	6-8 min	6.6 mg
2b	40 mg	10 mg/mL	4.0 mL	6-8 min	6.6 mg
3	65 mg	10 mg/mL	6.5 mL	13-15 min	12.5 mg
3b	65 mg	10 mg/mL	6.5 mL	13-15 min	12.5 mg
4	180 mg	40 mg/mL	4.5 mL	10-12 min	30.7 mg
4b	180 mg	40 mg/mL	4.5 mL	10-12 min	30.7 mg
5*	180 mg	40 mg/mL	4.5 mL	10-12 min	30.7 mg
6*#	490 mg (245 mg)	40 mg/mL	12.2 mL (6.1 mL)	30 min (15 min)	92.1 mg (46 mg)

*Doses for cohorts 5 and 6 represent the maximum potential dose, which may be decreased at the decision of the sponsor, based on existing data from prior cohorts.
*#Cohort 6 dose will require 2 nebulizations. Top line indicates total quantity and bottom line indicates quantity per nebulization (in parentheses)

6. STUDY OBJECTIVES AND ENDPOINTS

The objective of the study is to assess the safety, tolerability, pharmacokinetics of ARO-ENaC in normal healthy volunteers (NHVs) and patients with Cystic Fibrosis (CF).

6.1. Primary Endpoint

1. The incidence and frequency of adverse events over time through end of studyusing escalating multiple doses in NHVs and in CF patients.

6.2. Secondary Endpoints

1. Changes from pre-dose baseline in serum electrolytes in NHVs and CF patients as a safety measure.
2. Changes from pre-dose baseline in Forced Expiratory Volume (FEV1) in NHVs as a measure of safety.

3. Pharmacokinetics of ARO-ENaC in NHVs and in CF patients

6.3. Exploratory Endpoints

1. Changes from pre-dose baseline in FEV1 in CF patients
2. Changes from pre-dose baseline in lung clearance index (LCI) in CF patients
3. Changes from pre-dose baseline in the revised cystic fibrosis questionnaire (CFQ-R) in CF patients
4. Rate of pulmonary exacerbations in CF patients receiving ARO-ENaC versus PBO
5. Changes from pre-dose baseline in body mass index (BMI) in CF patients
6. Plasma metabolite identification in NHVs (reported in a separate report outside of this study)
7. Determination of urinary excretion and metabolite identification in NHVs (reported in a separate report outside of this study)
8. Changes from pre-dose baseline to Day 18 in expression of ENaC using bronchoscopic brush biopsy samples in NHVs
9. Changes from pre-dose baseline to Day 18 in expression of ENaC using bronchoalveolar lavage (BAL) samples in NHVs
10. Changes from pre-dose baseline to Day 18 in cytokine levels as well as cell count and differential in BAL fluid in NHVs

7. STUDY PLAN

7.1. Study Design

***Normal Healthy Volunteers**:*

While ARO-ENaC is intended for use in CF patients, these patients suffer from frequent disease exacerbations and are frequently on multiple concomitant medications. For this reason, this study initially evaluates each dose level in a healthy volunteer population to generate a baseline understanding of safety in a population with normal physiology and without the confounding factor of concomitant medication use.

For NHVs, 4 cohorts of 6 eligible subjects (4 active: 2 PBO) will be evaluated at each dose level starting with Cohort 1. Cohort 5 will include 12 eligible patients (8 active: 4 placebo) who will be treated with a dose equal to or less than that given to cohort 4, with the purpose of collecting bronchoscopic samples to evaluate for αENaC mRNA knockdown. Participants who have signed an IRB/EC approved informed consent form and have met all of the protocol eligibility criteria during screening, will be randomized to receive ARO-ENaC or PBO. NHV cohorts will receive a single cycle of three doses all at a fixed dose level administered daily on Days 1, 2, 3.

NHV cohorts 1-4 will begin with administration of ARO-ENaC or PBO to two sentinel participants (one ARO-ENaC, one PBO). Following the Day 4 evaluation in these participants, if there are no significant safety concerns based on PI's judgement, the remaining participants in the cohort will be dosed. Cohort 5 will not utilize sentinel participants as the dose evaluated in cohort 5 will be equal to or lower than doses used in cohorts 1-4. Dosing of participants will be staggered by at least 30 minutes such that no two participants will be dosed simultaneously. Blood samples will be drawn pre-dose on Day 1 for baseline measurements.

Based on observations for all NHV subjects in a cohort through Day 21, and experience from any prior cohorts (i.e. all cumulative available safety data), the DSC will meet to vote on the following (See Fig. 1):

1. Dose escalation to next NHV cohort
2. Initiation of CF cohort (Cohorts 2b, 3b, 4b) when applicable per **Figure 1**.

Escalation to the next highest dose level in NHVs will proceed in cohorts of 6 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.

Following an affirmative vote from the DSC meeting held during NHV cohort 4 allowing the opening of CF Cohort 4b, NHV cohort 5 may be subsequently opened for accrual. The primary purpose of cohort 5 will be to assess the effect of ARO-ENaC on αENaC mRNA expression, with samples obtained via bronchoscopy. The dose in cohort 5 will be equivalent to or less than the dose utilized in cohort 4. Based on available safety data reviewed during prior DSC meetings, the Sponsor or DSC may adjust the dose in cohort 5 downward.

The DSC will consist of at least one study Investigator, Sponsor Medical Monitor, Sponsor pharmacovigilance lead, and an independent physician experienced in pulmonary drug development and/or early stage clinical trials.

Cystic Fibrosis Patients:

Cohorts 2b, 3b, 4b will enroll eligible CF patients who have signed an IRB/EC approved informed consent form to receive two cycles of ARO-ENaC or PBO administered daily on Days 1, 2 and 3, then again on Days 22, 23 and 24. Screening for the CF cohorts can begin once Cohort 2 dosing has commenced but dose administration may not proceed until after the DSC has opened the designated CF patient cohort. CF patient cohorts 2b, 3b, and 4b will be opened for accrual once the corresponding NHV cohort receiving the same dose level has reached Day 21 and based on DSC vote that it is safe to proceed. CF patient cohorts 2b, 3b, and 4b will enroll in sequence with 2b enrolling first, followed by 3b and then Cohort 4b.

Once all subjects in CF cohort 4b have completed the Day 29 study visit, the DSC will meet to review all cumulative safety data to date and to vote on the initiation of CF cohort 6. Following an affirmative vote from this DSC meeting, CF cohort 6 may be opened for accrual. The purpose of cohort 6 is to assess the safety and efficacy of an alternate dosing regimen. In CF cohort 6, the 3- day dosing cycle used in cohort 4b is compressed to a single dose, which is given at 2 week intervals, such that subjects will be dosed on Days 1, 15, and 29. The dose given on each dosing day to a subject in CF cohort 6 (in respirable delivered dose [RDD] terms, which represents the amount of drug that reaches the lung, as opposed to the amount of drug loaded into the nebulizer) will be ≤ 3x the dose given on each dosing day to a subject in CF cohort 4b. Thus, the dose (in RDD terms) given on Day 1 to a subject in cohort 6 will be ≤ the total cumulative dosage given over Days 1, 2, and 3 to a subject in cohort 4b. Of note, the RDD does not scale identically to the nebulizer loaded dose (i.e. tripling the RDD does not necessarily result in tripling the nebulizer loaded dose).

Blinding (where applicable) will be preserved to the extent possible. However, treatment un-blinding or un-blinding 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. After all subjects in a cohort (except cohort 6) have completed an End-of-Study visit, Sponsor may be unblinded to that cohort. PI and study participants will remain blinded.

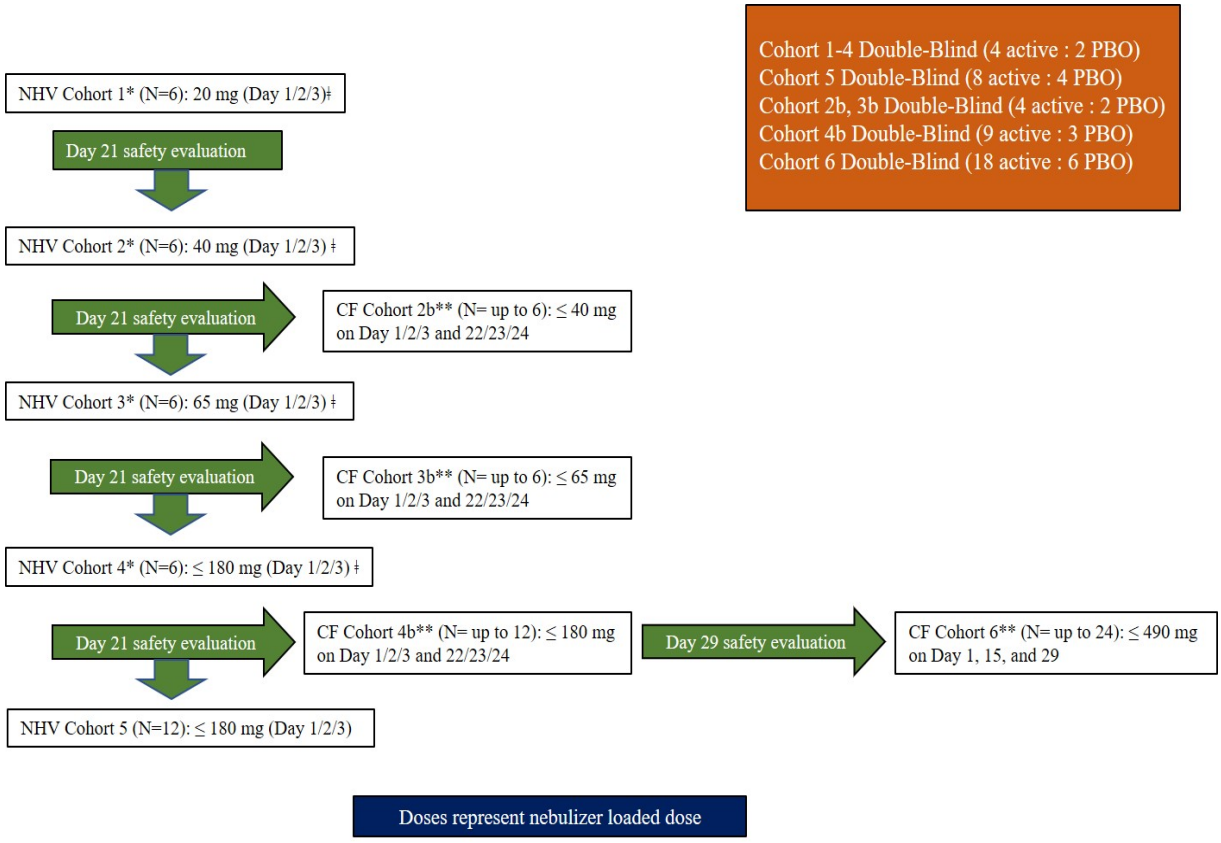
Primary analysis is planned once both all subjects in cohort 6 have completed the Day 43 visit and all subjects from other cohorts have completed EOS. PIs and study participants will remain blinded through EOS. Sponsor will be unblinded to analyze both safety and efficacy endpoints for all NHV and CF cohorts. Final Analysis is planned when all subjects ended study and database is locked. Safety and efficacy results will be updated based on complete database in final analysis.

Table 4: Cohort Summary

Cohort	Population	Blinding	# Subjects	Dosing Schedule
1	NHVs	Double-blind	6 (4 active: 2 PBO)	20 mg Day 1, 2, 3
2	NHVs	Double-blind	6 (4 active: 2 PBO)	40 mg Day 1, 2, 3
3	NHVs	Double-blind	6 (4 active: 2 PBO)	65 mg Day 1, 2, 3
4	NHVs	Double-blind	6 (4 active: 2 PBO)	≤ 180 mg Day 1, 2, 3
5	NHVs	Double-blind	12 (8 active: 4 PBO)	≤ 180 mg Day 1, 2, 3
2b	CF Patients	Double-blind	Up to 6 (4 active: 2 PBO)	≤ 40 mg dosed on Day 1, 2, 3; ≤ 40 mg dosed on Day 22, 23, 24
3b	CF Patients	Double-blind	Up to 6 (4 active: 2 PBO)	≤ 65 mg dosed on Day 1, 2, 3; ≤ 65 mg dosed on Day 22, 23, 24
4b	CF Patients (ppFEV1 > 70%)	Double-blind	Up to 12 (9 active: 3 PBO)	≤ 180 mg dosed on Day 1, 2, 3; ≤ 180 mg dosed on Day 22, 23, 24
6	CF Patients	Double-blind	Up to 24 (18 active: 6 PBO)	≤ 490 mg dosed on Day 1; ≤ 490 mg dosed on Day 15; ≤ 490 mg dosed on Day 29

The dose escalation schedule is outlined in **Figure 1.**

Figure 1: Dose Escalation Schedule



* Cohorts 1-4 each use 2 sentinel subjects dosed before the rest of the cohort. Where specified, dose represents the highest dose that may be used in the cohort. The DSC or Sponsor may adjust the dose downward if indicated based on available safety data.

ǂ specified dose given once on Day 1, Day 2 and Day 3
** Cohorts 2b, 3b, 4b, and 6 enroll in sequence per Figure 1 and after associated DSC approval.

7.2. Rationale for Study Design

While ARO-ENaC is intended for use in CF patients, these patients suffer from frequent disease exacerbations and are frequently on multiple concomitant medications. For this reason, this study initially evaluates each dose level in a healthy volunteer population to generate a baseline understanding of safety in a population with normal physiology and without the confounding factor of concomitant medication use.

This FIH study plans to investigate ARO-ENaC in normal healthy volunteers to evaluate the drug's safety and tolerability. The study initiates in healthy volunteers because the risk is considered low based on GLP animal toxicity testing. It is expected that treatment of CF with ARO-ENaC will require multiple doses; thus, this study in healthy volunteers transitions from a single cycle of three doses to two cycles of three doses each (on Days 1, 2, 3 and Days 22, 23, 24) in patients with CF if safety data is acceptable to DSC.

Cohort 5 is included in the study to evaluate for changes in αENaC expression following ARO- ENaC

dosing in NHVs, utilizing samples obtained via bronchoscopy, as there is no known serum marker of pharmacodynamic effect for ARO-ENaC.

CF cohort 6 is included in the study to compare the safety and efficacy of compressing the dosing regimen of ARO-ENaC from daily dosing over three days to one single, larger dose given every two weeks. The rationale for testing this dosing regimen is that sheep pharmacology studies indicate similar efficacy of ARO-ENaC when given via one single, larger dose as when given via three smaller daily doses. Additionally, sheep studies indicate a waning of drug effect at 3 weeks post-dose, providing the rationale for testing a 2 week dosing interval.

All cohorts in the study are double-blind to limit the occurrence of conscious and unconscious bias in trial conduct and interpretation. Blinding will be achieved using a PBO (0.9% normal saline). Inclusion of participants receiving PBO will reduce bias in the assessment of drug safety and tolerability. The patient cohorts are proposed to understand multiple dose safety, pharmacodynamics and pharmacokinetics which should assist with dose selection in later stage clinical studies.

LCI data will be collected for all subjects in cohorts 4b and 6 and for a subset of patients in cohorts 2b and 3b (the subset of patients with ppFEV1 >70%). LCI collection is feasible but time consuming in those with more severe lung disease; for this reason, CF cohorts 2b, 3b and 4b only assess LCI in those with ppFEV1 >70% and CF cohort 4b only enrolls patients with ppFEV1 > 70%. CF cohort 6 trials a different approach, and will collect LCI measurements on all subjects in the cohort, even those with ppFEV1 < 70%.

7.3. DSC and Criteria for Dose-escalation and Dose Limiting Toxicities

Escalation to the next cohort will proceed according to the study design until the highest planned dose cohort is completed, unless the trial is stopped early by the DSC, PI or Sponsor. Dose escalation will require approval by the DSC based on evaluation of all available safety and, when available, pharmacodynamic data through at least Day 21 of the most advanced NHV cohort. The DSC meeting held during CF cohort 4b will review all cumulative safety data to date, through the Day 29 visit of CF cohort 4b.

7.4. Stopping Rules & Data Safety Committee

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

1. A single Serious Adverse Event (SAE, defined in Section 11.1) considered at least possibly related to study drug
2. Two cases of a drop from baseline FEV1 (which must be confirmed on repeat within 48 hours) **in healthy volunteer cohorts** of ≥ 20% (absolute decline in % of predicted) considered to be at least possibly related to study drug by study investigator
3. One case of a drop from baseline FEV1 (which must be confirmed on repeat within 48 hours) **in cystic fibrosis cohorts** of ≥ 20% (absolute decline in % of predicted) considered to be probably related to study drug by study investigator.
4. A post-dose increase in serum potassium to > 5.7 milliequivalents/Liter which must be from a non-hemolyzed sample and confirmed by repeat blood draw within 48 hours of initial results.

If any such events occur, within 5 days of Sponsor being notified of the event, the DSC will meet to review the event and determine any necessary actions as detailed below. Further dosing of the subject who triggered the stopping rule will be paused until such DSC review occurs.

Central labs will be utilized. However local labs may be utilized as necessary (including for Screening) if needed by the investigator or for emergent situations, including time critical analytes such as serum potassium and repeat clinical chemistries.

The Sponsor or PI 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. Including, but not limited to the events listed above, the DSC **may** pause the study to 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
- The study is stopped immediately with no further dosing
- The study will continue until the current cohort is completed
- The study will continue, but the next dose escalation will be to a level midway between the current level and the next planned level
- The study will continue as planned

The DSC will consist of at least the Sponsor Medical Monitor, Sponsor pharmacovigilance lead, a study Investigator and an independent physician experienced in Phase 1 clinical trials.

7.5. Primary Analysis and Final Analysis

Primary analysis is planned once both all subjects in cohort 6 have completed the Day 43 study visit and all subjects from other cohorts have completed EOS. PIs and study participants will remain blinded through EOS. Sponsor will be unblinded to analyze both safety and efficacy endpoints for all NHV and CF cohorts. Final Analysis is planned when all subjects have ended the study and the database is locked. Safety and efficacy results will be updated based on complete database in final analysis.

7.6. Duration of the Study

For each NHV subject the approximate duration of the study clinic visits is a maximum of 8 weeks from the beginning of the Screening period to the Day 29 End-of-Study (EOS) examination.

For each CF patient, the duration of the study is approximately 20 weeks from the beginning of the Screening period to the Day 113 EOS examination.

8. PATIENT SELECTION

8.1. Number of Patients

Approximately 36 NHVs and up to 56 CF patients (including up to 2 extra patients per cohort in Screening and not including potential replacements) may be enrolled in the study.

8.2. Inclusion Criteria

Applicable to NHV and CF Cohorts

To be eligible for participation, NHVs and CF patients must meet all the following inclusion criteria:

1. Male or non-pregnant, non-lactating female volunteers 18-55 years of age, inclusive at time of Screening
2. Normal ppFEV1 (>80%) at Screening in NHVs only
3. Able and willing to provide written informed consent prior to the performance of any study specific procedures
4. Participants with a BMI between 18.0 and 35.0 kg/m2, inclusive, at Screening
5. A 12-lead ECG at Screening with no abnormalities that may compromise participant's safety in this study
6. Non-smoker (smoker is defined as smoking cigarettes daily for at least the past 12 months) with current non-smoking status confirmed by urine cotinine at Screening AND any previous smoking history prior to 12 months must be < 15 pack years. Patients may be on nicotine replacement (patch or gum). e-cigarettes (vapor) is not permitted. A positive urine cotinine result due to nicotine replacement is acceptable for enrollment at the discretion of the PI.
7. Participants of childbearing potential must agree to use highly-effective contraception during the study and for at least 12 weeks following the end of the study or last dose of study drug, whichever is later. Males must not donate sperm during the study and for at least 12 weeks following the end of the study or last dose of study drug, whichever is later. (See Section 10.6 for details regarding highly-effective contraception).
8. Participants who are willing and able to comply with all study assessments and adhere to the protocol schedule
9. No abnormal finding of clinical relevance at the Screening evaluation other than CF for CF patients
10. No planned significant dietary changes during the study period.

Additional Inclusion Criteria for CF Patients

To be eligible for participation, CF patients must meet all the following inclusion criteria:

1. All other treatments for CF (including CFTR modulators/correctors) have been stable for at least two months (meaning no new medications or changes in dose) prior to first dose and patient is willing to continue this treatment regimen without change for study duration.
2. Confirmed diagnosis of CF based on source verifiable medical record
3. Percent predicted FEV1 of ≥ 40 to ≤ 90% adjusted for age, sex, and height according to NHANES III at the Screening Visit. For Cohort 4b patients must have ppFEV1 > 70%.
4. Participants with a BMI between 18.0 and 35.0 kg/m^2, inclusive, at Screening. ***Participants with BMI between 15-18 kg/m^2 or between 35-40 kg/m^2 may be enrolled at the PI's discretion in consultation with Sponsor depending on co-morbidities.***

8.3. Exclusion Criteria

Applicable to NHV and CF Cohorts unless otherwise specified

A potential NHV and CF participant will be excluded from the study if any of the following criteria apply:

1. Acute lower respiratory infection within ***30 days*** of Screening (NHVs only)
2. Any history of anaphylaxis
3. Human immunodeficiency virus infection, as shown by the presence of anti-HIV antibody (sero-positive)
4. Seropositive for HBV or HCV (positive for anti-HCV antibody must be confirmed with positive HCV-RNA test for exclusion)
5. Uncontrolled hypertension (BP > 150/100 mmHg at Screening)
6. A history of torsades de pointes, ventricular rhythm disturbances (e.g., ventricular tachycardia or fibrillation), pathologic sinus bradycardia (<50 bpm with symptoms), heart block (excluding first-degree block, being PR interval prolongation only), congenital long QT syndrome or new ST segment elevation or depression or new Q wave on ECG. Participants with a history of atrial arrhythmias should be discussed with the Medical Monitor
7. A clinically significant history of hyperkalemia or hyperkalemia at Screening
8. A family history of congenital long QT syndrome or unexplained sudden cardiac death
9. Medications known to prolong the QTc interval (NHVs only, not applicable to CF patients)
10. Use of prescription diuretics (including amiloride, spironolactone, other potassium sparing diuretics), ACE inhibitors, angiotensin receptor blockers, trimethoprim, potassium supplements, digoxin, cyclosporine, or tacrolimus. CF patients may be eligible after 5-half-life washout period for applicable medication.
11. Symptomatic heart failure (per NYHA guidelines), unstable angina, myocardial infarction, severe cardiovascular disease (ejection fraction < 20%, transient ischemic attack (TIA) or cerebrovascular accident (CVA) within 6 months prior to study entry)
12. History of malignancy within the last 2 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 >2-year disease-free interval may be entered following approval by the Medical Monitor
13. History of major surgery within ***3 months*** of Screening
14. 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)
15. Use of illicit drugs (such as cocaine, phencyclidine [PCP]) within 1 year prior to the Screening visit 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)

16. Use of an investigational agent or device within 30 days prior to first dose or current participation in an investigational study
17. Blood donation (500 mL) within 7 days prior to first dose. Donation or loss of whole blood (excluding the volume of blood that will be drawn during the Screening procedures of this study) prior to administration of the study treatment 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 study treatment administration
18. Any concomitant medical or psychiatric condition or social situation that would make it difficult to comply with protocol requirements or put the participant at additional safety risk
19. Participants who are unable to return for all scheduled study visits
20. For NHV cohorts only, ALT or AST > ULN at Screening
21. Serum sodium < LLN or serum potassium > ULN at Screening
22. eGFR of < 60 mL/min/m2, at Screening
23. Platelet count < LLN at Screening (NHVs only)

Additional Exclusion Criteria for CF Patients

A potential participant will be excluded from the study if any of the following criteria apply:

1. CF exacerbation within ***30 days*** of first dose
2. History of solid organ transplant
3. ALT or AST > ***2.5 X*** ULN at Screening
4. Platelet count < 100,000 at Screening
5. Diagnosis of hepatic cirrhosis based on medical history and documented in medical record or other source document.

Notes:

- Sponsor Medical Monitor has the option to exclude the enrollment of a participant if, based upon the participant's medical history or Screening results, it is felt that a participant's safety may be at risk and/or if the safety data may confound or conflict with the study results.
- All spirometry tests used as inclusion criteria and all laboratory tests used as exclusion criteria may be repeated once during Screening, with the repeat value used for inclusion/exclusion purposes.

8.4. Participant Withdrawal Criteria

Participants will be advised that they are free to withdraw from the study at any time for any reason or, if necessary, the PI, or medically trained designee, may withdraw a participant from the study, per the following criteria, to protect the participant's health:

- the need to take medication which may interfere with study measurements
- intolerable/unacceptable AEs
- major violation of or deviation from study protocol procedures

- non-compliance of participant with protocol
- participant unwilling to proceed and/or consent is withdrawn
- withdrawal of the participant from the study if, in the PI's judgement, it is in the participant's best interest
- confirmed pregnancy (Note: within 24 hours of awareness of the pregnancy the PI must notify the Sponsor [see Section 11].)

The reasons for withdrawal will be recorded on the case report form (CRF) and included in the final clinical study report, along with any AEs and any necessary medical treatment.

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

Participants who are withdrawn or discontinue prior to EOS visit for reasons other than an adverse event, may be replaced at Sponsor discretion.

8.5. Restrictions and Concomitant Medications

- ***Confinement & Study visits:*** For NHV cohorts, clinical facility confinement will be approximately 4 days for single dose administration (Day -1 through Day 4 assessments in cohorts 1-4 and Day 0 through Day 4 in cohort 5). Subjects will be admitted to the facility on Day -1 (or Day 0 in cohort 5) and will undergo assessments at the facility on Days 1, 2, 3 and 4 prior to discharge. After Day 4 assessments NHV participants can be discharged and will return to the clinical facility starting on Day 5 for out-patient visits per the Schedule of Assessments (SOA). For CF patients, clinical facility confinement will be approximately 6 hours on dosing days unless additional monitoring at Principal Investigator (PI) discretion is needed for safety reasons. Patients will return to the clinical facility for outpatient visits. There is no planned overnight confinement for CF patients.

- ***Fasting:*** On the day of dosing or on other days with blood draws, participants will have fasted from food for at least 4 hours prior to study treatment administration or blood draw unless otherwise specified or as otherwise required by study procedures. Fasting from food and drink will be required beginning at midnight on the night prior to a scheduled bronchoscopy, or as otherwise required by study procedures.

- ***Recreational Drugs & Alcohol:*** Participants will be instructed to abstain from consuming alcohol for at least 48 hours prior to admission, and while confined to the clinical facility. In addition, participants will be instructed to refrain from use of alcohol above these limits: >14 units per week for women or >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. Participants must abstain from use of recreational drugs throughout the study.

- ***Concomitant Medications:*** NHVs and CF patients may not use prescription diuretics

(including amiloride, spironolactone, other potassium sparing diuretics), ACE inhibitors, angiotensin receptor blockers, trimethoprim, potassium supplements, digoxin, cyclosporine, or tacrolimus. Use of other concomitant medications may be approved by Sponsor Medical Monitor and PI. Patients will be instructed to inform the PI of the details (indication, dose and dates of administration) if they do take any medication, and these details will be recorded in the CRF. Medication wash-out to achieve study eligibility at screen constitutes a protocol procedure and will be preceded by informed consent. In CF patients and in NHV cohorts, protein powders, multi-vitamins (as long as they contain less than 40 mEq potassium taken per day) and fish oil are acceptable. Other supplements should be approved by the Medical Monitor.

9. INVESTIGATIONAL PRODUCT

9.1. Description, Identification and Dosage

Arrowhead Pharmaceuticals, Inc. is responsible for the supply of active drug supplies together with detailed instructions (in a pharmacy manual as well as a study procedure manual) describing preparation and administration of ARO-ENaC Inhalation. The PBO (0.9% normal saline) will be supplied by the clinical site.

Accordingly, ARO-ENaC Inhalation is provided as a sterile solution containing the API, an RNAi trigger molecule conjugated to a targeting moiety for the epithelial integrin αvβ6 (ADS-003), ready for inhalation by nebulization.

The PBO will be nebulized 0.9% normal saline.
Doses Administered per Dose Level:

Each single dose of either active drug (ARO-ENaC) or PBO (normal saline 0.9%), will be administered by inhaled nebulized solution. ARO-ENaC Inhalation is manufactured by dissolution of the lyophilized API into an aqueous normal saline solution which is then aseptically filled into glass vials. The resulting solution, ARO-ENaC Inhalation, is administered to the patient by inhaled nebulized solution.

There will be no dose escalation within a cohort (i.e., the same drug dose will be administered to each participant within a cohort). The randomization schedule for NHVs and CF patients where applicable will be provided to each clinical site and will be maintained along with any other materials that could jeopardize the blind in a secured area of the pharmacy.

The per protocol specified dose to be administered is the Nebulizer Loaded Dose. The nebulizer treatment should be administered until the full loaded volume for nebulization has been administered.

Cohort	Nebulizer Loaded Dose	Concentration	Loaded Volume for Nebulization	Approximate nebulization time	Calculated respirable delivered dose (RDD)
1	20 mg	10 mg/mL	2.0 mL	3-5 min	2.9 mg
2	40 mg	10 mg/mL	4.0 mL	6-8 min	6.6 mg
2b	40 mg	10 mg/mL	4.0 mL	6-8 min	6.6 mg
3	65 mg	10 mg/mL	6.5 mL	13-15 min	12.5 mg
3b	65 mg	10 mg/mL	6.5 mL	13-15 min	12.5 mg
4	180 mg	40 mg/mL	4.5 mL	10-12 min	30.7 mg
4b	180 mg	40 mg/mL	4.5 mL	10-12 min	30.7 mg
5*	180 mg	40 mg/mL	4.5 mL	10-12 min	30.7 mg
6*#	490 mg (245 mg)	40 mg/mL	12.2 mL (6.1 mL)	30 min (15 min)	92.1 mg (46 mg)

*Doses for cohorts 5 and 6 represent the maximum potential dose, which may be decreased at the decision of the Sponsor, based on existing data from prior cohorts.
*#Cohort 6 dose will require 2 nebulizations. Top line indicates total quantity and bottom line indicates quantity per nebulization (in parentheses).

Supply, Preparation, Storage, and Labelling of ARO-ENaC

ARO-ENaC Inhalation is an aqueous solution in a sterile, 10-mL type I glass vial with a fluorocarbon-lined butyl stopper and an orange flip-off seal. Each vial contains a nominal volume of 4.2 mL to provide a full 4.0 mL withdrawable volume.

Strength: 40 mg/mL

Volume: 4.0 mL

Appearance: Clear, colorless to yellow solution

Inactive ingredients: 0.9% saline (0.9% sodium chloride in water)

Shipment and storage: Refrigerated, 2-8 ºC

Stability of each lot of ARO-ENaC inhalation is monitored concurrently with the clinical program under both normal and accelerated conditions in accordance with ICH Guidelines.

ARO-ENaC will be prepared, per the Pharmacy Manual, by a pharmacist or qualified staff

member at the clinical sites. The time of preparation for active drug must be documented and tracked to demonstrate administration within prepared drug stability boundaries. Please refer to the Pharmacy Manual for more detailed instructions.

The investigational product vials will be labelled per Good Manufacturing Practice (cGMP)/Good Clinical Practice (cGCP).

Study drug supplies will be stored at clinical sites securely under the appropriate conditions as noted in the Pharmacy Manual.

9.2. Study Drug Handling

The Sponsor will provide the PI with a sufficient quantity of clinical drug supplies. A Pharmacy Manual will be prepared to define the procedures for ordering, storage, and dispensing. Procedures for study drug administration are included in the Study Procedure Manual.

The PI must ensure that deliveries of investigational product 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 recommended storage conditions. It is also the responsibility of the PI to ensure that the integrity of packaged study product not be jeopardized prior to dispensing.

Only participants enrolled in the study may receive study drug, in accordance with all applicable regulatory requirements. Only authorized and trained site staff may dispense or administer study drug. The study drug 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 specific requirements stated in the Pharmacy Manual.

ARO-ENaC will be supplied by Arrowhead Pharmaceuticals, Inc. and labelled with the drug name, batch number, lot number, expiration date (as applicable), and storage conditions. Individual doses will be dispensed by clinical trial site staff members on the day of dosing and recorded in the drug accountability records.

Procedures delineated in the Pharmacy Manual will be followed for the receipt, handling and accountability of the study drug.

9.3. Accountability of Study Supplies

All study related material supplied is only for use in this clinical trial and should not be used for any other purpose. The PI is responsible for the investigational product accountability, reconciliation and record maintenance at the investigational site. In accordance with all applicable regulatory requirements, the PI or designated site staff must maintain investigational product accountability records throughout the course of the study. This person will document the amount of investigational product received from Arrowhead Pharmaceuticals, Inc. and the amount administered to participants. A non-blinded Clinical Research Associate (CRA) will perform initial and ongoing study drug accountability. The non-blinded CRA will protect the integrity of the assignment blind and will not participate in data review for study participants. Used vials of ARO-ENaC will be retained sequestered per participant and cohort (where allowable by local policy) and made available to the non- blinded CRA during study drug reconciliation.

A Drug Dispensing Log must be kept current and will contain the following information:

- the identification of the participant to whom the drug was dispensed
- the date(s) and quantity of the drug dispensed to the participant.

The date and time of dose preparation and release will be maintained to support administration of study drug. The pharmacy will dispense the study medication and the clinical site staff will administer the study medication only to participants included in this study following the procedures set out in the study protocol and Pharmacy Manual. Study drug administration will be documented on the CRFs and/or other study drug accountability record. The inventory must be available for inspection by the CRA during the study. Drug supplies, excluding partially used or empty containers, will either be collected at the end of the study by the CRA or returned by the PI or designee to Arrowhead Pharmaceuticals Inc. or the designated Arrowhead approved depot.

9.4. Retention of Investigational Product Vials

For this study, used and partially used drug vials will be retained, if allowed by local policy, for an adequate period to allow accountability by the CRA. No additional study drug samples will be retained.

9.5. Allocation to Treatment

All potential participants who sign an informed consent at Screening will receive a unique 6-digit number (i.e. a Screening Number). The first 3 digits will represent the assigned site number and will be the same for each participant that screens at an individual site. The next 3 digits will be assigned sequentially (starting with 001). For patients who are deemed eligible, this 6-digit screening number will become the patient's permanent study ID number.

Eligible subjects in double-blind cohorts will be allocated a unique randomization number, in accordance with the randomization schedule. In each of NHV Cohorts 1-4, the first two subjects (sentinels) will be randomized separately to one active and one PBO. Each subject will be assigned to either active (ARO-ENaC) or PBO treatment. The allocation of active treatment or PBO will be performed using a block randomization algorithm.

Participants who drop out prior to their EOS visit for reasons other than an adverse event, may be replaced.

9.6. Blinding and Code-Break

Blinding of study drug/PBO assignment is critical to the integrity of this clinical trial. It is expected that in most cases, AEs can be properly managed without the need for unblinding. However, in the event of a medical event in which knowledge of an individual participant's assignment is considered relevant to the participant's well-being, treatment options and management, the PI, the Sponsor Medical Monitor or a documented designated treating physician may request unblinding of treatment assignment. PI requests to unblind will be directed to the Medical Monitor. The randomization schedules will be maintained under controlled access. The personnel involved in the dispensing of investigational products will be accountable for ensuring compliance to randomization schedules. The non-blinded CRA will review the randomization schedule in comparison to the dispensing log to verify correct randomization.

If the PI considers an adverse event 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 system. The 'Medical Emergency Unblinding' form in IWRS is only accessible to the designated unblinded Pharmacist, PI and Sub-I. The study monitor should be informed promptly.

If a participant requires emergent 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.

After the completion of the final study visit for each cohort, unblinding for Sponsor analysis will occur at Sponsor discretion. Additionally, after all subjects in CF cohort 6 have completed the Day 43 study visit and all subjects from other cohorts have completed EOS, Sponsor will be unblinded for the purpose of conducting a primary analysis. After all subjects in all cohorts have completed their EOS visit, all cohorts may be unblinded.

10. STUDY METHODS AND SCHEDULES

10.1. Overview of Procedures

Normal Healthy Volunteers

While ARO-ENaC is intended for use in CF patients, these patients suffer from frequent disease exacerbations and are frequently on multiple concomitant medications. For this reason, this study initially evaluates each dose level in a healthy volunteer population to generate a baseline understanding of safety in a population with normal physiology and without the confounding factor of concomitant medication use.

For NHVs, 4 cohorts of 6 eligible subjects (4 active: 2 PBO) will be evaluated at each dose level starting with Cohort 1. Cohort 5 will include 12 eligible subjects (8 active: 4 PBO) who will be treated with a dose equal to or less than that given to Cohort 4, with the purpose of collecting bronchoscopic samples to evaluate for αENaC mRNA knockdown. Participants who have signed an IRB/EC approved informed consent form and have met all of the protocol eligibility criteria during screening, will be randomized to receive ARO-ENaC or PBO. NHV cohorts will receive a single cycle of three doses all at a fixed dose level and administered daily on Days 1, 2, 3.

NHV cohorts 1-4 will begin with administration of ARO-ENaC or PBO to two sentinel participants (one ARO-ENaC, one PBO). Following the Day 4 evaluation in these participants, if there are no significant safety concerns based on PI's judgement, the remaining participants in the cohort will be dosed. Cohort 5 will not utilize sentinel participants as the dose evaluated in cohort 5 will be equal to or lower than doses used in cohorts 1-4. Dosing of participants will be staggered by at least 30 minutes such that no two participants will be dosed simultaneously, blood samples will be drawn pre-dose on Day 1 for baseline measurements.

Based on observations for all NHV subjects in each of cohorts 1-4 through Day 21, and experience from any prior cohorts (i.e. all cumulative available safety data), the DSC will meet to vote on the following (See Fig. 1):

1. Dose escalation to next NHV cohort
2. Initiation of CF cohort (Cohorts 2b-4b) when applicable per **Figure 1**.

Escalation to the next highest dose level in NHVs will proceed in cohorts of 6 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.

Following an affirmative vote from the DSC meeting held during NHV cohort 4 allowing the opening of CF Cohort 4b, NHV cohort 5 may also be subsequently opened for accrual. The primary purpose of cohort 5 will be to assess the effect of ARO-ENaC on αENaC mRNA expression, with samples obtained via bronchoscopy. The dose in cohort 5 will be equivalent to or less than the dose utilized in cohort 4. Based on available safety data reviewed during prior DSC meetings, the Sponsor or DSC may adjust the dose in cohort 5 downward.

The DSC will consist of at least one study Investigator, Sponsor Medical Monitor, Sponsor pharmacovigilance lead, and an independent physician experienced in Phase 1 clinicaltrials.

Cystic Fibrosis Patients

Cohorts 2b, 3b, 4b will enroll eligible CF patients who have signed an IRB/EC approved informed consent form to receive two cycles of ARO-ENaC or PBO administered daily on Days 1, 2 and3, then again on Days 22, 23 and 24. Screening for the CF cohorts can begin once Cohort 2 dosing has commenced but dose administration may not proceed until after the DSC has opened the designated CF patient cohort. CF patient cohorts 2b, 3b, and 4b will be opened for accrual once the corresponding NHV cohort receiving the same dose level has reached Day 21 and based on DSC vote that it is safe to proceed. CF patient cohorts 2b, 3b, and 4b will enroll in sequence with 2b enrolling first, followed by 3b, and then Cohort 4b.

Once all subjects in CF cohort 4b have completed the Day 29 study visit, the DSC will meet to review all cumulative safety data to date and to vote on initiation of CF cohort 6. Following an affirmative vote from this DSC meeting, CF cohort 6 may be opened for accrual. The purpose of cohort 6 is to assess the safety and efficacy of an alternate dosing regimen. In CF cohort 6, the 3- day dosing cycle used in cohort 4b is compressed to a single dose, which is given at 2 week intervals, such that subjects will be dosed on Days 1, 15, and 29. The dose given on each dosing day to a subject in CF cohort 6 (in respirable delivered dose [RDD] terms, which represents the amount of drug that reaches the lung, as opposed to the amount of drug loaded into the nebulizer) will be ≤ 3x the dose given on each dosing day to a subject in CF cohort 4b. Thus, the dose (in RDD terms) given on Day 1 to a subject in cohort 6 will be ≤ the total cumulative dosage given over Days 1, 2, and 3 to a subject in cohort 4b. Of note, the RDD does not scale identically to the nebulizer loaded dose (i.e. tripling the RDD does not necessarily result in tripling the nebulizer loaded dose).

Blinding (where applicable) will be preserved to the extent possible. However, treatment un- blinding may occur, at PI or Medical Monitor discretion, where deemed necessary for treatment of an AE or for a decision to be made regarding trial continuation. After all subjects in a cohort (except cohort 6) have completed an EOS visit, Sponsor may be unblinded to that cohort. PI and study participants will remain blinded.

Primary analysis is planned when both all subjects in cohort 6 have completed the Day 43 study visit and all subjects from other cohorts have completed EOS. PIs and study participants will remain blinded through EOS. Sponsor will be unblinded to analyze both safety and efficacy endpoints for all NHV and CF cohorts. Final Analysis is planned when all subjects ended the study and the database is locked. Safety and efficacy results will be updated based on complete database in final analysis.

Please refer to the dose escalation schedule outlined in **Figure 1.**

During the study (according to the SOA listed in Tables 1.1 – 1.4), participants will undergo the following evaluations at regular intervals: medical history review, physical examinations, vital sign measurements (blood pressure, temperature, heart rate, respiratory rate, pulse oximetry), weight measurement, AE monitoring, ECGs, Chest X-rays, Spirometry, urine pregnancy tests (females of childbearing potential), and concomitant medication review. Blood and urine samples will be collected for clinical laboratory analysis. Blood and urine samples will also be collected for pharmacokinetic and immunogenicity analysis, as well as for plasma metabolite identification and urine excretion and metabolite identification. In addition, NHV participants in Cohort 5 will undergo bronchoscopies. Furthermore, CF patients will be tested for Lung Clearance Index, assessment and reporting of CF pulmonary exacerbations, undergo CF genotyping and sweat chloride testing, and complete a CF questionnaire.

Patient visits to the clinical facility will occur as per the SOA. Clinically significant changes including AEs will be followed until resolution is achieved or considered medically stable. Refer to the SOA for additional information.

The PI (or medically qualified designee) will be required to remain within the clinical study facility for 2 hours on dosing days and will remain on call for the duration of the study. Participants should refrain from strenuous physical activities throughout the study.

10.2. Selection and Screening

Prior to commencement of any screening procedures, the PI, or designee, will inform the participant about the nature and purpose of the study, including the risks and benefits involved, possible AEs, the fact that their participation is voluntary and provide a copy of the EC/IRB - approved Informed Consent Form for review. Each participant will acknowledge receipt of this information by giving written informed consent for their involvement in the study in the presence of the PI, or designee, who will also sign and date the Informed Consent Form. Time of consent will be recorded in the site's source documents and reflected in the eCRFs. The original signed consent form will be retained by the PI and a copy of the original will be given to the participant. Informed consent will be performed per the Principles of the International Conference on Harmonisation (ICH) Good Clinical Practice (cGCP) procedures.

Having given Informed Consent, potential participants will undergo procedures outlined in the SOA, to be performed within 28 days of the scheduled dosing date, to determine that they meet the inclusion/exclusion criteria specified in Sections 8.2 and 8.3.

If a subject were to fail a particular Screening assessment due to a condition which may change over time, then the subject may be allowed to repeat the failed Screening assessment one additional time to establish eligibility and will be deemed not eligible if that Screening assessment is failed the second time. Any repeat Screening assessment must be performed within the specified 28-day Screening window. A subject who is deemed not eligible for the study following completion of Screening may subsequently re-enter Screening, with the opening of a new Screening window and full repeat of Screening assessments, at a future date.

10.3. On-Study Procedures/Assessments

10.3.1. Demographics/Medical History

Medical History will include previously diagnosed medical conditions, medication use over the previous 30 days, including vitamins, over-the-counter medications, prescription drugs,

recreational drugs or supplements and alcohol and tobacco use.

10.3.2. Physical Exam

A complete physical exam will be performed at Screening and Day 1 (pre-dose) with a symptom directed physical exam performed after Day 1. Areas examined include:

- Constitutional
- Skin
- HEENT
- Heart
- Lungs
- Back
- Abdomen
- Extremities
- Mental Status
- Neuro (gait/reflexes)
- Additional examination may be performed at PI discretion

10.3.3. Electrocardiogram

A single 12-lead ECG measurement will be obtained at time points outlined in the SOA after the participant is semi-supine for at least 3 minutes. On dosing days ECGs will be performed pre- dose and 2 hours post-dose and may be performed more frequently if indicated. ECGs should be performed prior to other invasive procedures such as blood draws. Any abnormal ECGs will be repeated in triplicate, with each measurement approximately 1 minute apart.

10.3.4. Chest X-ray

Chest radiographs will be performed as per SOA and in the event that a healthy volunteer or CF patient experiences AEs involving lower lung infection, dyspnea, bronchospasm or CF exacerbation.

10.3.5. Vital Sign Assessments

Systolic/diastolic blood pressure, temperature, heart rate, respiratory rate (breaths/min) and pulse oximetry will be obtained at time points outlined in the SOA after the participant is semi-supine for at least 3 minutes. Vitals signs will be obtained prior to venipuncture and other invasive procedures.

10.3.6. Clinical Laboratory Tests

Blood and urine samples will be collected to perform clinical laboratory tests. Participants will be required to fast at least 8 hours for the screening and at least 4 hours for other sample collections.

Within the screen period, up to 28 days prior to the first dose of study medication, a blood and urine sample will be collected for the laboratory tests detailed below to establish baseline data and eligibility for enrolment. The results will be assessed by the PI, or medically qualified designee, before study enrollment. Any abnormality in laboratory values i.e., those that would jeopardize the safety of the participant or impact on the validity of the study results (that are confirmed on repeat) deemed clinically significant by the PI, or medically qualified designee will result in exclusion of that participant. Clinical laboratory tests will be performed on participants' blood and urine at specified time-points listed in the SOA.

- ***Biochemistry:*** Sodium, potassium, chloride, bicarbonate, glucose, urea, creatinine (including

eGFR), creatine kinase, troponin-I, phosphate, total calcium, albumin, total protein, total bilirubin, lipase and amylase, conjugated bilirubin, gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), and hemoglobin A1C

- ***Hematology:*** Hemoglobin, red blood cell count (RBC), hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, white cell count, neutrophils, lymphocytes, monocytes, eosinophils and basophils
- ***Coagulation:*** Partial thromboplastin time (PTT), Prothrombin time (PT) with INR
- ***Urinalysis:*** Leucocytes, nitrites, urobilinogen, protein, pH, blood, specific gravity, ketone, bilirubin and glucose. Urine electrolytes will also be collected, stored and analyzed as needed.
- ***Microscopic urinalysis will be performed if indicated:*** white blood cells, red blood cells, epithelial cells, bacteria
- ***24 hour urine:*** Aldosterone, sodium, potassium
- ***Serology:*** Hepatitis B surface antigen, Hepatitis C antibody and HIV antibody screen. If necessary, participants will be counseled by the PI, or medically trained designee, concerning the blood tests for Hepatitis B surface antigen, Hepatitis C and HIV antibodies, and their subsequent results.
- ***FSH:*** Post-menopausal status will be confirmed by follicle-stimulating hormone (FSH) level consistent with post-menopausal state.
- ***Pregnancy:*** Females of childbearing potential will have a urine pregnancy test at Screening and pre-dose on the first dosing day of each cycle. If urine pregnancy test is positive, dosing should not occur, and the patient will be referred to their primary care provider for follow up.
- ***Drug and Alcohol Use Screen:*** Urine drug screen for Benzodiazepines, Amphetamines, Barbiturates, Methamphetamines, Methadone, Opiates, Phencyclidine, Cannabinoids, MDMA and Cocaine. Alcohol Breath Test will be done to test for alcohol consumption. A positive alcohol breath test should prompt further discussion about quantity and frequency of alcohol consumption to assess whether the subject meets the alcohol- related exclusion criteria.
- ***Immunogenicity:*** Specified subjects will be assessed for anti-drug antibodies as per SOA.
- ***Genotype:*** All CF patient cohorts will be assessed for mutations causative or otherwise known to influence Cystic Fibrosis, specifically CFTR and ENaC variants with the subject's written consent. If not completed at Screening, blood draws for genotype evaluation may be completed anytime during the study.
- ***Sweat chloride:*** All CF patients will undergo a sweat chloride assessment, if the test is available at the patient's study site. If not able to be completed at Day 1, sweat chloride evaluation may be completed anytime during the study as there is no expectation that study drug treatment should alter sweat chloride levels.

The Day 1 (pre-dose) value will be used as each participant's baseline value for data analysis

purposes or as otherwise specified. If Day 1 or as otherwise specified values are erroneous or not available and repeat blood draw is not possible, the pre-dose value closest to Day 1 (e.g., Screening) may be used as baseline.

10.3.7. Spirometry, CFQ-R and Lung Clearance Index

- ***Spirometry:*** Spirometry will be evaluated per SOA in healthy volunteers and in CF patients. Spirometry will be conducted in accordance with ATS-ERS guidelines (Graham 2019). Sites are required to perform spirometry measurements to achieve three acceptable FEV1 and FVC measurements at each timepoint noted in the protocol Schedule of Assessments (SOA). The largest FVC and the largest FEV1 observed from all of the acceptable values are to be reported in the clinical database, even if they do not come from the same maneuver. Their ratio is used for the FEV1/FVC ratio calculation. All measurements must be documented in the source/progress notes. The required spirometer range to use is NHANES III. In NHVs, spirometry will be performed at a single site. While multiple sites will be utilized to enroll CF patient cohorts, spirometry will be measured in an individual patient at the same facility and with the same spirometry equipment throughout the study.

  Bronchodilator will not be given to either NHVs or CF patients as part of the spirometry assessment. However, there may be NHVs and CF patients who use bronchodilators as concomitant medications during the study. With that in mind, pre-bronchodilator spirometry is defined as a spirometry measurement which is performed after subjects have both:

  - Withheld short-acting bronchodilator(s) (either short-acting beta-agonist or short-acting muscarinic antagonist) for at least 4 hours prior to spirometry
  - Withheld long-acting bronchodilator(s) (either long-acting beta-agonist or long- acting muscarinic antagonist) for at least 12 hours prior to spirometry

  Post-bronchodilator spirometry is defined as a spirometry measurement that is performed which does not meet these criteria (i.e. a subject has used a short-acting bronchodilator within the prior 4 hours and/or a long-acting bronchodilator within the prior 12 hours).

  For the Screening assessment, either pre-bronchodilator or post-bronchodilator spirometry is acceptable.

  For all other (i.e. non-Screening) assessments, the initial spirometry assessment of the visit should be a pre-bronchodilator spirometry measurement. If a subject fails to withhold bronchodilator(s) appropriately in order to accomplish a pre-bronchodilator measurement on a given assessment day, then the following considerations apply:

  - If a subject's Day 1 (pre-dose) baseline spirometry measurement is pre- bronchodilator but the subject fails to withhold bronchodilator on a subsequent visit day, then post-bronchodilator spirometry should be performed on that visit day only
  - If a subject fails to withhold bronchodilator on the Day 1 (pre-dose) baseline spirometry measurement, then the Day 1 spirometry measurement should proceed as a post-bronchodilator measurement. Importantly, all subsequent spirometry measurements for that subject should also be performed post-bronchodilator
  - Each spirometry assessment will be recorded in the source documents as pre- or post-

bronchodilator

Finally, the above considerations only apply to the initial spirometry assessment of a visit. On dosing days, subjects will also undergo repeat (post-dose) spirometry assessments. Those post-dose spirometry assessments may be either pre-bronchodilator or post-bronchodilator, which should be recorded in the source documents. In CF patients, the initial spirometry assessment of a visit will be used for efficacy assessments, while the post-dose spirometry measures will be used as safety assessments.

- **Cystic Fibrosis Questionnaire-Revised (CFQ-R):** The questionnaire provides information about demographics, quality of life and daily activities. Subjects will be asked to complete the CFQ-R in their native language if valid translations are available. If there is no validated translation then the subject will not complete the questionnaire. Copies of the CFQ-R will be provided in the study Procedural Manual.
- **Lung Clearance Index (LCI):** LCI is a measure derived from multiple breath washout (MBW) test which provides an assessment of ventilation heterogeneity, and is defined as the cumulative expired volume at the point where end-tidal inert gas concentration falls below 1/40th of the original concentration, divided by the functional residual capacity (FRC). LCI with be evaluated per SOA in CF patients only and LCI will be measured in any individual patient at the same site. For CF cohorts 2b, 3b, and 4b, at every study visit where LCI is measured per SOA, the site is asked to provide **three** acceptable tests. For CF cohort 6, at every study visit where LCI is measured per SOA, the site is asked to provide **two** acceptable tests (fewer tests in this cohort due to the increased length of the assessment in those with more severe lung disease). For data analysis, the results of all acceptable tests will be averaged. Thus, if a site provides either more or less than the requested number of acceptable tests, all acceptable data will be used for analysis. Test should be performed in accordance with the ERS/ATS consensus statement for inert gas washout measurement. This test will be performed at all sites when available.

  Bronchodilator will not be given to CF patients as part of the LCI assessment. However, there may be CF patients who use bronchodilators as concomitant medications during the study. With that in mind, pre-bronchodilator LCI is defined as a LCI measurement which is performed after subjects have both:

  - Withheld short-acting bronchodilator(s) (either short-acting beta-agonist or short-acting muscarinic antagonist) for at least 4 hours prior to LCI
  - Withheld long-acting bronchodilator(s) (either long-acting beta-agonist or long- acting muscarinic antagonist) for at least 12 hours prior to LCI

  Post-bronchodilator LCI is defined as a LCI measurement that is performed which does not meet these criteria (i.e. a subject has used a short-acting bronchodilator within the prior 4 hours and/or a long-acting bronchodilator within the prior 12 hours).

  All LCI assessments should be pre-bronchodilator. If a subject fails to withhold bronchodilator(s) appropriately in order to accomplish a pre-bronchodilator measurement on a given assessment day, then the following considerations apply:

  - If a subject's Day 1 (pre-dose) baseline LCI measurement is pre-bronchodilator but the subject fails to withhold bronchodilator on a subsequent visit day, then post-bronchodilator LCI should be performed on that visit day only

- If a subject fails to withhold bronchodilator on the Day 1 (pre-dose) baseline LCI measurement, then the Day 1 LCI measurement should proceed as a post-bronchodilator measurement. Importantly, all subsequent LCI measurements for that subject should also be performed post-bronchodilator
- Each LCI assessment will be recorded in the source documents as pre- or post-bronchodilator

10.3.8. Bronchoscopies

Subjects will be required to fast beginning at midnight the night before the procedure. Following sedation with fentanyl and midazolam (or as per local sedation standards), the bronchoscope will be introduced into the airway and topical anesthesia with xylocaine or lidocaine will be applied to the airway. Subsequently, bronchoalveolar lavage (BAL) and bronchial brushing samples will be obtained. Bronchial brushings will be obtained first.

Bronchial brushings: Small airway brushings will be obtained. The bronchoscope will be wedged into a subsegment of the right lower lobe (see Banerjee 2009 for reference). A sheathed cytology brush will be passed down the working channel of the bronchoscope and then unsheathed under fluoroscopic guidance with the brush tip lying 2-3 cm from the pleural surface. Small airway brushings (≤ 5 brushings) will be collected at this site. The cellular material will be washed off in saline. The liquid will be centrifuged and the cell pellet will be frozen and stored. The cell pellet will be utilized for measurement of αENaC expression.

BAL: The bronchoscope will be wedged into a subsegment of the right middle lobe and also into a subsegment of the lingula. In each subsegment, 2 x 50 mL aliquots of normal saline will be instilled and then withdrawn via suction (2 aliquots from right middle lobe and 2 aliquots from lingula). Thus, a total of 200 mL of saline will be instilled into the lungs. BAL fluid will be centrifuged and the resultant supernatant will be collected, frozen and stored. BAL cells will be utilized for cell count and differential. BAL supernatant will be used for measurement of cytokine concentrations (including IL-1β, IL-6, IL-8, and TNFα), as well as for measurement of αENaC expression in BAL exosomes. An aliquot of BAL fluid will be saved for future research purposes upon the decision of the Sponsor.

Data from the Day 0 bronchoscopy assessment will be used as the baseline value.

Further details on handling of bronchoscopy specimens will be available in the Central Laboratory Manual.

10.3.9. Pharmacokinetics

Samples (blood and urine) for analysis of circulating ARO-ENaC will be obtained at time points following study drug administration as outlined in the SOA. ARO-ENaC metabolites will be identified in pooled samples taken at timepoints per the SOA.

10.3.9.1 Blood Sample Collection, Processing, and Analysis

Blood samples from participants will be collected at time points outlined in the SOA through an indwelling cannula or through a fresh vein puncture. The actual blood collection time will be recorded in the source documents. In all such cases, appropriate time corrections, for the actual time of sample collection will be incorporated at the time of data analysis.

The actual sample times will be recorded in the eCRF and will be entered at the time of or as soon as possible after sampling. All times must be recorded in the 24-hour format. An explanation must be given for any blood sample taken outside of the set sampling times.

Whole blood will be collected and processed per the Laboratory Manual.

To avoid hemolysis which can yield a falsely elevated potassium level:

- Mix tubes with anticoagulant additives gently 5-10 times
- Avoid drawing blood from a hematoma
- Avoid drawing the plunger back too forcefully, if using a needle and syringe, or too small a needle, and avoid frothing of the sample
- Make sure the venipuncture site is dry
- Avoid a probing, traumatic venipuncture
- Avoid prolonged tourniquet application or fist clenching

*Any sample with suspected hemolysis should be redrawn and not processed.

Any patient with hyperkalemia reported should be called back for repeat blood draw immediately upon receipt of result.

10.3.9.2 Urine Sample Collection, Processing and Analysis

Urine samples from participants will be collected at time points outlined in the SOA. For spot urine collection, the actual urine collection time and volume will be recorded. For interval sample collections, the start and stop time of urine sample collection (along with volume) will be recorded with an attempt to void at the end of the collection interval. All times must be recorded in the 24-hour format. An explanation must be given for any sample taken outside of the set sampling times.

Urine will be collected and processed per the Laboratory Manual.

10.3.10. Concomitant Medications/Therapies

Participants will be instructed to inform the PI of the details (indication, dose and dates of administration) if they do take any medication, and these details will be recorded in the CRF. Paracetamol may be used during the study only as necessary. CFTR modulators/correctors and other CF standard of care medications are allowed but patients should stay on the same regimen for the duration of the study.

10.3.11. Early Termination Procedures

The reason for Early Termination will be documented in source documents and eCRF. Procedures as outlined in the SOA will be completed.

10.4. Allocation of Treatment

Participants will receive study drug by assigned cohort. Treatments will be administered per the randomized sequence (where applicable) kept by the pharmacy or in a secure place at the clinical site, under control of the un-blinded staff member.

Details of dose volume to be administered to achieve the specified pulmonary delivered dose are presented in the Pharmacy and Study Procedure Manuals.

10.5. Study Treatment Administration & Timing of Procedures

Appropriately trained employees of the clinical site will administer the study treatment. Each dose will be administered as inhaled nebulized solution. The date, time and location of administration will be recorded in the source notes.

Actual times of procedures for each participant will vary depending on scheduling and will be recorded in the CRF.

In the event of multiple procedures scheduled at the same time, non-invasive procedures (i.e., ECGs, AE assessment) will be conducted prior to invasive procedures (i.e., blood sample collection). Timing of activities may be adjusted slightly to accommodate all procedures.

10.6. Pregnancy Testing and Contraception Requirements

Female participants of childbearing potential will have urine pregnancy tests at the Screening and Day 1 (baseline) visits, prior to dosing throughout the study, and at completion of the study as indicated in Tables 1.1-1.4. Females not of childbearing potential must be either surgically sterile or postmenopausal (defined as cessation of regular menstrual periods for at least 12 months without an alternative medical cause) with supportive FSH consistent with postmenopausal state based on laboratory reference ranges. If a subject's urine pregnancy test is positive, the participant will be referred to their primary care provider for follow-up. Female participants with a positive pregnancy test at Screening or on Day 1 predose will not be enrolled in the study. Female participants who become pregnant after Day 1 will not receive any subsequent administration of IP but may otherwise continue in the study.

All participants (female participants of childbearing potential with male partners and male participants with female partners of childbearing potential) must consent to use highly effective contraception during the study and for at least 12 weeks following the end of study or last dose of IP, whichever is later. Males must not donate sperm during the study and for at least 12 weeks following the end of the study or last dose of IP, whichever is later. Highly effective contraception is:

- Using twice the normal protection of birth control by using a condom AND one other form of contraception; either birth control pills (The Pill), or injectable birth control, birth control patch or contraceptive implant associated with inhibition of ovulation, or intrauterine device;
- Surgical sterilization as a single form of birth control: ie, tubal ligation, hysterectomy, bilateral oophorectomy, vasectomy or equivalently effective surgical form of birth control;
- True sexual abstinence for the duration of the study and for at least 12 weeks following the end of study or after the last dose of IP, whichever is later, is acceptable only when in line with the preferred and usual lifestyle of the participant. 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.

Highly effective contraception is to begin at least 7 days prior to initial dosing of IP.

10.7. Safety Assessments

The safety of ARO-ENaC will be evaluated by collection of the following measurements performed at time points as specified in the SOA as well as CS changes from baseline to scheduled time points:

- Monitoring of AEs/SAEs
- Physical examinations
- Vital signs
- ECG measurements
- Chest X-rays
- Measurement of serum and urine electrolytes

- Spirometry
- Clinical laboratory tests (hematology, chemistry, coagulation, urinalysis)
- Concomitant medications/therapy
- Reasons for treatment discontinuation due to toxicity

The AE/SAE reporting period for an enrolled participant will begin when the participant provides informed consent. Treatment-Emergent AEs/SAEs will be those defined as following dose administration. All AEs/SAEs that occur during the AE reporting period specified in the protocol must be reported to Arrowhead Pharmaceuticals, Inc. regardless of the relationship of the AE to study treatment. Any known untoward event that occurs beyond the AE reporting period that the PI considers an SAE and possibly related to study treatment will be reported to Arrowhead.

11. ADVERSE EVENTS

The PI and clinical facility staff are responsible for detection, recording and reporting of events that meet the criteria and definition of various AEs as listed below. Adverse events will be recorded from time of signed consent through to EOS; only AEs that occur post-dose will be considered treatment-emergent. The PI and clinical facility staff are responsible for detection, recording and reporting of pregnancy and appropriate follow up. Any known pregnancy that occurs within 90 days following the end of study visit should be reported by the subject to the PI. Information regarding any reported pregnancy should be collected for at least 1 year after birth or longer if it is decided that additional follow-up is required or until the end of the pregnancy.

11.1. Definitions

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or clinical investigation patient 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 ICH E2a: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, 27 October 1994).

Treatment-emergent AEs will be defined as AEs with onset after administration of the study drug, or when a pre-existing medical condition increases in severity or frequency after study drug administration.

AEs 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 pre-existing 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)
- An overdose of either the investigational product or a concurrent medication without any resulting signs or symptoms

A **Serious Adverse Event (SAE)** is an AE that:

- Results in death

- Is life-threatening (NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event/reaction in which the participant was at immediate risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death, if it were more severe)
- Requires inpatient hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is a medically important event or reaction

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 participant 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, or development of drug dependency or drug abuse.

11.2. Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs

Abnormal assessments (e.g., ECGs and vital signs) that are judged by the PI as clinically significant (CS) 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 CS or results in clinical sequelae. All laboratory abnormalities reported as AEs should by recorded as CS in the laboratory database.

Clinically significant abnormal laboratory findings or other CS 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 CS.

11.3. Timing, Frequency, and Method of Detecting AEs

Any pre-existing conditions or signs and/or symptoms present in a participant prior to the start of the study (i.e., 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 post-dose will be considered treatment-emergent. All AEs must be recorded irrespective of whether they are considered drug-related. AEs will be collected through the EOS.

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

11.4. Recording of AEs

When an AE occurs, it is the responsibility of the PI or medically qualified designee to review all documentation (e.g., 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 CRF. Additional reporting requirements for an AE that meets serious criteria are

discussed in Section 11.7 below.

The PI or medically qualified designee will attempt to establish a diagnosis of the event basedon 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 patient's medical records to the Sponsor in lieu of completion of the appropriate AE eCRF pages.

11.5. Evaluating AEs

11.5.1. 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 participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities.

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; and 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 11.1.

11.5.2. Assessment of Causality

The PI (or medically qualified designee) is obligated to assess the relationship between investigational product 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 investigational product will be considered and investigated. The PI (or medically qualified designee) will also consult the Investigator's Brochure 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 three possible categories: Not Related, Possibly Related and Probably Related.

An AE will be considered "not related" to the use of the product if any of the following tests are met:

- An unreasonable temporal relationship between administration of the product and the onset of the AE (e.g., 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(e.g.,

death as a passenger in an automobile accident)

- A clearly more likely alternative explanation for the AE is present (e.g., typical adverse reaction to a concomitant drug and/or typical disease-related event)

An AE will be considered "Possibly related" when an event follows a reasonable temporal sequence from administration of the study drug, but which could also be explained by concurrent disease or other drugs or chemicals. Or an event that follows a known or expected response pattern to the drug but that could have been produced by a number of other factors.

An AE will be considered "Probably related" when an event follows a reasonable temporal sequence from administration of the IP, unlikely to be attributed to concurrent disease or other drugs or chemicals. Also, an event that follows a known or expected response pattern to the IP, or that is confirmed by stopping or reducing the dosage of the IP and that could not reasonably be explained by known characteristics of the participant's clinical state.

11.6. Follow-up of AEs

After the initial AE, the PI is required to proactively follow each participant and provide further information on the participant'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 participant 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 as may be indicated to elucidate the nature and/or causality of the AE or SAE. This 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.

11.7. Prompt Reporting of SAEs

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

11.7.1. Completion and Transmission of the SAE reports

Once an Investigator becomes aware that an SAE has occurred in a study participant, 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, he/she 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 11.5.2. However, as new information becomes

available, causality may be modified.

Email transmission of the SAE report form 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 participant's health or well-being will be reported to the Sponsor emergency contact listed below.

Primary Sponsor Emergency Contact
[redacted]

Back-up Sponsor Emergency Contact
[redacted]

11.7.2. Pregnancy Reporting

Pregnancy occurring in a participant or in the female partner of a male participant during the study (or within 90 days following EOS) must be reported on a pregnancy reporting form or on an SAE form to the designated Pharmacovigilance CRO within 24 hours of initially becoming aware of the pregnancy by the Investigator.

Pregnancies are not SAEs. However, pregnancy data will be collected at the initial notification, birth/termination of pregnancy, and for at least 1 year after birth or longer if it is decided that additional follow-up is required or until the end of the pregnancy.

Any SAE that occurs during pregnancy (eg, serious maternal complications, therapeutic abortion, ectopic pregnancy, stillbirth etc.) must be reported in accordance with the procedure for reporting SAEs.

11.7.3. Serious Adverse Event Reports to the IRB

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 EC/IRB.

11.8. Regulatory Requirements for Reporting of SAEs

The PI (or medically qualified designee) will promptly report all SAEs in accordance with the procedures detailed in Section 11.7. 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, Investigators, and IRBs/ethics committee in accordance with the Sponsor's procedures and local regulatory requirements.

11.9. Post-study AEs

A post-study AE is defined as any event that occurs outside of the AE detection period defined in Section 11.3.

Investigators are not obligated to actively seek AEs in former study participants. However, if the Investigator learns of any SAE at any time after a participant 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.

11.10. SAEs Related to Study Participation

An SAE considered related to study participation (e.g., procedures, invasive tests, a change in existing therapy), even if it occurs during the pre- or post-treatment period, will be reported promptly (refer Section 11.7).

12. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

In general, continuous data will be summarized by descriptive statistics, including number of subjects, mean, standard deviation, median, minimum, and maximum. Categorical data will be summarized by the number and percentage of subjects. Specifics of data analysis are provided in the Statistical Analysis Plan.

12.1. Sample Size Considerations & Stratification

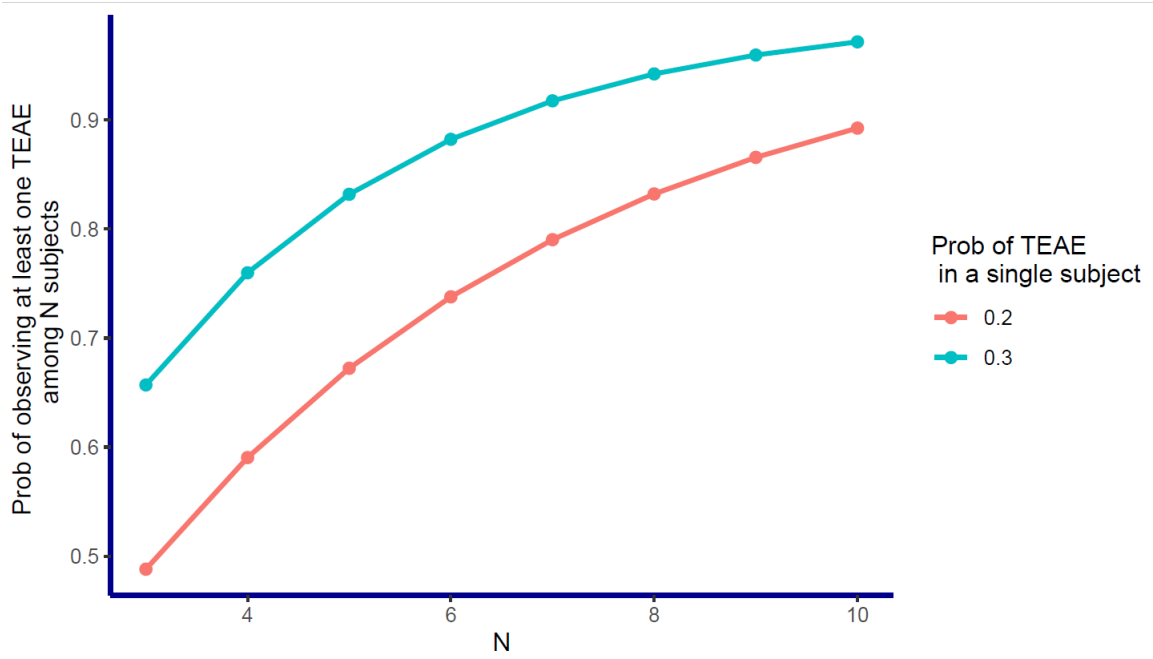
Sample sizes for NHV cohorts 1-5 and CF cohorts 2b, 3b, and 4b are decided based on the probability of observing at least one TEAE from each cohort. When probability of observing TEAE from a single subject who received active drug is 30%, chance of observing at least one TEAE will be higher than 75%, among 4 dosed subjects. With cohort size 8 or 9 (cohorts 4b and 5), probability of seeing at least one TEAE would be approximately 85% and 95% if assumed probabilities of observing AE from a single dosed subject are 20% and 30%, respectively. Table 5 below lists probabilities of observing at least one TEAE under various scenarios.

Table 5: Sample Size Calculation for Cohorts 1-5 and 2b, 3b and 4b

Probability of observing at least one TEAE from each dosed subject	Number of subjects treated per Cohort	Probability of observing at least one TEAE from Cohort
0.2	4	0.59
0.3	4	0.76
0.2	5	0.67
0.3	5	0.83
0.2	6	0.74
0.3	6	0.88
0.2	7	0.79
0.3	7	0.92
0.2	8	0.83
0.3	8	0.94

Probability of observing at least one TEAE from each dosed subject	Number of subjects treated per Cohort	Probability of observing at least one TEAE from Cohort
0.2	9	0.87
0.3	9	0.96
0.2	10	0.89
0.3	10	0.97

Figure 2: Relationship between Sample Size and Probability of Observing at Least One TEAE



Sample size for cohort 6 is calculated to have more than 80% power to observe 5% absolute change from baseline in ppFEV1 at Day 43 at significance level 0.05, with standard deviation 7%. Sub-group analysis of CF patients will be conducted according to the presence of CFTR modulator/corrector use at baseline as well as inhaled hypertonic saline use at baseline.

No stratification is planned in this study.

12.2. Screening Data

Demographics will be tabulated by participant and summarized by cohort and treatment group. Eligibility assessments at baseline, including medical/surgical history data and physical examination data (including height and weight), will be listed for each participant.

12.3. Safety/Tolerability Data

In general, safety analyses will be performed based on Safety Analysis Set, which include all enrolled subjects who receive at least one dose of study drug (placebo or active). Results will be summarized by population (NHV and CF) and cohort. Placebo subjects from each cohort will be pooled by population.

Treatment-emergent AEs will be summarized by System Organ Class (SOC) and Preferred Term (PT), classified from verbatim terms, with MedDRA version 23.0 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 Preferred Term will also be summarized. Causality (relationship to study treatment) will be summarized separately.

The incidence and frequency of treatment-emergent AEs, 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 Preferred Terms. All AEs, SAEs will also be summarized in listings. The duration of AEs will be determined and included in listings, along with the action taken and 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 patient listings.

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

12.4. Immunogenicity (Anti-drug Antibodies) Data

Changes from assay negative to positive will be summarized by dose and time to positive. Descriptive statistics of immunogenicity parameters will include mean, SD, minimum, and maximum.

12.5. Pharmacokinetic Data

Plasma concentrations of ARO-ENaC collected at specified time points post-dose from all participants at different dose levels will be used to calculate the following single dose pharmacokinetic parameters (Table 6):

Table 6: Definitions of Pharmacokinetic Parameters to be Assessed

AUC_{0-24}	The area under the plasma concentration versus time curve from 0 to 24 hours
AUC_{inf}	The area under the plasma concentration versus time curve from zero to infinity
C_{max}	The maximum plasma concentration will be obtained directly from the plasma concentration time profile
t_{max}	The time to maximum plasma concentration will be obtained by inspection
$t_{1/2}$	The half-life will be calculated by the equation $t_{1/2} = \ln(2)/k_{el}$

Additional PK parameters may be calculated as needed. The pharmacokinetic parameters will be determined using non-compartmental method(s). Descriptive statistics of pharmacokinetic parameters will include mean, standard deviation (SD), and coefficient of variation (CV), minimum and maximum. Dose-related trends in pharmacokinetic parameters will be assessed.

Pharmacokinetic parameters will be tabulated and summarized by dose level. The concentration-time profiles for each participant and the mean concentration-time profiles by dose level will be plotted with concentration presented on both linear and logarithmic scales.

Statistical analysis will be performed on the pharmacokinetic parameters using validated statistical software.

12.6. Pharmacodynamic/Efficacy Data

Descriptive statistics will be performed by scheduled study visit on the following measures of efficacy and pharmacodynamic activity:

- *Spirometry (ppFEV1)*
- *Revised Cystic Fibrosis Questionnaire (respiratory domain)*
- *Lung Clearance Index*
- *Rate of CF Exacerbations*
- *BMI*
- *ENaC mRNA expression from bronchoscopic samples*

Efficacy data for CF patients will be analyzed by cohort. Placebo subjects from each cohort will be pooled. Efficacy analyses will be completed based on Safety Analysis Set, which include all enrolled subjects who receive at least one dose of study drug (placebo or active). CF patients will be analyzed in aggregate and also separately based on CFTR mutation status and based on use or no use of a stable regimen (last 2 months) of CFTR modulators/correctors as well as use or no use of inhaled hypertonic saline.

12.7. Data Recording and Quality Control

Source documents must be maintained for each participant in the study, consisting of all demographic and medical information, including clinical laboratory data, and entered into the EDC system within five days of the subject visit. A copy of the signed informed consent form must be retained. All information on the eCRFs must be traceable to these source documents in the participant's file.

Data recorded in all participants' eCRFs will be subjected to a quality control review.

12.8. Handling Missing and Incomplete Data

Partial or missing dates of adverse events and concomitant medications will be imputed. Adverse events with missing severity and/or possible relationship to treatment will be included in the all adverse events analyses, except by severity grade and treatment-related. Every effort will be made to obtain complete dates for deaths. Details of the imputation algorithms will be specified in the statistical analysis plan.

13. STUDY APPROVAL AND CONDUCT

The following conditions will be met.

13.1. Regulatory Approval

The requirements for the conduct of clinical trials in accordance with local applicable regulations will be met before commencement of this study.

13.2. Ethics Committee (EC)/ Institutional Review Board (IRB) Approval

Prior to initiation of the study, written EC/IRB approval of the Protocol and Informed Consent Forms, based on the principles of ICH cGCP procedures, will be received. A copy of the signed and dated letter of approval will be provided to the clinical site and ArrowheadPharmaceuticals,

Inc. prior to study commencement. Any written information and/or advertisements to be used for volunteer recruitment will be approved by the EC/IRB prior to use. A list of the EC/IRB voting members, their titles or occupations, FWA number (where applicable) and their institutional affiliations will be requested before study initiation.

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

13.3. Ethical Considerations

This study will be carried out per the Declaration of Helsinki 1964, as modified by the 64th World Medical Assembly, Fortaleza, Brazil, October 2013, the Notes for Guidance on Good Clinical Practice (cGCP) (2000) (CPMP/ICH/135/95), and the Principles of the ICH cGCP. The protocol will be submitted for approval to the EC/IRB, and written approval obtained before subjects are enrolled. The composition of the EC/IRB will also be provided to the Sponsor. If approval is suspended or terminated by the IRB, the PI will notify the Sponsor immediately

Where applicable, the clinical site and Arrowhead Pharmaceuticals, Inc. 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 and is subject to the proposed recipient signing a full and complete release of the company from all claims, damages and costs.

13.4. Written Informed Consent

Informed consent will be obtained before the patient 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 wash-out 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 participants 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.

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

All eligible participants 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 participant will acknowledge receipt of this information by giving written informed consent for participation in the study. The volunteer will be given a copy of the signed Informed Consent Form to retain.

13.5. Emergency Contact with Principal Investigator

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

13.6. Notification of General Practitioner

It is the responsibility of the PI or designee, to notify, where applicable, with the consent of the participant, the general practitioner of the patient's participation in the trial, by sending a letter stating the nature of the trial, treatments, expected benefits or adverse events and concomitant drugs to be avoided.

13.7. 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 laboratory, which will perform the test for the study.

13.8. 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 EC/IRB of an amendment, except where necessary to eliminate an immediate hazard to study patients.

Deviations may result from the action or inaction of the participant, 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, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. – either tests not done, incorrect tests done, or not done within the time frame specified in the protocol

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

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

13.9. Termination of the Study

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 for safety reasons at any time in collaboration with the Sponsor.

14. STUDY ADMINISTRATION

14.1. Study Monitoring

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

In accordance with applicable regulations, cGCP, and Arrowhead Pharmaceuticals, Inc. procedures, Arrowhead Pharmaceuticals, Inc. will be responsible for assigning a study monitor (CRA) who will contact the site to organize a visit prior to participant enrolment to review the protocol and data collection procedures with site staff. In addition, the assigned study monitor will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrolment 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 investigational product 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 participants are being protected.
- Study is conducted in accordance with the currently approved protocol (and any amendments), cGCP 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 Pharmaceuticals, Inc
- Data queries
- Accountability, reconciliation and arrangements for unused investigational product(s)
- Inventory and final disposition (e.g., destruction, shipping to repository, etc.)
- Review of site study records for completeness

14.2. Quality Assurance

To ensure compliance with cGCP and all applicable regulatory requirements, Arrowhead Pharmaceuticals, Inc. 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.

14.3. Records Retention

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 (e.g., audit or inspection) and whenever feasible, to allow 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 (e.g., 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 re-generating a hard copy, if required. Furthermore, the PI must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

Arrowhead Pharmaceuticals, Inc. 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 Pharmaceuticals, Inc. standards/procedures; otherwise, the retention period will default to 15 years.

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 approval, letter of constitution of the IRB and copies of any other correspondence relevant to the study with the IRB or regulatory authorities
- The IRB approved Informed Consent Form
- Current *curriculum vitae* (signed and dated) of the PI and co-workers with major responsibilities in the trial
- Site Signature and Delegation of Responsibility Log
- FDA Form 1572 (where applicable)
- Financial Disclosure Form(s)
- Blank CRF/eCRF
- Signed participant informed consent forms
- Laboratory reference ranges (signed and dated)
- The completed CTN Application Form (where applicable)
- The Final Study Report
- Clinical raw data including the Source Data Forms, all clinical laboratory report forms, patient CRFs, drug accountability forms, and dispensing records, etc.

15. INFORMATION DISCLOSURE AND INVENTIONS

15.1. Ownership

[redacted]

[redacted]

15.2. Confidentiality

[redacted]

15.3. Publication

[redacted]

16. APPENDICES

16.1. Definition of Pulmonary Exacerbations (PEx) in CF Patients

A PEx is defined as a new initiation of or change in antibiotic therapy (IV, inhaled, or oral) for any 4 or more of the following signs/symptoms:

- Change in sputum (New or increased)
- Hemoptysis
- Increased cough
- Increased dyspnea
- Increased malaise, fatigue, or lethargy
- Temperature above 38°C (equivalent to approximately 100.4°F)
- Anorexia or weight loss
- Sinus pain or tenderness

- Change in sinus discharge
- Change in physical examination (PE) of the chest
- Decrease in pulmonary function by 10%
- Radiographic changes indicative of pulmonary infection
- Increased respiratory rate or dyspnea at rest

16.2. Guideline for Evaluation and Treatment of Hyperkalemia for NHVs and CF Patients

Hyperkalemia related to renal ENaC inhibition is not expected in this clinical study. However, as a precaution, the following guideline (derived from D B Mount et al., Treatment and Prevention of Hyperkalemia in adults, UpToDate, 2020), is provided to monitor and treat patients with treatment emergent hyperkalemia.

1. Any otherwise unexplained (e.g. no hemolysis) hyperkalemia (K+ > ULN per central lab reference range) will result in:
    a. Immediate return to clinic or local healthcare provider for repeat K+ and ECG. Confirmed hyperkalemia will further result in:
        i. Patients with K+ > 6.5 mmol/L or with any symptoms of muscle weakness or paralysis or new clinically significant cardiac conduction abnormalities on ECG consistent with hyperkalemia (as compared to pre-dose ECG) should result in treatment with therapies to stabilize myocardium, and remove potassium from circulation (intravenous calcium in addition to intravenous insulin and dextrose). Also consider use of diuretics and/or cation exchangers and possibly hospital admission.
        ii. If potassium is > ULN but < 6.5 mmol/L in the absence of symptoms or ECG findings, treatment for elevated potassium may include only diuretics or cation exchangers. Consider hospital admission.
        iii. Patient should be followed at least weekly until K+ < ULN.
        iv. Confirmed elevated potassium will result in discontinuation of dosing in the individual patient.

Local labs may be utilized as necessary (including for Screening) if needed by the investigator or for emergent situations, including time critical analytes such as serum potassium and repeat clinical chemistries.

17. REFERENCES

Amaral MD. Novel personalized therapies for cystic fibrosis: treating the basic defect in all patients. J Intern Med. 2015;277(2):155-66

Banerjee B, et al. Successful establishment of primary small airway cell cultures in human lung transplantation. Respir Res 2009; 10:99.

Bangel-Ruland N, Tomczak K, Weber WM. Targeting ENaC as a molecular suspect in cystic fibrosis. Curr Drug Targets. 2015;16(9):951-7

Boucher RC. Human airway ion transport. Part one. Am J Respir Crit Care Med 1994;150(1): 271-81

Boucher RC. Human airway ion transport. Part two. Am J Respir Crit Care Med 1994;150(2): 581-93

Choi JY, Muallem D, Kiselyov K, et al. Aberrant CFTR-dependent HCO3- transport in mutations associated with cystic fibrosis. Nature. 2001;410(6824):94-7

Cystic Fibrosis Foundation Patient Registry: 2015 Annual Data Report; ©2016 Cystic Fibrosis Foundation: Bethesda, 2016; pp. 1–94. Available online: https://www.cff.org/Our-Research/CF-Patient-Registry/2015-Patient-Registry-Annual-Data-Report.pdf (accessed on 19 June 2017)

Facciolongo N, et al. Incidence of complications in bronchoscopy. Multicentre prospective study of 20,986 bronchoscopies. Monaldi Arch Chest Dis. 2009;71:8-14.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. Potent and specific genetic interference by double-stranded RNA in C. elegans. Nature. 1998; 391:806–811.

Graham BL, et al. Standardization of spirometry 2019 update. Am J Respir Crit Care Med 2019; 8:e70-88.

Greger R, Mall M, Bleich M, Ecke D, Warth R, Riedemann N, Kunzelmann K. Regulation of epithelial ion channels by the cystic fibrosis transmembrane conductance regulator. J Mol Med (Berl). 1996 Sep;74(9):527-34.

Hobbs CA, Da Tan C, Tarran R. Does epithelial sodium channel hyperactivity contribute to cystic fibrosis lung disease? The Journal of physiology. 2013;591(18):4377-87

Kerem E., et al. Pulmonary Epithelial Sodium-Channel Dysfunction and Excess Airway Liquid in Pseudohypoaldosteronism. N Engl J Med 1999; 341:156-162

Mall MA, Mayer-Hamblett N, Rowe, SM. Cystic Fibrosis: Emergence of Highly Effective Targeted Therapeutics and Potential Clincal Implications. Am J Respir Crit Care Med. 2019 Dec 20.

Maselli DJ, Keyt H, Restrepo MI. Inhaled Antibiotic Therapy in Chronic Respiratory Diseases.

International journal of molecular sciences. 2017;18(5).

Middleton PG, Mall MA, et al. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. N Engl J Med. 2019 Nov 7;381(19):1809-1819.

O'Riordan TG, et al. Acute hyperkalemia associated with inhalation of a potent ENaC antagonist: Phase 1 trial of GS-9411. J Aerosol Med Pulm Drug Deliv 2014; 27: 200–8.

Pue CA, Pacht ER. Complications of fiberoptic bronchoscopy at a university hospital. Chest 1995; 107: 430-432.

Quinton PM. Chloride impermeability in cystic fibrosis. Nature. 1983 Feb 3;301(5899):421-2.

Quon BS, Rowe SM. New and emerging targeted therapies for cystic fibrosis. BMJ. 2016;352:i859

Rauh R, et al. A mutation of the epithelial sodium channel associated with atypical cystic fibrosis increases channel open probability and reduces Na+ self inhibition. J Physiol 588.8 (2010) pp 1211–1225

Robinson PD, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests, European Respiratory Journal 2013; 41: 507-522

Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med. 2015;372(4):351-62

Tepper JS, Kuehl PJ, Cracknell S, Nikula KJ, Pei L5, Blanchard JD. Symposium Summary: "Breathe In, Breathe Out, Its Easy: What You Need to Know About Developing Inhaled Drugs" Int J Toxicol. 2016 Jul;35(4):376-92.

Vallières E, Elborn JS. Cystic fibrosis gene mutations: evaluation and assessment of disease severity. Advances in Genomics and Genetics. 2014;4:161-72

Willumsen NJ, Boucher RC. Transcellular sodium transport in cultured cystic fibrosis human nasal epithelium. Am J Physiol. 1991 Aug;261(2 Pt 1):C332-41.

Willumsen NJ, Davis CW, Boucher RC. Cellular Cl- transport in cultured cystic fibrosis airway epithelium. Am J Physiol. 1989 May;256(5 Pt 1):C1045-53.