Statistical Analysis Plan

Protocol 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
Protocol Number:	AROENaC1001 (Amendment 4.0)
NCT Number:	NCT04375514
Sponsor:	Arrowhead Pharmaceuticals, Inc. 177 East Colorado Boulevard, Suite 700, Pasadena, CA 91105
Authors:	[redacted] EMB Statistical Solutions, LLC [redacted] Arrowhead Pharmaceuticals, Inc.
SAP Date:	17 May 2021

Version Number	Date (DDMMYYYY)	Summary of Changes
Original (v1.0)	17052021	Original SAP

Statistical Analysis Plan Approval

Author

[redacted]

17 May 2021
Date

EMB Statistical Solutions, LLC

Approved by

[redacted]

17 May 2021
Date

Arrowhead Pharmaceuticals, Inc.

Table of Contents

List of abbreviations and definitions of terms

Abbreviation or Term	Definition/Explanation
ADA	Anti-drug antibody
AE	Adverse event
AUC0-24	The area under the plasma concentration versus time curve from the zero to 24 hours
AUCinf	The area under the plasma concentration versus time curve from zero to infinity
AUClast	The area under the plasma concentration versus time curve from the zero to the last quantifiable plasma concentration
BAL	Bronchoalveolar lavage
BLQ	Below the limit of quantification
BMI	Body mass index
CF	Cystic Fibrosis
CFQ-R	Revised cystic fibrosis questionnaire
CFTR	CF transmembrane conductance regulator
CI	Confidence interval
Cmax	The maximum plasma concentration
DSC	Data Safety Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
ENaC	Epithelial sodium (Na+) channel
EOS	End of study
FEV1	Forced Expiratory Volume in the first second
FRC	Functional residual capacity
FSH	Follicle-stimulating hormone
LCI	Lung clearance index
LLOQ	Lower limit of quantification
LOD	Limit of detection
MedDRA	Medical Dictionary for Regulatory Activities
CTCAE	Common Terminology Criteria for Adverse Events
NHV	Normal healthy volunteers
PBO	Placebo
PI	Principal investigator
PK	Pharmacokinetic
ppFEV1	Percent Predicted FEV1
PT	Preferred term
RDD	Respirable delivered dose
SDTM	Standard Data Tabulation Model
SI	International System of Units
SOC	System organ class
$t_{1/2}$	The half-life
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event
Tmax	The time to maximum plasma concentration
WHODrug	World Health Organization Drug Dictionary

1. INTRODUCTION

This statistical analysis plan (SAP) is designed in compliance with ICH E9 to outline the methods to be used in the analysis of study data in order to evaluate the study objective(s) from Arrowhead Pharmaceuticals, Inc. (Sponsor) protocol AROENaC1001 Version 4.0, dated 09 April 2021. Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial.

An approved and signed SAP is required prior to database lock and prior to the planned unblinding and analysis of an ongoing cohort. Completed cohorts may be unblinded without a signed SAP.

The formats for the tables, figures, listings (TFLs) described in this SAP are provided in a companion document. A table of contents for the TFLs will be included.

EMB Statistical Solutions, LLC (EMB) will have responsibility for performing the analyses outlined in this SAP. The Sponsor will have responsibility for performing the PK noncompartmental analysis (NCA) (Appendix B).

2. OBJECTIVES, ENDPOINTS AND HYPOTHESES

2.1 Objectives and Endpoints/Estimands

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

Endpoints
Primary
• The incidence and frequency of adverse events (AEs) over time through end of study using escalating multiple doses in NHVs and in CF patients.
Secondary
• 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
• 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 placebo (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

2.2 Hypotheses and/or Estimations

No formal hypothesis testing will be performed.

3. STUDY OVERVIEW

3.1 Study Design

This is a phase 1/2a first-in-human dose-ranging study to assess the safety, tolerability and PK of ARO-ENaC in NHV and in patients with CF.

Normal Healthy Volunteers:

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. 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 through Day 21, and experience from any prior cohorts, the Data Safety Committee (DSC) will meet to vote on dose escalation to next NHV cohort and initiation of CF cohorts, when applicable.

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.

Cystic Fibrosis Patients:

Cohorts 2b, 3b, 4b will enroll eligible CF patients 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. 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. Each cohort 2b and 3b will enroll 4 subjects to receive active drug and 2 subjects to receive placebo. Cohort 4b will enroll 9 subjects to receive active drug and 3 subjects to receive placebo.

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. 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). Cohort 6 will enroll 18 subjects to receive active drug and 6 subjects to receive placebo.

Table 4 from Protocol: 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 Day 1, 2, 3; ≤ 40 mg Day 22, 23, 24
3b	CF Patients	Double-blind	Up to 6 (4 active: 2 PBO)	≤ 65 mg Day 1, 2, 3; ≤ 65 mg Day 22, 23, 24
4b	CF Patients (ppFEV > 70%)	Double-blind	Up to 12 (9 active: 3 PBO)	≤ 180 mg Day 1, 2, 3; ≤ 180 mg Day 22, 23, 24
6	CF Patients	Double-blind	Up to 24 (18 active: 6 PBO)	≤ 490 mg Day 1; ≤ 490 mg Day 15; ≤ 490 mg Day 29

Principal investigators (PIs) and study participants will remain blinded through End-of-Study (EOS). EMB may be unblinded prior to the completion of a cohort to provide unblinded interim analyses to a designated unblinded team at the Sponsor (outlined in the Matrix of Access to Restricted Data). With the exception of Cohort 6, the Sponsor (apart from the designated unblinded

team) will remain blinded until all subjects in a cohort have completed the EOS visit (or discontinued early) at which time they may be unblinded to that cohort. For Cohort 6, the Sponsor may be unblinded prior to completion of the cohort. Apart from the unblinding detailed above, blinding (where applicable) will be preserved to the extent possible. However, treatment unblinding of an individual participant may occur, at the discretion of the PI or Medical Monitor, where deemed necessary for treatment of an AE or for a decision to be made regarding trial continuation.

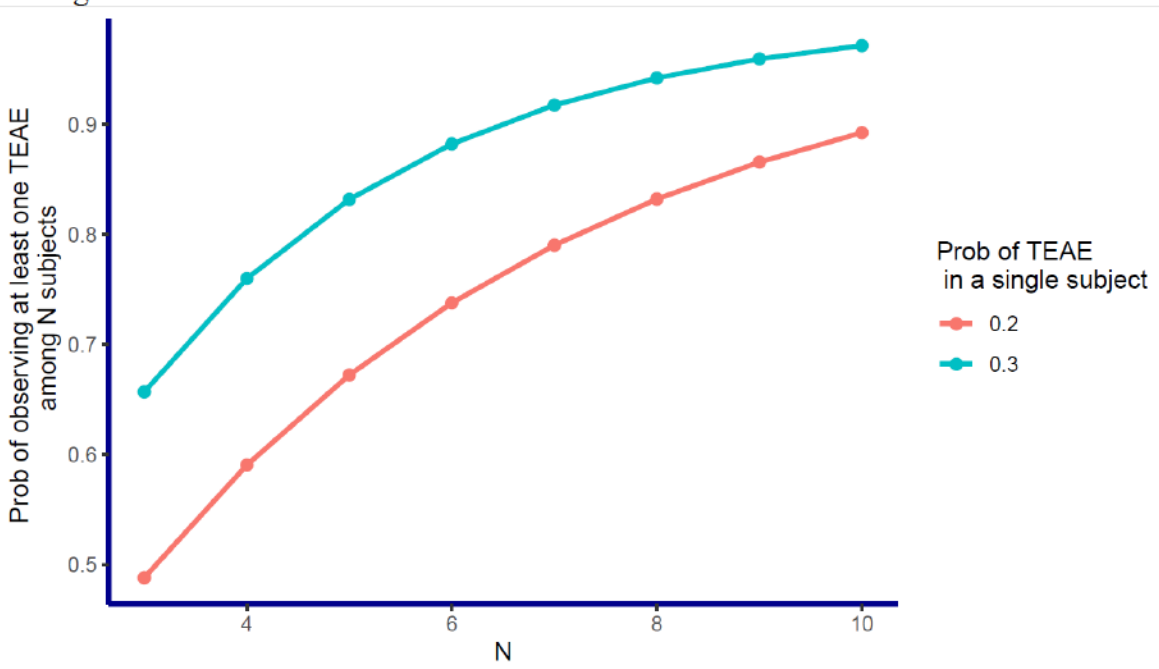
3.2 Sample Size

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. The table below lists probabilities of observing at least one TEAE under various scenarios.

Table 5 from Protocol: 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
0.2	9	0.87
0.3	9	0.96
0.2	10	0.89
0.3	10	0.97

Figure 2 from Protocol: Relationship between Sample Size for a Cohort and Probability of Observing at Least One TEAE within a Cohort



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%.

No stratification is planned in this study.

4. COVARIATES AND SUBGROUPS

4.1 Planned Covariates

There are no predetermined covariates.

4.2 Subgroups

Subgroup analyses of Spirometry and LCI for CF patient cohorts will be categorized as follow.

- CFTR mutation status
- Use of CFTR modulators at baseline (Y/N)
- Use of inhaled hypertonic saline at baseline (Y/N)

5. DEFINITIONS

Study Day:
Day of study relative to the date of first dose of active drug or placebo. First dose date will be considered as Day 1. Calculated prior to first dose date as (date of measurement – date of first dose) and calculated after first dose date as (date of measurement – date of first dose + 1).

Baseline:
The last observation prior to the first dose of active drug or placebo. The baseline value may be an unscheduled or repeated measurement.

Change (absolute) from baseline:
The arithmetic difference between a post-baseline value and baseline for a given time point: change (absolute) from baseline = (post-dose value – baseline value).

Percent change from baseline:
The percent change between a post-baseline value and baseline for a given time point: [(post-dose value – baseline value) / baseline value] *100%

Treatment-emergent AEs (TEAEs):
TEAE will be derived as any AEs occurring on or after the first dose.

Treatment-emergent SAEs (TESAEs):
TESAE will be derived as any serious AEs occurring on or after the first dose.

Concomitant Medications:
Concomitant medications will be derived as any medications with an end date on or after the first dose.

Use of medications at baseline:
For each of the following medications: CFTR modulators, inhaled hypertonic saline, inhaled bronchodilators, chronic inhaled antibiotics, inhaled corticosteroids, dornase alfa, and azithromycin, any use within 30 days prior to first dose will be considered as use at baseline. Dornase alfa and azithromycin will be identified by the Preferred Drug Name as coded by WHODrug. All other medications will be identified as recorded on the CF-Concomitant Medications eCRF.

Sputum pseudomonas status at baseline:
Any pseudomonas (as collected on the Airway Infection eCRF) present within 30 days prior to first dose will be considered as positive at baseline.

6. ANALYSIS SETS

6.1 Full Analysis Set

This analysis set includes all enrolled subjects who are randomized.

Disposition data will be summarized and listed using the Full Analysis Set. The Full Analysis Set will be analyzed as randomized.

6.2 Safety Analysis Set

This analysis set includes all enrolled subjects who receive at least one dose of active drug or placebo.

All safety, treatment exposure, demographic and baseline characteristic data, and efficacy data will be summarized and listed using the Safety Analysis Set. The Safety Analysis Set will be analyzed as treated.

6.3 Pharmacokinetic Analysis Set

This analysis set includes all enrolled subjects who receive at least one dose of active drug (ARO-ENaC) and have measurable PK concentration data. Subjects may be excluded if they have protocol deviations which would impact PK analysis.

PK concentrations and parameters will be summarized and listed using the PK Analysis Set. The PK Analysis Set will be analyzed as treated.

7. PLANNED ANALYSES

7.1 Data Safety Committee and Early Stopping Guidelines

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, principal investigator (PI), or Sponsor. Dose escalation will require approval by the DSC based on evaluation of all available safety and, when available, PD data through at least Day 21 of the most advanced NHV cohort.

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 the stopping rules stated in the Protocol.

7.2 Interim Analysis

For NHV Cohorts 1 to 5, interim analyses may occur after completion of each NHV cohort (after all subjects within a cohort complete or discontinue early). After completion of each cohort, the Sponsor will be unblinded and EMB may create unblinded TFLs.

For CF Cohorts 2b to 4b, interim analyses may occur when all subjects within each cohort complete their Day 37 visit (or discontinue early). EMB will create both blinded and unblinded TFLs. Unblinded TFLs will be kept in a secure location and will be handled and viewed only by designated unblinded personnel, independent of the study team. Designated unblinded personnel are documented in the Matrix of Access to Restricted Data. Blinded TFLs will be summarized by cohort, not separated by active and placebo arms. After completion of each CF cohort, the Sponsor may be unblinded to that cohort.

7.3 Primary Analysis

Primary analysis will occur when all subjects in Cohort 6 have completed their Day 43 visit (or have discontinued early) and all other cohorts have completed. EMB and the Sponsor may be unblinded after the data have been entered and cleaned.

7.4 Final Analysis

Final analysis will occur upon completion of all cohorts in the study, after the study has been locked and all cohorts have been unblinded. The purpose of final analysis is to provide safety and efficacy analyses using complete study data.

8. DATA SCREENING AND ACCEPTANCE

8.1 Data Handling and Electronic Transfer of Data

The Sponsor will be responsible for providing all data to be used in the planned analyses. Data will be provided to EMB as Standard Data Tabulation Model (SDTM) datasets.

8.2 Handling of Missing and Incomplete Data

Missing values will not be imputed, except in the case of determining treatment-emergent status for AEs, concomitant status for medication and procedures, and for missing or partial dates where a complete date is required for calculations.

Missing	Imputation	Exception
Day	01	Default to Study Day 1 if an AE, medication/procedure starts the same year and month as Study Day 1 and the end date has same year and month as Study Day 1 or later.
Day / Month	01 / Jan	Default to Study Day 1 if an AE, medication/procedure starts the same year as Study Day 1 and the end date has same year as Study Day 1 or later.
Day / Month / Year	To calculate a missing start date: 1. If a partial or complete stop date is present: a. Stop date < first dosing date: impute January 1 of the stop year b. Stop date ≥ first dosing date: impute the date of first dose 2. If it is unknown whether the stop date is < or ≥ first dosing date due to a missing or partial stop date, or if a stop date is not	

	collected: impute date of first dose.	

Medications and procedures with missing or partial end dates will be assumed to be concomitant unless a partial end date documents it as ending prior to treatment.

The original missing or partial date, the imputed complete date, and an indicator variable that indicates which dates were imputed will be retained in the database. Dates will be presented as collected in all listings. Imputed dates will not be presented in listings but may be used for summary tables.

8.3 Outliers

Descriptive statistics may be used to identify outliers of key variables. However, no statistical analysis or adjustment for outliers is planned; outliers will not be omitted from summaries.

8.4 Distributional Characteristics

Before implementation of parametric methods of analysis, the distribution of analysis variables will be examined to determine if model assumptions are satisfied. Transformations or nonparametric methods of analysis may be used if warranted. However, in some cases, nonparametric analysis may be the initially proposed method due to the expected distribution of response. Whenever alternative methods of analysis are required, the description of the new method along with the rationale for its use will be documented in the CSR.

8.5 Validation of Statistical Analyses

All computations for statistical analyses will be performed using SAS® software Version 9.4 or later. All SAS programs used in the production of statistical summary outputs will be validated with independent programming prior to finalization. In addition, all program outputs will be independently reviewed. The validation process will be used to confirm that all data manipulations and calculations were accurately done. Once validation is complete, a statistical reviewer will perform a final review of the documents to ensure the accuracy and consistency with this plan and consistency within tables. Upon completion of validation and quality review procedures, all documentation will be collected and filed by the project statistician or designee.

9. STATISTICAL METHODS OF ANALYSIS

For all summary tables and figures described in this SAP, NHV Cohorts 1-5 and CF patient Cohorts 2b-4b and 6 will be summarized separately. Subjects will be grouped by dose and dosing regimen within each table and figure, with subjects receiving placebo pooled across cohorts. All data will be combined in listings, which will be sorted by cohort and subject number.

9.1 General Considerations

Descriptive statistics will be provided for selected demographic, safety, PK, and efficacy data. Descriptive statistics of continuous data will include the number of subjects, mean, median,

standard deviation, minimum & maximum. Descriptive summaries of categorical data will use frequency counts and percentages. Graphical summaries of the data may be also presented.

9.2 Subject Accountability

The number and percent of subjects in each analysis set (Full Analysis Set, Safety Analysis Set, and PK Analysis Set), who complete or discontinue treatment (including reasons for discontinuing), and who complete or discontinue from the study (including reasons for discontinuing) will be summarized using the Full Analysis Set.

9.3 Important Protocol Deviations

Protocol deviations will be recorded during the conduct of the study and those identified as important will be listed by subject using the Full Analysis Set.

9.4 Demographic and Baseline Characteristics

9.4.1 Subject Demographics

Descriptive summaries of subject demographics and baseline characteristics will be produced using the Safety Analysis Set. Demographic data will include:

- Race: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, and Other. If one subject has more than once race is reported, the subject will be categorized as More than One Race Reported.
- Ethnicity: Not Hispanic or Latino, Hispanic or Latino, Not Reported, Unknown, and Other.
- Gender: Male and Female
- Age at Screening in years
- Baseline weight
- Baseline height
- Baseline BMI

Additionally, demographic and baseline characteristic data for CF patient cohorts will include:

- Region (US vs Other)
- FEV1 (L)
- Percent predicted FEV1 (ppFEV1) (%)
- ppFEV1 categories (≤40%, >40% - ≤70%, >70% - ≤90%, >90%)
- FVC (L)
- Percent predicted FVC (ppFVC) (%)
- FEV1/FVC

- Sweat chloride
- CFQ-R Respiratory Symptoms domain
- CFTR mutation status
- Use of CFTR modulators at baseline (Y vs N)
- Use of inhaled hypertonic saline at baseline (Y vs N)
- Use of inhaled bronchodilator at baseline (Y vs N)
- Use of chronic inhaled antibiotic at baseline (Y vs N)
- Use of dornase alfa at baseline (Y vs N)
- Use of inhaled corticosteroids at baseline (Y vs N)
- Use of azithromycin at baseline (Y vs N)
- Sputum pseudomonas status at baseline (Positive vs Negative)

All demographic data will be listed by subject.

9.4.2 Eligibility

Inclusion and exclusion eligibility criteria not met will be listed by subject for all screen failures.

9.4.3 Medical History

The Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 or later will be used to code medical history events to a system organ class (SOC) and a preferred term (PT). Descriptive statistics summaries of subject incidence of medical history events will be provided by SOC and PT.

Medical history will be listed by subject.

9.5 Safety Analyses

All safety summaries outlined in this section will be created using the Safety Analysis Set.

9.5.1 Adverse Events

AEs will be recorded from time of signed consent through to EOS. The PI and clinical facility staff are responsible for detection, recording and reporting of events that meet the criteria and definition of AEs. The investigator will determine the severity and relationship of an AE associated with use of the study drug, and will identify serious adverse events (SAEs). All AE data summaries will report these as recorded on the eCRF.

MedDRA version 23.0 or later will be used to code all events categorized as AEs to a SOC and PT.

An overall summary of AEs will be provided. The overall summary will include the number and percent of subjects experiencing an event as well as the number of events in each of the following

categories: AEs, TEAEs, treatment-related TEAEs, SAEs, TESAEs, treatment-related TESAEs, and AEs resulting in study drug withdrawal, study discontinuation, and death. Categories are not mutually exclusive.

Descriptive statistics summaries of subject incidence of AEs will be summarized for all TEAEs, treatment-related TEAEs, TESAEs, treatment-related TESAEs, and AEs resulting in study drug withdrawal or study discontinuation will be tabulated by SOC in alphabetical order and PT in descending order of frequency. Summaries will include count and percent of subjects as well as number of events.

Descriptive statistics summaries of subject incidence of TEAEs by SOC, PT, and relationship to study drug will be provided. Subjects will be counted once under the closest relationship reported within each SOC and PT. Events will be counted the number of times they occur within each SOC and PT.

Descriptive statistics summaries of subject incidence of TEAEs by SOC, PT, and severity will be provided. Subjects will be counted once under the highest severity reported within each SOC and PT. Events will be counted the number of times they occur within each SOC and PT.

All AEs will be listed by subject. Separate by-subject listings of SAEs and of AEs resulting in study drug withdrawal or study discontinuation will be provided.

9.5.2 Laboratory Test Results

Laboratory assessments include clinical chemistry, coagulation, hematology, urinalysis, and urine electrolytes. Microscopic urinalysis will also be performed as needed, with results listed by subject but not otherwise summarized. Clinical laboratory values will be expressed using the International System of Units (SI).

Descriptive statistics summaries of quantitative laboratory tests will be provided by scheduled visit. Derived changes and percent changes from baseline to each scheduled postdose visit will be included. Additionally, descriptive statistics for the peak post-baseline values will be included for the chemistry lab tests: ALT, AST, ALP, and BILI, and descriptive statistics for the lowest post-baseline values and lowest overall values will be provided for the hematology lab tests: HGB and PLT. Laboratory test values above or below the limits of detection will be set to the upper or lower limit values, respectively, for inclusion in the descriptive statistics summaries; values will be reported without imputation in the listings.

Plots of mean (+/- SD) key laboratory test results over time will be provided by study day. The key laboratory tests to be included will be: alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatine kinase, creatinine, potassium, and sodium. SD bars below 0 will be set to 0.

Scatter plots of peak post-baseline total bilirubin [divided by upper limit of normal (ULN)] vs peak post-baseline alanine aminotransferase [divided by ULN] will be provided to visually evaluate drug-induced serious hepatotoxicity.

Additionally, select laboratory tests from serum chemistry, coagulation, and hematology will be programmatically graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading criteria (Appendix A). Laboratory test values above or below the limits of detection will be set to the upper or lower limit values, respectively, for programmatic grading. Using the CTCAE grades, shift tables describing changes from baseline to the maximum post-baseline grade will be provided for clinical chemistry, coagulation, and hematology laboratory results. The number and percent of subjects will be reported in each shift category. Assessments which are missing a baseline value or which are not graded at baseline will be summarized as unknown.

All laboratory data will be listed by subject. A separate listing of abnormal and out-of-range laboratory values will also be provided. Both listings will include normal reference ranges and flags (low, high, abnormal).

9.5.3 Vital Signs

Vital signs will include systolic and diastolic blood pressure, temperature, heart rate, respiratory rate (breaths/min), and pulse oximetry. Vital sign reporting will also include weight and BMI for CF patient cohorts.

Descriptive statistics summaries of vital signs will be provided by scheduled visit. Derived changes from baseline will be included in the summary.

Plots of mean (+/- SD) pulse oximetry (%) over time will be provided by study day. SD bars above 100% or below 0% will be set to 100% and 0%, respectively.

All vital sign data will be listed by subject.

9.5.4 Electrocardiograms

Descriptive statistics summaries of ECG parameters (heart rate, QT interval, PR interval, QRS interval, RR interval, QTcF, QTcB, and clinical interpretation) will be provided by scheduled timepoint. Derived changes from baseline will be included for quantitative parameters. The ECG clinical interpretation will be summarized using counts and percentages of subjects in each category (Normal, Abnormal NCS, Abnormal CS, Not Done).

All ECG data will be listed by subject.

9.5.5 Physical Measurements

All physical examination data will be listed by subject.

9.5.6 Chest X-Ray Findings

Descriptive statistics summaries of chest X-ray findings will be provided by scheduled visit using counts and percentages of subjects in each category (Normal, Abnormal NCS, Abnormal CS, Not Done).

All chest X-ray data will be listed by subject.

9.5.7 Antibody Formation

Descriptive statistics summaries of anti-drug antibodies (ADA) will be provided by scheduled visit.

All ADA data will be listed by subject.

9.5.8 Exposure to Investigational Product

Descriptive statistics summaries of study drug administration will include the number and percent of subjects receiving each study drug at each scheduled dosing. The total dose given (mg) and extent of exposure (days) will also be provided. Extent of exposure will be calculated as the date of last dose – the date of first dose + 1.

All study drug administration data will be listed by subject.

9.5.9 Exposure to Concomitant Mediation

Medications with an end date prior to the first dose of study drug will be identified as prior medications; medications with an end date on or after the first dose of study drug will be identified as concomitant medications. Similarly, procedures with a date prior to the first dose of study drug will be identified as prior procedures; procedures with a date on or after the first dose of study drug will be identified as concomitant procedures. Prior medications and prior procedures will be included in listings only.

Medications will be coded using WHODrug version Mar 2020 or later and will be reported by Anatomical Therapeutic Chemical (ATC) classification level 4 and Preferred Drug Name. Procedures will be coded using MedDRA version 23.0 or later to a SOC and PT.

Descriptive statistics summaries of the number and percent of subjects who receive each concomitant medication will be summarized. Similarly, descriptive statistics summaries of the number and percent of subjects who receive each concomitant procedure will also be summarized by SOC and PT.

Additionally, concomitant medications and procedures related to CF will be recorded separately. Listings of prior and concomitant medications and therapies will be provided by subject.

9.6 Efficacy Analyses

All efficacy summaries outlined in this section will be created using the Safety Analysis Set.

9.6.1 Spirometry

Spirometry will be evaluated in NHVs and CF patients. In general, spirometry for NHVs are considered a safety measurement and spirometry for CF patients are considered both a safety and efficacy measurement. For CF patient cohorts, use of an inhaled bronchodilator will be recorded at each spirometry assessment. All CF patient spirometry summaries will identify use of an inhaled bronchodilator as recorded on the Spirometry eCRF.

Descriptive statistics summaries of pulmonary function parameters obtained from the spirometry assessments will be provided by scheduled timepoint. Absolute changes and percentage changes from baseline will be included. In addition to an overall summary, subgroup analyses (as defined in Section 4.2) will be provided.

A sensitivity analysis of FEV1 and ppFEV1 will be performed for CF patient cohorts if use of an inhaled bronchodilator at any post-baseline assessment does not match use of an inhaled bronchodilator at the baseline assessment for any CF patients. The sensitivity analysis will consist of a summary of FEV1 and ppFEV1 values with absolute and percent changes from baseline by scheduled visit, excluding any post-baseline spirometry assessments where the bronchodilator status does not match the bronchodilator status at baseline. Subgroup analyses will not be provided for the sensitivity analysis.

Plots of mean (+/- SD) FEV1 (L) and ppFEV1 (%) over time will be provided by study day from baseline to the EOS visit. Plots for CF patient cohorts will additionally include a plot from baseline to Day 37 (Cohorts 2b-4b) and a plot from baseline to Day 43 (Cohort 6).

Additionally, a plot will be provided for CF patient cohorts to visually assess the relationship between baseline sweat chloride level and ppFEV1 absolute change from baseline value. For Cohorts 2b-4b, the Day 37 ppFEV1 absolute change from baseline value will be used. For Cohort 6, the Day 43 ppFEV1 absolute change from baseline value will be used. Pearson correlation coefficients may be provided within the plot.

All pulmonary function data obtained from the spirometry assessment will be listed by subject.

9.6.2 Lung Clearance Index

The LCI will be assessed in CF patients with baseline ppFEV1 >70% in Cohorts 2b- 4b and for all subjects in Cohort 6. In CF Cohorts 2b-4b, three acceptable LCI values at each study visit are planned to be collected. In Cohort 6, two acceptable LCI values at each study visit are expected. At each visit, the average value of acceptable LCI measurements will be used. If only one acceptable value is collected, then the single acceptable value will be used. Additionally, use of an inhaled bronchodilator will be recorded at each LCI assessment. All LCI summaries will identify use of an inhaled bronchodilator as recorded on the LCI eCRF.

Descriptive statistics summaries of LCI will be provided by scheduled visit. Absolute changes from baseline will be included. In addition to an overall summary, subgroup analyses (as defined in Section 4.2) will be provided.

A sensitivity analysis of LCI will be performed if use of an inhaled bronchodilator at any post-baseline assessment does not match use of an inhaled bronchodilator at the baseline assessment for any CF patients. The sensitivity analysis will consist of a summary of LCI values with absolute changes from baseline by scheduled visit, excluding any post-baseline LCI assessments where the bronchodilator status does not match the bronchodilator status at baseline. Subgroup analyses will not be provided for the sensitivity analysis.

Plots of mean (+/- SD) LCI over time will be provided by study day from baseline to the EOS visit. Plots will additionally include a plot from baseline to Day 37 (Cohorts 2b-4b) and a plot from baseline to Day 43 (Cohort 6).

All LCI data will be listed by subject.

9.6.3 Revised Cystic Fibrosis Questionnaire

The CFQ-R will be collected at scheduled timepoints for CF patients only. The questionnaire provides information about demographics, quality of life, and daily activities.

CFQ-R has a total of 50 questions to form 12 domains. Question 43, which is scored 1, 2, 3, 4, or 5, is not used in calculating any domain; all the other 49 questions are scored 1, 2, 3, or 4.

To calculate the score for each domain, the response scores on the negatively phrased questions are reversed (reversed scores = 5 – response scores) so that 1 always represents the worst condition and 4 always represents the best condition.

The scaled score for each domain ranges from 0 (worst condition) to 100 (best condition) and will be calculated as follows:

Scaled score for a domain = 100 × [mean(scores of all questions in that domain) – 1] / 3

The scaled score for a specific domain will not be calculated if more than half of the questions in the domain have missing scores.

The table below provides the questions included in each domain, the questions with the reversed scores, as well as the maximum number of missing questions for a domain score to be calculated.

CFQ-R for Adolescents and Adults

Domain	Questions		Reversed Questions	Max # of missing Questions
	Total	Individual		
Physical	8	1, 2, 3, 4, 5, 13, 19, 20	13	4
Role	4	35, 36, 37, 38	35	2
Vitality	4	6, 9, 10, 11	6,10	2
Emotion	5	7, 8, 12, 31, 33	-	2
Social	6	22, 23, 27, 28, 29, 30	23,28,30	3
Body	3	24, 25, 26	-	1
Eat	3	14, 21, 50	-	1
Treatment Burden	3	15, 16, 17	15,17	1
Health perceptions	3	18, 32, 34	18,32,34	1
Weight	1	39	-	0
Respiration*	6	40, 41, 42, 44, 45, 46	43	3
Digestion	3	47, 48, 49	-	1

*Question 43 not used to calculate a domain

A descriptive statistics summary of CFQ-R domain scores and absolute changes from baseline by scheduled timepoint will be provided.

All CFQ-R data will be listed by subject.

9.6.4 Rate of Pulmonary Exacerbations

Pulmonary exacerbation assessments will be collected at scheduled timepoints for CF patients. CF-related exacerbation rates are important clinical events associated with disease progression, and they remain a significant issue even in patients receiving CFTR modulator therapy (Middleton 2019).

A descriptive statistics summary displaying the number and percent of subjects reporting pulmonary exacerbation assessments (Y/N), as well as the total number of exacerbations reported will be provided by scheduled visit.

All pulmonary exacerbation assessment data will be listed by subject.

9.6.5 Body Mass Index

Height and weight measurements will be collected at scheduled timepoints per the Protocol, and BMI will be derived. BMI measurements will be included in vital signs reporting, described in Section 9.5.3.

9.6.6 αENaC mRNA Expression

Only NHVs from Cohort 5 will undergo bronchoscopies to evaluate αENaC mRNA knockdown. Bronchoscopies will be performed at baseline and on Day 18.

A descriptive statistics summary of αENaC mRNA expression will be provided by scheduled visit. Derived changes from baseline will be included.

All bronchoscopy data will be listed by subject.

9.7 Pharmacokinetic Analyses

All PK summaries outlined in this section will be created using the PK Analysis Set. PK summaries will additionally include the geometric mean and the geometric coefficient of variation (CV). The geometric CV will be calculated as:

geometric CV = sqrt(e^[(SD of log-transformed data)^2] – 1) * 100%

The Sponsor will provide NCA of PK data (Appendix B).

9.7.1 Plasma Concentrations

Descriptive statistics summaries of plasma concentrations will be provided by scheduled timepoint. Concentrations below the limit of quantification (BLQ) will be set to zero for summary statistics.

Mean plasma concentrations (+SD) will be plotted on a linear and semi-logarithmic scale versus nominal time points by treatment. Individual plasma concentrations will be plotted on a linear and semi-logarithmic scale versus actual sampling times. For each cohort, spaghetti plots of individual plasma concentrations on a linear and semi-logarithmic scale will also be presented. A reference line indicating the lower limit of quantification (LLOQ) will be included in plots, as appropriate.

Actual sampling times that are outside the sampling window (> ±10% of nominal time) will be listed, but will be excluded from descriptive statistic summaries of concentration data.

All plasma concentration data will be listed by subject.

9.7.2 Urine Amounts Excreted

Descriptive statistics summaries of urine amounts excreted will be provided by scheduled collection interval.

All urine amounts excreted will be listed by subject.

9.7.3 Pharmacokinetic Parameters

Plasma PK parameters will be calculated for Day 1, Day 3, and Day 1_3. Urine PK parameters will be calculated for Day 3 only.

Parameter	Definition
Plasma PK Parameters	
Cmax	Maximum observed concentration
Tmax	Time to reach maximum plasma concentration
AUC0-24	The area under the plasma concentration versus time curve from the zero to 24 hours
AUClast	The area under the plasma concentration versus time curve from the zero to the time of last quantifiable plasma concentration
AUCinf	The area under the plasma concentration versus time curve from zero to infinity
t1/2	Apparent terminal elimination halflife
RACmax	Apparent drug accumulation calculated by Cmax of last dose vs first dose
RAAUC	Apparent drug accumulation calculated by AUC of last dose vs first dose
Urine PK Parameters	
Ae0_24	Amount recovered in urine over 0 - 24 hours post dose (Day 3 only)
Fe0_24	Percentage of the administrated drug recovered in urine over 0 - 24 hours (Day 3 only)

CLr	Renal clearance over a time interval calculated by Ae/AUC at the same time interval (0 - 24 hours, Day 3 only)

Additional PK parameters in each part may be determined where appropriate.

Plasma and urine PK parameters will be listed and summarized descriptively by treatment. Summary statistics of Tmax will not include geometric mean and geometric CV. Diagnostic PK parameters will be listed only.

9.7.4 Dose Proportionality Analysis

The Cmax and AUCinf ARO-ENaC PK parameters from Day 1 and Day 1_3 will be compared across each dose level (RDD) to assess dose proportionality. If AUCinf cannot be determined reliably for all subjects or treatments, an alternative AUC measure, such as AUC to a fixed timepoint or AUClast, may be used in the statistical analysis of dose proportionality. Analyses for NHV and CF patients will be performed separately. Statistical analyses will be done using a power model with the following general form:

$$\ln(\text{PK parameter}) = \alpha + \beta * \ln(\text{dose}) + \varepsilon$$

where α is the y-intercept, β is the slope, and ε is the error. Estimates of α and β, and 90% confidence intervals (CIs) for β, will be provided for each PK parameter. Dose proportionality will be indicated if the Cmax and AUC 90% CIs for β all contain 1. The SAS code will be similar to the following:

```
proc reg data=adpp;
     by parameter;
     model ln(aval) = ln(dose) / clb alpha=0.1;
run;
```

10. CHANGES FROM PROTOCOL-SPECIFIED ANALYSES

There are no major changes between the protocol-defined statistical analyses and those presented in this statistical plan.

11. LITERATURE CITATIONS/REFERENCES

Clinical Study Protocol. A Phase 1/2a Dose-Escalating Study to Evaluate the Safety, Tolerability and Pharmacokinetic Effects of AROENaC in Normal Healthy Volunteers and Safety, Tolerability and Efficacy in Patients with Cystic Fibrosis. Version 4.0. Final, 09 Apr 2021.

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.

SAS Institute, Inc., SAS® Version 9.4 software, Cary, NC.

12. DATA NOT COVERED BY THIS PLAN

Not Applicable.

13. APPENDIX A: GRADING LABORATORY VALUES ACCORDING TO CTCAE VERSION 5

	Grade 1	Grade 2	Grade 3	Grade 4
Hematology				
Eosinophils (Eosinophilia)	> ULN and > baseline		Steroids initiated	
Hemoglobin, increased	Increase in > 0 - 2 g/dL	Increase in > 2 - 4 g/dL	Increase in > 4 g/dL	
Hemoglobin, decreased (Anemia)	< LLN - 10.0 g/dL	< 10.0 – 8.0 g/dL	< 8.0 g/dL OR transfusion indicated	Life-threatening consequences or urgent intervention required
Leukocytes, increased (Leukocytosis)			> 100,000/mm3	Clinical manifestations of leucostasis or urgent intervention indicated
Leukocytes, decreased	< LLN – 3000/mm3	< 3000 – 2000/mm3	< 2000 – 1000/mm3	< 1000/mm3
Lymphocytes, increased		> 4000 – 20,000/mm3	> 20,000/mm3	
Lymphocytes, decreased	< LLN – 800/mm3	< 800 – 500/mm3	< 500 – 200/mm3	< 200/mm3
Neutrophils, decreased	< LLN – 1.5 x 10^9/L	< 1.5 x – 1.0 x 10^9/L	< 1.0 – 0.5 x 10^9/L	< 0.5 x 10^9/L
Platelet count, decreased	< LLN – 75.0 x 10^9/L	< 75.0 x – 50.0 x 10^9/L	< 50.0 x – 25.0 x 10^9/L	< 25.0 x 10^9/L
Serum Chemistry				
Albumin, low (Hypoalbuminemia)	< LLN – 30 g/L	< 30 – 20 g/L	< 20 g/L	Life-threatening consequences or urgent intervention required
Amylase, increased	> ULN – 1.5 x ULN	> 1.5 x – 2.0 x ULN OR > 2.0 x – 5.0 x ULN without symptoms	> 2.0 x – 5.0 x ULN with signs or symptoms OR > 5.0 x ULN without symptoms	> 5.0 x ULN with signs or sympto ms
Alanine aminotransferase, increased	Normal baseline: > ULN – 3.0 x ULN Abnormal baseline: 1.5 x – 3.0 x baseline	Normal baseline: > 3.0 x – 5.0 x ULN Abnormal baseline: > 3.0 x – 5.0 x baseline	Normal baseline: > 5.0 x – 20.0 x ULN Abnormal baseline: > 5.0 x – 20.0 x baseline	Normal baseline: > 20.0 x ULN Abnormal baseline: > 20.0 x baseline
Alkaline Phosphatase, increased	Normal baseline: > ULN – 2.5 x ULN Abnormal baseline: 2.0 x – 2.5 x baseline	Normal baseline: > 2.5 x – 5.0 x ULN Abnormal baseline: > 2.5 x – 5.0 x baseline	Normal baseline: > 5.0 x – 20.0 x ULN Abnormal baseline: > 5.0 x – 20.0 x baseline	Normal baseline: > 20.0 x ULN Abnormal baseline: > 20.0 x baseline
Aspartate aminotransferase, increased	Normal baseline: > ULN – 3.0 x ULN Abnormal baseline: 1.5 x – 3.0 x baseline	Normal baseline: > 3.0 x – 5.0 x ULN Abnormal baseline: > 3.0 x – 5.0 x baseline	Normal baseline: > 5.0 x – 20.0 x ULN Abnormal baseline: > 5.0 x – 20.0 x baseline	Normal baseline: > 20.0 x ULN Abnormal baseline: > 20.0 x baseline
Bicarbonate, decreased	< LLN and no intervention initiated			

	Grade 1	Grade 2	Grade 3	Grade 4
Calcium (corrected), high (Hypercalcemia)	> ULN – 2.9 mmol/L	> 2.9 – 3.1 mmol/L or symptomatic	> 3.1 – 3.4 mmol/L or hospitalization indicated	> 3.4 mmol/L or life-threatening consequences
Calcium (corrected), low (Hypocalcemia)	< LLN – 2.0 mmol/L	< 2.0 – 1.75 mmol/L or symptomatic	< 1.75 – 1.5 mmol/L or hospitalization indicated	< 1.5 mmol/L or life-threatening consequences
Creatinine, increased	> ULN – 1.5 x ULN	Normal baseline: > 1.5 – 3.0 x ULN Abnormal baseline: > 1.5 x – 3.0 x baseline	> 3.0 – 6.0 x ULN OR > 3.0 x baseline	> 6.0 x ULN
Creatine phosphokinase, increased	> ULN – 2.5 x ULN	> 2.5 – 5.0 x ULN	> 5 – 10 x ULN	> 10 x ULN
Gamma glutamyl transferase, increased	Normal baseline: > ULN – 2.5 x ULN Abnormal baseline: 2.0 x – 2.5 x baseline	Normal baseline: > 2.5 x – 5.0 x ULN Abnormal baseline: > 2.5 x – 5.0 x baseline	Normal baseline: > 5.0 x – 20.0 x ULN Abnormal baseline: > 5.0 x – 20.0 x baseline	Normal baseline: > 20.0 x ULN Abnormal baseline: > 20.0 x baseline
Glucose, high (Hyperglycemia)	Abnormal glucose above baseline with no medical intervention	Change in daily management from baseline for a diabetic or oral antiglycemic agent initiated or workup for diabetes	Insulin therapy initiated or hospitalization indicated	Life-threatening consequences or urgent intervention indicated
Glucose, low (Hypoglycemia)	< LLN - 3.0 mmol/L	< 3.0 - 2.2 mmol/L	< 2.2 - 1.7 mmol/L	< 1.7 mmol/L or life-threatening consequences or seizures
Potassium, high (Hyperkalemia)	> ULN – 5.5 mmol/L	> 5.5 – 6.0 mmol/L or intervention indicated	> 6.0 – 7.0 mmol/L or hospitalization indicated	> 7.0 mmol/L or life-threatening consequences
Potassium, low (Hypokalemia)	< LLN – 3.0 mmol/L without symptoms	< LLN – 3.0 mmol/L with symptoms or intervention indicated	< 3.0 – 2.5 mmol/L or hospitalization indicated	< 2.5 mmol/L or life-threatening consequences
Sodium, high (Hypernatremia)	> ULN – 150 mmol/L	> 150 - 155 mmol/L or intervention initiated	> 155 – 160 mmol/L or hospitalization indicated	> 160 mmol/L or life-threatening consequences
Sodium, low (Hyponatremia)	< LLN – 130 mmol/L	125-129 mmol/L without symptoms	125-129 mmol/L with symptoms OR 120-124 mmol/L regardless of symptoms	< 120 mmol/L or life-threatening consequences
Total bilirubin, increased	Normal baseline: > ULN – 1.5 x ULN Abnormal baseline: > 1.0 x – 1.5 x baseline	Normal baseline: > 1.5 x – 3.0 x ULN Abnormal baseline: > 1.5 x – 3.0 x baseline	Normal baseline: > 3.0 x – 10.0 x ULN Abnormal baseline: > 3.0 x – 10.0 x baseline	Normal baseline: > 10.0 x ULN Abnormal baseline: > 10.0 x baseline
Coagulation				

	Grade 1	Grade 2	Grade 3	Grade 4
INR, increased	Not on anticoagulant: > 1.2 – 1.5 On anticoagulant: > 1.0 x – 1.5 x baseline OR monitoring indicated	Not on anticoagulant: > 1.5 – 2.5 On anticoagulant: > 1.5 x – 2.5 x baseline OR dose adjustment indicated	Not on anticoagulant: > 2.5 On anticoagulant: > 2.5 x baseline OR bleeding	
Activated Partial Thromboplastin Time, prolonged	> ULN – 1.5 x ULN	> 1.5 x – 2.5 x ULN	> 2.5 x ULN or bleeding	

14. APPENDIX B: NONCOMPARTMENTAL PHARMACOKINETIC ANALYSIS

14.1.1 Handling Missing or Non-Quantifiable Data

For NCA, BLQ plasma concentrations will be assigned a value of 0. The following rules apply with special situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If a predose plasma concentration is missing, it may be set to zero by default, for first dose only.
- if an embedded BLQ value is considered anomalous within the concentration time profile, this value will be set as missing and excluded from the summary statistics.

For Urine PK parameters calculation, all BLQ will be set to zero.

14.1.2 Pharmacokinetic Parameters Calculation

Standard PK parameters will be determined, where possible, from the plasma and urine concentrations of ARO-ENaC using NCA methods in the validated software program Phoenix WinNonlin (Certara USA, Inc. version 8.1 or higher). The PK parameters will be calculated for Day 1, Day 3, and Day 1_3.

PK analysis will be carried out, where possible, using actual blood sampling times postdose. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

Cmax and Tmax will be obtained directly from the concentration-time profiles. For multiple peaks, the highest postdose concentration will be reported as Cmax. In the case that multiple peaks are of equal magnitude, the earliest Tmax will be reported.

14.1.2.1 Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λz) will only be calculated when a reliable estimate can be obtained using at least 3 data points, not including Cmax, and the adjusted coefficient for determination of exponential fit (R2-adj) of the regression line is ≥ 0.7. Parameters requiring λz for their calculation (eg, AUCinf, t1/2, CL, and Vz) will only be calculated if the R2-adj value of the regression line is ≥ 0.7.

The following regression-related diagnostic PK parameters will be determined, when possible.

Parameter	Units	Definition
λz	1/h	apparent terminal elimination rate constant
λz N	NA	number of data points included in the log-linear regression
λz Span Ratio	NA	time period over which λz was determined as a ratio of t1/2
R2-adj	NA	adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λz (ie, the difference between λz Upper and λz Lower) should be ≥2 half-lives. If the λz Span Ratio is <2, the robustness of the t1/2 values will be discussed in the PK report.

14.1.2.2 Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of area under the concentration-time curve (AUC) will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow Cmax.

If the extrapolated area is >20%, AUCinf (and derived parameters) may be excluded from the summary statistics and statistical analysis at the discretion of Sponsor clinical pharmacology representative.

14.1.3 Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data must have strong justification and will be documented in the CSR.

Any quantifiable predose concentration value before the first dose will be considered anomalous and set to missing for the PK analysis.