Statistical Analysis Plan

A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Clinical Study with ALN-AS1.

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ABBREVIATIONS

Abbreviation	Definition
AAR	Annualized attack rate
ADA	Antidrug antibody
ADaM	Analysis Data Model
AE	Adverse event
AECI	Adverse event of clinical interest
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ATC	Anatomic Therapeutic Class
BMI	Body mass index
BPI-SF	Brief Pain Inventory - Short Form
CDISC	Clinical Data Interchange Standards Consortium
cERD	Circulating extracellular RNA detection assay
CI	Confidence interval
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DILI	Drug Induced Liver Injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDISH	Evaluation of drug-induced serious hepatotoxicity
eGFR	Estimated Glomerular Filtration Rate
EQ-5D	EQ-5D Quality of Life questionnaire
EOS	End of Study
GalNac	N-acetyl glactosamine
HLT	High level term
ISR	Injection site reaction
LFT	Liver function test
LLOQ	Lower Limit of Quantification
mBMI	Modified body mass index
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger ribonucleic acid
ms	Millisecond
NCA	Non-compartmental analysis
PBG	Porphobilinogen

Abbreviation	Definition
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred term
Q1	First quartile
Q3	Third quartile
QOL	Quality of life
QTc	Corrected QT interval
QTcB	QTc using Bazett's formula
QTcF	QTc using Fridericia's formula
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SEM	Standard error of the mean
SI	Standard International (unit)
SMQ	Standardized MedDRA Query
SOC	System organ class
SRC	Safety Review Committee
ULN	Upper limit of normal
WHO	World Health Organization

1. INTRODUCTION

This Statistical Analysis Plan (SAP) has been written based upon clinical protocol (amendment 4.0, date: 02 August 2017) and outlines the planned analyses for safety, Pharmacodynamic (PD), clinical activity and Pharmacokinetic (PK) in patients with acute intermittent porphyria (AIP).

This SAP supersedes the statistical considerations described in the clinical protocol (amendment 4.0; date: 02 August 2017). If there are differences between the statistical considerations in this SAP compared to the protocol, then this will be identified in the clinical study report (CSR).

The purpose of this SAP is to summarize key analyses to be conducted for each objective and presented in the CSR. Population PK or PK-PD analysis is out of scope and will be described in another document. Tables, Listings, and Figures mock shells will also reside in a separate document.

2. STUDY DESIGN

Study ALN-AS1-002 is an open-label, extension study designed to evaluate the long-term safety, clinical activity of givosiran in patients with AIP. The number of patients included up to 16 patients from Part C in the parent study ALN-AS1-001.

Eligible patients who completed Part C in the parent study either received the same dosing regimen as the pivotal Phase 3 study (e.g. 2.5 mg/kg every month) or had 1 or more dosing regimen changes across studies. The change in dosing regimen for a patient may occur due to emerging data and decisions from the Safety Review Committee (SRC). After a patient has completed 12 months of givosiran administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including givosiran administration may take place at the patient's home by a healthcare professional. The expected duration of treatment for each patient is up to 36 months (3 years) followed by a post-treatment visits at either month 39/Early Termination visit or month 42.

3. OBJECTIVES OF THE STUDY

3.1. Primary Objective:

- Evaluate the long-term safety and tolerability of givosiran in patients with AIP

3.2. Secondary Objectives:

- Assess the PD effect of ALN-AS1 over time
- Assess the clinical activity of ALN-AS1 over time

3.3. Exploratory Objectives:

- Characterize the PK profile of ALN-AS1
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

4. POPULATIONS

Population	Definitions
Safety Analysis Set	All patients who receive any amount of study drug
PD Analysis Set	All patients who receive any amount of study drug and who have at least 1 post-dose blood sample for PD
PK Analysis Set	All patients who receive any amount of study drug and have at least 1 post-dose blood sample for PK and who have evaluable PK data

Safety Analysis Set will be used to analyze safety and clinical activity data. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

5. MISSING DATA

For continuous and categorical variables, data will not be imputed unless otherwise specified.

There will be no imputation used for partial and/or completely missing dates for events defined as 'porphyria attacks' and for dates of hemin medication. For Delta-aminolevulinic acid (ALA)/ Porphobilinogen (PBG) biomarkers, if any value is recorded as <Lower Limit of Quantification (LLOQ) then the assigned value used for calculations will be assigned a value of LLOQ/2.

The following rules will be applied to incomplete dates to determine the reporting of adverse events (AEs) or medications. These imputed dates will not be displayed in listings because these dates are used to determine if the medication was prior or concomitant to first dose in this study and if the AE occurred on or after the first dose in this study. The rules below do not apply to hemin medications or AEs defined as 'porphyria attacks'.

Imputation rules for Start Date of AE:

- If start date is completely missing, then start date will be imputed to be the date of the first dose of study drug
- For a partial start date (day is missing, month is missing or both day and month are missing):
  - partial date < the first dose date: the first day/month
  - partial date = the first dose date: the first dose date
  - partial date > the first dose date: the first day/month

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date.

For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug in this study; medications will be classified as prior or concomitant depending on the medication stop dates. If any medications have a completely missing stop date, then the medication will be assumed as ongoing.

6. STATISTICAL METHODOLOGY

6.1. General Considerations:

As this is an extension study, formal statistical analyses will not be performed. All summaries will be descriptive.

Data will be presented in by-patient data listings. The listings will contain study days as described below:

> Study day: relative to the first dose of givosiran in this study. The first dose date is designated as Day 1. On treatment study days will be calculated as: evaluation date – first dose date+1. Pre-treatment study days will be calculated as: evaluation date – first dose date.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), median, quartiles (first quartile [Q1], third quartile [Q3]), minimum value, maximum value, and when specified, standard error of the mean (SEM). The precision of the measurement for each continuous variable will be used to determine the number of decimal places to present in tables, figures and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean, median, SD, quartiles, and SEM will be reported to 1 greater decimal place. Any values that require transformation to standard units (metric or Standard International [SI]) will be converted with the appropriate corresponding precision.

Frequencies and percentages will be presented for categorical and ordinal variables. If there are missing values, the number of missing values will be presented, but without a percentage. Percentages will be based on the number of non-missing values.

For assessments with repeated collection at a given study visit (e.g. electrocardiogram [ECG] parameters), the mean will represent the value at that visit unless otherwise noted.

6.2. Computing Environment

Analysis will be performed using SAS Version 9.4 or higher. Use of other software will be described in the CSR. Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) Analysis Model Version 2.1 or higher and CDISC ADaM Implementation Guide Version 1.0 or higher.

6.3. Baseline Definitions

Baseline will be defined as the derived baseline value in the parent study ALN-AS1-001 (Refer to ALN-AS1-001 SAP; dated 29 September 2017).

6.4. Study Groups

All tables and figures will be presented by the following study groups:

- Randomized treatment arm in the parent study/Current treatment arm (i.e., column for givosiran/givosiran vs. placebo/givosiran)
- Total (pooling regardless of randomized treatment arm in the parent study)

Summaries of key efficacy (e.g. PD and Clinical Activity) will also include study group:

- Intended Dose Group (i.e., givosiran 2.5 mg/kg monthly in ALN-AS1-002)

The following rules will be used:

- In analyses on PD measurements using the Intended Dose group, summaries at each postbaseline visit will be based on patients who are on 2.5 mg/kg at that visit.
- In analyses on clinical activity assessments (porphyria attacks and hemin use using the Intended Dose group, only events that occur between the first day of dosing and the last day of dosing with the intended dose regimen (2.5 mg/kg once monthly) are included.

An intended dose period will be defined as starting from the first dose of 2.5mg/kg Givosiran monthly.

Summaries of key safety (e.g. AEs) will also include study groups based upon initial dose in ALN-AS1-002:

- Initial dose of 2.5 mg/kg (monthly)
- Initial dose of 5 mg/kg (at any frequency)

6.5. Visit Windows

Data will be tabulated and analyzed per the visit as recorded in the electronic case report form (eCRF).

Data collected at unscheduled visits will be included in by-subject listings and figures, but no assignment to a scheduled visit will be made for the purposes of by-visit summary tabulations. However, unscheduled visits will be considered for any categorical shifts summaries (e.g. shifts from baseline to 'worst' post-baseline value).

For patients who discontinued treatment, the time window for AEs, PD (ALA/PBG/ALAS1), attack and hemin use will be up to 28 days from the last dose.

6.6. Interim Analyses

Interim analyses will be performed to support regulatory activities and publications. These analyses will be descriptive in nature and will not involve any formal hypothesis testing.

For an interim analysis, as this study will be ongoing, a cut-off approach will be implemented to ensure data quality. The interim analysis will include data entered on or prior to a pre-specified cutoff date. For assessments with starting/end dates (e.g., Exposure, AEs, medical history, medications), the starting date will be compared to the pre-specified cut-off date.

7. STATISTICAL METHODOLOGY

7.1. Patient Disposition

Patient disposition will be summarized and include the total number and percentage of patients in the following categories:

- Enrolled Patients (signed informed consent and met eligibility)
- Treated Patients
- Completed Patients
- Safety Analysis Set
- PD Analysis Set
- PK Analysis Set

Summaries of the number and percentage who discontinued study treatment based upon primary reason for discontinuation (e.g. AE, Death, Lost to Follow-up, Physician Decision, etc.) will be presented. Summaries of the number and percentage who withdrew from study prematurely based upon primary reason for withdrawal (e.g. AE, Death, Lost to Follow-up, Physician Decision, etc.) will also be presented. Per patient data listings will be presented displaying the primary reason the patient discontinued treatment and/or went off study.

7.2. Protocol Deviations

Protocol violations will be classified into major or minor deviation by medical review. A major deviation is defined as a deviation that may significantly impact the completeness, accuracy and/or reliability of the trial data; that may significantly affect a patient's rights, safety and well-being (ICH.E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013). Deviations not classified as major will be assigned as minor. All protocol deviations will be presented in a listing.

7.3. Baseline Characteristics

Baseline characteristics will be based upon the derived baseline in the parent study ALN-AS1-001 (Refer to ALN-AS1-001 SAP; dated 29 September 2017). These characteristics include but are not limited to age, sex, race, ethnicity, body weight, height, and body mass index (BMI) as well as genotype, prior hemin prophylaxis status, baseline ALA, PBG, and 5-aminolevulinic acid synthase 1 (ALAS1) levels.

Data recorded in medical history in this study and data recorded in the medical history of the parent study ALN-AS1-001 will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC), high level term (HLT) and preferred term (PT). A patient only contributes once to the count for a given condition (overall, by SOC, by HLT, by preferred term). Medical history and pregnancy test results will also be presented in data listings.

7.4. Safety Analyses

The primary objective of this study is to evaluate the long-term safety and tolerability of givosiran in patients with AIP. All analyses will use the Safety Analysis Set. All clinical safety

laboratory data collected from the central laboratory will be used to evaluate safety. For summaries of ECG data, these will be performed separately based upon data collected locally and centrally.

Parameters: Safety and tolerability assessments for givosiran will include study drug exposure, AEs, clinical laboratory parameters (hematology, serum chemistry, liver function test [LFT], urinalysis, coagulation), vital signs, 12-lead ECGs, concomitant medication, and physical examinations.

Exposure: Summaries will include descriptive statistics of the duration of treatment (days) and total number of doses received. Additional summaries of the cumulative number of patients (percentage) that completed study treatment intervals (e.g. ≥1 day, ≥1 month, ≥3 month, etc.) and the number of patients (percentage) that completed study treatment at the latest visit interval will also be displayed. Additionally, cumulative number of doses received at home will be displayed.

The date of the last exposure to study drug is defined as the earliest day of the following dates:

- Last dose date + 27 days
- Analysis cut-off date
- The date of EOS

Duration of exposure (days) is defined as date of the last exposure – date of the first dose +1. Dose interruptions and compliance will not be considered when calculating of duration of exposure.

A separate per-patient exposure listing will be generated to display the dosing regimen in the parent study (ALN-AS1-001) and actual dosing (mg/kg) regimen(s) in this study.

Adverse Events: AEs will be coded using the MedDRA coding system (version 21.0 or later) and displayed in tables and data listing by SOC and PT. Patients who report multiple occurrences of the same PT will be classified according to the most related or most severe category. Patients with a missing severity or missing relationship to study drug will be classified to the most related or most severe category.

Summaries of AEs will consist of events which occur or worsen (including date/time when applicable) after the first dose of givosiran in this study. Note: Porphyria attacks are recorded on the AE eCRF, however they will not be reported as AEs but rather as an endpoint of clinical activity (see section 7.5.2); therefore, the term “AE” excludes porphyria events.

An overview of the frequency (percentage) of AEs including the total number of events will be tabulated (summaries such as but not limited to, the number of patients with at least 1 AE, the number of patients with at least 1 AE related to study drug, the number of patients with at least 1 serious AE [SAE], the number of patients who discontinued study drug to an AE). Separate tabulations will be generated by SOC and PT for all AEs, AEs by maximum severity, related AEs, related AEs by maximum severity, SAEs, discontinuation from study drug due to AEs, interruption due to AEs, and deaths. Patient listings will be generated separately for any patient who died, discontinued from study drug and reported any SAEs.

An additional set of AE outputs will also be generated by study groups defined by the initial dose of givosiran [i.e. Patients with an initial dose of 2.5 mg/kg (monthly) in ALN-AS1-002 and Patients with an initial dose of 5 mg/kg (at any time) in ALN-AS1-002].

Adverse Events of Clinical Interest (AECIs):

Injection Site Reactions [ISRs]: AEs mapping to the HLT="Injection Site Reactions" using MedDRA dictionary will be included in the summary. An ISR may consist of one or more signs or symptoms. Key summaries such as but not limited to will be generated: Number of patients with at least 1 ISR, percent of injections complicated by ISRs, frequency (percentages) of ISRs by PT (corresponds to individual sign or symptom). A separate listing will be generated to display all patients who reported ISRs.

Hepatic AEs, including LFT abnormalities considered clinically significant by the investigator: AEs mapping to the Standardized MedDRA Query (SMQ) Drug-related hepatic disorders - comprehensive search (includes all narrow and broad terms). The following key summaries will be generated: Frequency (percentages) of Hepatic SMQ Events by SOC and PT. A separate listing will be generated to display all patients who reported hepatic AEs.

Acute pancreatitis: AEs mapping to Acute Pancreatitis SMQ (narrow terms) plus the following PT terms (lipase increased, lipase abnormal, amylase increased, amylase abnormal, hyperlipasemia, hypermylasaemia, pancreatic enzyme abnormality, pancreatic enzymes abnormal, pancreatic enzymes increased). The following key summaries will be generated: Frequency (percentages) of Acute Pancreatitis Events by SOC and PT. A separate listing will be generated to display all patients who reported acute pancreatitis.

Anaphylactic Reaction: AEs mapping to the anaphylactic reaction SMQ. The following key summaries will be generated: Frequency (percentages) of anaphylactic reaction by search category (narrow and broad) and PT. A separate listing will be generated to display AEs that meet SMQ criteria.

Other Adverse Events:

Malignancies: AEs mapping to Malignant or Unspecified Tumors SMQ. The following key summaries will be generated: Frequency (percentages) of Malignancies by HLT and PT.

Acute Renal Failure: AEs mapping to the acute renal failure SMQ. The following key summaries will be generated: Frequency (percentages) of acute renal failure by SOC and PT.

Clinical Laboratory Parameters: Clinical laboratory parameters will be expressed in SI units. Laboratory data collected and recorded as below the LLOQ will be set to the lower limit of detection for calculation of summary statistics. Summaries generally include data from central laboratory. Certain summaries include data from both central and local laboratory (e.g., LFT).

Summary data for each laboratory parameter (hematology, serum chemistry, LFT, and coagulation), which are continuous variables, will have a tabular summary of descriptive statistics (n, mean, SD, median, quartiles, minimum, and maximum; see Appendix 2 for list of laboratory parameters). Descriptive statistics of actual value, change from baseline and percentage change from baseline at each scheduled visit will be displayed.

For each continuous parameter, severity will be categorized based upon Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Shift tables will be generated to summarize shifts from baseline category to the worst post-baseline category. Worst post-baseline category

will be considered the highest CTCAE grade or highest shift in ULN category unless otherwise specified (e.g. estimated glomerular filtration rate [eGFR]).

For hematology and chemistry laboratories, summary tables of potentially clinically significant abnormalities will also be provided.

The lipase and amylase (when applicable) will be categorized into the following categories: ≤ULN, >ULN and ≤1.5xULN, >1.5xULN and ≤2xULN, >2xULN and ≤5xULN, >5xULN. A tabular summary will be generated to summarize the number and associated percentage of patient in each of the categories at any post-baseline visit. A by-patient listing of lipase values will be generated.

The eGFR (mL/min/1.73 m^2) will be categorized into the following categories: ≥90,60-89, 45-59, 30-44, 15-29 and <15. A shift table of baseline to worst post-eGFR (i.e. category with the lowest value) will be presented.

All laboratory data will be presented in data listings, with potentially clinically significant values flagged. Out of range laboratory results will be identified in listings.

Liver Function Tests (LFTs): A listing will be generated for all patients with abnormal liver function tests as defined by ALT>3xULN, AST>3xULN or total bilirubin >2x ULN at any visit.

A tabular summary will be generated to summarize the number and associated percentage of patient in each of the categories at any post-baseline visit, shift tables will be presented for ALT, AST, total bilirubin:

- 1<ALT≤3, 3<ALT≤5, >5<ALT≤10, <10<ALT≤20, ALT>20xULN
- 1<AST≤3, 3<AST≤5, >5<AST≤10, <10<AST≤20, AST>20xULN
- 1<ALT or AST≤3, 3<ALT or AST≤5, >5<ALT or AST≤10, <10<ALT or AST≤20, AST or ALT>20xULN
- ALP>1.5xULN
- 1.5<Total Bilirubin ≤2, 2<Total Bilirubin≤3, 3<Total Bilirubin≤5 and Total Bilirubin>5
- ALT or AST > 3xULN and Concurrent Total Bilirubin > 2xULN
- ALT or AST > 3xULN and Concurrent Total Bilirubin > 1.5xULN

Evaluation of drug-induced serious hepatotoxicity (eDISH) plots of peak bilirubin at any time versus peak ALT or AST at any time will also be presented. Spaghetti plots for patients who had worst ALT > 3xULN or total bilirubin > 2xULN will be presented (include data from both central and local laboratory).

Vital Signs and Physical Examination: Descriptive statistics for each vital sign (weight, height, oral body temperature, blood pressure, heart rate, and respiration rate) will be summarized at scheduled visits. In addition, a tabular summary of potentially clinically significant post-baseline abnormalities in vital signs will be presented. Per patient listing of vital signs results will be generated.

ECG: Summaries of ECG data will be generated separately for data collected locally and centrally. Note that baseline ECG data is only available based on local collection.

ECG findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, corrected QT (QTc) interval. For each visit, the results will be the mean of the measurement from the triplicate ECG values for each patient at a single visit.

Descriptive statistics will be summarized at each scheduled visit. Change from baseline to each post-baseline assessment will be summarized (local ECG only). The number and percentage of patients with normal, abnormal or clinical significant abnormal results at each visit will also be summarized.

An outlier analysis will be performed for QTc using Bazett's (QTcB) and Fridericia's (QTcF) formula.

For local ECGs, categorical analysis via a shift table will be summarized as:

- The number and percentage of patients with maximum increase from baseline in QTcB/QTcF (≤30, >30-60, >60 milliseconds [ms]).
- The number and percentage of patients with maximum post-baseline QTcB/QTcF (≤450, >450-480, >480-500, >500 ms)

For central ECGs, categorical analysis will be summarized as follows:

- The number and percentage of patients with maximum post-baseline QTcB/ QTcF (≤450, >450-480, >480-500, >500 ms)

A per-patient listing of all ECG data will be generated for local and central data collection. A separate per patient listing with any post-baseline QTc value of > 500 ms or any patient with an increase from baseline of > 60 ms will also be generated.

Prior and Concomitant Medications: Concomitant medications will be coded using the WHO Drug Dictionary (March 2015 or later). Concomitant medications will be defined as any medication taken after the first dose of study drug in this study and any medication that started prior to the first dose in this study and was ongoing on or after the date of the first dose of study drug. If the end date of medication is missing or incomplete such that it cannot be determined whether it was prior to the first dose of study drug, it will be identified as concomitant.

Concomitant medications will be tabulated by Anatomic Therapeutic Class (ATC) and Preferred Term and by overall decreasing frequency of patients who took the medications. A patient listing will be provided for all prior and concomitant medications.

7.5. Secondary Analyses

7.5.1. Pharmacodynamic Biomarkers

One of the key secondary objectives is to evaluate the long-term PD effect of ALN-AS1 on urine levels of ALA and PBG.

ALA and PBG: Analysis of ALA and PBG concentrations will be performed on the PD Analysis Set. Values will be based upon urine concentrations collected from the central laboratory. In all analyses of urine ALA and PBG, the concentrations will be expressed as mmol/mol of urine creatinine (e.g, creatinine normalized values). Any values recorded as <LLOQ will be assigned a value of LLOQ/2.

Data will be summarized descriptively (n, mean, SD, SEM, median, quartiles, minimum, and maximum) at baseline and each post-baseline scheduled visits. The analysis will be performed separately for scheduled assessments collected not during attacks and all scheduled timepoints. Tabular summaries of actual value, change from baseline and knockdown values (knockdown= -1*percentage change) will be generated at each scheduled visit. Plots of the group means (±SEM) of actual values and knockdown values will also be generated. Per-patient plots of actual values over time will be generated.

7.5.2. Clinical Activity

Clinical activity of ALN-AS1 will be assessed by the number of porphyria attacks and by the number of doses of hemin administered.

Analysis of Clinical Activity will be performed on the Safety Analysis Set.

Porphyria attacks: An attack will be defined as any event with preferred term=”Porphyria” recorded on Adverse Event eCRF. Attacks which occur within the same date (i.e., end date of attack is the same date as start date of the next attack) will be counted as one attack. When collapsing attacks that occurred on the same date, the following rules will be applied for severity and location:

1. Severity will be assigned the maximum severity among the records.
2. Location will be assigned ‘hospital’ if ≥ 1 attack with location=hospital.
   Location will be assigned ‘outpatient’ if ≥ 1 attack with location=outpatient
   Location will be assigned ‘home’ if all the records state location=home.
   Location will be assigned ‘other’ if all the records state location=other.

The number of porphyria attacks during the treatment period (attacks with start dates on or after date of first dose) will be summarized. Annualized attack rate (AAR) per patient will be calculated as the total number of porphyria attacks divided by the total person-days at risk and multiplied by the total number of days in a year (365.25). Person-days at risk will be defined as: (date patient went off study – date of first dose+1). For an interim snapshot, the person-days will be defined by date of the first dose until the minimum of the date of the interim snapshot cut-off date or date the patient went off study. For calculation of AAR, only records with non-missing start and stop dates will be considered.

Porphyria attacks will be summarized separately in each subgroup:

1. Composite attacks: Attacks requiring hospitalization, urgent health care (i.e. outpatient clinic, Emergency Department, etc.) or IV hematin at home.
2. Attacks requiring hospitalization
3. Attacks requiring urgent health care (i.e. Outpatient)
4. Attacks required treatment with IV hematin at home
5. Attacks not treated with IV hematin at home
6. All attacks

For each subgroup, the total number of events, total person-years (total days/365.25), mean rate (total number of events/total person-years) and SEM will be presented for each study group. The

SEM will be calculated using Cochran's estimate of SEM for weighted mean [1]. Attacks for each subgroup will display the rates separately for each phase of study: run-in (ALN-AS1-001 study) and treatment phase (ALN-AS1-002). A tabular summary of percentage change (%) in AAR rate from run-in to treatment phase will be also calculated per cohort. In addition, a by-patient listing will summarize the porphyria attacks in each of the subgroups. An exploratory analysis of AAR based on 6 months of givosiran dosing and beyond 6 months may be explored.

In addition, clinical activity will be assessed by duration and severity of attack (mild/moderate/severe). Reduction from run-in period (ALN-AS1-001 study) in composite to all attacks ratios will be assessed.

Hemin: Dosing with hemin will be summarized for doses during the treatment period (hemin start dates on or after the first dose of givosiran in this study). Hemin dosing will be identified as medication with standardized medication terms such as 'hematin', 'haem arginate' or 'hemin'. Annualized rate of hemin dosing will be summarized in the same manner as porphyria attacks (i.e., mean rate, SEM, person-years, total events). For the calculation of annualized rate of hemin, only records with non-missing start and stop dates will be considered. These summaries will also be presented in the same manner (i.e. separately for each cohort displaying results from each phase of study: run-in (ALN-AS1-001 study) and treatment phase (ALN-AS1-002). An exploratory analysis of Annualized rate of hemin dosing based on 6 months of givosiran dosing and beyond 6 months may be explored.

7.6. Exploratory Analyses

7.6.1. Characterize the PK profile of ALN-AS1

Non-compartmental analysis (NCA) for PK will be summarized in this analysis plan. For all the PK analyses, the PK Analyses Set will be used.

Plasma concentration-time data for givosiran and its metabolite, 3'(N-1) givosiran (if analyzed) will be summarized using descriptive statistics (n, mean, SD, median, quartiles, minimum, maximum and %CV). Concentrations will be summarized by scheduled study visit and time collection (e.g. $C_{p,\ 2h}$, $C_{p,6h}$, etc.). Other pharmacokinetic parameters will not be determined due to the sparse PK sampling in the study.

Antidrug antibody (ADA): Results will be presented in a listing. For each patient, the collection of ADA screening status, confirmatory status and titer value (if applicable) will be displayed at each visit. A tabular summary of ADA titer values may also be generated.

7.6.2. Changes in health-related Quality of Life (QOL)

Quality of Life will be measured via the EQ-5D-5L instrument [2]. Measurements will be summarized for each of the 5 domains (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), total health today score (EQ Visual Analogue Scale) and index score at each scheduled visit. To calculate the index score based on the 5 domains, the United States will be used as the reference country. Safety Analysis Set will be used for the analysis.

Assessments performed during attacks and not during attacks will be analyzed separately. Patients are instructed to only complete the EQ-5D form once if they experience an attack during the treatment period (but not more than 1 form within a 6-month timeframe). If there is more than 1 record per patient for an attack, the worst score will be assigned for each of the domains

and the lowest value of total health today will also be assigned. Categorical summaries of the number and percentage of patients reporting each ordinal response within the domain will be presented. Descriptive statistics will be presented to summarize the total health score and index score at each visit.

Assessments from Brief-Pain Inventory-Short Form (BPI-SF) are collected weekly during the first 9 months, then every 3 months to the end of study. Average of individual pain intensity score (at its "worst" and "least" in the past week, and "average") over treatment period will be calculated for each patient, descriptive summary will be provided for each individual pain intensity score. In addition, descriptive summary will be provided for "average" pain intensity score in the past week collected during the run-in period of the parent study.

7.6.3. Characterize analytes related to targeting heme biosynthesis pathway

One of the exploratory biomarkers is serum and/or urinary ALAS1 messenger ribonucleic acid (mRNA) using a circulating extracellular RNA detection (cERD) assay.

ALAS1 mRNA: Analysis of ALAS1 mRNA concentrations will be performed on the PD Analysis Set based upon central laboratory data collection. Tabular summaries of descriptive statistics of actual value, change from baseline and knockdown value will be presented at each scheduled visit. Plots of the group means (±SEM) of actual values and knockdown values at each scheduled visit will be generated. Per-patient plots will be generated as well. Separate analyses will be done for data collected in serum and urine (Urine ALAS1 is considered as a part of secondary analysis on pharmacodynamic biomarkers, and serum ALAS1 by cERD analysis is exploratory).

Measurements of other exploratory endpoints will be summarized in the same manner as ALAS1 mRNA.

8. CHANGES TO PLANNED ANALYSES FROM PROTOCOL

Acute pancreatitis SMQ has been added to the list of AECIs but not specified in the protocol. See Section 7.4.

9. REFERENCES

[1] Gatz, DF and Smith L. "The Standard Error of a Weighted Mean Concentration -I. Bootstrapping vs. Other methods." Atmospheric Environment 29, no 11 (June 11, 1995):

1185-93.

[2] Calculating the U.S. Population-based EQ-5D Index Score. August 2005. Agency for Healthcare Research and Quality, Rockville, MD.

Appendix 1: Selected Clinical Laboratory Evaluation Parameters

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Phosphate
Potassium	Albumin
Blood urea nitrogen (BUN)	Calcium
Creatinine and eGFR	Carbon dioxide
Uric acid	Chloride
Total Protein	Bicarbonate
Glucose	Lipase
Liver Function Tests	
Aspartate transaminase (AST)	Alkaline phosphatase (ALP)
Alanine transaminase (ALT)	Bilirubin
GGT	
Coagulation Studies	
Prothrombin time (PT)	International Normalized Ratio (INR)
Immunogencity	
Antidrug Antibodies	
Urinalysis	
pH (dipstick)	Bilirubin
Specific gravity	Nitrite
Ketones	Red blood cells
Protein	Urobilinogen
Glucose	Leukocytes
Visual inspection of appearance and color	Microscopy (if clinically indicated)
Albumin	
Inflammation	
C-reactive protein	
Pregnancy Testing	
β- human chorionic gonadotropin	

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Statistical Analysis Plan

A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Clinical Study with ALN-AS1.

Protocol Number:	ALN-AS1-002
Protocol Version and Date:	Original: 19 July 2016 Amendment 0.1 20 September 2016 Amendment 1: 10 November 2016 Amendment 2: 01 December 2016 Amendment 3: 03 February 2017 Amendment 4: 02 August 2017 Amendment 5: 03 May 2018
Name of Test Drug:	Giovsiran (ALN-AS1)
Phase:	Phase 1/2
Methodology:	Open-label in Patients with Acute Intermittent Porphyria
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Tel: PPD Fax: PPD
Sponsor Representative:	PPD
Analysis Plan Date:	September 7, 2018
Analysis Plan Version:	Amendment 1.0

AUTHOR SIGNATURE PAGE

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Clinical Study with ALN-AS1.
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street, Cambridge, MA 02142 USA
Protocol Number:	ALN-AS1-002
Document Date / Version:	September 7, 2018 / Amendment 1.0

APPROVAL SIGNATURE PAGE

This document has been approved and signed electronically on the final page by the following:

PPD

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Definition
AAR	Annualized attack rate
ADA	Antidrug antibody
ADaM	Analysis Data Model
AE	Adverse event
AECI	Adverse event of clinical interest
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ATC	Anatomic Therapeutic Class
BMI	Body mass index
BPI-SF	Brief Pain Inventory - Short Form
CDISC	Clinical Data Interchange Standards Consortium
cERD	Circulating extracellular RNA detection assay
CI	Confidence interval
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DILI	Drug Induced Liver Injury
ECG	Electrocardiogram
eCRF	Electronic case report form
eDISH	Evaluation of drug-induced serious hepatotoxicity
eGFR	Estimated Glomerular Filtration Rate
EQ-5D	EQ-5D Quality of Life questionnaire
EOS	End of Study
GalNac	N-acetyl glactosamine
HLT	High level term
ISR	Injection site reaction
LFT	Liver function test
LLOQ	Lower Limit of Quantification
mBMI	Modified body mass index
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger ribonucleic acid
ms	Millisecond
NCA	Non-compartmental analysis
PBG	Porphobilinogen

Abbreviation	Definition
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred term
Q1	First quartile
Q3	Third quartile
QOL	Quality of life
QTc	Corrected QT interval
QTcB	QTc using Bazett's formula
QTcF	QTc using Fridericia's formula
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SEM	Standard error of the mean
SI	Standard International (unit)
SMQ	Standardized MedDRA Query
SOC	System organ class
SRC	Safety Review Committee
ULN	Upper limit of normal
WHO	World Health Organization

1. INTRODUCTION

This Statistical Analysis Plan (SAP) has been written based upon clinical protocol (amendment 4.0, date: 02 August 2017) and outlines the planned analyses for safety, Pharmacodynamic (PD), clinical activity and Pharmacokinetic (PK) in patients with acute intermittent porphyria (AIP).

This SAP supersedes the statistical considerations described in the clinical protocol (amendment 4.0; date: 02 August 2017). If there are differences between the statistical considerations in this SAP compared to the protocol, then this will be identified in the clinical study report (CSR).

The purpose of this SAP is to summarize key analyses to be conducted for each objective and presented in the CSR. Population PK or PK-PD analysis is out of scope and will be described in another document. Tables, Listings, and Figures mock shells will also reside in a separate document.

2. STUDY DESIGN

Study ALN-AS1-002 is an open-label, extension study designed to evaluate the long-term safety, clinical activity of givosiran in patients with AIP. The number of patients included up to 16 patients from Part C in the parent study ALN-AS1-001.

Eligible patients who completed Part C in the parent study either received the same dosing regimen as the pivotal Phase 3 study (e.g. 2.5 mg/kg every month) or had 1 or more dosing regimen changes across studies. The change in dosing regimen for a patient may occur due to emerging data and decisions from the Safety Review Committee (SRC). After a patient has completed 12 months of givosiran administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including givosiran administration may take place at the patient's home by a healthcare professional. The expected duration of treatment for each patient is up to 36 months (3 years) followed by a post-treatment visits at either month 39/Early Termination visit or month 42.

3. OBJECTIVES OF THE STUDY

3.1. Primary Objective:

- Evaluate the long-term safety and tolerability of givosiran in patients with AIP

3.2. Secondary Objectives:

- Assess the PD effect of ALN-AS1 over time
- Assess the clinical activity of ALN-AS1 over time

3.3. Exploratory Objectives:

- Characterize the PK profile of ALN-AS1
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

4. POPULATIONS

Population	Definitions
Safety Analysis Set	All patients who receive any amount of study drug
PD Analysis Set	All patients who receive any amount of study drug and who have at least 1 post-dose blood sample for PD
PK Analysis Set	All patients who receive any amount of study drug and have at least 1 post-dose blood sample for PK and who have evaluable PK data

Safety Analysis Set will be used to analyze safety and clinical activity data. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

5. MISSING DATA

For continuous and categorical variables, data will not be imputed unless otherwise specified.

There will be no imputation used for partial and/or completely missing dates for events defined as 'porphyria attacks' and for dates of hemin medication. For Delta-aminolevulinic acid (ALA)/ Porphobilinogen (PBG) biomarkers, if any value is recorded as <Lower Limit of Quantification (LLOQ) then the assigned value used for calculations will be assigned a value of LLOQ/2.

The following rules will be applied to incomplete dates to determine the reporting of adverse events (AEs) or medications. These imputed dates will not be displayed in listings because these dates are used to determine if the medication was prior or concomitant to first dose in this study and if the AE occurred on or after the first dose in this study. The rules below do not apply to hemin medications or AEs defined as 'porphyria attacks'.

Imputation rules for Start Date of AE:

- If start date is completely missing, then start date will be imputed to be the date of the first dose of study drug
- For a partial start date (day is missing, month is missing or both day and month are missing):
  - partial date < the first dose date: the first day/month
  - partial date = the first dose date: the first dose date
  - partial date > the first dose date: the first day/month

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date.

For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug in this study; medications will be classified as prior or concomitant depending on the medication stop dates. If any medications have a completely missing stop date, then the medication will be assumed as ongoing.

6. STATISTICAL METHODOLOGY

6.1. General Considerations:

As this is an extension study, formal statistical analyses will not be performed. All summaries will be descriptive.

Data will be presented in by-patient data listings. The listings will contain study days as described below:

> Study day: relative to the first dose of givosiran in this study. The first dose date is designated as Day 1. On treatment study days will be calculated as: evaluation date – first dose date+1. Pre-treatment study days will be calculated as: evaluation date – first dose date.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), median, quartiles (first quartile [Q1], third quartile [Q3]), minimum value, maximum value, and when specified, standard error of the mean (SEM). The precision of the measurement for each continuous variable will be used to determine the number of decimal places to present in tables, figures and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean, median, SD, quartiles, and SEM will be reported to 1 greater decimal place. Any values that require transformation to standard units (metric or Standard International [SI]) will be converted with the appropriate corresponding precision.

Frequencies and percentages will be presented for categorical and ordinal variables. If there are missing values, the number of missing values will be presented, but without a percentage. Percentages will be based on the number of non-missing values.

For assessments with repeated collection at a given study visit (e.g. electrocardiogram [ECG] parameters), the mean will represent the value at that visit unless otherwise noted.

6.2. Computing Environment

Analysis will be performed using SAS Version 9.4 or higher. Use of other software will be described in the CSR. Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) Analysis Model Version 2.1 or higher and CDISC ADaM Implementation Guide Version 1.0 or higher.

6.3. Baseline Definitions

Baseline will be defined as the derived baseline value in the parent study ALN-AS1-001 (Refer to ALN-AS1-001 SAP; dated 29 September 2017).

6.4. Study Groups

All tables and figures will be presented by the following study groups:

- Randomized treatment arm in the parent study/Current treatment arm (i.e., column for givosiran/givosiran vs. placebo/givosiran)
- Total (pooling regardless of randomized treatment arm in the parent study)

Summaries of key efficacy (e.g. PD and Clinical Activity) will also include study group:

- Intended-Dose Period (2.5 mg/kg monthly in ALN-AS1-002)

Summaries of key safety (e.g. AEs) will also include study groups based upon initial dose in ALN-AS1-002:

- Initial dose of 2.5 mg/kg (monthly)
- Initial dose of 5 mg/kg (at any frequency)

For summaries in the Intended-Dose Period, the data included for these summaries is defined in Section 6.5 Visit Windows.

6.5. Visit Windows

Data will be tabulated and analyzed per the visit as recorded in the electronic case report form (eCRF).

For summaries based upon Intended-Dose Period, the following rules will be used:

- Data collected at scheduled visits (e.g. ALA, PBG, etc.) will be displayed if the collection was during the intended dose period (i.e., first date of intended dose ≤ collection date ≤ last date of intended dose)
- Data not collected at scheduled visits (e.g. AEs, attack, hemin) will be summarized if the start date was during the intended dose period (i.e., first date of intended dose ≤ start date ≤ last date of intended dose)

Data collected at unscheduled visits will be included in by-subject listings and figures, but no assignment to a scheduled visit will be made for the purposes of by-visit summary tabulations. However, unscheduled visits will be considered for any categorical shifts summaries (e.g. shifts from baseline to ‘worst’ post-baseline value).

6.6. Interim Analyses

Interim analyses will be performed to support regulatory activities and publications. These analyses will be descriptive in nature and will not involve any formal hypothesis testing.

For an interim analysis, as this study will be ongoing, a cut-off approach will be implemented to ensure data quality. The interim analysis will include data entered on or prior to a pre-specified cutoff date. For assessments with starting/end dates (e.g., Exposure, AEs, medical history, medications), the starting date will be compared to the pre-specified cut-off date.

7. STATISTICAL METHODOLOGY

7.1. Patient Disposition

Patient disposition will be summarized and include the total number and percentage of patients in the following categories:

- Enrolled Patients (signed informed consent and met eligibility)
- Treated Patients
- Completed Patients
- Safety Analysis Set
- PD Analysis Set
- PK Analysis Set

Summaries of the number and percentage who withdrew from study prematurely based upon primary reason for withdrawal (e.g. AE, Death, Lost to Follow-up, Physician Decision, Withdrawal by Subject, etc.) will also be presented. Per patient data listings will be presented displaying the primary reason the patient went off study.

7.2. Protocol Deviations

Protocol violations will be classified into major or minor deviation by medical review. A major deviation is defined as a deviation that may significantly impact the completeness, accuracy and/or reliability of the trial data; that may significantly affect a patient's rights, safety and well-being (ICH.E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013). Deviations not classified as major will be assigned as minor. All protocol deviations will be presented in a listing.

7.3. Baseline Characteristics

Baseline characteristics will be based upon the derived baseline in the parent study ALN-AS1-001 (Refer to ALN-AS1-001 SAP; dated 29 September 2017). These characteristics include but are not limited to age, sex, race, ethnicity, body weight, height, and body mass index (BMI) as well as genotype, prior hemin prophylaxis status, baseline ALA, PBG, and 5-aminolevulinic acid synthase 1 (ALAS1) levels.

Data recorded in medical history in this study and data recorded in the medical history of the parent study ALN-AS1-001 will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC), high level term (HLT) and preferred term (PT). A patient only contributes once to the count for a given condition (overall, by SOC, by HLT, by preferred term). Medical history and pregnancy test results will also be presented in data listings.

7.4. Safety Analyses

The primary objective of this study is to evaluate the long-term safety and tolerability of givosiran in patients with AIP. All analyses will use the Safety Analysis Set. All clinical safety laboratory data collected from the central laboratory will be used to evaluate safety. For

summaries of ECG data, these will be performed separately based upon data collected locally and centrally.

Parameters: Safety and tolerability assessments for givosiran will include study drug exposure, AEs, clinical laboratory parameters (hematology, serum chemistry, liver function test [LFT], urinalysis, coagulation), vital signs, 12-lead ECGs, concomitant medication, and physical examinations.

Exposure: Summaries will include descriptive statistics of the duration of treatment (days) and total number of doses received. Additional summaries of the cumulative number of patients (percentage) that completed study treatment intervals (e.g. ≥1 day, ≥1 month, ≥3 month, etc.) and the number of patients (percentage) that completed study treatment at the latest visit interval will also be displayed. Additionally, cumulative number of doses received at home will be displayed.

Duration of treatment (days) will be defined as = [(date of the last dose + 84) – date of the first dose + 1 days]. Treatment duration calculation will consider observed dosing at the time of the snapshot (e.g. patient only received the first dose of givosiran on the day of the interim snapshot, then the exposure will be 1 day rather than 84 days). Dose interruptions and compliance will not be considered when calculating of duration of exposure.

A separate per-patient exposure listing will be generated to display the dosing regimen in the parent study (ALN-AS1-001) and actual dosing (mg/kg) regimen(s) in this study.

Adverse Events: AEs will be coded using the MedDRA coding system (version 21.0 or later) and displayed in tables and data listing by SOC and PT. Patients who report multiple occurrences of the same PT will be classified according to the most related or most severe category. Patients with a missing severity or missing relationship to study drug will be classified to the most related or most severe category.

Summaries of AEs will consist of events which occur or worsen (including date/time when applicable) after the first dose of givosiran in this study. Note: Porphyria attacks are recorded on the AE eCRF, however they will not be reported as AEs but rather as an endpoint of clinical activity (see section 7.5.2); therefore, the term “AE” excludes porphyria events.

An overview of the frequency (percentage) of AEs including the total number of events will be tabulated (summaries such as but not limited to, the number of patients with at least 1 AE, the number of patients with at least 1 AE related to study drug, the number of patients with at least 1 serious AE [SAE], the number of patients who discontinued study drug to an AE). Separate tabulations will be generated by SOC and PT for all AEs, AEs by maximum severity, related AEs, related AEs by maximum severity, SAEs, discontinuation from study drug due to AEs, interruption due to AEs, and deaths. Patient listings will be generated separately for any patient who died, discontinued from study drug and reported any SAEs.

An additional set of AE outputs will also be generated by study groups defined by the initial dose of givosiran [i.e. Patients with an initial dose of 2.5 mg/kg (monthly) in ALN-AS1-002 and Patients with an initial dose of 5 mg/kg (at any time) in ALN-AS1-002].

Adverse Events of Clinical Interest (AECIs):

> **Injection Site Reactions [ISRs]**: AEs mapping to the HLT=”Injection Site Reactions” using MedDRA dictionary will be included in the summary. An ISR may consist of one or more signs or symptoms. Key summaries such as but not limited to will be generated:

Number of patients with at least 1 ISR, percent of injections complicated by ISRs, frequency (percentages) of ISRs by PT (corresponds to individual sign or symptom). A separate listing will be generated to display all patients who reported ISRs.

Hepatic AEs, including LFT abnormalities considered clinically significant by the investigator: AEs mapping to the Standardized MedDRA Query (SMQ) Drug-related hepatic disorders - comprehensive search (includes all narrow and broad terms). The following key summaries will be generated: Frequency (percentages) of Hepatic SMQ Events by SOC and PT. A separate listing will be generated to display all patients who reported hepatic AEs.

Acute pancreatitis: AEs mapping to Acute Pancreatitis SMQ (narrow terms) plus the following PT terms (lipase increased, lipase abnormal, amylase increased, amylase abnormal, hyperlipasemia, hypermylasaemia, pancreatic enzyme abnormality, pancreatic enzymes abnormal, pancreatic enzymes increased). The following key summaries will be generated: Frequency (percentages) of Acute Pancreatitis Events by SOC and PT. A separate listing will be generated to display all patients who reported acute pancreatitis.

Anaphylactic Reaction: AEs mapping to the anaphylactic reaction SMQ. The following key summaries will be generated: Frequency (percentages) of anaphylactic reaction by search category (narrow and broad) and PT. A separate listing will be generated to display AEs that meet SMQ criteria.

Other Adverse Events:

Malignancies: AEs mapping to Malignant or Unspecified Tumors SMQ. The following key summaries will be generated: Frequency (percentages) of Malignancies by HLT and PT.

Acute Renal Failure: AEs mapping to the acute renal failure SMQ. The following key summaries will be generated: Frequency (percentages) of anaphylactic reaction by SOC and PT.

Clinical Laboratory Parameters: Clinical laboratory parameters will be expressed in SI units. Laboratory data collected and recorded as below the LLOQ will be set to the lower limit of detection for calculation of summary statistics. Summaries generally include data from central laboratory. Certain summaries include data from both central and local laboratory (e.g., LFT).

Summary data for each laboratory parameter (hematology, serum chemistry, LFT, and coagulation), which are continuous variables, will have a tabular summary of descriptive statistics (n, mean, SD, median, quartiles, minimum, and maximum; see Appendix 2 for list of laboratory parameters). Descriptive statistics of actual value, change from baseline and percentage change from baseline at each scheduled visit will be displayed.

For each continuous parameter, severity will be categorized based upon Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Shift tables will be generated to summarize shifts from baseline category to the worst post-baseline category. Worst post-baseline category will be considered the highest CTCAE grade or highest shift in ULN category unless otherwise specified (e.g. estimated glomerular filtration rate [eGFR]).

For hematology and chemistry laboratories, summary tables of potentially clinically significant abnormalities will also be provided. . A by-patient listing of lipase values will be generated.

The lipase and amylase (when applicable) will be categorized into the following categories: ≤ULN, >ULN and ≤1.5xULN, >1.5xULN and ≤2xULN, >2xULN and ≤5xULN, >5xULN. A tabular summary will be generated to summarize the number and associated percentage of patient in each of the categories at any post-baseline visit.

The eGFR (mL/min/1.73 m^2) will be categorized into the following categories: ≥90,60-89, 30-59, 15-29 and <15. A shift table of baseline to worst post-eGFR (i.e. category with the lowest value) will be presented.

All laboratory data will be presented in data listings, with potentially clinically significant values flagged. Out of range laboratory results will be identified in listings.

Liver Function Tests (LFTs): A listing will be generated for all patients with abnormal liver function tests as defined by ALT>3xULN, AST>3xULN or total bilirubin >2x ULN at any visit.

A tabular summary will be generated to summarize the number and associated percentage of patient in each of the categories at any post-baseline visit, shift tables will be presented for ALT, AST, total bilirubin:

- 1<ALT≤3, 3<ALT≤5, >5<ALT≤10, <10<ALT≤20, ALT>20xULN
- 1<AST≤3, 3<AST≤5, >5<AST≤10, <10<AST≤20, AST>20xULN
- 1<ALT or AST≤3, 3<ALT or AST≤5, >5<ALT or AST≤10, <10<ALT or AST≤20, AST or ALT>20xULN
- ALP>1.5xULN
- 1.5<Total Bilirubin ≤2, 2<Total Bilirubin≤3, 3<Total Bilirubin≤5 and Total Bilirubin>5
- ALT or AST > 3xULN and Total Bilirubin > 2xULN

Evaluation of drug-induced serious hepatotoxicity (eDISH) plots of peak bilirubin at any time versus peak ALT or AST at any time will also be presented.

Vital Signs and Physical Examination: Descriptive statistics for each vital sign (weight, height, oral body temperature, blood pressure, heart rate, and respiration rate) will be summarized at scheduled visits. In addition, a tabular summary of potentially clinically significant post-baseline abnormalities in vital signs will be presented. Per patient listing of vital signs results will be generated.

ECG: Summaries of ECG data will be generated separately for data collected locally and centrally. Note that baseline ECG data is only available based on local collection.

ECG findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, corrected QT (QTc) interval. For each visit, the results will be the mean of the measurement from the triplicate ECG values for each patient at a single visit.

Descriptive statistics will be summarized at each scheduled visit. Change from baseline to each post-baseline assessment will be summarized (local ECG only). The number and percentage of patients with normal, abnormal or clinical significant abnormal results at each visit will also be summarized.

An outlier analysis will be performed for QTc using Bazett's (QTcB) and Fridericia's (QTcF) formula.

For local ECGs, categorical analysis via a shift table will be summarized as:

- The number and percentage of patients with maximum increase from baseline in QTcB/QTcF (≤30, >30-60, >60 milliseconds [ms]).
- The number and percentage of patients with maximum post-baseline QTcB/QTcF (≤450, >450-480, >480-500, >500 ms)

For central ECGs, categorical analysis will be summarized as follows:

- The number and percentage of patients with maximum post-baseline QTcB/ QTcF (≤450, >450-480, >480-500, >500 ms)

A per-patient listing of all ECG data will be generated for local and central data collection. A separate per patient listing with any post-baseline QTc value of > 500 ms or any patient with an increase from baseline of > 60 ms will also be generated.

Prior and Concomitant Medications: Concomitant medications will be coded using the WHO Drug Dictionary (March 2015 or later). Concomitant medications will be defined as any medication taken after the first dose of study drug in this study and any medication that started prior to the first dose in this study and was ongoing on or after the date of the first dose of study drug. If the end date of medication is missing or incomplete such that it cannot be determined whether it was prior to the first dose of study drug, it will be identified as concomitant.

Concomitant medications will be tabulated by Anatomic Therapeutic Class (ATC) and Preferred Term and by overall decreasing frequency of patients who took the medications. A patient listing will be provided for all concomitant medications. A separate listing will also be generated for any medication that was recorded prior to the first dose in this study and not identified as concomitant.

7.5. Secondary Analyses

7.5.1. Pharmacodynamic Biomarkers

One of the key secondary objectives is to evaluate the long-term PD effect of ALN-AS1 on urine levels of ALA and PBG.

ALA and PBG: Analysis of ALA and PBG concentrations will be performed on the PD Analysis Set. Values will be based upon urine concentrations collected from the central laboratory. In all analyses of urine ALA and PBG, the concentrations will be expressed as mmol/mol of urine creatinine (e.g, creatinine normalized values). Any values recorded as <LLOQ will be assigned a value of LLOQ/2.

Data will be summarized descriptively (n, mean, SD, SEM, median, quartiles, minimum, and maximum) at baseline and each post-baseline scheduled visits. The analysis will be performed separately for scheduled assessments collected not during attacks and all scheduled timepoints. Tabular summaries of actual value, change from baseline and knockdown values (knockdown= -1*percentage change) will be generated at each scheduled visit. Plots of the group

means (±SEM) of actual values and knockdown values will also be generated. Per-patient plots of actual values over time will be generated.

7.5.2. Clinical Activity

Clinical activity of ALN-AS1 will be assessed by the number of porphyria attacks and by the number of doses of hemin administered.

Analysis of Clinical Activity will be performed on the Safety Analysis Set.

Porphyria attacks: An attack will be defined as any event with preferred term=”Porphyria” recorded on Adverse Event eCRF. Attacks which occur within the same date (i.e., end date of attack is the same date as start date of the next attack) will be counted as one attack. When collapsing attacks that occurred on the same date, the following rules will be applied for severity and location:

1. Severity will be assigned the maximum severity among the records.
2. Location will be assigned ‘hospital’ if ≥ 1 attack with location=hospital.
   Location will be assigned ‘outpatient’ if ≥ 1 attack with location=outpatient
   Location will be assigned ‘home’ if all the records state location=home.
   Location will be assigned ‘other’ if all the records state location=other.

The number of porphyria attacks during the treatment period (attacks with start dates on or after date of first dose) will be summarized. Annualized attack rate (AAR) per patient will be calculated as the total number of porphyria attacks divided by the total person-days at risk and multiplied by the total number of days in a year (365.25). Person-days at risk will be defined as: (date patient went off study – date of first dose+1). For an interim snapshot, the person-days will be defined by date of the first dose until the minimum of the date of the interim snapshot cut-off date or date the patient went off study. For calculation of AAR, only records with non-missing start and stop dates will be considered.

Porphyria attacks will be summarized separately in each subgroup:

1. Attacks requiring hospitalization, urgent health care (i.e. outpatient clinic, Emergency Department, etc.) or IV hematin at home.
2. Attacks requiring hospitalization
3. Attacks requiring urgent health care (i.e. Outpatient)
4. Attacks required treatment with IV hematin at home
5. Attacks not treated with IV hematin at home
6. All attacks

For each subgroup, the total number of events, total person-years (total days/365.25), mean rate (total number of events/total person-years) and SEM will be presented for each study group. The SEM will be calculated using Cochran’s estimate of SEM for weighted mean [1]. Attacks for each subgroup will display the rates separately for each phase of study: run-in (ALN-AS1-001 study) and treatment phase (ALN-AS1-002). A tabular summary of percentage change (%) in AAR rate from run-in to treatment phase will be also calculated per cohort. In addition, a

by-patient listing will summarize the porphyria attacks in each of the subgroups. An exploratory analysis of AAR based on 6 months of givosiran dosing and beyond 6 months may be explored.

In addition, clinical activity will be assessed by duration and severity of attack (mild/moderate/severe).

Hemin: Dosing with hemin will be summarized for doses during the treatment period (hemin start dates on or after the first dose of givosiran in this study). Hemin dosing will be identified as medication with standardized medication terms such as 'hematin', 'haem arginate' or 'hemin'. Annualized rate of hemin dosing will be summarized in the same manner as porphyria attacks (i.e., mean rate, SEM, person-years, total events). For the calculation of annualized rate of hemin, only records with non-missing start and stop dates will be considered. These summaries will also be presented in the same manner (i.e. separately for each cohort displaying results from each phase of study: run-in (ALN-AS1-001 study) and treatment phase (ALN-AS1-002). An exploratory analysis of Annualized rate of hemin dosing based on 6 months of givosiran dosing and beyond 6 months may be explored.

7.6. Exploratory Analyses

7.6.1. Characterize the PK profile of ALN-AS1

Non-compartmental analysis (NCA) for PK will be summarized in this analysis plan. For all the PK analyses, the PK Analyses Set will be used.

Plasma concentration-time data for givosiran and its metabolite, 3'(N-1) givosiran (if analyzed) will be summarized using descriptive statistics (n, mean, SD, median, quartiles, minimum, maximum and %CV). Concentrations will be summarized by scheduled study visit and time collection (e.g. $C_{p,\,2h}$, $C_{p,6h}$, etc.). Other pharmacokinetic parameters will not be determined due to the sparse PK sampling in the study.

Antidrug antibody (ADA): Results will be presented in a listing. For each patient, the collection of ADA screening status, confirmatory status and titer value (if applicable) will be displayed at each visit. A tabular summary of ADA titer values may also be generated.

7.6.2. Changes in health-related Quality of Life (QOL)

Quality of Life will be measured via the EQ-5D-5L instrument [2]. Measurements will be summarized for each of the 5 domains (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), total health today score (EQ Visual Analogue Scale) and index score at each scheduled visit. To calculate the index score based on the 5 domains, the United States will be used as the reference country. Safety Analysis Set will be used for the analysis.

Assessments performed during attacks and not during attacks will be analyzed separately. Patients are instructed to only complete the EQ-5D form once if they experience an attack during the treatment period (but not more than 1 form within a 6-month timeframe). If there is more than 1 record per patient for an attack, the worst score will be assigned for each of the domains and the lowest value of total health today will also be assigned. Categorical summaries of the number and percentage of patients reporting each ordinal response within the domain will be presented. Descriptive statistics will be presented to summarize the total health score and index score at each visit.

Assessments from Brief-Pain Inventory-Short Form (BPI-SF) are collected weekly during the first 9 months, then every 3 months to the end of study. Average of individual pain intensity score (at its "worst" and "least" in the past week, and "average") over treatment period will be calculated for each patient, descriptive summary will be provided for each individual pain intensity score. In addition, descriptive summary will be provided for "average" pain intensity score in the past week collected during the run-in period of the parent study.

7.6.3. Characterize analytes related to targeting heme biosynthesis pathway

One of the exploratory biomarkers is serum and/or urinary ALAS1 messenger ribonucleic acid (mRNA) using a circulating extracellular RNA detection (cERD) assay.

ALAS1 mRNA: Analysis of ALAS1 mRNA concentrations will be performed on the PD Analysis Set based upon central laboratory data collection. Tabular summaries of descriptive statistics of actual value, change from baseline and knockdown value will be presented at each scheduled visit. Plots of the group means (±SEM) of actual values and knockdown values at each scheduled visit will be generated. Per-patient plots will be generated as well. Separate analyses will be done for data collected in serum and urine (Urine ALAS1 is considered as a part of secondary analysis on pharmacodynamic biomarkers, and serum ALAS1 by cERD analysis is exploratory).

Measurements of other exploratory endpoints will be summarized in the same manner as ALAS1 mRNA.

8. CHANGES TO PLANNED ANALYSES FROM PROTOCOL

Acute pancreatitis SMQ has been added to the list of AECIs but not specified in the protocol. See Section 7.4.

9. REFERENCES

[1] Gatz, DF and Smith L. "The Standard Error of a Weighted Mean Concentration -I. Bootstrapping vs. Other methods." Atmospheric Environment 29, no 11 (June 11, 1995):

1185-93.

[2] Calculating the U.S. Population-based EQ-5D Index Score. August 2005. Agency for Healthcare Research and Quality, Rockville, MD.

Appendix 1: Selected Clinical Laboratory Evaluation Parameters

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Phosphate
Potassium	Albumin
Blood urea nitrogen (BUN)	Calcium
Creatinine and eGFR	Carbon dioxide
Uric acid	Chloride
Total Protein	Bicarbonate
Glucose	Lipase
Liver Function Tests	
Aspartate transaminase (AST)	Alkaline phosphatase (ALP)
Alanine transaminase (ALT)	Bilirubin
GGT	
Coagulation Studies	
Prothrombin time (PT)	International Normalized Ratio (INR)
Immunogencity	
Antidrug Antibodies	
Urinalysis	
pH (dipstick)	Bilirubin
Specific gravity	Nitrite
Ketones	Red blood cells
Protein	Urobilinogen
Glucose	Leukocytes
Visual inspection of appearance and color	Microscopy (if clinically indicated)
Albumin	
Inflammation	
C-reactive protein	
Pregnancy Testing	
β- human chorionic gonadotropin	

Signature Page for VV-CLIN-002514 v1.0

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Statistical Analysis Plan

A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Clinical Study with ALN-AS1.

Protocol Number:	ALN-AS1-002
Protocol Version and Date:	Original: 19 July 2016 Amendment 0.1 20 September 2016 Amendment 1: 10 November 2016 Amendment 2: 01 December 2016 Amendment 3: 03 February 2017 Amendment 4: 02 August 2017
Name of Test Drug:	Giovsiran (ALN-AS1)
Phase:	Phase 1/2
Methodology:	Open-label in Patients with Acute Intermittent Porphyria
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Tel: PPD Fax: PPD
Sponsor Representative:	PPD
Analysis Plan Date:	April 9, 2018
Analysis Plan Version:	Version 1.0

AUTHOR SIGNATURE PAGE

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Clinical Study with ALN-AS1.
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street, Cambridge, MA 02142 USA
Protocol Number:	ALN-AS1-002
Document Date / Version:	April 9, 2018 / Version 1.0

APPROVAL SIGNATURE PAGE

Signatories:

PPD

Date: 13 APR 2018

PPD

Date: 13 APR 2018

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
AIP	Acute intermittent porphyria
ALAS1	Delta-aminolevulinic acid
ATC	Anatomic Therapeutic Class
BPI-SF	Brief Pain Inventory- Short Form
cERD	Circulation extracellular RNA detection assay
CI	Confidence interval
CTCAE	Common Terminology for Clinical Adverse Events
CYP	Cytochrome P450
DILI	Drug Induced Liver Injury
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
EQ-5D	EQ-5D Quality of Life questionnaire
EOS	End of Study
GalNac	N-acetyl glactosamine
ISR	Injection Site Reaction
LFT	Liver Function Test
mBMI	Modified body mass index
PBG	Porphobilinogen
PD	Pharmacodynamic (s)
PK	Pharmacokinetic(s)
QOL	Quality of life
SAE	Serious adverse event
SEM	Standard error of the mean
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal

1. INTRODUCTION

This Statistical Analysis Plan (SAP) has been written based upon clinical protocol (amendment 4.0, date: 02 August 2017) and outlines the planned analyses for safety, Pharmacodynamic (PD), clinical activity and Pharmacokinetic (PK) in patients with acute intermittent porphyria (AIP).

This SAP supersedes the statistical considerations described in the clinical protocol (amendment 4.0; date: 02 August 2017). If there are differences between the statistical considerations in this SAP compared to the protocol, then this will be identified in the clinical study report.

The purpose of this Statistical Analysis Plan is to summarize key analyses to be conducted for each objective and presented in the clinical study report. Population PK or PK-PD analysis is out of scope and will be described in another document. Tables, Listings, and Figures will also reside in a separate document.

2. STUDY DESIGN

Study ALN-AS1-002 is an open-label, extension study designed to evaluate the long-term safety, clinical activity of givosiran in patients with acute intermittent porphyria (AIP). The number of patients included up to 16 patients from Part C in parent study ALN-AS1-001.

Eligible patients who completed Part C in the parent study either received the same dosing regimen as the parent study (e.g. 2.5 mg/kg every month) or had 1 or more dosing regimen changes across studies. The change in dosing regimen for a patient may occur due to emerging data and decisions from the Safety Review Committee (SRC). After a patient has completed 12 months of givosiran administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including givosiran administration may take place at the patient's home by a healthcare professional. The expected duration of treatment for each patient is up to 36 months (3 years) followed by a post-treatment visits at either month 39/Early Termination visit or month 42.

3. OBJECTIVES OF THE STUDY

3.1. Primary Objective:

- Evaluate the long-term safety and tolerability of givosiran in patients with AIP

3.2. Secondary Objectives:

- Assess the PD effect of ALN-AS1 over time
- Assess the clinical activity of ALN-AS1 over time

3.3. Exploratory Objectives:

- Characterize the PK profile of ALN-AS1
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

4. POPULATIONS

Population	Definitions
Safety Analysis Set	All patients who receive any amount of study drug
PD Analysis Set	All patients who receive any amount of study drug and who have at least 1 post-dose blood sample for PD
PK Analysis Set	All patients who receive any amount of study drug and have at least 1 post-dose blood sample for PK and who have evaluable PK data

Safety Analysis Set will be used to analyze safety and clinical activity data. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

5. MISSING DATA

For continuous and categorical variables, data will not be imputed unless otherwise specified.

There will be no imputation used for partial and/or completely missing dates for events defined as 'porphyria attacks' and for dates of hemin medication. For ALA/PBG biomarkers, if any value is recorded as <Lower Limit of Quantification (LLOQ) then the assigned value used for calculations will be assigned a value of LLOQ/2.

The following rules will be applied to incomplete dates to determine the reporting of adverse events or medications. These imputed dates will not be displayed in listings because these dates are used to determine if the medication was prior or concomitant to first dose in this study and if the adverse event occurred on or after the first dose in this study. The rules below do not apply to hemin medications or adverse events defined as 'porphyria attacks'.

Imputation rules for Start Date of AE:

- If start date is completely missing, then start date will be imputed to be the date of the first dose of study drug
- For a partial start date (day is missing, month is missing or both day and month are missing):
  - partial date < the first dose date: the last day/month
  - partial date = the first dose date: the first dose date
  - partial date > the first dose date: the first day/month

Imputation rules for End Date of AE:

- If end date is completely missing, then end date remains missing
- For a partial end date (day is missing, or month is missing or both day and month are missing) then the last day/month will be imputed.

If the imputed AE start date is after the imputed AE end date, then the start date will be set to the imputed AE end date.

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date.

For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug in this study; medications will be classified as prior or concomitant depending on the medication stop dates. If any medications have a completely missing stop date, then the medication will be assumed as ongoing.

6. STATISTICAL METHODOLOGY

6.1. General Considerations:

As this is an extension study, formal statistical analyses will not be performed. All summaries will be descriptive.

Data will be presented in by-patient data listings. The listings will contain 2 study days as described below:

1. Study day: relative to the first dose of givosiran in this study. The first dose date is designated as Day 1. On treatment study days will be calculated as: evaluation date – first dose date+1. Pre-treatment study days will be calculated as: evaluation date – first dose date.
2. Parent study day: the study day relative to the first dose of study drug/placebo in the parent study (ALN-AS1-001).

Descriptive statistics (n, mean, standard deviation, median, quartiles, minimum, and maximum) will be presented for continuous variables.

Frequencies and percentages will be presented for categorical and ordinal variables. If there are missing values, the number of missing values will be presented, but without a percentage. Percentages will be based on the number of non-missing values.

Means and medians will be reported to one decimal place more than the precision of the recorded data. Standard deviations will be reported to one decimal place more than the recorded data. Minimum and maximum values will be reported to the same precision as recorded in eCRF or external data.

For assessments with repeated collection at a given study visit (e.g. ECG Parameters), the mean will represent the value at that visit unless otherwise noted.

6.2. Computing Environment

Analysis will be performed using SAS Version 9.4 or higher. Use of other software will be described in the Clinical Study Report. Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) Analysis Model Version 2.1 or higher and CDISC ADaM Implementation Guide Version 1.0 or higher.

6.3. Baseline Definitions

Baseline will be defined as the derived baseline value in the parent study ALN-AS1-001 (Refer to ALN-AS1-001 SAP; dated 29 September 2017).

6.4. Study Groups

All tables and figures will be presented by the following study groups:

- Randomized treatment arm in parent study/Current treatment arm (i.e., column for givosiran/givosiran vs. placebo/givosiran)
- Total (pooling regardless of prior randomized treatment)

Summaries of key efficacy (e.g. PD and Clinical Activity) will also include study group:

- Target Dose Period (2.5 mg/kg monthly in ALN-AS1-002)

Summaries of key safety (e.g. AEs) will also include study groups based upon initial dose in ALN-AS1-002:

- Initial dose of 2.5 mg/kg (monthly)
- Initial dose of 5 mg/kg (at any time)

For summaries in the Target Dose Period, the data included for these summaries is defined in Section 6.5 Visit Windows.

6.5. Visit Windows

Data will be tabulated and analyzed per the visit as recorded in the electronic case report form (eCRF).

For summaries based upon Target Dose Period, the following rules will be used:

- Data collected at scheduled visits (e.g. ALA, PBG, etc.) will be displayed if the collection was during the target dose period (i.e., first date of target dose ≤ collection date ≤ last date of target dose)
- Data not collected at scheduled visits (e.g. AEs, attack, hemin) will be summarized if the start date was during the target dose period (i.e., first date of target dose ≤ start date ≤ last date of target dose)

Data collected at unscheduled visits will be included in by-subject listings and figures, but no assignment to a scheduled visit will be made for the purposes of by-visit summary tabulations. However, unscheduled visits will be considered for any categorical shifts summaries (e.g. shifts from baseline to ‘worst’ post-baseline value).

6.6. Interim Analyses

Interim analyses will be performed to support regulatory activities and publications. These analyses will be descriptive in nature and will not involve any formal hypothesis testing.

For an interim analysis, as this study will be ongoing, a cut-off approach will be implemented to ensure data quality. The interim analysis will include data entered on or prior to a pre-specified cutoff date. For assessments with starting/end dates (e.g., Exposure, AEs, medical history, medications), the starting date will be compared to the pre-specified cut-off date. For any

assessments with multiple replicates associated with the single visit, the earliest of the dates will be compared to the cut-off date; if this date is on or before the data cut-off, the replicates will be included in the analysis.

7. STATISTICAL METHODOLOGY

7.1. Patient Disposition

Patient disposition will be summarized and include the total number and percentage of patients in the following categories:

- Enrolled Patients (signed informed consent and met eligibility)
- Treated Patients
- Completed Patients
- Safety Analysis Set
- PD Analysis Set
- PK Analysis Set

Summaries of the number and percentage who withdrew from study prematurely based upon primary reason for withdrawal (e.g. AE, Death, Lost to Follow-up, Physician Decision, Withdrawal by Subject, etc.) will also be presented. Per patient data listings will be presented displaying the primary reason the patient went off study.

7.2. Protocol Deviations

Protocol violations will be classified into major or minor deviation by medical review. A major deviation is defined as a deviation that may significantly impact the completeness, accuracy and/or reliability of the trial data; that may significantly affect a patient's rights, safety and well-being (ICH.E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013). Deviations not classified as major will be assigned as minor. All protocol deviations will be presented in a listing.

7.3. Baseline Characteristics

Baseline characteristics will be based upon the derived baseline in the parent study ALN-AS1-001 (Refer to ALN-AS1-001 SAP; dated 29 September 2017). These characteristics include but are not limited to age, sex, race, ethnicity, body weight, height, and body mass index (BMI) as well as genotype, prior hemin prophylaxis status, baseline ALA, PBG, and ALAS1 levels.

Data recorded in medical history in this study and data recorded in the medical history of the parent study ALN-AS1-001 will be summarized by MedDRA system organ class (SOC), high level term (HLT) and preferred term (PT). A patient only contributes once to the count for a given condition (overall, by SOC, by HLT, by preferred term). Medical history and pregnancy test results will also be presented in data listings.

7.4. Safety Analyses

The primary objective of this study is to evaluate the long-term safety and tolerability of givosiran in patients with AIP. All analyses will use the Safety Analysis Set. All clinical safety lab data collected from the central laboratory will be used to evaluate safety. For summaries of ECG data, these will be performed separately based upon data collected locally and centrally.

Parameters: Safety and tolerability assessments for givosiran will include study drug exposure, adverse events (AEs), clinical laboratory parameters (hematology, serum chemistry, liver function tests, urinalysis, coagulation), vital signs, 12-lead electrocardiograms (ECGs), concomitant medication, and physical examinations.

Exposure: Summaries will include descriptive statistics of the duration of treatment (days) and total number of doses received. Additional summaries of the cumulative number of patients (percentage) that completed study treatment intervals (e.g. < 84 months, 84-168 days, etc.) and the number of patients (percentage) that completed study treatment at the latest visit interval will also be displayed. Additionally, the number of patients (percentage) who receive treatment at home and a categorical summary of the total number of doses administered at home for each patient will be displayed [e.g. 1 dose at home, 2-6 doses at home, 6-12 doses, 12-18 doses, ≥ 18 doses].

Duration of treatment (days) will be defined as = [date of the last dose – date of the first dose + 84 days]. Treatment duration calculation will consider observed dosing at the time of the snapshot (e.g. patient only received the first dose of givosiran on the day of the interim snapshot, then the exposure will be 1 day rather than 84 days). Dose interruptions and compliance will not be considered when calculating of duration of exposure.

A separate per-patient exposure listing will be generated to display the dosing regimen in the parent study (ALN-AS1-001) and actual dosing (mg/kg) regimen(s) in this study. For each patient, the treatment duration will be presented by total duration of givosiran across studies (ALN-AS1-001 and ALN-AS1-002) and separate total duration of givosiran in ALN-AS1-001 and ALN-AS1-002 studies.

Adverse Events: AEs will be coded using the MedDRA coding system (version 21.0 or later) and displayed in tables and data listing by System Organ Class (SOC) and Preferred Terms (PT). Patients who report multiple occurrences of the same preferred term will be classified according to the most related or most severe category. Patients with a missing severity or missing relationship to study drug will be classified to the most related or most severe category.

Summaries of AEs will consist of events which occur or worsen (including date/time) after the first dose of givosiran in this study. Note: Porphyria attacks are recorded on the AE eCRF, however they will not be reported as AEs but rather as an endpoint of clinical activity (see section 7.5.2); therefore, the term “AE” excludes porphyria events.

An overview of the frequency (percentage) of AEs including the total number of events will be tabulated (summaries such as but not limited to, the number of patients with at least 1 AE, the number of patients with at least 1 AE related to study drug, the number of patients with at least 1 SAE, the number of patients who discontinued study drug to an AE). Separate tabulations will be generated by SOC and PT for all AEs, AEs by maximum severity, related AEs, related AEs by maximum severity, serious adverse events (SAEs), discontinuation from study drug due to AEs, interruption due to AEs, and deaths. Patient listings will be generated separately for any patient who died, discontinued from study drug and reported any SAEs.

An additional set of AE outputs will also be generated by study groups defined by the initial dose of givosiran [i.e. Patients with an initial dose of 2.5 mg/kg (monthly) in ALN-AS1-002 and Patients with an initial dose of 5 mg/kg (at any time) in ALN-AS1-002].

Adverse Events of Clinical Interest (AECIs):

Injection Site Reactions [ISRs]: AEs mapping to the High Level Term (HLT)="Injection Site Reactions" using MedDRA dictionary will be included in the summary. Key summaries such as but not limited to will be generated: Frequency (percentages) of ISRs by SOC and PT, Frequency (percentages) of ISRs by maximum severity by SOC and PT. A separate listing will be generated to display all patients who reported ISRs. An additional summary of ISRs by SOC and PT over time may also be generated.

Hepatic AEs, including LFT abnormalities considered clinically significant by the investigator: AEs mapping to the Standardized MedDRA Query (SMQ) Drug-related hepatic disorders - comprehensive search (includes all narrow and broad terms). The following key summaries will be generated: Frequency (percentages) of Hepatic SMQ Events by SOC and PT, Frequency (percentages) of Hepatic SMQ by maximum severity by SOC and PT. A separate listing will be generated to display all patients who reported hepatic AEs.

Acute pancreatitis: AEs mapping to Acute Pancreatitis SMQ (narrow terms) plus the following PT terms (lipase increased, lipase abnormal, amylase increased, amylase abnormal, hyperlipasemia, hypermylasaemia, pancreatic enzyme abnormality, pancreatic enzymes abnormal, pancreatic enzymes increased). The following key summaries will be generated: Frequency (percentages) of Acute Pancreatitis Events by SOC and PT, Frequency (percentages) of Acute Pancreatitis Events by maximum severity by SOC and PT. A separate listing will be generated to display all patients who reported acute pancreatitis.

Clinical Laboratory Parameters: Clinical laboratory parameters will be expressed in Standard International (SI) units. Laboratory data collected and recorded as below the lower limit of quantification (LLQ) will be set to the lower limit of detection for calculation of summary statistics. Summaries will only include data from central laboratory.

Summary data for each lab parameter (hematology, serum chemistry, coagulation, and urinalysis), which are continuous variables, will have a tabular summary of descriptive statistics (n, mean, standard deviation, median, quartiles, minimum, and maximum; see Appendix 1 for list of lab parameters). Descriptive statistics of actual value, change from baseline and percentage change from baseline at each scheduled visit will be displayed

For each continuous parameter, severity will be categorized based upon CTCAE (version 4.03). Shift tables will be generated to summarize shifts from baseline category to the worst post-baseline category. Worst post-baseline category will be considered the highest CTCAE grade or highest shift in ULN category unless otherwise specified (e.g. eGFR).

For hematology and chemistry labs, summary tables of potentially clinically significant abnormalities will also be provided. Listings of any of the patients included with these abnormalities will be generated as well. A listing of patients with abnormal lipase values (defined as lipase > 3 x ULN and/or 3 times the level of the actual baseline where baseline was abnormal) will be generated.

The estimated glomerular filtration rate (eGFR mL/min/1.73 m^2) will be categorized into the following categories: ≥90,60-89, 30-59, 15-29 and <15. A shift table of baseline to worst post-eGFR (i.e. category with the lowest value) will be presented.

All laboratory data will be presented in data listings. Separate listings will be included for those laboratory data from local labs. Out of range laboratory results will be identified in listings.

Liver Function Tests (LFTs): A listing will be generated for all patients with abnormal liver function tests as defined by ALT>3xULN, AST>3xULN and total bilirubin >2x ULN at any visit.

A tabular summary will be generated to summarize the number and associated percentage of patient in each of the categories at any post-baseline visit:

- 1<ALT≤3, 3<ALT≤5, >5<ALT≤10, <10<ALT≤20, ALT>20xULN
- 1<AST≤3, 3<AST≤5, >5<AST≤10, <10<AST≤20, AST>20xULN
- 1<ALT or AST≤3, 3<ALT or AST≤5, >5<ALT or AST≤10, <10<ALT or AST≤20, AST or ALT>20xULN
- ALP>1.5xULN
- 1.5<Total Bilirubin ≤2, 2<Total Bilirubin≤3, 3<Total Bilirubin≤5 and Total Bilirubin>5
- INR>1.2

eDISH plots of peak bilirubin at any time versus peak ALT or AST at any time will also be presented.

Vital Signs and Physical Examination: Descriptive statistics for each vital sign (weight, height, oral body temperature, blood pressure, heart rate, and respiration rate) will be summarized at scheduled visits. In addition, a tabular summary of potentially clinically significant post-baseline abnormalities in vital signs will be presented. Per patient listing of vital signs results and assessments of physical examinations will be generated.

ECG: Summaries of ECG data will be generated separately for data collected locally and centrally. Note that baseline ECG data is only available based on local collection.

ECG findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, QTc interval. For each visit, the results will be the mean of the measurement from the triplicate ECG values for each patient at a single visit.

Descriptive statistics will be summarized at each scheduled visit. Change from baseline to each post-baseline assessment will be summarized (local ECG only). The number and percentage of patients with normal, abnormal or clinical significant abnormal results at each visit will also be summarized.

An outlier analysis will be performed for corrected QT interval (QTc) using Bazett's formula (QTcB) and Fridericia (QTcF).

For local ECGs, categorical analysis will be summarized as:

- The number and percentage of patients with maximum increase from baseline in QTcB/QTcF (≤30, >30-60, >60 ms).
- The number and percentage of patients with maximum post-baseline QTcB/QTcF (≤450, >450-480, >480-500, >500 ms)

For central ECGs, categorical analysis will be summarized as follows:

- The number and percentage of patients with maximum post-baseline QTcB/ QTcF (≤450, >450-480, >480-500, >500 ms)

A per-patient listing of all ECG data will be generated separately for local and central data collection. A separate per patient listing with any post-baseline value of > 500 ms or any patient with an increase from baseline of > 60 ms will also be generated.

Prior and Concomitant Medications: Concomitant medications will be coded using the WHO Drug Dictionary (March 2015 or later). Concomitant medications will be defined as any medication taken after the first dose of study drug in this study and any medication that started prior to the first dose in this study and was ongoing on or after the date of the first dose of study drug. If the end date of medication is missing or incomplete such that it cannot be determined whether it was prior to the first dose of study drug, it will be identified as concomitant.

Concomitant medications will be tabulated by Anatomic Therapeutic Class (ATC) and Preferred Term and by overall decreasing frequency of patients who took the medications. A patient listing will be provided for all concomitant medications. A separate listing will also be generated for any medication that was recorded prior to the first dose in this study and not identified as concomitant.

7.5. Secondary Analyses

7.5.1. Pharmacodynamic Biomarkers

One of the key secondary objectives is to evaluate the long-term PD effect of ALN-AS1 on urine levels of delta aminolevulinic acid (ALA) and porphobilinogen (PBG).

ALA and PBG: Analysis of ALA and PBG concentrations will be performed on the PD analysis set. Values will be based upon urine concentrations collected from the central laboratory. In all analyses of urine ALA and PBG, the concentrations will be expressed as mmol/mol of urine creatinine (e.g, creatinine normalized values). Any values recorded as <Lower Limit of Quantification (LLOQ) will be assigned a value of LLOQ/2.

Data will be summarized descriptively (n, mean, standard deviation, median, quartiles, minimum, and maximum) at baseline and each post-baseline scheduled visits. The analysis will be performed separately for scheduled assessments collected not during attacks and all scheduled timepoints. Tabular summaries of actual value, change from baseline and knockdown values (knockdown= -1*percentage change) will be generated at each scheduled visit. Plots of the group means (±SEM) of actual values and knockdown values will also be generated. Per-patient plots of actual values over time will be generated.

7.5.2. Clinical Activity

Clinical activity of ALN-AS1 will be assessed by the number of porphyria attacks and by the number of doses of hemin administered.

Analysis of Clinical Activity will be performed on the Safety Analysis Set.

Porphyria attacks: An attack will be defined as any event with preferred term=”Porphyria” recorded on Adverse Event eCRF. Attacks which occur within the same date (i.e., end date of attack is the same date as start date of the next attack) will be counted as one attack. When collapsing attacks that occurred on the same date, the following rules will be applied for severity and location:

1. Severity will be assigned the maximum severity among the records.
2. Location will be assigned ‘hospital’ if ≥ 1 attack with location=hospital.
   Location will be assigned ‘outpatient’ if ≥ 1 attack with location=outpatient
   Location will be assigned ‘home’ if all the records state location=home.
   Location will be assigned ‘other’ if all the records state location=other.

The number of porphyria attacks during the treatment period (attacks with start dates on or after date of first dose) will be summarized. Annualized attack rate (AAR) per patient will be calculated as the total number of porphyria attacks divided by the total person-days at risk and multiplied by the total number of days in a year (365.25). Person-days at risk will be defined as: (date patient went off study – date of first dose+1). For an interim snapshot, the person-days will be defined by date of the first dose until the minimum of the date of the interim snapshot cut-off date or date the patient went off study. For calculation of AAR, only records with non-missing start and stop dates will be considered.

Porphyria attacks will be summarized separately in each subgroup:

1. Attacks requiring hospitalization, urgent health care (i.e. outpatient clinic, Emergency Department, etc.) or IV hematin at home.
2. Attacks requiring hospitalization
3. Attacks requiring urgent health care (i.e. Outpatient)
4. Attacks required treatment with IV hematin at home
5. Attacks not treated with IV hematin at home
6. All attacks

For each subgroup, the total number of patients with an event, total number of events, total person-years (total days/365.25), mean rate (total number of events/total person-years) and SEM will be presented for each study group. The SEM will be calculated using Cochran’s estimate of SEM for weighted mean [1]. Attacks for each subgroup will display the rates separately for each phase of study: run-in (ALN-AS1-001 study) and treatment phase (ALN-AS1-002). A tabular summary of percentage change (%) in AAR rate from run-in to treatment phase will be also calculated per cohort. In addition, a by-patient listing will summarize the porphyria attacks in each of the subgroups. An exploratory analysis of AAR based on 6 months of givosiran dosing and beyond 6 months may be explored.

In addition, clinical activity will be assessed by:

- Duration and severity of attack (mild/moderate/severe)
- Number and duration of attacks that required hospitalizations.

Hemin: Dosing with hemin will be summarized for doses during the treatment period (hemin start dates on or after the first dose of givosiran in this study). Hemin dosing will be identified as medication with standardized medication terms such as 'hematin', 'haem arginate' or 'hemin'. Annualized rate of hemin dosing will be summarized in the same manner as porphyria attacks (i.e., mean rate, SEM, person-years, total events, total patients with an event). For the calculation of annualized rate of hemin, only records with non-missing start and stop dates will be considered. These summaries will also be presented in the same manner (i.e. separately for each cohort displaying results from each phase of study: run-in (ALN-AS1-001 study) and treatment phase (ALN-AS1-002). An exploratory analysis of Annualized rate of hemin dosing based on 6 months of givosiran dosing and beyond 6 months may be explored.

7.6. Exploratory Analyses

7.6.1. Characterize the PK profile of ALN-AS1

Non-compartmental analysis (NCA) for PK will be summarized in this analysis plan. For all the PK analyses, the PK analyses set will be used.

Plasma concentration-time data for givosiran and its metabolite, 3'(N-1) givosiran (if analyzed) will be summarized using descriptive statistics (n, mean, standard deviation, median, quartiles minimum, maximum and %CV). Concentrations will be summarized by scheduled study visit and time collection (e.g. $C_{p,\ 2h}$, $C_{p,6h}$, etc.). Other pharmacokinetic parameters will not be determined due to the sparse PK sampling in the study.

Antidrug antibody (ADA): Results will be presented in a listing. For each patient, the collection of ADA screening status, confirmatory status and titer value (if applicable) will be displayed at each visit. A tabular summary of ADA titer values may also be generated.

7.6.2. Changes in health-related Quality of Life (QOL)

Quality of Life will be measured via the EQ-5D-5L instrument [2]. Measurements will be summarized for each of the 5 domains (mobility, self-care, usual activities, pain/discomfort and anxiety/depression), total health today score (EQ Visual Analogue Scale) and index score at each scheduled visit. To calculate the index score based on the 5 domains, the United States will be used as the reference country. Safety Analysis Set will be used for the analysis.

Assessments performed during attacks and not during attacks will be analyzed separately. Patients are instructed to only complete the EQ-5D form once if they experience an attack during the treatment period (but not more than 1 form within a 6-month timeframe). If there is more than 1 record per patient for an attack, the worst score will be assigned for each of the domains and the lowest value of total health today will also be assigned. Categorical summaries of the number and percentage of patients reporting each ordinal response within the domain will be presented. Descriptive statistics will be presented to summarize the total health score and index score at each visit. In addition, a table which summarizes the number and percentage of patients who improved, worsened or had no change from baseline in each of the 5 domains will be presented as well.

Assessments from Brief-Pain Inventory-Short Form (BPI-SF) Porphyria will summarized during this study. Total number and percentage of patients reporting pain at least once on study and the location(s) of pain will be summarized over time intervals. If a patient has more than 1 response within a time interval window which differ, the most severe response will be presented (i.e. patient reports both pain in neck='Y' and 'N' will be assigned a 'Y' during that interval). In addition to location, the level of pain felt will be summarized as well. If there are multiple responses for a patient within an interval, the maximum ordinal response (0 to 10; where 0 is mild and 10 is the worst) for pain will be assigned. A per patient listing of responses from the BPI-SF and, diary entries will be generated.

7.6.3. Characterize analytes related to targeting heme biosynthesis pathway

One of the exploratory biomarkers is serum and/or urinary 5-aminolevulinic acid synthase 1 (ALAS1) messenger ribonucleic acid (mRNA) using a circulating extracellular RNA detection (cERD) assay.

ALAS1 mRNA: Analysis of ALAS1 mRNA concentrations will be performed on the PD analysis set based upon central laboratory data collection. Tabular summaries of descriptive statistics of actual value, change from baseline and knockdown value will be presented at each scheduled visit. Plots of the group means (±SEM) of actual values and knockdown values at each scheduled visit will be generated. Per-patient plots will be generated as well. Separate analyses will be done for data collected in serum and urine.

Measurements of other exploratory endpoints will be summarized in the same manner as ALAS1 mRNA.

8. CHANGES TO PLANNED ANALYSES FROM PROTOCOL

Acute pancreatitis SMQ has been added to the list of AECIs but not specified in the protocol. See Section 7.4.

9. REFERENCES

[1] Gatz, DF and Smith L. "The Standard Error of a Weighted Mean Concentration -I. Bootstrapping vs. Other methods." Atmospheric Environment 29, no 11 (June 11, 1995): 1185-93.

[2] Calculating the U.S. Population-based EQ-5D Index Score. August 2005. Agency for Healthcare Research and Quality, Rockville, MD.

Appendix 1: Selected Clinical Laboratory Evaluation Parameters

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Phosphate
Potassium	Albumin
Blood urea nitrogen (BUN)	Calcium
Creatinine and eGFR	Carbon dioxide
Uric acid	Chloride
Total Protein	Bicarbonate
Glucose	Lipase
Liver Function Tests	
Aspartate transaminase (AST)	Alkaline phosphatase (ALP)
Alanine transaminase (ALT)	Bilirubin
GGT	
Coagulation Studies	
Prothrombin time (PT)	International Normalized Ratio (INR)
Immunogencity	
Antidrug Antibodies	
Urinalysis	
pH (dipstick)	Bilirubin
Specific gravity	Nitrite
Ketones	Red blood cells
Protein	Urobilinogen
Glucose	Leukocytes
Visual inspection of appearance and color	Microscopy (if clinically indicated)
Albumin	
Inflammation	
C-reactive protein	
Pregnancy Testing	
β- human chorionic gonadotropin	