Statistical Analysis Plan

PROTOCOL NUMBER:	AROHSD1001
STUDY TITLE:	A Phase 1/2a Single and Multiple Dose-Escalating Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamic Effects of ARO-HSD in Normal Healthy Volunteers as well as in Patients with NASH or Suspected NASH
NCT NUMBER:	NCT04202354
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PROTOCOL VERSION:	7.0
SAP VERSION:	1.0
SAP Date:	16 July 2021

Version Number	Date	Summary of Changes
V1.0	16 July 2021	NA (Original SAP)

Table of Contents

List of abbreviations and definitions of terms

Abbreviation or Term	Definition/Explanation
AE	Adverse event
Ae_{0_24h}	Amount of unchanged drug recovered in urine over 0 - 24 hours post dose
ALT	Alanine aminotransferase
AST	Aspartate transaminase
AUC_{0-24}	The area under the plasma concentration versus time curve from the zero to 24 hours
AUC_{inf}	Area under the curve from time 0 to infinity
AUC_{last}	The area under the plasma concentration versus time curve from the zero to the last quantifiable plasma concentration
C_{max}	Concentration maximum (peak)
CL_R	Renal clearance over a time interval calculated by Ae/AUC at the same time interval (0-24 h in this study).
CL/F	Apparent clearance (CL/F) calculated following extravascular drug administration
CRO	Contract Research Organization
DSC	Data Safety Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELF	Enhanced Liver Fibrosis (Score)
EOS	End of Study
fe_{0_24h}	Percentage of the administrated drug recovered in urine over 0 -24 hours postdose
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
HSD	Hydroxysteroid dehydrogenase
LISR	Local injection site reactions
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
NASH	Non-alcoholic steatohepatitis
NHV	Normal healthy volunteers
PBO	Placebo
PD	Pharmacodynamic
PK	Pharmacokinetic
PT	Preferred Term
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
RNAi	RNA interference
SOC	System Organ Class
$t_{1/2}$	terminal elimination half-life
T_{max}	Time to maximum observed plasma concentration (C_{max})
TEAE	Treatment-emergent adverse event
TESAE	Treatment-emergent serious adverse event

ULN	Upper Limit of Normal
Vz/F	Apparent terminal-phase volume of distribution following extravascular drug administration

1. INTRODUCTION

AROHSD1001 is a first in human dose-ranging study to assess the safety and tolerability of ARO-HSD in healthy volunteers and then in patients with NASH. Treatment with ARO-HSD is expected to reduce hepatic production of the HSD17β13 protein via RNAi.

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 AROHSD1001 Version 7.0, dated 12 March 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.

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

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

Primary Endpoint
• The incidence and frequency of adverse events (AEs) possibly or probably related to treatment after single doses in healthy volunteers and after multiple doses in patients with NASH or suspected NASH.
Secondary Endpoints
• Pharmacokinetics of ARO-HSD
Exploratory Endpoints
(All Cohorts) • Change in fasting LDL-C, Total Cholesterol, non-HDL-C, HDL-C, VLDL-C, and triglycerides compared to baseline
(Patient cohorts 1b, 3b and 4b) • Change in ALT and in AST as a marker of ARO-HSD activity compared to baseline • Change in cytokeratin-18 compared to baseline • Change in IL-1β, IL-6, IL-10, INF-gamma, and TNF-α compared to baseline • Change in retinol and retinol binding protein compared to baseline • Change in liver fat using MRI-PDFF compared to baseline • Change in blood exosomal HSD17β13 mRNA levels (if feasible) compared to baseline • Change in blood exosomal PNPLA3 mRNA levels (if feasible) compared to baseline • Change in Enhanced Liver Fibrosis (ELF) Score at EOS compared to baseline

- Change in Pro-C3 at EOS compared to baseline
- Liver biopsy derived HSD17β13 mRNA expression compared to baseline
- Liver biopsy derived HSD17β13 protein expression compared to baseline in response to multiple doses of ARO-HSD (if scientifically feasible)
- Liver biopsy derived change in PNPLA3 mRNA expression compared to baseline
- Liver biopsy derived change in PNPLA3 protein expression compared to baseline in response to multiple doses of ARO-HSD (if scientifically feasible)

2.2 Hypotheses and/or Estimations

No formal statistical hypotheses will be tested in this study. Any reported statistical tests will be considered exploratory.

3. STUDY OVERVIEW

3.1 Study Design

The study will be conducted in approximately 50 adult males and females, aged 18 through 65 years (minimum of 19 years of age for NASH or suspected NASH patients) with BMI between 18.0 and 40.0 kg/m² enrolled and dosed as described in the table below. Cohorts 1, 2, 3, and 4 (NHVs) are double-blind, randomized with a 1:1 (active:PBO) ratio to reduce bias. Cohorts 1b, 3b and 4b are open-label with all subjects receiving active treatment and include patients with NASH or suspected NASH.

Cohort Summary

Cohort	Population	Blinding	# Subjects	Dosing Schedule
1	NHVs	Double blind	8 (4 active: 4 PBO)	25 mg Day 1 only
1b	Patients with suspected NASH (biopsies on screen and Day 71)	Open-Label	up to 6 (all active)	25 mg Days 1, 29
2	NHVs	Double blind	8 (4 active: 4 PBO)	50 mg Day 1 only
3	NHVs	Double blind	8 (4 active: 4 PBO)	≤ 100 mg Day 1 only
4	NHVs	Double blind	8 (4 active: 4 PBO)	≤ 200 mg Day 1 only
3b	Patients with suspected NASH (biopsies on screen and Day 71)	Open-Label	up to 6 (all active)	≤100 mg Days 1, 29
4b	Patients with suspected NASH (biopsies on screen and Day 71)	Open-Label	up to 6 (all active)	≤200 mg Days 1, 29

For NHV subjects in Cohorts 1 through 4, the duration of the study clinic visits is approximately 158 days from screening to the End-of-Study (EOS) visit. For subjects in Cohorts 1b, 3b and 4b, the duration of the study clinic visits is approximately up to 173 days from screening to the EOS visit.

The final analysis will be completed following database lock after the last subject's last visit. Individual cohorts may be summarized prior to database release; however, the final analysis will be based on all available data collected in the study.

3.2 Sample Size

This study represents a proof of principle study, and as such no formal sample size calculation was performed.

Approximately 50 subjects may be enrolled in the study (not including replacements or optional expansion cohort).

4. COVARIATES AND SUBGROUPS

4.1 Planned Covariates

There are no predetermined covariates.

4.2 Subgroups

There are no planned subgroup analyses.

5. DEFINITIONS

Day 1:
For each subject, Day 1 is defined as the first day that protocol-specified study treatment is administered to the subject.

End of Study (EOS) Date:
For each subject, the EOS date is the study completion/early termination date recorded on the eCRF.

Study Day:
For each subject, and for a given date of interest, study day is defined as the number of days relative to Day 1:

- After first dose date: study day = date of interest – Day 1 date + 1
- Prior to first dose date: study day = date of interest – Day 1 date, so that the day prior to Day 1 is Day -1

Study End Date:
The study end date is when the last subject finishes the EOS visit.

Baseline Values:
For all variables, baseline is defined as the last non-missing value obtained prior to the first dose administration on Day 1.

For ECG, if a triplicate is taken prior to the first dose administered on Day 1, baseline is defined as the mean over the set of triplicate assessments.

Analysis Day:
Selected endpoints will be summarized based on Analysis Day defined using Study Day as below.

Scheduled Visit/ Analysis Day	Day 8	Day 15	Day 29	Day 43	Day 57	Day 71	Day 85	Day 99	Day 113
Study Day Window Cohort 1-4	[6,10]	[13,17]	[27,31]	[41, 45]	[55,59]	[69,73]	[83,87]	[97,101]	[111,115]
Study Day Window Cohort 1b,3b,4b	NA	[13,17]	[27,31]	NA	[55,59]	[66,76]	[83,87]	NA	[108,118]

If there is more than one record in a study day window, the analytical record for that specific analysis day will be defined as the record closest to the scheduled visit day. If two records are equidistant from the scheduled visit day, then the earlier record will be chosen. If there are multiple records on the same day, the last record will be used.

Duration of study:
EOS date – Day 1 date + 1

Treatment-Emergent Adverse Event (TEAE):
TEAEs are defined as adverse events occurring on or after the first dose of study treatment through EOS or pre-treatment existing conditions that worsen after the investigational product administration.

Treatment-emergent SAEs (TESAEs):
TESAEs are defined as serious adverse events occurring on or after the first dose of study treatment through EOS or pre-treatment existing conditions that worsen after the investigational product administration.

TEAE at Injection Site:
TEAE occur at the injection site. MedDRA Preferred Terms which are included in LISR are listed in Protocol Section 16.2.

TEAE Related to Local Injection Site Reaction (LISR):
This is defined as adverse events at the injection site which start on the day after the injection of the investigational product and persist for at least 48 hours. MedDRA Preferred Terms which are included in LISR are listed in Protocol Section 16.2.

6. ANALYSIS SETS

6.1 Full Analysis Set

For cohorts 1-4, the full analysis set includes all enrolled subjects who are randomized. For cohorts 1b, 3b, and 4b, the full analysis set includes all subjects who sign the informed consent form and receive at least one dose of investigational product.

Disposition data will be summarized and listed using the Full Analysis Set.

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 (PK) Analysis Set

This analysis set includes all subjects who receive at least one dose of active study treatment and have sufficient plasma concentration data to characterize PK profile. Subjects may be excluded if they have protocol deviations which would affect the PK results.

7. PLANNED ANALYSES

7.1 Interim Analysis and Early Stopping Guidelines

An external independent DSC will formally review the accumulating data from this study. Analyses for the DSC are provided by the Independent Biostatistical Center (IBC), which is external to Arrowhead.

7.2 Final Analysis

Final Analysis will be performed upon completion of all cohorts in the study, following database lock and unblinding.

8. DATA SCREENING AND ACCEPTANCE

8.1 General Principles

The objective of data screening is to assess the quantity, quality and statistical characteristics of the data related to the requirements of the planned analyses.

8.2 Data Handling and Electronic Transfer of Data

Arrowhead Pharmaceutical Inc. (Arrowhead) Data Management will provide all data to be used in the planned analyses. This study will use the RAVE database.

All data collected in the eCRF will be extracted from RAVE. Protocol deviations will be transferred from the Clinical Trial Management System. Unblinded randomization list will be transferred from Novotech to Arrowhead Statistical Programming (or a CRO delegate) upon Database Lock (DBL).

Data will be provided to EMB as Standard Data Tabulation Model (SDTM) datasets.

8.3 Handling of Missing and Incomplete Data

Missing dates 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.

8.4 Detection of Bias

Lack of protocol compliance may introduce potential bias in the estimation of protocol endpoints. Clinical study team will assess incidence of important protocol deviation. Clinical study team will also identify and document the criteria for important protocol deviations.

8.5 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.

8.6 Distributional Characteristics

Distributional assumptions for the secondary endpoints may be assessed. Prior to implementation of parametric methods of analysis, if the assumptions of the analysis are not met, then alternative methods may be utilized. The use of alternative methods will be fully justified in the CSR.

8.7 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

9.1 General Considerations

Descriptive statistics will be provided for selected demographic, safety and efficacy data. Descriptive statistics on continuous data will include the number of subjects, mean, median, standard deviation, minimum & maximum while categorical data will be summarized using frequency counts and percentages. Graphical summaries of the data may be also presented.

Unless otherwise noted, all summaries and figures will be presented by treatment, with NHV Cohorts 1-4 and Patient Cohorts 2b, 3b, and 4b presented separately. Subjects receiving placebo will be pooled across cohorts.

9.2 Subject Disposition

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

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 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.

- Sex: Male and Female
- Age at screening in years
- Baseline weight
- Baseline height
- Baseline BMI

Additionally, demographic and baseline characteristic data for patients with NASH or suspected NASH cohorts will include:

- HSD17B13 Genotype (T/T vs T/TA)
- PNPLA3 Genotype (C/C vs G/G)

9.5 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. Medical history will be summarized both by number and proportion of participants receiving therapies of interest presented by system organ class (SOC) and preferred term (PT) as coded by the Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 or later.

9.6 Safety Analyses

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

9.6.1 **Adverse Events**

Adverse event (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, the latest available version) and data will be summarized by System Organ Class (SOC) and a Preferred Term.

A table providing an overall summary of AEs will be produced which will include the number of TEAEs; the number and percentage of participants reporting at least one: TEAE, TESAE, grade 3 (severe) or higher TEAE, TEAE related to study treatment (possibly or probably related to ARO-HSD), TESAE related to study treatment, grade 3 or higher TEAE related to the investigational product and TEAE leading to drug or study withdrawal.

Descriptive statistics summaries of subject incidence will be summarized for all TEAEs, treatment-related TEAEs, TESAEs, treatment-related TESAEs, and TEAEs resulting in investigational product withdrawal or study discontinuation, treatment emergent fatal AEs, TEAE at injection site and TEAE related to LISR will be tabulated by SOC in alphabetical order and PT in descending order of frequency. Descriptive summaries will include count and percent of subjects as well as number of events..

Descriptive statistics summaries of subject incidence of TEAEs, TEAE at injection site and TEAE related to LISR by SOC, PT, and severity will be provided. Subjects will be counted once under the highest severity reported within each SOC and PT. The 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 relationship to investigational product 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.

A by-subject AE data listing, including verbatim term, MedDRA SOC and PT, TEAE flag, seriousness, severity, outcome and relationship to study treatment will be provided. Separate listings will be generated for SAEs.

9.6.2 Laboratory Test Results

Laboratory assessments include biochemistry, coagulation, hematology, urinalysis. 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.

Tables of shifts from baseline to the worst post-baseline grade (graded according to the NCI Common Toxicity Grading Criteria in Appendix A) will be provided for the following laboratory parameters: ALT, AST, GGT, ALP, total bilirubin, and CK. Unscheduled assessments will be included in the shift tables.

An eDISH plot will be created between peak Total Bilirubin vs. peak ALT, both in multiples of ULN, with horizontal and vertical lines indicating Hy's law thresholds: ALT = 3 × ULN and total bilirubin = 2 × ULN. For Cohorts 1-4, a single plot will be produced, with individual subjects noted as receiving either active treatment or PBO (different tick marks).

9.6.3 Analyses of Lipid Parameter

Descriptive statistics summaries will be provided for following parameters: triglycerides, HDL-C, LDL-C, total cholesterol, non-HDL-C, and VLDL-C. Derived changes and percent changes from baseline to each scheduled postdose visit will be included.

9.6.4 Vital Signs

Descriptive statistics summaries of vital sign parameters (systolic blood pressure, diastolic blood pressure, pulse rate, body temperature and respiratory rate) will be provided by scheduled visit. Derived changes and percent changes from baseline to each scheduled postdose visit will be included.

All vital sign parameters will be listed per cohort for active drug participants, for placebo participants pooled across cohorts, as well as for all participants (active drug and placebo) considered collectively, for all time points assessed.

9.6.5 Physical Examinations

Descriptive statistics summaries of physical examination will be provided by scheduled visit, body system and abnormality.

By-participant data listings will be generated for all the physical examination data, including the Investigator assessment of clinical significance for abnormal findings, for all time points assessed.

9.6.6 Electrocardiogram

Descriptive statistics summaries of electrocardiogram (ECG) parameters (heart rate, PR interval, QRS interval, QT interval, and QTcF interval) will be provided by scheduled visit. Derived changes and percent changes from baseline to each scheduled postdose visit will be included. If more than one measure was collected at a scheduled visit (maximum 3), then the average value will be used.

The overall interpretation of 12-lead ECG results will be classified using frequency counts and percentages for the categories of Normal, Abnormal NCS and Abnormal CS for each treatment group for all time points assessed.

Tables of shifts from baseline to the worst post-baseline clinical significance will be provided for the overall interpretation of 12-lead ECG assessments. Additionally, subjects will be categorized and summarized per their maximum post-baseline absolute QTcF interval using limits of 450ms, 480 msec and 500 msec. They will also be categorized per their maximum change from baseline QTcB and QTcF interval using limits of 30 msec and 60 msec.

All ECG data will be presented in the by-subject data listings.

9.6.7 Anti-drug Antibodies

Descriptive statistics summaries (number and percent of subject with positive ADA) of anti-drug antibodies (ADA) will be provided by scheduled visit for each treatment group, pooled placebo and pooled treatment, as data applicable. Also, the titer values will be summarized descriptively for each time point by treatment if data available.

If ADA positive were observed at baseline, the postdose titer values will be compared with baseline and plotted vs time point to see if a treatment-boosted ADA happening to the subject.

If any ADA positive observed, spaghetti plots of individual plasma concentrations versus actual time will be presented by ADA category for each treatment group for PK analysis set.

All ADA data will be listed by subject for safety analysis set which has at least one ADA data reported.

9.6.8 Exposure to Investigational Product

The number of subjects receiving study treatment will be summarized by the total number of injections received and the duration of study. Duration of study will be calculated in weeks as: (EOS date – date of first dose + 1)/7.

9.6.9 Exposure to Concomitant Mediation

Medications with an end date on or after the first dose of investigational product will be identified as concomitant medications. Medications will be coded using World Health Organization Drug Dictionary (WHO-DD, the latest available version). and will be reported by Anatomical Therapeutic Chemical (ATC) classification level 4 and Preferred Drug Name.

Participants who take the same medication more than once will be counted only once for that preferred term.

Descriptive statistics summaries of the number and percent of subjects who receive each concomitant medication will be summarized.

9.7 Other Analyses

9.7.1 Analyses of Pharmacokinetic Endpoints

All PK summaries outlined in this section will be created using the PK Analysis Set. Plasma PK concentration and PK parameters (except Tmax) summaries will additionally include the geometric mean and the geometric coefficient of variation (CV).

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 and used for PK parameters calculation but will be excluded from summary of concentration data.

All plasma concentration data will be listed by subject.

Urine PK concentration data will be listed and summarized.

The following PK parameters will be calculated for ARO-HSD PK concentration data for NHV and patients with suspected NASH, whenever data applicable:

Parameter	Definition
C_{max}	Maximum observed plasma concentration
T_{max}	Time to maximum observed plasma concentration (C_{max})
AUC_{0-24}	The area under the plasma concentration versus time curve from the zero to 24 hours
AUC_{last}	The area under the plasma concentration versus time curve from the zero to the last quantifiable plasma concentration
AUC_{inf}	The area under the plasma concentration versus time curve from zero to infinity
$t_{1/2}$	Apparent terminal elimination half-life
CL/F	Apparent clearance (CL/F) calculated following extravascular drug administration

V_Z/F	Apparent terminal-phase volume of distribution following extravascular drug administration
Ae_{0_24h}	Amount of unchanged drug recovered in urine over 0 - 24 hours post dose
fe_{0_24h}	Percentage of the administrated drug recovered in urine over 0 -24 hours postdose
CL_R	Renal clearance over a time interval calculated by Ae/AUC at the same time interval (0-24 h in this study).

Additional PK parameters such as dose/body weight normalized PK parameters may also be calculated as applicable.

The Sponsor will provide NCA analysis of plasma and urine PK data. Details about the PK parameter calculations are located in Appendix B.

The plasma and urine PK parameters will be listed and summarized descriptively by treatment. The diagnostic PK parameters listed in Appendix B will be listed only.

The Cmax and AUCs (AUClast and AUCinf) PK parameters from Cohort 1- 4 Day 1 will be compared across each dose level to assess dose proportionality. 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;
```

9.7.2 **Analyses of Pharmacodynamic Endpoints**

Pharmacodynamic endpoints will be analyzed for cohorts 1b, 3b and 4b and will be created using the Safety Analysis Set.

For NASH biomarkers, descriptive statistics summaries will be provided for following biomarkers:

- ELF which consists of Hyaluronic acid (HA), Procollagen III amino terminal peptide (PIIINP), and Tissue inhibitor of metalloproteinase 1 (TIMP-1)]
- Cytokeratin-18
- PRO-C3
- IL-1β, IL-6, IL-10
- INF-gamma, and TNF-α
- Liver Fibrosis Score
- Retinol binding protein

- Tumor Necrosis Factor
- Vitamin A

Derived changes and percent changes from baseline to each scheduled postdose visit will be included.

For hepatic imaging (MRI-PDFF and FibroScan), descriptive statistics summaries will be provided. Derived changes and percent changes from baseline to each scheduled postdose visit will be included.

Relative expression for HSD17B13 will be summarized and plotted using a dot plot. Relative expression is defined as $\left[2^{-(X_n - X_1)} - 1\right] * 100\%$

10. CHANGES FROM PROTOCOL-SPECIFIED ANALYSES

As of this date, there have been no 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 Single and Multiple Dose-Escalating Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamic Effects of ARO-HSD in Normal Healthy Volunteers as well as in Patients with NASH or Suspected NASH. Version 7.0. Final, 12 March 2021.

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

12. APPENDIX

12.1 Appendix A: Grading Laboratory Values according to CTCAE Version 5

	Grade 1	Grade 2	Grade 3	Grade 4
Serum Chemistry				
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
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
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

12.2 APPENDIX B: Noncompartmental Pharmacokinetic Analysis

12.2.1 Handling Missing or Non-Quantifiable Data

For Noncompartmental analysis (NCA), plasma concentrations below the limit of quantification (BLQ) 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 and summary, all BLQ will be set to zero.

12.2.2 Pharmacokinetic Parameter Calculation

Standard PK parameters will be determined, where possible, from the plasma and urine concentrations of ARO-HSD using noncompartmental methods (NCA) in validated software program Phoenix WinNonlin (Certara USA, Inc. version 8.1 or higher).

Pharmacokinetic 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.

AUC will be estimated using the linear trapezoidal rule for increasing concentrations and the log-trapezoidal rule for decreasing concentrations (i.e., “linear up / log down” trapezoidal rule in Phoenix WinNonlin).

12.2.2.1 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 ≥ 0.8. 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 ≥0.8.

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.

12.2.2.2 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 Arrowhead clinical pharmacology representative and EMB.

12.2.3 **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.