Official Title:	**ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias**
NCT Number:	**NCT03338816**
Document Date:	**Statistical Analysis Plan, 13 Feb 2019**

STATISTICAL ANALYSIS PLAN

ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias

Protocol Number:	ALN-AS1-003
Protocol Version and Date:	Protocol Amendment 3: 21 September 2018 Original Protocol: 06 September 2017
Investigational Drug:	ALN-AS1 (Givosiran)
Phase:	Phase 3
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Tel: [redacted] Fax: [redacted]
Sponsor Representative:	[redacted]
Analysis Plan Version and Date:	SAP Amendment 2: 13 February 2019 SAP Amendment 1: 11 September 2018 Original SAP: 05 December 2017

APPROVAL SIGNATURE PAGE

ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias

Protocol Number: ALN-AS1-003

Analysis Plan Version and Date: Amendment 2: 13 February 2019

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

[redacted]
Alnylam Pharmaceuticals, Inc.

[redacted]
Alnylam Pharmaceuticals, Inc

TABLE OF CONTENTS

APPROVAL SIGNATURE PAGE 2
TABLE OF CONTENTS 3
LIST OF TABLES 5
LIST OF FIGURES 5
LIST OF ABBREVIATIONS 6
1. INTRODUCTION 8
2. STUDY OVERVIEW 8
2.1. Synopsis of Study Design 8
2.2. Randomization Methodology 9
2.3. Blinding 9
2.4. Study Procedures 10
3. OBJECTIVES AND ENDPOINTS 10
3.1. Objectives 10
3.1.1. Primary Objective 10
3.1.2. Secondary Objectives 10
3.1.3. Exploratory Objectives 11
3.2. Endpoints 11
3.2.1. Primary Endpoint 11
3.2.2. Secondary Endpoints 11
3.2.3. Exploratory Endpoints 12
3.2.4. Safety Endpoints 12
4. PATIENT POPULATION 13
5. GENERAL STATISTICAL METHODS 14
5.1. Determination of Sample Size 14
5.2. General Considerations 14
5.3. Computing Environment 15
5.4. Baseline Definitions 15
5.5. Randomization Stratification Factors 16
5.6. Visit Windows 16
5.7. Multiple Comparisons/Multiplicity 17
5.8. Analysis Cutoff and Database Lock 17

5.9. Interim Analyses 18
5.10. Analyses for the Entire Study 18
6. STATISTICAL ANALYSIS 21
6.1. Patient Disposition 21
6.2. Demographics and Baseline Characteristics 21
6.3. Protocol Deviations 21
6.4. Drug Exposure 22
6.5. Efficacy Analysis 22
6.5.1. Primary Endpoint 23
6.5.1.1. Primary Analysis using Negative Binomial Regression Model 23
6.5.1.2. Component Analysis 24
6.5.1.3. Analysis in the Per Protocol Set 24
6.5.1.4. Sensitivity Analysis 24
6.5.2. Secondary Endpoints 25
6.5.3. Exploratory Endpoints 26
6.5.4. Subgroup Analysis 27
6.6. Pharmacodynamic Analysis 28
6.7. Pharmacokinetic Analysis 28
6.8. Safety Analysis 28
6.8.1. Adverse Events 29
6.8.2. Laboratory Data 30
6.8.3. Vital Signs and Physical Examination 31
6.8.4. Electrocardiogram 31
6.8.5. Prior and Concomitant Medications 32
6.9. Anti-Drug Antibody 32
7. CHANGES FROM PREVIOUS VERSION(S) 33
7.1. Changes in SAP Amendment 1 33
7.2. Changes in SAP Amendment 2 34
8. REFERENCES 35
9. APPENDICES 36
9.1. Details of Interim Analysis 36
9.1.1. Interim Analysis Population 36
9.1.2. Rationale for Month 3 ALA Endpoint 36

9.1.3. Analysis of ALA Endpoint 37
9.1.3.1. Primary Analysis Using ANCOVA/Multiple Imputation 37
9.1.3.2. Additional Analysis 38
9.1.3.3. Durability of ALA Lowering 38
9.2. Sample SAS Code for Interim Analysis of ALA Data 38
9.3. Details of the Pattern Mixture Model for ALA 40
9.4. Details of the Pattern Mixture Model for Attack Data 41
9.5. Analgesic medications 43

LIST OF TABLES

Table 1: Derived Analysis Visit Windows 16

LIST OF FIGURES

Figure 1: Study Design 9
Figure 2: ALA Levels over Time (in Days) for Individual AIP Patients in the Givosiran Phase 1 Study (Cohort C) 37

LIST OF ABBREVIATIONS

Abbreviation	Definition
6m-DB	6-month double-blind placebo-controlled
AAR	Annualized attack rate
ADP	ALA dehydratase deficient porphyria
AE	Adverse event
ALA	Aminolevulinic acid
ALAS1	Aminolevulinic acid synthase
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Class
AUC	Area under curve
BMI	Body mass index
CI	Confidence interval
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DB	Double-blind
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form
HBMS	Hydroxymethylbilane Synthase
HCP	Hereditary Coproporphyria
HLT	High Level Term
HRQOL	Health-related quality of life
IA	Interim analysis
ICH	International Council for Harmonisation
IRS	Interactive response system
ISR	Injection site reaction
LLN	Lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed effect model repeated measurement

Abbreviation	Definition
mRNA	messenger RNA
NCI	National Cancer Institute
OLE	Open label extension
PBG	Porphobilinogen
PD	Pharmacodynamics
PGIC	Patient Global Impression of Change
PK	Pharmacokinetics
PMM	Pattern Mixture Model
PPS	Per Protocol Set
PT	Preferred Term
Q1	First quartile
Q3	Third quartile
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of mean
SMQ	Standardized MedDRA Query
SOC	System organ class
SSR	Sample size reassessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
VP	Variegate porphyria
WHO	World Health Organization

1. INTRODUCTION

Acute hepatic porphyrias (AHPs) are a family of rare, serious and life-threatening disorders characterized by acute and severe neurovisceral attacks, often requiring hospitalization or urgent healthcare visit, as well as by chronic debilitating symptoms. Patients are treated with intravenous hemin during acute attacks, but there is a high unmet need for safe and efficacious therapies to prevent attacks and decrease the chronic symptoms in between attacks. Givosiran is an investigational RNA interference (RNAi) agent in development for the treatment of AHPs in adult and adolescent patients. It acts to inhibit synthesis of liver aminolevulinic acid synthase (*ALAS1*) messenger RNA (mRNA) with consequent reductions in aminolevulinic acid (ALA) and porphobilinogen (PBG) levels, the neurotoxic intermediates that are causal in this disease. Givosiran is formulated for administration via subcutaneous (SC) injection.

The ENVISION Study (ALN-AS1-003) is a Phase 3 study designed to evaluate the efficacy and safety of SC-administered givosiran in patients with AHPs. This statistical analysis plan (SAP) outlines the methods to be used in the analysis of study data in order to address the study objectives of Study ALN-AS1-003. Any change to the data analysis methods described in the protocol, as well as the justification for the change, will be described in the SAP and clinical study report (CSR).

An original version of the SAP was signed off on Dec 05, 2017. The SAP amendment 1 provided updated plan for analysis of the data and was signed off on Sep 11, 2018, prior to the interim analysis. This SAP amendment 2 provides detailed planned analyses for data in the OLE periods and will be finalized and signed off before the final analysis of the 6-month double-blind (6m-DB) data and the unblinding of treatment assignments. The changes in these SAP amendments are documented in Section 7 .

2. STUDY OVERVIEW

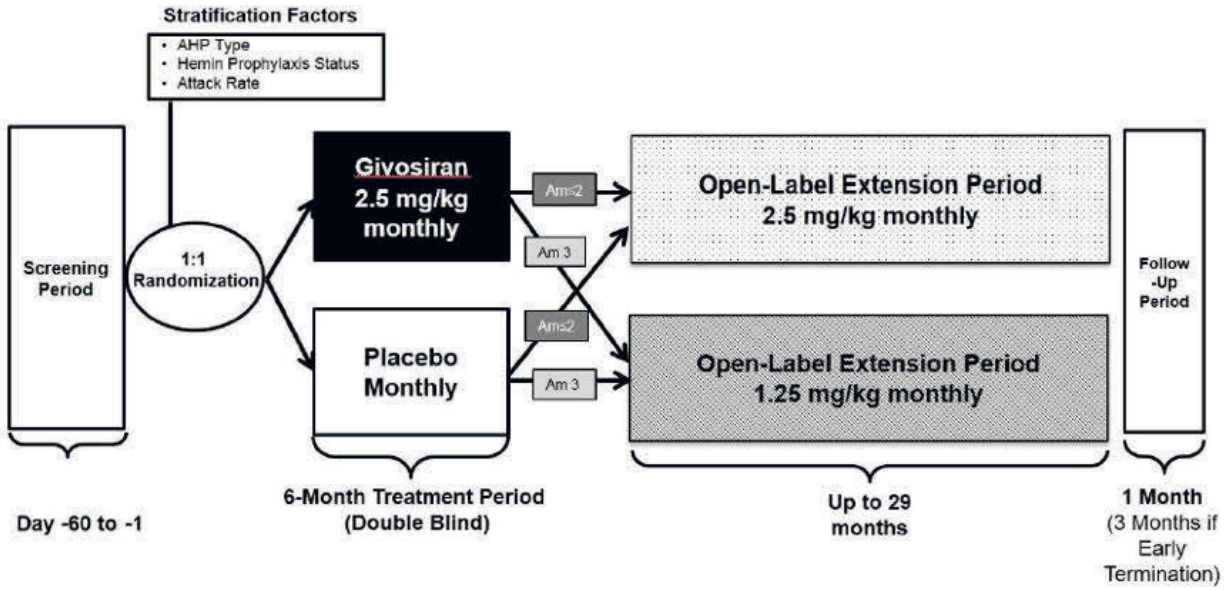
2.1. Synopsis of Study Design

The ENVISION Study (ALN-AS1-003) is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of givosiran in approximately 74 patients with AHPs; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 6 months, followed by an open-label extension (OLE) period of up to 30 months to evaluate the long-term safety and efficacy of givosiran.

Patients who crossed over to the OLE period prior to the implementation of the protocol amendment 3 (i.e., under amendment version 1 or 2) and are receiving a 2.5 mg/kg once monthly givosiran dose will remain on that dose. Upon entry to the OLE period under amendment version 3, patients will cross over to receive a 1.25 mg/kg once monthly dose of givosiran. Starting at Month 13 (when 6 months of open-label givosiran dosing have been completed), patients in the once monthly 1.25 mg/kg treatment group who experience inadequate disease control may have their monthly dose increased to 2.5 mg/kg, upon discussion and agreement by the Investigator and medical monitor and demonstration of tolerability to givosiran and if ALA criteria are met (urine ALA levels (mmol/mol Cr) are not stably maintained ≤ULN or are inducible).

The study design schema is presented in Figure 1.

Figure 1: Study Design



Abbreviations: Am ≤2=original protocol (06 Sept 2017), protocol amendment 1 (04 May 2018), and protocol amendment 2.0 (26 July 2018); Am 3=protocol amendment 3 (21 Sept 2018)

2.2. Randomization Methodology

Patients will be randomized 1:1 to the givosiran treatment arm and the placebo arm in a double-blinded manner. Treatment will be stratified at randomization by AHP type (acute intermittent porphyria (AIP) [with genetic evidence of mutation in the hydroxymethylbilane synthase *(HMBS)* gene] vs hereditary coproporphyria (HCP), variegate porphyria (VP), ALA dehydratase deficient porphyria (ADP), or any AHP without identified mutation in a porphyria-related gene).

Randomization for AIP patients will be further stratified by each patient's use of hemin prophylaxis regimen at the time of screening and by each patient's historical annualized attack rate prior to randomization. Patients on a hemin prophylaxis regimen prior to study entry will be stratified by their historical annualized attack rate: <7 attacks vs ≥7 attacks. Patients who were not on a hemin prophylaxis regimen prior to study entry will be stratified by their historical annualized attack rate: <12 attacks vs ≥12 attacks.

2.3. Blinding

Treatment assignments will be maintained by the Interactive Response System (IRS) which has controlled access limited to supply chain team members. Any unplanned unblinding occurring during the 6-month double-blind placebo-controlled treatment period (referred to as the 6m-DB period hereafter) will be documented and reported in the CSR.

The independent (external) data monitoring committee (DMC) and an independent (external) biostatistics group supporting the DMC will have access to subject level treatment assignments throughout the study.

An unblinded interim analysis (IA) will be conducted when approximately 40 AIP patients have completed at least 3 months of the double-blind treatment period. This IA will be based on creatinine normalized urinary ALA (referred to as ALA hereafter) data at 3 months for regulatory filing, and the analysis will be performed by the ALA IA independent statistician and programmer. The IA Filing Decision Committee at Alnylam will review data provided by the ALA IA independent statistician and programmer and decide whether to proceed to filing based on the IA results. If the committee makes an affirmative filing decision based on the IA, an Alnylam unblinded IA filing team will be formed and will have access to treatment assignment information in patients included for IA. They will not have access to post-IA patient-level study data until the 6m-DB period data are locked and unblinded, except for access to unblinded Suspected Unexpected Serious Adverse Reaction (SUSAR) safety data as shared with the agency via regular pharmacovigilance (PV) process, and safety data on the approximately 40 AIP patients included in the IA needed to prepare the 90-day safety update.

A blinded study conduct team will be in place throughout the 6m-DB period of the study regardless of the ALA interim analysis results. The blinded study conduct team will not have access to treatment assignment or any information that could reveal treatment assignment (e.g. PK, ALA, PBG, and ALAS1) until the study is unblinded for the primary efficacy analysis at Month 6.

Details about the specifics of the blinding aspects for the whole, including before, during, and after the IA are outlined in the Blinding Plan. Details about the measures taken for the unblinded IA, including concepts and plans for how the IA will be conducted and maintenance of data integrity are outlined in the Data Integrity Plan (Amendment 3, 07Sep2018).

2.4. Study Procedures

The schedule of assessments is described in the study protocol (Table 1, Table 2, and Table 3).

3. OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary Objective

- Evaluate the effect of givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP

3.1.2. Secondary Objectives

- Evaluate the effects of givosiran, compared to placebo, on urinary ALA levels in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on urinary PBG levels in patients with AIP

- Evaluate the effects of givosiran, compared to placebo, on hemin usage in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP
- Evaluate the effects of givosiran compared to placebo in patients with AIP on the symptoms of pain, nausea, and fatigue
- Evaluate the effects of givosiran, compared to placebo, in patients with AIP on the Physical Component Summary (PCS) of the 12-item Short-Form Health Survey (SF-12)
- Evaluate the safety and tolerability of givosiran in patients with any AHP

3.1.3. Exploratory Objectives

- Evaluate the effects of givosiran, compared to placebo, in patients with AIP and in patients with any AHP over the 6m-DB period on:
  - Rate of all porphyria attacks (requiring hospitalization, urgent healthcare visit, IV hemin administration at home, or treated at home without IV hemin)
  - Urinary ALAS1 mRNA levels
  - Analgesic usage (opioid and non-opioid)
  - Additional quality of life (QOL) measures, including missed days of work/school
- Assess the treatment effect of givosiran at evaluated doses over the OLE period in patients with AIP and in patients with any AHP who had previously been randomized to placebo treatment
- Assess the long-term treatment effect of givosiran in patients with AIP and in patients with any AHP
- Characterize the PK of and assess the antidrug antibodies (ADA) of givosiran in patients with any AHP

3.2. Endpoints

3.2.1. Primary Endpoint

Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6m-DB period.

3.2.2. Secondary Endpoints

- Urinary ALA levels in patients with AIP at 3 months
- Urinary ALA levels in patients with AIP at 6 months
- Urinary PBG levels in patients with AIP at 6 months

- Annualized rate of administered hemin doses (evaluated by annualized days of hemin use, see Section 6.5.2 for details) in patients with AIP over the 6m-DB period
- Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP over the 6m-DB period
- Daily worst pain score as measured by Brief Pain Inventory-Short Form (BPI-SF) numeric rating scale (NRS) in patients with AIP over the 6m-DB period
- Daily worst fatigue score as measured by Brief Fatigue Inventory-Short Form (BFI-SF) NRS in patients with AIP over the 6m-DB period
- Daily worst nausea score as measured by NRS in patients with AIP over the 6m-DB period
- Change from baseline in the Physical Component Summary (PCS) of the 12-item Short-Form Health Survey (SF-12) in patients with AIP at 6 months

3.2.3. Exploratory Endpoints

Exploratory endpoints will be measured in patients with AIP and in patients with any AHP over the 6m-DB period or over the OLE period:

- Rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home
- Rate of all porphyria attacks
- Rate of administered hemin doses (evaluated by annualized days of hemin use, see Section 6.5.2 for details)
- Urinary ALA and PBG levels
- Urinary ALAS1 mRNA levels
- Daily worst pain, daily worst fatigue, and daily worst nausea scores over 12 months
- PCS of the SF-12
- EQ-5D-5L index score
- Patient Global Impression of Change (PGIC)
- Porphyria Patient Experience Questionnaire (PPEQ)
- Analgesic usage (opioid and non-opioid)
- Plasma PK parameters of givosiran
- Incidence and titer of ADAs

3.2.4. Safety Endpoints

The primary safety parameter is the adverse events (AEs) that occurred on or after the time of the first dose of study drug is administered. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. The primary summaries of the safety of givosiran versus placebo will be based on safety parameters assessed during the 6m-DB period. The

frequency of AEs will also be evaluated over the entire study including the 6m-DB and OLE periods. Since porphyria attacks will be recorded for efficacy assessment of the study drug, they will not be treated as AEs or SAEs.

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received at least one dose of study drug. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- AIP patients in the Full Analysis Set (FAS_{AIP}): All randomized AIP patients (with identified mutation in the *HMBS* gene) who received at least one dose of study drug. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- Per Protocol Analysis Set (PPS): All randomized AIP patients (with identified mutation in the *HMBS* gene) who received at least 4 doses (>60%) of study drug during the 6-month double-blind period, were followed for collection of attack data through 6 months (≥162 days) and did not experience major protocol deviations that may impact the primary efficacy results (e.g., not meeting the key inclusion/exclusion criteria). Patients will be analyzed according to their randomly assigned treatment group.
- Safety Analysis Set (SAS): All patients who received at least one dose of study drug, grouped according to the treatment actually received.
- PK Analysis Set: All patients who received at least one dose of study drug and have evaluable PK data contributing to the estimation of PK parameter.
- PD Analysis Set: All patients who received at least one dose of study drug and who have baseline and at least one post-dose urine sample for the determination of ALA or PBG.
- All Givosiran Treated Set: All patients who received at least one dose of givosiran, including patients who took givosiran during the 6m-DB period and patients who first took placebo during the 6m-DB period and switched to givosiran during the OLE period.

Both FAS and FAS_{AIP} will be used to evaluate efficacy endpoints. The primary endpoint will also be analyzed using the Per Protocol Set. Safety during the 6m-DB period will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively. The All Givosiran Treated Set will be used to summarize long-term efficacy and safety data during givosiran treatment. See Section 5.10 for details. Number of patients included in all analysis sets will be provided.

5. GENERAL STATISTICAL METHODS

5.1. Determination of Sample Size

The planned total enrollment for the study is approximately 74 patients, including approximately 70 AIP patients.

Seventy patients will yield at least 90% power to detect a 45% reduction in the annualized attack rate at a 2-sided 5% significance level assuming a mean annualized attack rate of 8, a standard deviation (SD) of 5 in the placebo arm (mean/SD of 4/2.9 for 6 months to preserve the over-dispersion of 3.8), and a mean annualized attack rate of 4.4 with SD of 3 in the givosiran arm (mean/SD of 2.2/1.8 for 6 months to preserve the over-dispersion of 4.2), using a negative binomial model. This study design will still have at least 80% power even if the dropout rate is as high as 15% under the same assumptions.

5.2. General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, SD, median, interquartile range (Q1, Q3), minimum value (min), and maximum value (max). 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, Q1 and Q3 will be reported to one greater decimal place. Any values that require transformation to standard units (metric or SI) will be converted with the appropriate corresponding precision.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For ALA, PBG and urinary creatinine, the assessments collected and recorded as lower than the lower limit of quantification/detection will be replaced by half of the lower limit of quantification/detection. For other safety laboratory parameters, the assessments collected and recorded as lower than the lower limit of quantification/detection will be replaced by the lower limit of quantification/ detection. Any assessment collected and recorded as greater than the upper limit of quantification will be replaced by the upper limit of quantification.

For all analysis sets except for the All Givosiran Treated Set, summaries will be presented by treatment arm (givosiran and placebo).

For the All Givosiran Treated Set, summaries will be presented by the following groups:

- Givosiran/Givosiran: all patients who received givosiran during the 6m-DB including patients who continued to receive givosiran during the OLE period and patients who discontinued treatment during the 6m-DB period;
- Placebo/Givosiran: all patients who received placebo during the 6m-DB period and switched to givosiran in the OLE period;
- All Givosiran: all patients who received at least one dose of givosiran during either 6m-DB or OLE period.

5.3. Computing Environment

All statistical analyses will be performed using validated SAS statistical software Version 9.4 (or later), unless otherwise noted. Figures may be generated using R version 3.4 (or later).

5.4. Baseline Definitions

For ALA, PBG, and ALAS1 mRNA, collection of the screening urine samples must occur when the patient is not having an attack, and ≥4 days after prophylactic hemin discontinuation and after their last hemin dose. Two screening urine samples (collected on 2 different days) should be collected for ALA/PBG; one screening urine sample should be collected for ALAS1 mRNA. On Day 1 visit, before the first dose of study drug is administered, the third urine sample for ALA/PBG is scheduled to be collected. If a patient is having an attack or it has been less than 4 days after the last hemin dose on Day 1, the third urine sample will not be collected on Day 1 and will be postponed until at least 4 days after the last hemin dose. The baseline creatinine normalized ALA/PBG/ALAS1 values will be defined as the median of measurements taken on or prior to Day 1. Any samples taken during an attack or within 3 days after receiving hemin on or prior to Day 1 will be excluded from the baseline calculation. For a patient, if all available samples on or prior to Day 1 meet the exclusion criteria, the last non-missing value on or prior to Day 1 will be used as the baseline value for the patient.

Daily worst scores in pain, nausea and fatigue, as well as analgesic medication use at home, are collected using an electronic diary (eDiary) by patients or caregivers. During the Screening period, the eDiary variables are collected when patients are not experiencing a porphyria attack. For each eDiary variable collected in the Screening period, the average of a minimum of 4 days and a maximum of 7 days (consecutive days not required) is defined as the baseline value. If there are more than 7 days collected for any variable, the measurements closest to Day 1 will be used. All measurements used for the baseline value must be taken when a patient is not experiencing a porphyria attack. If a patient has less than 4 days of measurements on non-attack days prior to Day 1, the average of the available non-attack days will be used for the baseline value.

For all other measures, baseline will be defined as the last non-missing value available on or prior to the first dose of study drug, unless otherwise specified.

For the All Givosiran Treated Set, baseline for patients who switched from placebo to givosiran will be redefined as the values prior to the first dose of Givosiran. For ALA, PBG, and ALAS1 mRNA, the redefined baseline will be calculated as the median of all values after the first dose in the 6m-DB period and prior to the first dose in the OLE period, excluding samples during an attack or within 3 days after receiving hemin. For all the other endpoints, the redefined baseline will be derived in the similar way as described above for the baseline for the 6m-DB period.

5.5. Randomization Stratification Factors

Randomization for this study is first stratified by AHP type: AIP [with genetic evidence of a mutation in the *HMBS* gene] vs non-AIP [HCP, VP, ADP, or any AHP without an identified mutation in a porphyria-related gene].

The AIP patient stratum is further stratified by the use of hemin prophylaxis or not immediately prior to Screening, and on the historical AAR. Patients who had been on hemin prophylaxis will be stratified by their historical AAR of <7 vs ≥7. Patients who had not been on a hemin prophylaxis will be stratified by their historical AAR: <12 vs ≥12. This historical AAR is calculated based on the medical record reviewed for inclusion criterion of at least 2 attacks requiring hospitalization, urgent healthcare visit or IV hemin administration at home within the 6 months prior to randomization.

Since only a few patients in the non-AIP stratum of HCP, VP, ADP, or any AHP without identified mutation in a porphyria-related gene are anticipated to be enrolled in the study, there is no further stratification within the stratum.

The historical AAR prior to randomization will be classified into high versus low when used as a categorical covariate. For patients on a hemin prophylaxis regimen at the time of screening, high attack rate is defined as AAR ≥7. For patients not on a hemin prophylaxis regimen at screening, AAR ≥12 is considered as high attack rates.

Stratification factors are recorded in both the IRS and the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database. In the presence of stratification errors, the stratification used in analysis may not match that in the IRS. In addition, for non-AIP patients, the two AIP-specific stratification factors hemin prophylaxis regimen and historical AAR are collected in the clinical database and will be used as covariates in the analysis of AHP population.

5.6. Visit Windows

For PD and clinical laboratory parameters, analysis visits will be derived for the 6m-DB period based on the study day and visit windows. Target days and the acceptable range of study days for each visit are defined in Table 1 below. If more than one visit falls within a visit window, the visit occurring closest to the target day will be selected. If there are multiple visits with the same distance from the scheduled visit day, the later visit will be selected. The derived visits will be used for all analyses.

Table 1: Derived Analysis Visit Windows

Study Visit	Target Day	Window
Screening	Day -60 to -1	≤ -1
Day 1	1	1
Week 2	15	2 – 22
Month 1	29	23 – 43
Month 2	57	44 – 71

Study Visit	Target Day	Window
Month 3	85	72 – 106
Month 4	113	107 – 127
Month 5	141	128 – 155
Month 6	169	156 - the earlier of (Day 183, and the day of the first dose of OLE)

For PD and laboratory parameters assessed during the OLE period and for vital signs and ECG measurements during the entire study, no windowing rules will be implemented, and the reported clinical visits will be used for analysis.

For QOL assessments including SF-12, EQ-5D-5L and missed days of work/school, data are collected every 3 months up to 12 months, then every 6 months afterwards. If the scheduled visits are not performed, any unscheduled and/or discontinuation visits will be mapped to the corresponding scheduled visits if the visit dates fall into below windows. The midpoint of two visits will be included in the time window of the preceding visit.

Scheduled visits (Month 3, 6, 9, 12) ± 6 weeks

Scheduled visits (Month 18, 24, 30, 36) ± 12 weeks

For patients who switched from placebo to givosiran, study visits will be mapped based on the redefined baseline.

The derived visits will be used for analyses.

5.7. Multiple Comparisons/Multiplicity

At the time of the unblinded interim analysis, only the first secondary efficacy endpoint ALA levels at Month 3 will be assessed. A significance level of 0.001 will be used when comparing the ALA levels between the two treatment arms using an ANCOVA model.

For the final analyses of the 6m-DB primary treatment period, a significance level of 0.049 will be used to test the efficacy endpoints, reflecting a penalty of 0.001 for the unblinded interim analysis. A fixed-sequence testing strategy for the primary and secondary endpoints will be implemented to control the overall type I error rate. The primary endpoint will be compared between treatment arms at the significance level of 0.049. If the test is statistically significant, then the secondary endpoints will each be tested at the same significance level of 0.049 in the order specified in the efficacy endpoint section (Section 3.2). If the test of an endpoint in the sequence is not statistically significant, the testing of remaining endpoints in the sequence will stop and the null hypotheses for the subsequent tests will not be rejected.

There will be no multiplicity adjustment for exploratory endpoints.

5.8. Analysis Cutoff and Database Lock

For the interim analysis and the final 6m-DB analysis, as this study will be ongoing, the study database will be soft-locked with all data up to a prespecified cutoff date quality controlled, i.e. data in EDC will be frozen and external data such as laboratory data will be QA’d and cleaned.

The soft lock is an interim database lock. Additional details regarding the soft lock process are located in the study Data Management Plan.

The interim and the final 6m-DB analysis will include data on or prior to this prespecified cutoff date. For assessments with starting/ending dates (e.g., AEs, medications, medical history), the starting date will be compared with the pre-specified cutoff date.

After the study is completed, i.e., all patients completed the OLE period and the safety follow-up visits, the database will be hard locked and all data collected will be used for analysis.

5.9. Interim Analyses

An unblinded interim analysis will be conducted when approximately 40 AIP patients have completed the Month 3 visit. The data for these ~40 patients will be soft-locked for an interim database lock and transferred in support of the interim analysis. A cutoff analysis approach will be implemented in the analysis datasets as specified in Section 5.8 . The details of the interim analyses are discussed in Appendix 9.1

5.10. Analyses for the Entire Study

The study design includes a 6m-DB period and an OLE period. The primary objective is to evaluate the efficacy and safety of givosiran compared with placebo during the 6m-DB period. In addition, the long-term efficacy and safety of givosiran during the entire givosiran treatment period (beyond 6m-DB) will be characterized for the All Givosiran Treated Set. For patients who received placebo in the 6m-DB period and givosiran in the OLE period, the intra-patient comparison of the two treatment periods will be conducted to assess the trajectory changes for selected efficacy parameters.

The detailed definitions for different treatment periods are as the following.

- **6m-DB Period**

  The treatment comparison of givosiran versus placebo will focus on the 6m-DB period, defined as below:

  1) For patients who received at least one dose of givosiran during the OLE period, all assessments collected prior to the first dose of givosiran in the OLE period will be included in the 6m-DB period. All data will be included in the summary tables and figures.
  2) For patients who discontinued treatment and did not receive any givosiran dose in the OLE period, all assessments will be included in the 6m-DB period. Assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only unless specified otherwise (e.g., AEs in Section 6.8).

Endpoint	**Windowing rule for patients who did not enter OLE**
Safety and PD endpoints	Assessments with onset date within 28 days of the last dose [1]
Attacks and hemin use related endpoints	Data collected on or before the earlier of 1) Day 162 which is the start day of Month 6 visit window; and 2) end of study (EOS) date.

Endpoint	Windowing rule for patients who did not enter OLE
Other clinical efficacy endpoints	Assessments collected on or before Day 183.

[1] In a rare situation that a patient did not discontinue 6m-DB and did not enter OLE, all data will be included in summary. For AE summary, related AEs will be considered as TEAE and included in summary tables regardless of time window.

- **OLE Period**

  The start day of the OLE period is defined as the day when the first dose of the OLE period is administered. The assessments collected or AEs with onset after the administration of the first dose of the OLE period will be included in the OLE period. When assessments or AE onset dates are exactly the first OLE dose date with time missing, the records will be included in the OLE period.

- **During Givosiran Treatment**

  For all patients who received at least one dose of givosiran, data will be summarized for the "during givosiran treatment" period, defined as below.

  1) For patients who received givosiran in the 6m-DB period, all assessments collected after the first dose of givosiran during the entire study including both the 6m-DB and OLE periods (if available) will be included in the "during givosiran treatment".
  2) For patients who received placebo in the 6m-DB period and switched to givosiran in the OLE period, all assessments collected after the first dose of givosiran in the OLE period will be included in the "during givosiran treatment".
  3) For patients who discontinued givosiran treatment during the 6m-DB period, the data handling will follow the same rules as discussed above for "6m-DB period". For discontinuation in the OLE period, assessments within 28 days of the last dose of givosiran will be included in summaries. For ongoing patients, all data are included.

Selected efficacy parameters will be summarized over the entire study including the 6m-DB and OLE periods for all patients to show the trajectory changes comparing the placebo experience versus the givosiran experience, for example, summary of creatinine normalized urinary ALA by visit. In these summaries, the treatment groups are "Givosiran" or "Placebo" based on randomization during the 6m-DB period.

Intra-patient comparison for ALA, AAR and annualized days of hemin use

For patients who received placebo during the 6m-DB period and crossed over to receive givosiran during the OLE period, intra-patient comparisons for ALA, AAR and annualized days of hemin use will be performed to evaluate the efficacy of givosiran versus placebo.

For AAR and annualized days of hemin use, a negative binomial regression model with period as a main effect and patient as a random effect (PROC GENMOD with the REPEATED statement) will be fitted to compare the mean rate during the 6m-DB period while on placebo and that of the OLE period while on Givosiran treatment. In addition, individual patient's AAR and annualized days of hemin use during the 6m-DB and the OLE periods will be calculated separately and compared graphically. To avoid an unstable estimation due to limited duration of follow-up, the

AAR and annualized days of hemin use will only be calculated for patients who have at least 85 days of follow-up during the OLE period.

For creatinine normalized ALA, Wilcoxon signed rank test will be used to make intra-patient comparisons on the medians over the 6m-DB period versus the OLE period, Month 3 vs. Month 9, and Month 6 vs. Month 12, when sufficient data are collected during the OLE period.

Treatment sequence

Patients are assigned to receive either 2.5 mg/kg or 1.25 mg/kg once monthly dose of givosiran upon entry to the OLE period through the end of Month 12 (At Month 13, patients on 1.25 mg/kg once monthly dose may receive a dose adjustment). After sufficient data are collected during the OLE period, summaries for the All Givosiran Treated Set will be provided by the following subgroups:

- Givosiran/Givosiran:
  - Givosiran 2.5 mg/kg/Givosiran 2.5 mg/kg: all patients who received givosiran 2.5 mg/kg in 6m-DB and OLE periods.
  - Givosiran 2.5 mg/kg/ Givosiran 1.25 mg/kg: all patients who received givosiran 2.5 mg/kg in the 6m-DB period and givosiran 1.25 mg/kg in the OLE period;
  - Overall: all patients who received givosiran at any dose level in the 6m-DB, including patients who discontinued during 6m-DB and did not receive dose in OLE.
- Placebo/Givosiran:
  - Placebo/Givosiran 2.5 mg/kg: all patients who received placebo in the 6m-DB period and givosiran 2.5 mg/kg in the OLE period;
  - Placebo/ Givosiran 1.25 mg/kg: all patients who received placebo in the 6m-DB period and givosiran 1.25 mg/kg in the OLE period.
  - Overall: all patients who received placebo during the 6m-DB period and switched to givosiran at either dose level in the OLE period;
- Givosiran 2.5 mg/kg: all patients who received givosiran 2.5 mg/kg in either 6m-DB or OLE periods;
- All Givosiran: all patients who received any dose of givosiran during either 6m-DB or OLE period.

To assess the effect of givosiran 2.5 mg/kg and 1.25 mg/kg on ALA reduction separately, for the two subgroups Placebo/Givosiran 2.5 mg/kg and Placebo/Givosiran 1.25 mg/kg, Wilcoxon signed rank test will be conducted for intra-patient comparison on the medians over the 6m-DB period versus the OLE period, Month 3 vs. Month 9, and Month 6 vs. Month 12, when sufficient data are collected during the OLE period.

6. STATISTICAL ANALYSIS

6.1. Patient Disposition

Number and percentage of patients in the following categories will be summarized by treatment arm and overall as appropriate:

- Randomized
- Treated
- Completed the Months 3 and 6 visits
- Discontinued treatment and primary reasons for treatment discontinuation
- Withdrew from the study and primary reasons for withdrawal
- Entered the OLE period.

The number and percentage of patients enrolled by country and site will be summarized by randomized treatment arm and overall. The number and percentage of patients in each level of each randomization stratification factor recorded in IRS, and a comparison of the number and percentage of patients in each randomization stratification factor in IRS versus the clinical database will be summarized by randomized treatment arm and overall.

6.2. Demographics and Baseline Characteristics

Demographic, background (e.g. medical history) and baseline disease characteristics will be summarized by treatment arm and overall.

Age, height, weight, and body mass index (BMI) will be summarized by descriptive statistics. Age group, sex, race, ethnicity, and region will be summarized by presenting the numbers and percentages of patients in each category.

The following baseline disease characteristics will also be summarized: age at diagnosis, time from diagnosis to randomization, prior hemin prophylaxis status (Y/N) at screening, number of attacks requiring hospitalization, urgent healthcare visits or IV hemin administration at home in the last 6 months prior to the study, prior chronic symptoms when not having attacks (Y/N) and prior chronic opioid medication usage when not having attacks (Y/N).

6.3. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to primary analysis and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the final protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol

deviation and whether or not this deviation is classified as a major protocol deviation. In addition, each major deviation will be clearly identified as to whether or not it warrants exclusion from the Per Protocol Set, based on the potential impact on the primary efficacy results according to the judgment of the Sponsor. This file will be finalized prior to study unblinding.

6.4. Drug Exposure

Exposure to study medication in days and the number of study drug SC administrations received will be summarized by treatment arm.

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

- Last dose date + 27 days
- Analysis cutoff date
- The date of EOS

Duration of exposure is defined as the last date of exposure to study drug – date of the first dose +1. The exposure during the 6m-DB period is right censored by the date of the first OLE dose, i.e. the last exposure day in the 6m-DB period is no later than the day before the first OLE dose. Similarly, the exposure during the OLE is left censored by the date of the first OLE dose, i.e. the Day 1 of the OLE is the day of the first OLE dose.

Dose interruptions and compliance are not taken into account for duration of exposure.

6.5. Efficacy Analysis

All efficacy endpoints will be analyzed in both AIP and AHP patients. Model-based analysis for AIP and AHP patients will adjust for the two stratification factors, i.e. use of hemin prophylaxis at screening (Y/N) and historical AAR prior to randomization (High/Low), unless otherwise specified. For analysis of AHP patients, AHP type (AIP versus non-AIP) will not be included in the model due to the small number of non-AIP patients.

Porphyria Attacks

Porphyria patients experiencing acute attacks present with highly morbid and potentially life-threatening symptoms relating to dysfunction across the central, peripheral, and autonomic nervous system. The most commonly seen signs and symptoms of a porphyria attack include diffuse, severe neurovisceral pain mostly in the abdomen, back, or limbs, nausea and vomiting, fatigue, hypertension, tachycardia, motor weakness.

Attacks can be reported by patients through eDiary or communicated with investigators. Protocol defined criteria for porphyria attacks include investigator-confirmed attacks requiring hospitalization, urgent healthcare visit, IV hemin administration at home, or treated at home not requiring hemin use. For data collection, each attack is reported with one associated outcome in the hierarchical order, e.g., if an attack requires both hospitalization and urgent healthcare, the attack will be reported as requiring hospitalization only. The porphyria attacks in the primary endpoint include those attacks requiring hospitalization, urgent healthcare visits or IV hemin administration at home.

The start time of a porphyria attack is defined as the time at which acute and sustained worsening of the patient's porphyria manifestations beyond normal day-to-day variability. The end of

porphyria attacks is characterized by recovery back to within a patient's normal day-to-day variability. Attacks that occur on the same calendar day of the last treated attack will be considered a part of the original attack and will count as one attack in the calculation of the AAR. Any attacks that begin on the next day from the last treated attack ends will constitute a new attack.

6.5.1. Primary Endpoint

The primary endpoint of the study is the annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the initial 6m-DB period. The primary analysis set for the primary endpoint is FAS_{AIP} (defined in Section 4).

The number of qualifying porphyria attacks is annualized for each patient using below formula:

$$\text{Annualized attack rate (AAR)} = \frac{\text{total number of porphyria attacks}}{\text{total number of days in the treatment period}} \times 365.25.$$

Let λ_G represent the mean AAR for givosiran arm and λ_O represent the mean AAR for the placebo arm. The hypotheses of the primary analysis are:

$$H_o: \frac{\lambda_G}{\lambda_O} = 1 \qquad H_A: \frac{\lambda_G}{\lambda_O} \neq 1$$

The statistical significance level for the primary analysis of the primary endpoint is 2-sided 0.049. An estimated AAR ratio : $\frac{\lambda_G}{\lambda_O} < 1$ and 2-sided p-value < 0.049 will lead to rejection of H_o.

6.5.1.1. Primary Analysis using Negative Binomial Regression Model

The primary analysis will be performed using a negative binomial regression model that include fixed effects of the treatment arms, the stratification factors for the AIP patients, which include the hemin prophylaxis regimen prior to the study (yes vs no) and historical AAR (high vs low). The logarithm of the amount of time (in the units of year) that each patient spends in the 6m-DB period will be included in the model as an offset variable. With the offset variable, the negative binomial regression model essentially models the AAR for each patient based on the length of time in the treatment period, and hence will be able to account for the different lengths of follow-up time.

For patients who discontinue the study treatment early (before the end of the 6m-DB period), best efforts will be made to continue the collection of attack and hemin use data. The primary efficacy analysis will be based on all qualified attacks occurring in the 6m-DB period, including those that occur after treatment discontinuation. No imputation of attack data is planned for the primary analysis.

In addition to p-value, an estimated ratio of mean AARs between the two treatment arms with the corresponding 95% confidence interval will be estimated from the negative binomial regression model. Descriptive statistics for the median and interquartile range of the annualized attack rate will also be presented by treatment arm.

6.5.1.2. Component Analysis

The annualized rate of porphyria attacks is a composite endpoint including three components: 1) attacks requiring hospitalization; 2) attacks requiring urgent healthcare visit; or 3) attacks requiring IV hemin administration at home. The AAR of each of the three components will be analyzed using the same negative binomial regression model and the estimated ratio of mean AARs between treatment arms with corresponding 95% confidence interval will be provided. Zero-inflated negative binomial regression model will also be used to analyze each component to address the potential issue of an "excessive" number of patients with zero attack. When there are less than 10 patients experiencing attacks in a component, model-based analysis will not be conducted for that component.

6.5.1.3. Analysis in the Per Protocol Set

The analysis of the primary endpoint using negative binomial regression model will also be conducted for the Per Protocol Set (PPS) (defined in Section 4).

6.5.1.4. Sensitivity Analysis

The attack data collection is on calendar days, i.e. start and stop times by hours are not collected. To evaluate the impact of potential under- or over-counting of attacks due to the 1-day window, the following two sensitivity analyses will be performed for attacks meeting the primary endpoint definition:

- Count all discrete attacks even if they overlap during a day.
- Extend the attack counting windows to a 2-day window, i.e. attacks that occurs on the same calendar day, or are separated by one calendar day will be counted as one attack.

An Andersen-Gill model using the same fixed effects as the primary model will be performed treating the attacks as recurrent events.

If the number of zero attacks are high, a zero inflated negative binomial model will be performed to provide further assurance of the results.

Attack data could be missing if a patient stops attack data collection before the end of the 6m-DB period, e.g. due to study discontinuation. The missing data period is defined as the time (days) between the end of attack data collection and Day 162 which is the earliest expected end of the 6m-DB period of a patient, inclusive. For patients who provide attack data through the end of 6m-DB period, no missing data imputation will be performed. If ≥ 5% patients on givosiran arm have missing attack data, a pattern mixture model (PMM) with MI will be performed to assess the robustness of primary analysis results to the possible violation of the MAR missingness assumption. The details of the PMM approach for attack data is described in Section 9.4 .

In addition, AAR based on the following revised definitions will also be analyzed using the primary analysis method:

- All investigator-confirmed attacks: all attacks meeting the protocol defined criteria, including attacks requiring hospitalization, urgent healthcare visit, IV hemin administration at home, or treated at home not requiring hemin use.

- Potential attacks: in addition to the investigator-confirmed attacks, attacks not confirmed by investigator but were deemed as potential attacks will also be included. Attacks that were reported due to entry errors will be excluded. An Alnylam medical monitor reviewed the reasons that investigators decided a reported attack was not a protocol-defined attack to determine which could be considered potential attacks (e.g., exclusion of unique potential attacks due to duplicate or accidental entry). This was done in a blinded manner prior to the 6m-DB period interim database lock and unblinding.

For these analyses, attacks with duplicated or overlapping dates will be counted as one attack.

6.5.2. Secondary Endpoints

The annualized-event-rate type of endpoints include the annualized rate of hemin administration in AIP patients (FAS_{AIP}) and the AAR requiring hospitalization, urgent care visits or IV hemin treatment at home in AHP patients (FAS) over the 6m-DB period. The number of events for each patient is annualized in the same way as AAR defined for the primary endpoint, divided by treatment period (in days) and multiplied by a year (365.25 days). These endpoints will be analyzed using a negative binomial regression model similar as the one used for the primary endpoint. Since hemin is administered as a one dose per day regimen, the annualized rate of hemin administration will be evaluated by the annualized days of hemin use.

The biomarker endpoints include the ALA levels in AIP patients (FAS_{AIP}) at 3 months and 6 months, the urinary PBG levels in AIP patients (FAS_{AIP}) at 6 months. Any postbaseline ALA/PBG values measured within 3 days after hemin use during the 6m-DB period will be treated as missing and excluded from analysis. For each biomarker secondary endpoint, the biomarker levels at the specified time point will be estimated and compared between two treatment arms using an MMRM model. The model will include baseline of the corresponding biomarker level as a continuous covariate, stratification factors (prior hemin prophylaxis status and historical attack rates), visit, treatment, and visit by treatment as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the unstructured covariance structure fails to converge, a compound symmetry covariance structure will be used. The least squares (LS) means and the corresponding SEM and 95% CIs for each treatment arm, along with the LS mean difference between two treatment arms at each post-baseline visit will be presented. The p-value for the treatment difference for each endpoint (ALA at Month 3 and Month 6; PBG at Month 6) will be presented. In addition, the overall p values measuring the average treatment effect on ALA and PBG during the entire 6m-DB period will also be reported to assess the durability of the treatment effect. If normal assumptions are violated, a non-parametric Wilcoxon rank sum test will be performed. For ALA at Month 3 and Month 6, if the drop-out rate during the 6m-DB period is ≥ 5% on the givosiran arm, sensitivity analyses will be performed using a PMM approach as described in Section 9.3.

Daily worst scores for pain measured by BPI-SF in Numeric Rating Scale (NRS), fatigue measured by BFI-SF in NRS, and nausea measured by NRS are collected through eDiary. For each of the symptoms (pain, fatigue and nausea), daily eDiary entries will be averaged into a weekly (i.e. 7 day) score. The change from baseline in weekly mean scores is defined as the post-baseline weekly mean score minus the baseline score. The AUC of change from baseline over 6 months in the respective measure will be calculated for each patient based on change from

baseline in weekly mean scores. For each of the measures, the AUC of change from baseline will be compared between two treatment arms using ANCOVA model with fixed effects of treatment arms and two stratification factors. In addition, the average weekly score change from baseline over 6 months will also be calculated and analyzed using ANCOVA.

Symptoms are expected to be elevated during porphyria attacks. This AUC approach encapsulates the peaks of these symptoms during attacks as well as lingering chronicity of these symptoms throughout the entire treatment period. AUC approaches have been used in other episodic conditions to compare both the severity and duration of pain across groups.

The weekly mean score will be calculated using the following algorithm:

1. A completed week will be defined as a week where a patient completes 4 or more daily entries in a week, which is consistent with other diary-based PRO research. The available eDiary entries for a completed week will be averaged to determine the weekly symptom score.
2. A missing week will be defined as a week where a patient is missing 4 or more daily entries. In a missing week, if there is one or more recorded attack days during the week, the mean score will be derived based on the following rules:
   - If there are 1 or more completed attack day diary entries, the mean weekly score will be computed as an average of the daily entries for that week.
   - If there is no completed attack day diary entry, the weekly mean score will be imputed using the average of all non-missing attack-week weekly mean scores within the same treatment period for the patient. If there's no non-missing attack week score, the weekly mean score will be imputed by the worst weekly mean scores within the same treatment period for the patient.

   Patients are expected to have elevated symptom scores during porphyria attacks, which can span over multiple days. This algorithm intends to preserve any completed eDiary entries collected during days associated with attacks.
3. In a missing week without any attack days, the weekly mean score will be imputed by the last observed non-missing week without any attack days. If there is no last observed non-missing week without any attack days, the imputation will be based on baseline observation carried forward (BOCF).

The SF-12 scores are obtained using the Optum (PRO CoRE 1.3 Smart Measurement® System) software with the 2009 U.S. general population t-scores applied. For the Physical Component Summary (PCS) of SF-12 score, change from baseline at Month 6 in AIP patients (FAS_{AIP}) will be analyzed using an MMRM model with baseline score as a continuous covariate, fixed effect terms including treatment arms, stratification factors, visit (Month 3 or Month 6), and visit by treatment interaction.

All secondary endpoints defined for the AIP patients will also be analyzed for the AHP patients using similar methods.

6.5.3. Exploratory Endpoints

The continuous exploratory endpoints in AIP patients for the 6m-DB period including EQ-5D-5L index score will be analyzed using an MMRM model similar to the one employed for the secondary endpoint PCS SF-12.

Patient Global Impression of Change (PGIC) at Month 6 and Month 12 will be summarized descriptively with number and proportion of patients in each category, as well as the two combined categories of "Improved" and "No Change or Worsening". The category of "Improved" includes minimally improved, much improved, and very much improved. The category of no change or worsening includes no change, very much worse, much worse, and minimally worse. Mantel–Haenszel chi-square test will be used to compare the proportion of "Improved" between the two treatment arms. Odds ratio will be estimated.

Missed days of work/school in the past 4 weeks at Month 6 will be summarized descriptively.

Porphyria Patient Experience Questionnaire (PPEQ) will be summarized descriptively.

For endpoints assessed in patients with any AHP (FAS) over the 6m-DB period, the analyses will be conducted using methods similar as those employed for the AIP patients (FAS_{AIP}).

For EQ-5D-5L, a categorical summary of the numbers and percentages of patients reporting each ordinal response within each EQ-5D domain will also be presented.

Analgesic medication use during the 6-month DB period will be summarized as follows:

- Number (%) of patients who took at least one analgesic medication by category (opioid, non-opioid, either opioid or non-opioid) and PT during periods of screening, Month 1 to 3, Month 4 to the end of 6m-DB period, and during the 6m-DB period.
- Descriptive summary on proportion of days with analgesic use during the 6m-DB period by category (opioid, non-opioid, either opioid or non-opioid).

Refer to Appendix 9.5 for the detailed rules in categorizing analgesic medication and opioid/non-opioid.

Data collected during the OLE period will be summarized descriptively. For daily worst pain/nausea/ fatigue scores collected during the OLE period, change from baseline in weekly mean scores will be summarized descriptively.

6.5.4. Subgroup Analysis

For the primary endpoint, subgroup analyses will be conducted to assess the consistency of treatment effect for the AIP and AHP patients during the 6m-DB period. A negative binomial regression model will be fit to estimate the treatment effect within each subgroup. The model includes treatment arms as a fixed effect and each patient's time on study as an offset variable. An estimated ratio of mean AARs between the two treatment arms with the corresponding 95% CI will be estimated for each subgroup. If the number of patients in either treatment arm of a subgroup is less than 10, only descriptive statistics will be presented.

- Age at Screening (< or ≥ median age in the overall population)
- Race (White or Non-white)
- Sex (Female or Male)
- Region group 1: North America (including US and Canada) or Other (outside North America)
- Region group 2: Europe or Other (outside Europe)

- Baseline BMI (<25 or ≥25)
- Prior hemin prophylaxis status (Yes or No)
- Historical attack rates prior to randomization based on the hemin prophylaxis status prior to the study (high or low)
- For patients on a hemin prophylaxis regimen at the time of screening, if AAR ≥7, the patient is considered having high attack rates prior to the study. For patients who were not on a hemin prophylaxis regimen at screening, AAR ≥12 is considered with high attack rates.
- Prior chronic opioid use when not having attacks (Yes or No)
- Prior chronic symptoms when not having attacks (Yes or No)

Other subgroups may be examined, if deemed appropriate.

The subgroup analyses may also be performed for other secondary endpoints.

The number of non-AIP patients is anticipated to be only a few. Descriptive statistics on the primary and secondary endpoints will be provided by treatment arm for non-AIP patients.

6.6. Pharmacodynamic Analysis

Analyses of secondary endpoints relating to ALA and PBG levels are described in Section 6.5.2. In addition, ALA, PBG, and ALAS1 levels will be summarized descriptively at each scheduled visit.

Population PK/PD analyses will be described in a separate population PK/PD analysis plan.

6.7. Pharmacokinetic Analysis

Plasma concentrations of givosiran and its major metabolite, AS(N-1)3' givosiran, will be summarized by nominal sampling time.

In patients at East Asian study centers with intense PK sampling, plasma and urine PK parameters of givosiran and AS(N-1)3' givosiran will be determined using non-compartmental methods. PK parameters include maximum plasma concentration (Cmax), time to maximum plasma concentration (tmax), elimination half-life ($t_{½\beta}$), and area under the concentration-time curve (AUC). Other parameters may be calculated, if deemed necessary. PK parameters will be described using summary statistics.

Population PK analysis will be used to describe plasma PK of givosiran and AS(N-1)3' givosiran across all patients. The population PK analysis will be described in a separate modeling and simulation analysis plan.

6.8. Safety Analysis

An adverse event (AE) is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the AEs that first occurs or worsens after the first dose of study drug. Safety parameters also include vital

signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Time windows for safety data to be analyzed for the 6m-DB period and the entire study including both 6m-DB and OLE periods as well as safety follow-up are described in Section 5.10 . All safety data regardless of time windows will be listed and summarized for selected endpoints, i.e. AEs by system organ class (SOC) and preferred term (PT), SAEs by SOC and PT, and selected laboratory parameters.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

No inferential safety analysis is planned.

6.8.1. Adverse Events

AEs will be classified by the MedDRA coding system (version 21.0 or later) and displayed in tables and data listings using SOC and PT.

AEs will be summarized by the numbers and percentages of patients reporting at least one AE, having at least one AE by primary SOC and PT. A patient with multiple occurrences of an AE will be counted only once in the respective AE category. Patients who report multiple occurrences of the same AE (PT) will be classified according to the most related or most severe occurrence, respectively.

Injection site reaction (ISR) events will be thoroughly summarized. The numbers and percentages of patients reporting at least one ISR event and the numbers and percentages of patients with different ISR symptoms will be summarized. Note that multiple symptoms of a single ISR will not be counted as multiple ISRs. In addition, ISRs will be characterized by injection. The incidence rate of ISR, defined as the number of ISRs divided by total number of injections will also be summarized. If there are multiple ISRs that occur between two consecutive injections, these events are considered caused by the earlier injection and counted as one ISR.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment (possibly related or definitely related), any severe AE, any severe AE related to treatment, any serious AE (SAE), any SAE related to treatment, any AE/SAE of clinical interest; any AE/SAE leading to treatment discontinuation, any study drug related AE/SAE leading to treatment discontinuation, any AE/SAE leading to study withdrawal, any study drug related AE/SAE leading to study withdrawal, and any deaths.

Tabulations by SOC and PT may be produced for the following. The SOC and PT within each SOC will be presented alphabetically.

- All AEs;
- AEs by severity;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;
- AEs leading to temporary treatment interruption;

- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AEs leading to study withdrawal;
- SAEs leading to study withdrawal.

Tabulations by PT in decreasing order in frequency in the givosiran arm will be produced for the following.

- All AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;

AEs and SAEs will also be summarized by maximum relationship to study drug and by maximum severity.

AEs mapping to the MedDRA high level term (HLT) of injection site reaction will be summarized by preferred term. AEs mapping to the standard MedDRA query (SMQ) Drug Related Hepatic Disorder will be summarized by SOC and preferred term. Adverse event mapping to a customized Acute Pancreatitis SMQ will be summarized by SOC and preferred term (PT). AEs mapping to the Acute Renal Failure SMQ will be summarized by SOC and PT. AEs mapping to the Anaphylactic Reaction SMQ will be summarized by PT. AEs mapping to the SMQ Malignant or Unspecified Tumors will be summarized by high level term and preferred term. Other SMQs or AE groupings may be evaluated.

AEs mapping to a modified Acute Pancreatitis SMQ were summarized by SOC and PT. This custom SMQ strategy was developed in such a way as to capture more possible pancreatitis cases than the standard MedDRA SMQ. This SMQ included all AEs from the acute pancreatitis SMQ (narrow terms) plus the PTs for lipase increased, lipase abnormal, amylase increased, amylase abnormal, hyperlipasaemia, hyperamylasaemia, pancreatic enzyme abnormality, pancreatic enzymes abnormal, pancreatic enzymes increased.

All AEs collected will be listed along with the information collected on those AEs, e.g. AE relationship to study drug, AE outcome etc. By-patient listings will also be provided for the following: all deaths, all SAEs, and all AEs leading to treatment discontinuation or study withdrawal, all AEs leading to death.

A listing of ISRs will be presented including descriptions, onset and resolution date, severity, treatment given, and event outcome.

6.8.2. Laboratory Data

Knowledge of several clinical laboratory tests, including ALA, PBG, and ALAS1, has the potential to unblind the reviewer. Procedures to protect the blind, including permissions to view these lab values, are described in the Data Integrity Plan (Amendment 3, 07Sep2018).

Clinical laboratory values will be expressed in Standard International (SI) units. Missing laboratory data will not be imputed.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category for selected parameters.

A listing will be produced for amylase and lipase in all patient with amylase/lipase observations above 3×ULN flagged. A listing for all patients with abnormal liver function tests defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point will also be provided.

A table will be produced to summarize the number and percentage of patients in each of below category at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALP > 1.5×ULN,
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN,
- Total Bilirubin > 1.5×ULN concurrent with ALT or AST > 3×ULN,
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN

EDISH plots for ALT, AST, and total bilirubin will also be provided.

For hematology and blood chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the NCI CTCAE Version 4.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

All laboratory data will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.8.3. Vital Signs and Physical Examination

Descriptive statistics by visit and treatment arm will be provided for each variable.

Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

6.8.4. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, and QTc interval. For post-baseline assessments where ECG is performed in triplicate, the average of the 3 (or all available) readings will be used for analysis.

Corrected QT interval (QTc), if not collected, will be calculated using both Fridericia's and Bazett's correction formula.

Bazett's square-root corrected QT: QTcB (ms) = QT (ms) $\times \sqrt{\frac{HR(bpm)}{60}}$

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) $\times \sqrt[3]{\frac{HR(bpm)}{60}}$.

PR, QRS, QT, QTc (ie, QTcB and QTcF) and RR intervals and their change from pre-dose baseline will be summarized for each treatment group by scheduled visit. Subjects will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTc interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTc interval. The number and percentage of subjects in each category will be summarized for each treatment group.

All ECG data for each patient will be provided in a data listing.

6.8.5. Prior and Concomitant Medications

Prior medications are those medications taken prior to the first dose of study drug. If the medication end date is before the date of first dose of study drug, the medication will be summarized as prior medication regardless of whether the start date is missing or not.

Concomitant medications are medications, other than study drug, taken at or after the first dose of study drug, as well as medications with a start date prior to first dose of study drug and are ongoing after first dose of study drug. If medication start date is on or after date of first dose of study drug, the medication will be summarized as concomitant medication regardless of whether the medication end date is missing or not. If the end date of a medication is missing or incomplete such that it cannot be determined whether it is before first study drug dose, it will be counted as a concomitant medication.

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2018). Results will be tabulated by anatomical therapeutic class (ATC) and preferred term.

6.9. Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time during study as well as at each scheduled visit will be summarized. The titer results for patients with confirmed positive ADA results will also be summarized using descriptive statistics.

ADA data and patients with confirmed positive ADA results will be presented in data listings.

7. CHANGES FROM PREVIOUS VERSION(S)

7.1. Changes in SAP Amendment 1

The SAP Amendment 1 includes more details previously not discussed in the original SAP. Some changes to analysis methods are made following regulatory feedback and further assessments. Amendment 1 was signed off before the interim analysis. The details of changes and rationales are listed in below table.

Summary of Changes	**Rationale**
In the protocol and original SAP, an interim sample size reassessment (SSR) was planned but was not conducted during study.	An SSR was originally planned to be performed at the time of the interim analysis to potentially increase the number of AIP patients in the study from approximately 70 to 90. Due to fast enrollment of the study leading to greater than expected numbers of patients randomized, an interim analysis for SSR was therefore no longer necessary.
For the interim analysis of ALA at Month 3, the missing data imputation was mLOCF in the original SAP; in this amendment, the imputation will be based on MI. Additional sensitivity analyses are added including a PMM and a non-parametric method.	Updates made following regulatory feedback.
For the analysis of ALA/PBG at the end of 6m-DB period, the primary analysis was based on ANCOVA/mLOCF in the original SAP; in this amendment, the primary analysis for ALA/PBG will be based on MMRM and a sensitivity analysis using PMM is added for ALA.	Both MMRM and MI/ANCOVA are based on MAR assumption and MMRM is often considered a more simple and powerful method. PMM is added to assess the robustness of the results when missingness mechanism is MNAR.
For the analysis of primary endpoint attack rate, PMM is added to address potential missing attack data and other sensitivity analyses are added (Section 6.5.1.3).	These sensitivity analyses are added to assess the robustness of the primary analysis results.
The median instead of the mean of valid pre-dose values is used to derive baseline ALA/PBG.	Due to the variability with PD measures, the median is less affected by extreme values than the mean.
For the secondary endpoint PCS SF-12, the primary analysis method is changed from ANCOVA to MMRM.	This endpoint is measured at Month 3 and Month 6. MMRM implicitly imputes missing data using data from both time points while ANCOVA does not address missing data.
For the secondary endpoints daily worst score of pain, nausea, and fatigue, the AUC over 6 months will be derived by first calculating the weekly mean scores (Section 6.5.2).	Updated to address regulatory feedback on missing data handling and justification of AUC method.

7.2. Changes in SAP Amendment 2

In the SAP Amendment 2, detailed analyses for the entire study including OLE period are added. Amendment 2 will be signed off before the final analysis of the 6-month double-blind (6m-DB) data and the unblinding of treatment assignments.

In this version, a new analysis population All Givosiran Treated Set is added, additional subgroups of the All Givosiran Treated Set were defined to accommodate analysis of the different givosiran doses allowed in the OLE period with Protocol Amendment 3, and different treatment periods are defined to allow for the analysis of data in the overall study. In addition, Per Protocol Set is defined to allow for an evaluation of the primary endpoint in the subset of patients who fulfill the protocol in terms of the eligibility requirements, adherence to the study drug and outcome assessments. A few minor updates are made to the analysis of the 6m-DB period, e.g., zero-inflated negative binomial regression model is added to analyze components of attacks. For the analysis of worst scores of pain, fatigue, and nausea, the following updates are made:

- The AUC of weekly scores is updated as the AUC of change from baseline in weekly scores. Note that with an ANCOVA analysis adjusted for baseline, using the actual score or change from baseline as a response variable will yield the same estimate of treatment difference and p value. This update is made to allow for a direct estimate of the LS mean change from baseline in each treatment arm.
- The mean weekly score change from baseline over 6 months is defined for analysis, which is mathematically similar to the AUC of change from baseline in weekly score but is clinically meaningful and interpretable.
- The testing order of daily worst scores of fatigue and nausea are switched because the mean nausea scores at baseline are low therefore less likely to show improvement with treatment.

8. REFERENCES

Keene ON, Roger JH, Hartley BF, Kenward MG. Missing data sensitivity analysis for recurrent event data using controlled imputation. *Pharm Stat*. 2014;13(4):258-264. doi:10.1002/pst.1624

Rubin, D.B. (1987), Multiple Imputation for Nonresponse in Surveys, New York: John Wiley & Sons, Inc.

Rubin, D.B. (1996), "Multiple Imputation After 18+ Years," Journal of the American Statistical Association, 91, 473–489.

Siddiqui, O. (2011). MMRM versus MI in Dealing with Missing Data – A Comparison Based on 25 NDA Data Sets, Journal of Biopharmaceutical Statistics, 21: 423–436, 2011.

9. APPENDICES

9.1. Details of Interim Analysis

At the interim, the only efficacy endpoint to be assessed is the Month 3 ALA level in AIP patients. The other efficacy endpoints will not be analyzed. ALA data beyond 3 months will be summarized descriptively by treatment arm for each scheduled visit during the 6m-DB period. Patient disposition, demographic and baseline characteristics, drug exposure, safety, PK, and PD will be summarized as described in Section 6 .

9.1.1. Interim Analysis Population

The analysis sets for the interim analysis are defined as follows:

- Interim Full Analysis Set (IFAS): a randomized patient will be included in the IFAS if
  1) the patient was randomized more than 92 days before the cutoff date; OR,
  2) the patient was randomized between 78 and 92 days before the cutoff date and both criteria below are met
     a. the patient has finished the Month 3 visit on or before the cutoff date
     b. all patients who have a randomization date before this patient are included

  Patients will be grouped by their randomly assigned treatment group (i.e. as randomized) for the efficacy analyses.

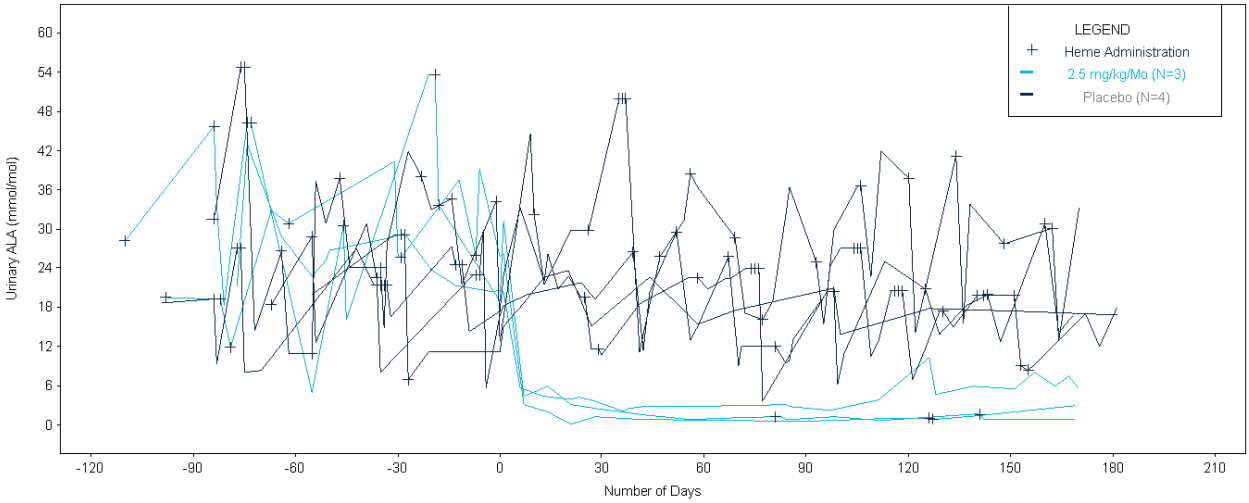
  - AIP patients in the Interim Full Analysis Set ($IFAS_{AIP}$)
  - Interim Safety Analysis Set (ISAS): includes the same cohort of patients as in the IFAS but grouped according to the treatment actually received.

The primary efficacy analysis set for the ALA data is $IFAS_{AIP}$. The ALA data of the non-AIP patients who have finished the Month 3 visit in the IFAS will be presented in a listing. The safety analyses will be based on the ISAS and will also be performed for the AIP and non-AIP patients separately. PK analysis at interim will be include patients who have evaluable PK data contributing to the estimation of PK parameters.

9.1.2. Rationale for Month 3 ALA Endpoint

Three months of treatment is considered an appropriate time point for assessment of the efficacy of givosiran based on ALA levels observed in the givosiran Phase 1 study. In the Phase 1 study, there were 40 individual patients including 17 AIP patients with recurrent attacks, the patient population of the ENVISION study and 23 asymptomatic high excreters (ASHE, or chronic high excreters CHE). Among the 17 AIP patients with recurrent attacks, 3 patients were treated with givosiran at the Phase 3 dose (2.5mg/kg monthly) for three months and 4 patients were treated with placebo. The ALA levels for both treatment (N=3) and placebo (N=4) patients were plotted over time (in days) in Figure 2 below. All three patients receiving givosiran experienced a rapid and robust decrease in ALA levels starting at Day 7 after the first dose, with plateau in ALA levels at Day 21 that was maintained throughout the entire dosing period (3 months).

Figure 2: ALA Levels over Time (in Days) for Individual AIP Patients in the Givosiran Phase 1 Study (Cohort C)



The planned sample size for the interim analysis is based on the following power considerations. Assuming the mean ALA levels (SD) at 3 months in the placebo and givosiran arms are 20.0 (11.1) and 2.3 (1.1) mmol/mol (based on Phase 1 study data), 40 AIP patients will yield more than 90% power in the ALA comparison at α level of 0.001.

9.1.3. Analysis of ALA Endpoint

9.1.3.1. Primary Analysis Using ANCOVA/Multiple Imputation

The primary analysis to evaluate the treatment effect in ALA at Month 3 is an ANCOVA model, with multiple imputation (MI) assuming missing at random (MAR) in case of missing data at Month 3. The ANCOVA model includes baseline ALA as a continuous covariate and fixed effect terms including treatment arm and stratification factors for AIP patients (prior hemin prophylaxis status and historical attack rates). The significance level for the comparison at the interim is 0.001 (2-sided). Any ALA values measured within 3 days after hemin use will be excluded from the ALA analysis and will be treated as missing. The Least Square (LS) means, SEMs and 95% confidence intervals (CIs) for the LS means, difference in LS Means, and corresponding 95% CIs and p-value will be presented.

Missing data at Month 3 will be multiply imputed separately for each treatment arm using the Markov Chain Monte Carlo (MCMC) method following the MAR assumption. Treatment arm, the stratification factors, ALA measurements at baseline, Week 2, Month 1 and Month 2 will be included in the imputation model. One hundred imputed datasets will be generated using the SAS PROC MI procedure. Each of the imputed datasets will then be analyzed via the primary ANCOVA model. The LS means and standard errors will be combined using SAS PROC MIANALZYE to produce inferences including treatment difference in LS means, 95% CI for the treatment difference, and the p-value. Point estimates (LS means and treatment difference) will be calculated as the average of the 100 complete-data estimates. A total variance estimate will be calculated as a weighted sum of within-imputation variance, which is the average of the complete-

data variance estimates, and a between-imputation variance term. The sample code for performing the primary analysis is provide in Section 9.1.

9.1.3.2. Additional Analysis

When there is missing data at Month 3, the following sensitivity analyses will be conducted to assess the robustness of the primary analysis.

- Pattern mixture model (PMM) approach which accommodates situations where the missingness mechanism is missing not at random (MNAR). The details are described in Section 9.2 .
- Completer analysis: the primary ANCOVA model will be applied to analyze the subset of $IFAS_{AIP}$ patients with non-missing Month 3 ALA value.

The upper limit normal (ULN) of the ALA level observed in the healthy volunteers is 1.5 mmol/mol Cr. The proportions of patients with Month 3 ALA levels below the thresholds of 1.5, 2, 3, and 4 times of the ULN in each treatment arm will be calculated. The difference between the proportions and the 95% exact confidence interval for the difference will be calculated. Patients with missing Month 3 ALA values will be included in the denominator.

Q-Q plot of the residual of the ANCOVA completer analysis will be provided for a visual examination of the normality assumption.

A non-parametric Wilcoxon rank sum test will be performed as a sensitivity analysis of the primary model.

9.1.3.3. Durability of ALA Lowering

The primary ANCOVA model (without missing data imputation) will be applied to evaluate the treatment effect at Week 2, Month 1 and Month 2. The LS mean ALA levels over time will be plotted for each treatment arm to assess the longitudinal treatment effect of Givosiran on ALA lowering. Spaghetti plots for individual patient will also be provided.

In the givosiran-treated arm, it is expected that there will be substantial ALA knockdown starting at Month 1 and maintained throughout the treatment duration. To assess the durability of treatment effect in ALA knockdown, the overall treatment effect over 3 post-baseline visits Month 1, Month 2 and Month 3 will be evaluated through a mixed effect model repeated measurement (MMRM). The MMRM model includes baseline ALA level as a continuous fixed covariate, stratification factors (prior hemin prophylaxis status and historical attack rates), visit and treatment as fixed factors, and patient as a random factor. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the unstructured covariance structure fails to converge, a compound symmetry covariance structure will be used. The overall LS mean treatment difference measuring the average treatment effect on ALA from Month 1 to 3 and associated p value will be estimated.

9.2. Sample SAS Code for Interim Analysis of ALA Data

At the interim analysis, ANCOVA/MI is specified as the primary analysis of ALA data. The analysis is performed in 3 steps with sample SAS code provided below.

Step 1. 100 datasets will be imputed. Negative imputed values will be replaced by 0.10.

```
PROC MI data=&dsin. seed=1234 nimpute=100 out=&method._out;
        by treatment;
        em maxiter=300 converge=1e-4 itprint outem=outem;
        var &cov. &base. &varlist. &resp.;
        mcmc chain=multiple initial=em;
run;
*Apply truncations to negative imputed values;
data &method._out;
        set &method._out;
        if m3 < 0 then m3 = 0.10;
        if m2 < 0 then m2 = 0.10;
        if m1 < 0 then m1 = 0.10;
        if m0_5 < 0 then m0_5 = 0.10;
        if m0 < 0 then m0 = 0.10;
run;
```

Notes:

dsin=alydsv: an input dataset with treatment group, subject id, covariates (2 stratification factors in 0/1 format), creatinine normalized ALA at baseline (m0), week 2(m0_5), Month 1 (m1), Month 2 (m2), and Month 3 (m3).
resp: m3
base: m0
trt: trt01p
cov: 2 stratification factors in 0/1 format
varlist: values at week 2, month 1 and 2 (m0_5, m1, m2)

Step 2. The imputed datasets will be analyzed using ANCOVA model.

```
PROC MIXED data=&method._out method=reml;
        by _Imputation_;
        class &trt. (ref='Placebo') &cov.;
        model &resp.= &trt. &base. &cov. /ddfm=kr;
        lsmeans &trt. / pdiff=control('Placebo') cl diff;
        ods output lsmeans = &method._lsm;
        ods output diffs = &method._diff;
run;
```

Step 3. Combine the analysis results using Rubin's formula through PROC MIANALYZE.

```
PROC MIANALYZE data=&method. lsm;
        by &trt.;
        modeleffects estimate;
        stderr;
        ods output ParameterEstimates=&method._LSest;
run;
```

9.3. Details of the Pattern Mixture Model for ALA

For the IA analysis of ALA level at Month 3, if there are missing data at Month 3, the PMM sensitivity analysis will be performed. Patients with missing data at Month 3 will be categorized into 3 mutually exclusive patterns as described below:

1. Placebo patients: the missing Month 3 ALA value is considered MAR and will be imputed using MI estimated from placebo patients.
2. Givosiran patients who have taken at least one dose within 56 days of Month 3: due to the long PD effect observed in the AS1-001 study, treatment effect of givosiran is assumed to maintain for up to 56 days. Therefore, the missing Month 3 ALA value will be imputed using the distribution of observed givosiran data assuming MAR.
3. Givosiran patients who have not taken any dose within 56 days of Month 3: a conservative approach will be taken to assume the givosiran treatment effect is similar to that of placebo at Month 3. Therefore, the missing Month 3 ALA value will be imputed using the distribution of observed placebo data using copy reference (CR) approach.

Missing values will be imputed 100 times to generate 100 complete datasets. The same ANCOVA model applied to the ALA data at the interim analysis will be fit to each complete dataset for the ALA level at Month 3 (Section 9.1.3.1). The resulting estimates from the 100 analyses will be combined using Rubin's formulae.

The detailed steps of implementation are provided below with sample SAS code.

Step 0. Assign a missing pattern to each patient whose ALA at Month 3 is missing.

Step 1. One hundred datasets will be imputed. MCMC method will be used to impute missing data for Patterns 1 and 2 with MAR. FCS method will be applied to Pattern 3 with MNAR.

Step 1.1 For Patterns 1 and 2, MI with MAR assumption will be performed.

```
PROC MI data=DATAIN seed=1234 nimpute=100 out=DATA_STEP1_1;
        by treatment;
        em maxiter=300 converge=1e-4 itprint outem=outem;
        var &cov. &base. &varlist. &resp.;
        mcmc chain=multiple initial=em;
run;
```

Step 1.2 For Pattern 3 patients, replace the ALA in DATA_STEP1_1 with data from DATAIN and denote the updated data DATA_STEP1_2. Impute missing values for Pattern 3 using the copy reference (CR) method.

```
proc mi data=DATA_STEP1_2 seed=&seed. nimpute=1 out=DATA_STEP2;
        by _imputation_;
        class treatment &cov.;
        fcs nbiter=30 reg (&resp. = &cov. &base. &varlist.);
        mnar model (&resp. / modelobs= (treatment='Placebo'));
        var &cov. &base. &varlist. &resp.;
run;
```

Negative imputed values will be replaced by 0.10 following Section 9.1 .

Steps 2 and 3. Follow Section 9.1 .

At the final analysis, if the drop-out rate during the 6m-DB period is > 5%, the PMM will also be performed as a sensitivity analysis to evaluate the treatment effect in ALA at Month 3 and Month 6, respectively. The implementation steps of PMM are similar as those described above.

9.4. Details of the Pattern Mixture Model for Attack Data

Patients with missing attack data will be categorized into one of the following missing patterns. MI approach for each missing pattern is described as below (Keene, 2014).

1. Patients treated with placebo during the 6m-DB period.

   The missing data are considered MAR and will be imputed based on the attack rate of the placebo arm.

2. Early withdrawals treated with givosiran during the 6m-DB period.

   Givosiran patients who withdraw on or before 56 days after the first dose of givosiran are considered as not having obtained positive treatment effect. Therefore, missing data will be imputed based on the attack rate of the placebo arm using copy reference (CR) approach.

3. Non-early withdrawals treated with givosiran during the 6m-DB period.

   Givosiran patients who withdraw more than 56 days after the first dose of givosiran are considered as having achieved some treatment effect during the on-treatment period (on or before 56 days after the last dose of givosiran), and the effect will be lost during the off-treatment period (more than 56 days after the last dose of givosiran). Missing data during the on-treatment period are considered MAR and will be imputed based on the attack rate of the givosiran arm. Missing data during the off-treatment period will be imputed based on the attack rate of the placebo arm using unconditional reference (UR) approach assuming this period represents a new episode for the patient.

The analysis model is the same as the primary negative binomial regression model. The primary model will be fitted to each complete dataset with imputed values. Rubin's rules will be applied to combine the estimates.

This sensitivity analysis can be implemented in the following steps.

Step 0. Assign a missing pattern to each patient whose duration of follow-up period (observed period before withdrawal) is less than 162 days and calculate the duration of missing period. For non-early withdrawals treated with givosiran (missing pattern 3), there are 2 missing periods, i.e. the on-treatment period and the off-treatment period. Duration of each missing period needs to be derived.

Let dur be the duration of the follow-up period. The duration of missing period for missing patterns 1 and 2 is 162 days-dur. The duration of missing periods for missing pattern 3 also depends on the last day of on-treatment period (denoted as x), which is the last dosing day+55 days (56 days from administration of the last dose).

Scenarios	Duration of missing period on-treatment (days)	Duration of missing period off-treatment (days)	Total duration of missing period (days)
$x \leq dur$	0	$162 - dur$	$162 - dur$
$dur < x \leq 162$	$x - dur$	$162 - x$	$162 - dur$
$x > 162$	$162 - dur$	0	$162 - dur$

Step 1. Fit the imputation model, derive and sample from the posterior distribution for each coefficient and the over-dispersion parameter. The following example SAS code will be applied to generate 100 posterior draws.

```
PROC GENMOD data=data_attack;
        class treatment strat_1 strat_2;
        model attack_observed=treatment strat_1 strat_2 / link=log dist=negbin
        offset=logfollowup_observed;
        bayes outpost=data_out thinning=20 nmc=2000 nbi=1000;
run;
```

Step 2. For each draw (after thinning) the expected number of events is calculated for the follow-up period and the missing period(s) after withdrawal for each patient, based on the duration of the follow-up period and the missing period(s). The design matrices for an individual patient are modified to assign the withdrawal to the desired treatment arm for the follow-up and the missing period(s) depending on the imputation method, i.e. MAR, CR, and UR. The number of attacks from missing period(s) can be sampled based on the conditional/marginal distribution of the number attacks from missing period(s) given that from the follow-up period. The total number of numbers of attacks is the sum of the observed and the imputed number of attacks. (Keene, 2014)

Missing Pattern	Number of attacks observed during the follow-up period	Number of attacks imputed during the missing period(s)	Imputation method	Total number of attacks
1	y_1	$\widehat{y_2}$	MAR	$y_1 + \widehat{y_2}$
2	$y1$	$\widehat{y_2}$	CR	$y_1 + \widehat{y_2}$
3	$y1$	$\widehat{y_{21}}$ $\widehat{y_{22}}$	MAR UR	$y_1 + \widehat{y_{21}} + \widehat{y_{22}}$

Step 3. Fit the total number of attacks using the primary analysis model and 162 days being the total duration of the follow-up period for all patients with missing attack data. Derived parameters from the model will be combined across 100 imputation datasets using Rubin's formula as

implemented in the MIANALYZE procedure. The estimated treatment difference and their confidence intervals will be transformed to the original scale.

```
PROC GENMOD data=data_attack;
        by _Imputation_;
        class treatment strat_1 strat_2;
        model attack_total=treatment strat_1 strat_2 / link=log dist=negbin
        offset=logfollowup_total;
run;
```

9.5. Analgesic medications

Analgesic medications, including opioids (synthetic and non-synthetic substances [narcotics]) or non-steroid anti-inflammatory medications (NSAIDs) are permitted for the management of porphyria and for porphyria attacks. All analgesia usage as taken by the patient at home will be captured by the patient or caregiver via the eDiary through Month 12 of the study. Additional medications, including analgesics, taken at a healthcare facility (eg, during an attack) will be captured in concomitant medications eCRF. After Month 12, usage of any analgesic medications will be recorded in the concomitant medications eCRF, along with all other medications used.

In supporting analgesic usage (opioid and non-opioid) analysis, the following criteria (based on ATC codes and indication as appropriate) will be used in categorization of analgesic medication and opioid/non-opioid.

Opioid:

- ATC3CD: N02A
- ATC4CD: N01AH
- ATC4CD: N01AX where CMINDC="PAIN" or "PORPHYRIA ACUTE ATTACK"
- ATC4CD: R05DA where CMINDC="PAIN" or "PORPHYRIA ACUTE ATTACK"

Non-opioid:

- ATC4CD: M01AB, M01AE, M02AA, N02BA, N02BE, N02BG, N02CC
- ATC4CD: N02CX where CMINDC="PAIN" or "PORPHYRIA ACUTE ATTACK" or "MIGRAINES"
- ATC4CD: N03AX where CMINDC="PAIN" or "PORPHYRIA ACUTE ATTACK"
- ATC4CD: N06AX where CMINDC="PAIN" or "PORPHYRIA ACUTE ATTACK"

Signature Page for VV-CLIN-003117 v2.0

Approval	[redacted]-2019 18:26:37 GMT+0000

Approval	[redacted]-2019 19:47:14 GMT+0000

Signature Page for VV-CLIN-003117 v2.0

STATISTICAL ANALYSIS PLAN

ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias

Protocol Number:	ALN-AS1-003
Protocol Version and Date:	Protocol Amendment 1: 04 May 2018 Original Protocol: 06 September 2017
Investigational Drug:	ALN-AS1 (Givosiran)
Phase:	Phase 3
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Tel: [redacted] Fax: [redacted]
Sponsor Representative:	[redacted]
Analysis Plan Version and Date:	SAP Amendment 1: 11 September 2018 Original SAP: 05 December 2017

Confidentiality Statement

APPROVAL SIGNATURE PAGE

ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias

Protocol Number: ALN-AS1-003

Analysis Plan Version and Date: Amendment 1: 11 September 2018

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

[redacted]

Alnylam Pharmaceuticals, Inc.

[redacted]

Alnylam Pharmaceuticals, Inc

TABLE OF CONTENTS

1 Introduction 8
2 Study Overview 9
2.1 Synopsis of Study Design 9
2.2 Randomization Methodology 9
2.3 Blinding 10
2.4 Study Procedures 10
3 Objectives and Endpoints 11
3.1 Objectives 11
3.1.1 Primary Objective 11
3.1.2 Secondary Objectives 11
3.1.3 Exploratory Objectives 11
3.2 Endpoints 12
3.2.1 Primary Endpoint 12
3.2.2 Secondary Endpoints 12
3.2.3 Exploratory Endpoints 12
3.2.4 Safety Endpoints 13
4 Patient Population 14
5 General Statistical Methods 15
5.1 Determination of Sample Size 15
5.2 General Considerations 15
5.3 Computing Environment 16
5.4 Baseline Definitions 16
5.5 Randomization Stratification Factors 16
5.6 Visit Windows 17
5.7 Multiple Comparisons/Multiplicity 18
5.8 Analysis Cutoff and Database Lock 18
5.9 Interim Analyses 18
5.9.1 Interim Analysis Population 19
5.9.2 Rationale for Month 3 ALA Endpoint 19
5.9.3 Analysis of ALA Endpoint 20
5.9.3.1 Primary Analysis Using ANCOVA/Multiple Imputation 20
5.9.3.2 Additional Analysis 21

5.9.3.3 Durability of ALA Lowering 21
6 Statistical Analysis 22
6.1 Patient Disposition 22
6.2 Demographics and Baseline Characteristics 22
6.3 Protocol Deviations 22
6.4 Drug Exposure 23
6.5 Efficacy Analysis 23
6.5.1 Primary Endpoint 23
6.5.1.1 Primary Analysis using Negative Binomial Regression Model 24
6.5.1.2 Component Analysis 24
6.5.1.3 Sensitivity Analysis 24
6.5.2 Secondary Endpoints 25
6.5.3 Exploratory Endpoints 27
6.5.4 Subgroup Analysis 27
6.6 Pharmacodynamic Analysis 28
6.7 Pharmacokinetic Analysis 28
6.8 Safety Analysis 28
6.8.1 Adverse Events 28
6.8.2 Laboratory Data 30
6.8.3 Vital Signs and Physical Examination 31
6.8.4 Electrocardiogram 31
6.8.5 Prior and Concomitant Medications 31
6.9 Anti-Drug Antibody 32
7 Changes From Previous Version(s) 33
8 References 35
9 Appendices 36
9.1 Sample SAS Code for Interim Analysis of ALA Data 36
9.2 Details of the Pattern Mixture Model for ALA 37
9.3 Details of the Pattern Mixture Model for Attack Data 38

List of Tables

Table 1: Derived Analysis Visit Windows 17

List of Figures

Figure 1: Study Design 9

Figure 2: ALA Levels over Time (in Days) for Individual AIP Paitents in the Givosiran Phase 1 Study (Cohort C) 20

ABBREVIATIONS

Abbreviation	Definition
6m-DB	6-month double-blind placebo-controlled
AAR	Annualized attack rate
ADP	ALA dehydratase deficient porphyria
AE	Adverse event
ALA	Aminolevulinic acid
ALAS1	Aminolevulinic acid synthase
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Class
AUC	Area under curve
BMI	Body mass index
CI	Confidence interval
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DB	Double-blind
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form
HBMS	Hydroxymethylbilane Synthase
HCP	Hereditary Coproporphyria
HLT	High Level Term
HRQOL	Health-related quality of life
IA	Interim analysis
ICH	International Council for Harmonisation
IRS	Interactive response system
ISR	Injection site reaction
LLN	Lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed effect model repeated measurement
mRNA	messenger RNA
NCI	National Cancer Institute
OLE	Open label extension
PBG	Porphobilinogen

Abbreviation	Definition
PD	Pharmacodynamics
PGIC	Patient Global Impression of Change
PK	Pharmacokinetics
PMM	Pattern Mixture Model
PT	Preferred Term
Q1	First quartile
Q3	Third quartile
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of mean
SOC	System organ class
SSR	Sample size reassessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
VP	Variegate porphyria
WHO	World Health Organization

1 INTRODUCTION

Acute hepatic porphyrias (AHPs) are a family of rare, serious and life-threatening disorders characterized by acute and severe neurovisceral attacks, often requiring hospitalization or urgent healthcare visit, as well as by chronic debilitating symptoms. Patients are treated with intravenous hemin during acute attacks, but there is a high unmet need for safe and efficacious therapies to prevent attacks and decrease the chronic symptoms in between attacks. Givosiran is an investigational RNA interference (RNAi) agent in development for the treatment of AHPs in adult and adolescent patients. It acts to inhibit synthesis of liver aminolevulinic acid synthase (*ALAS1*) messenger RNA (mRNA) with consequent reductions in aminolevulinic acid (ALA) and porphobilinogen (PBG) levels, the neurotoxic intermediates that are causal in this disease. Givosiran is formulated for administration via subcutaneous (SC) injection.

The ENVISION Study (ALN-AS1-003) is a Phase 3 study designed to evaluate the efficacy and safety of SC-administered givosiran in patients with AHPs. This statistical analysis plan (SAP) outlines the methods to be used in the analysis of study data in order to address the study objectives of Study ALN-AS1-003. Any change to the data analysis methods described in the protocol, as well as the justification for the change, will be described in the SAP and clinical study report (CSR).

The original statistical analysis plan (SAP) Version 1.0 was submitted to the Agency FDA on 07 Dec 2017 (Original SAP: 05 December 2017; Serial # 0023). A statistical information amendment document addressing the FDA comments received on 07 Feb 2018 was submitted to the FDA on 06 April 2018 (Serial #0032).

This SAP amendment 1 provides updated plan for analysis of the data from this study and is finalized and signed off before the interim analysis.

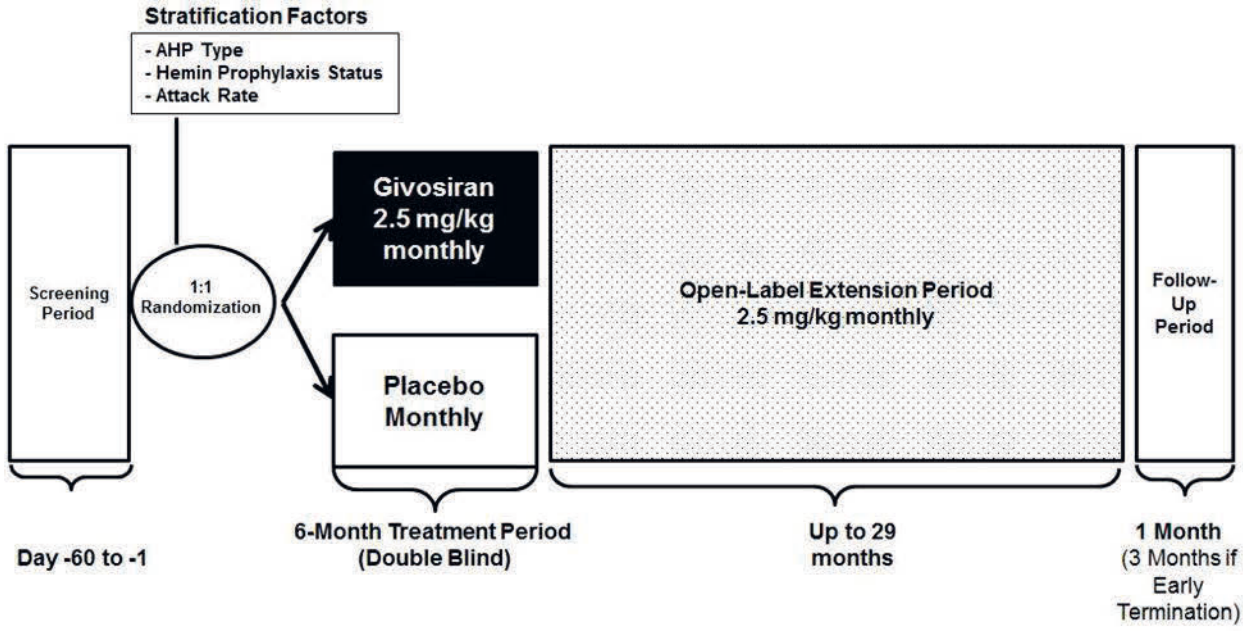
2 STUDY OVERVIEW

2.1 Synopsis of Study Design

The ENVISION Study (ALN-AS1-003) is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of givosiran in approximately 74 patients with AHPs; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 6 months, followed by an open-label extension (OLE) study of up to 30 months to evaluate the long-term safety and efficacy of givosiran.

The study design schema is presented in Figure 1.

Figure 1: Study Design



2.2 Randomization Methodology

Patients will be randomized 1:1 to the givosiran treatment arm and the placebo arm in a double-blinded manner. Treatment will be stratified at randomization by AHP type (acute intermittent porphyria (AIP) [with genetic evidence of mutation in the hydroxymethylbilane synthase *(HMBS)* gene] vs hereditary coproporphyria (HCP), variegate porphyria (VP), ALA dehydratase deficient porphyria (ADP), or any AHP without identified mutation in a porphyria-related gene).

Randomization for AIP patients will be further stratified by each patient's use of hemin prophylaxis regimen at the time of screening and by each patient's historical annualized attack rate. Patients on a hemin prophylaxis regimen prior to study entry will be stratified by their historical annualized attack rate: <7 attacks vs ≥7 attacks. Patients who were not on a hemin prophylaxis regimen prior to study entry will be stratified by their historical annualized attack rate: <12 attacks vs ≥12 attacks.

2.3 Blinding

Treatment assignments will be maintained by the Interactive Response System (IRS) which has controlled access limited to supply chain team members. Any unplanned unblinding occurring during the 6-month double-blind placebo-controlled treatment period (referred to as the 6m-DB period hereafter) will be documented and reported in the CSR.

The independent (external) data monitoring committee (DMC) and an independent (external) biostatistics group supporting the DMC will have access to subject level treatment assignments throughout the study.

An unblinded interim analysis (IA) will be conducted when approximately 40 AIP patients have completed at least 3 months of the double-blind treatment period. This IA will be based on creatinine normalized urinary ALA (referred to as ALA hereafter) data at 3 months for regulatory filing, and the analysis will be performed by the ALA IA independent statistician and programmer. The IA Filing Decision Committee at Alnylam will review data provided by the ALA IA independent statistician and programmer and decide whether to proceed to filing based on the IA results. If the committee makes an affirmative filing decision based on the IA, an Alnylam unblinded IA filing team will be formed and will have access to treatment assignment information in patients included for IA. They will not have access to post-IA patient-level study data until the 6m-DB period data are locked and unblinded, except for access to unblinded Suspected Unexpected Serious Adverse Reaction (SUSAR) safety data as shared with the agency via regular pharmacovigilance (PV) process, and safety data on the approximately 40 AIP patients included in the IA needed to prepare the 90-day safety update.

A blinded study conduct team will be in place throughout the 6m-DB period of the study regardless of the ALA interim analysis results. The blinded study conduct team will not have access to treatment assignment or any information that could reveal treatment assignment (e.g. PK, ALA, PBG, and ALAS1) until the study is unblinded for the primary efficacy analysis at Month 6.

Details about the specifics of the blinding aspects for the whole, including before, during, and after the IA are outlined in the Blinding Plan. Details about the measures taken for the unblinded IA, including concepts and plans for how the IA will be conducted and maintenance of data integrity are outlined in the Data Integrity Plan (Amendment 3, 07Sep2018).

2.4 Study Procedures

The schedule of assessments is described in the study protocol (Table 1, Table 2, and Table 3).

3 OBJECTIVES AND ENDPOINTS

3.1 Objectives

3.1.1 Primary Objective

- Evaluate the effect of givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP

3.1.2 Secondary Objectives

- Evaluate the effects of givosiran, compared to placebo, on urinary ALA levels in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on urinary PBG levels in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on hemin usage in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP
- Evaluate the effects of givosiran compared to placebo in patients with AIP on the symptoms of pain, nausea, and fatigue
- Evaluate the effects of givosiran, compared to placebo, in patients with AIP on the Physical Component Summary (PCS) of the 12-item Short-Form Health Survey (SF-12)
- Evaluate the safety and tolerability of givosiran in patients with any AHP

3.1.3 Exploratory Objectives

- Evaluate the effects of givosiran, compared to placebo, in patients with AIP and in patients with any AHP over the 6m-DB period on:
  - Rate of all porphyria attacks (requiring hospitalization, urgent healthcare visit, IV hemin administration at home, or treated at home without IV hemin)
  - Urinary ALAS1 mRNA levels
  - Analgesic usage (opioid and non-opioid)
  - Additional quality of life (QOL) measures, including missed days of work/school
- Assess the within-patient treatment effect of givosiran over the OLE period in patients with AIP and in patients with any AHP who had previously been randomized to placebo treatment
- Assess the long-term treatment effect of givosiran in patients with AIP and in patients with any AHP

- Characterize the PK of and assess the antidrug antibodies (ADA) of givosiran in patients with any AHP

3.2 Endpoints

3.2.1 Primary Endpoint

Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6m-DB period.

3.2.2 Secondary Endpoints

- Urinary ALA levels in patients with AIP at 3 months
- Urinary ALA levels in patients with AIP at 6 months
- Urinary PBG levels in patients with AIP at 6 months
- Annualized rate of administered hemin doses in patients with AIP over the 6m-DB period
- Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP over the 6m-DB period
- Daily worst pain score as measured by Brief Pain Inventory-Short Form (BPI-SF) numeric rating scale (NRS) in patients with AIP over the 6m-DB period
- Daily worst nausea score as measured by NRS in patients with AIP over the 6m-DB period
- Daily worst fatigue score as measured by Brief Fatigue Inventory-Short Form (BFI-SF) NRS in patients with AIP over the 6m-DB period
- Change from baseline in the Physical Component Summary (PCS) of the 12-item Short-Form Health Survey (SF-12) in patients with AIP at 6 months

3.2.3 Exploratory Endpoints

Exploratory endpoints will be measured in patients with AIP and in patients with any AHP over the 6m-DB period or over the OLE period:

- Rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home
- Rate of all porphyria attacks
- Rate of administered hemin doses
- Urinary ALA and PBG levels
- Urinary ALAS1 mRNA levels
- Daily worst pain, daily worst nausea, and daily worst fatigue scores over 12 months
- PCS of the SF-12
- EQ-5D-5L index score
- Patient Global Impression of Change (PGIC)

- Porphyria Patient Experience Questionnaire (PPEQ)
- Analgesic usage (opioid and non-opioid)
- Plasma PK parameters of givosiran
- Incidence and titer of ADA

3.2.4 Safety Endpoints

The primary safety parameter is the adverse events (AEs) that occurred on or after the time of the first dose of study drug is administered. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. The primary summaries of the safety of givosiran versus placebo will be based on safety parameters during the 6m-DB period. Since porphyria attacks will be recorded for efficacy assessment of the study drug, they will not be treated as AEs or SAEs.

4 PATIENT POPULATION

The populations (analysis sets) for the 6m-DB phase are defined as follows:

- Full Analysis Set (FAS): All randomized patients (AHP) who received at least one dose of study drug. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- Full Analysis Set in AIP patients (FAS_{AIP}): All randomized AIP patients (with identified mutation in the *HMBS* gene) who received at least one dose of study drug. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- Safety Analysis Set: All patients who received at least one dose of study drug, grouped according to the treatment actually received. Patients who received at least one dose of givosiran will be included in the givosiran arm.
- PK Analysis Set: All patients who received at least one dose of study drug and have evaluable PK data contributing to the estimation of PK parameter.
- PD Analysis Set: All patients who received at least one dose of study drug and who have at least one postdose urine sample for the determination of ALA or PBG will be included in the PD analyses.

The primary population used to evaluate efficacy will be the FAS_{AIP} for the primary endpoint and secondary endpoints in AIP patients, and FAS for the secondary endpoint of annualized attack rate in AHP patients. Safety will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

5 GENERAL STATISTICAL METHODS

This SAP focuses on the primary analyses assessing the treatment effect of givosiran versus placebo during the 6m-DB period. Further details on the descriptive analyses of the OLE data will be provided in an amendment to this SAP.

5.1 Determination of Sample Size

The planned total enrollment for the study is approximately 74 patients, including approximately 70 AIP patients.

Seventy patients will yield at least 90% power to detect a 45% reduction in the annualized attack rate at a 2-sided 5% significance level assuming a mean annualized attack rate of 8, a standard deviation (SD) of 5 in the placebo arm, and a mean annualized attack rate of 4.4 with SD of 3 in the givosiran arm, using a negative binomial model. This study design will still have at least 80% power even if the dropout rate is as high as 15% under the same assumptions.

5.2 General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, SD, median, interquartile range (Q1, Q3), minimum value (min), and maximum value (max). 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, Q1 and Q3 will be reported to one greater decimal place. Any values that require transformation to standard units (metric or SI) will be converted with the appropriate corresponding precision.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For ALA, PBG and urinary creatinine, the assessments collected and recorded as lower than the limit of quantification/detection will be replaced by half of the limit of quantification/detection. For other safety laboratory parameters, the assessments collected and recorded as lower than the limit of quantification/detection will be replaced by the limit of quantification/ detection. Any assessment collected and recorded as greater than the limit of quantification will be replaced by the limit of quantification.

All summaries will be presented by treatment arm for the 6m-DB period. For efficacy endpoints defined for the AIP patients, the data for non-AIP patients will be presented in listings. For other analyses, the summaries will be presented by porphyria type and overall for each treatment arm.

All data recorded on the CRF will be included in data listings.

5.3 Computing Environment

All statistical analyses will be performed using validated SAS statistical software Version 9.4 (or later), unless otherwise noted. Access to SAS software and datasets will be secured and limited to qualified team members only. Additionally, access to folders containing datasets, codes and outputs will be controlled per blinded or unblinded team members (Section 2.3).

5.4 Baseline Definitions

For ALA, PBG, and ALAS1 mRNA, collection of the screening urine samples must occur when the patient is not having an attack, and ≥4 days after prophylactic hemin discontinuation and after their last hemin dose. Two screening urine samples (collected on 2 different days) should be collected for ALA/PBG; one screening urine sample should be collected for ALAS1 mRNA. On Day 1 visit, before the first dose of study drug is administered, the third urine sample for ALA/PBG is scheduled to be collected. If a patient is having an attack or it has been less than 4 days after the last hemin dose on Day 1, the third urine sample will not be collected on Day 1 and will be postponed until at least 4 days after the last hemin dose. The baseline ALA/PBG/ALAS1 values will be defined as the median of measurements taken on or prior to Day 1. Any samples taken during an attack or within 3 days after receiving hemin on or prior to Day 1 will be excluded from the baseline calculation. For a patient, if all available samples on or prior to Day 1 meet the exclusion criteria, the last non-missing value on or prior to Day 1 will be used as the baseline value for the patient.

Daily worst scores in pain, nausea and fatigue, as well as analgesic medication use at home, are collected using an electronic diary (eDiary) by patients or caregivers. During the Screening period, the eDiary variables are collected when patients are not experiencing a porphyria attack. For each eDiary variable collected in the Screening period, the average of a minimum of 4 days and a maximum of 7 days (consecutive days not required) is defined as the baseline value. If there are more than 7 days collected for any variable, the measurements closest to Day 1 will be used. All measurements used for the baseline value must be taken when a patient is not experiencing a porphyria attack.

For all other measures, baseline will be defined as the last non-missing value available on or prior to the first dose of study drug, unless otherwise specified.

5.5 Randomization Stratification Factors

Randomization for this study is first stratified by AHP type: AIP [with genetic evidence of a mutation in the *HMBS* gene] vs HCP, VP, ADP, or any AHP without an identified mutation in a porphyria-related gene).

The AIP patient stratum is further stratified by the use of hemin prophylaxis or not immediately prior to Screening, and on the historical AAR. Patients who had been on hemin prophylaxis will be stratified by their historical AAR of <7 vs ≥7. Patients who had not been on a hemin prophylaxis will be stratified by their historical AAR: <12 vs ≥12. This historical AAR is calculated based on the medical record reviewed for inclusion criterion of at least 2 attacks requiring hospitalization, urgent healthcare visit or IV hemin administration at home within the 6 months prior to randomization.

As only a few patients in the stratum of HCP, VP, ADP, or any AHP without identified mutation in a porphyria-related gene are anticipated to be enrolled in the study, there is no further stratification within the stratum.

The historical AAR will be classified into high versus low when used as a categorical covariate. For patients on a hemin prophylaxis regimen at the time of screening, high attack rate is defined as AAR ⩾7. For patients not on a hemin prophylaxis regimen at screening, AAR ≥12 is considered as high attack rates.

Stratification factors are recorded in both the IRS and the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database. In the presence of stratification errors, the stratification used in analysis may not match that in the IRS.

5.6 Visit Windows

For PD and clinical laboratory parameters, analysis visits will be derived for the 6m-DB period based on the study day and visit windows. Target days and the acceptable range of study days for each visit are defined in Table 1 below. If more than one visit falls within a visit window, the visit occurring closest to the target day will be selected. If there are multiple visits with the same distance from the scheduled visit day, the later visit will be selected. The derived visits will be used for all analyses.

Table 1: Derived Analysis Visit Windows

Study Visit	Target Day	Window
Screening	Day -60 to -1	≤ -1
Day 1	1	1
Week 2	15	2 – 22
Month 1	29	23 – 43
Month 2	57	44 – 71
Month 3	85	72 – 106
Month 4	113	107 – 127
Month 5	141	128 – 155
Month 6	169	156 - the earlier of (Day 183, and the day of the first dose of OLE)

For PD and laboratory parameters assessed during the OLE period and for vital signs and ECG measurements during the entire study, no windowing rules will be implemented and the reported clinical visits will be used for analysis.

For QOL assessments including SF-12, EQ-5D-5L and missed days of work/school, data are collected every 3 months up to 12 months, then every 6 months afterwards. If the scheduled visits are not performed, any unscheduled and/or discontinuation visits will be mapped to the corresponding scheduled visits if the visit dates fall into below windows. The midpoint of two visits will be included in the time window of the preceding visit.

Scheduled visits (Month 3, 6, 9, 12) ± 6 weeks

Scheduled visits (Month 18, 24, 30, 36) ± 12 weeks

The derived visits will be used for analyses.

5.7 Multiple Comparisons/Multiplicity

At the time of the unblinded interim analysis, only the first secondary efficacy endpoint ALA levels at Month 3 will be assessed. A significance level of 0.001 will be used when comparing the ALA levels between the two treatment arms using an ANCOVA model.

For the final analyses of the 6m-DB primary treatment period, a significance level of 0.049 will be used to test the efficacy endpoints, reflecting a penalty of 0.001 for the unblinded interim analysis. A fixed-sequence testing strategy for the primary and secondary endpoints will be implemented to control the overall type I error rate. The primary endpoint will be compared between treatment arms at the significance level of 0.049. If it is statistically significant, then the secondary endpoints will each be tested at the same significance level of 0.049 in the order specified in the efficacy endpoint section (Section 3.2). If the test of an endpoint in the sequence is not statistically significant, the testing of remaining endpoints in the sequence will stop and the null hypotheses for the subsequent tests will not be rejected.

There will be no multiplicity adjustment for exploratory endpoints.

5.8 Analysis Cutoff and Database Lock

For the interim analysis and the final 6m-DB analysis, as this study will be ongoing, the study database will be soft-locked with all data up to a prespecified cutoff date quality controlled, i.e. data in EDC will be frozen and external data such as laboratory data will be QA'd and cleaned. The soft lock can also be thought of as an interim database lock. Additional details regarding the soft lock process are located in the study Data Management Plan.

The interim and the final analysis will include data on or prior to this prespecified cutoff date. For assessments with starting/ending dates (e.g., AEs, medications, medical history), the starting date will be compared with the pre-specified cutoff date.

After the study is completed, e.g., all patients completed the OLE period and the follow-up visits, the database will be hard locked and all data collected will be used for analysis.

5.9 Interim Analyses

An unblinded interim analysis will be conducted when approximately 40 AIP patients have completed the Month 3 visit. The data for these ~40 patients will be soft-locked and transferred in support of the interim analysis. A cutoff analysis approach will be implemented in the analysis datasets as specified in Section 5.8

At the interim, the only efficacy endpoint to be assessed is the Month 3 ALA level in AIP patients. The other efficacy endpoints will not be analyzed. ALA data beyond 3 months will be

summarized descriptively by treatment arm for each scheduled visit during the 6m-DB period. Patient disposition, demographic and baseline characteristics, drug exposure, safety, PK, and PD will be summarized as described in Section 6.

5.9.1 Interim Analysis Population

The analysis sets for the interim analysis are defined as follows:

- Interim Full Analysis Set (IFAS): a randomized patient will be included in the IFAS if
  1) the patient was randomized more than 92 days before the cutoff date; OR,
  2) the patient was randomized between 78 and 92 days before the cutoff date and both criteria below are met
     a. the patient has finished the Month 3 visit on or before the cutoff date
     b. all patients who have a randomization date before this patient are included

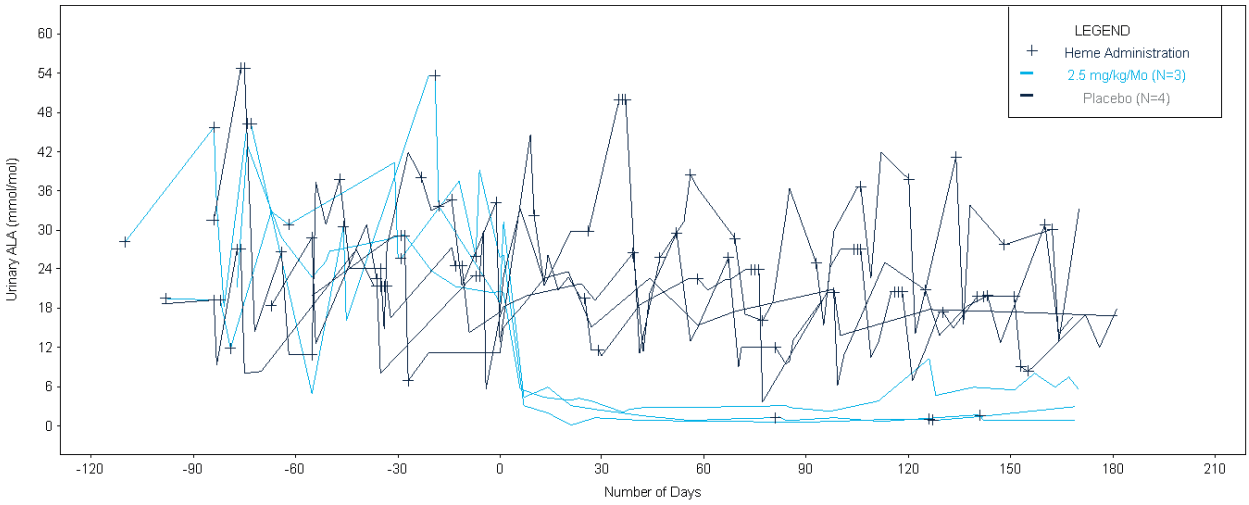
  Patients will be grouped by their randomly assigned treatment group (i.e. as randomized) for the efficacy analyses.
- AIP patients in the Interim Full Analysis Set ($IFAS_{AIP}$)
- Interim Safety Analysis Set (ISAS): includes the same cohort of patients as in the IFAS but grouped according to the treatment actually received.

The primary efficacy analysis set for the ALA data is $IFAS_{AIP}$. The ALA data of the non-AIP patients who have finished the Month 3 visit in the IFAS will be presented in a listing. The safety analyses will be based on the ISAS and will also be performed for the AIP and non-AIP patients separately. PK analysis at interim will be include patients who have evaluable PK data contributing to the estimation of PK parameters.

5.9.2 Rationale for Month 3 ALA Endpoint

Three months of treatment is considered an appropriate time point for assessment of the efficacy of givosiran based on ALA levels observed in the givosiran Phase 1 study. In the Phase 1 study, there were 40 individual patients including 17 AIP patients with recurrent attacks, the patient population of the ENVISION study and 23 asymptomatic high excreters (ASHE, or chronic high excreters CHE). Among the 17 AIP patients with recurrent attacks, 3 patients were treated with givosiran at the Phase 3 dose (2.5mg/kg monthly) for three months and 4 patients were treated with placebo. The ALA levels for both treatment (N=3) and placebo (N=4) patients were plotted over time (in days) in Figure 2 below. All three patients receiving givosiran experienced a rapid and robust decrease in ALA levels starting at Day 7 after the first dose, with plateau in ALA levels at Day 21 that was maintained throughout the entire dosing period (3 months).

Figure 2: ALA Levels over Time (in Days) for Individual AIP Paitents in the Givosiran Phase 1 Study (Cohort C)



The planned sample size for the interim analysis is based on the following power considerations. Assuming the mean ALA levels (SD) at 3 months in the placebo and givosiran arms are 20.0 (11.1) and 2.3 (1.1) mmol/mol (based on Phase 1 study data), 40 AIP patients will yield more than 90% power in the ALA comparison at α level of 0.001.

5.9.3 Analysis of ALA Endpoint

5.9.3.1 Primary Analysis Using ANCOVA/Multiple Imputation

The primary analysis to evaluate the treatment effect in ALA at Month 3 is an ANCOVA model, with multiple imputation (MI) assuming missing at random (MAR) in case of missing data at Month 3. The ANCOVA model includes baseline ALA as a continuous covariate and fixed effect terms including treatment arm and stratification factors for AIP patients (prior hemin prophylaxis status and historical attack rates). The significance level for the comparison at the interim is 0.001 (2-sided). Any ALA values measured within 3 days after hemin use will be excluded from the ALA analysis and will be treated as missing. The Least Square (LS) means, SEMs and 95% confidence intervals (CIs) for the LS means, difference in LS Means, and corresponding 95% CIs and p-value will be presented.

Missing data at Month 3 will be multiply imputed separately for each treatment arm using the Markov Chain Monte Carlo (MCMC) method following the MAR assumption. Treatment arm, the stratification factors, ALA measurements at baseline, Week 2, Month 1 and Month 2 will be included in the imputation model. One hundred imputed datasets will be generated using the SAS PROC MI procedure. Each of the imputed datasets will then be analyzed via the primary ANCOVA model. The LS means and standard errors will be combined using SAS PROC MIANALZYE to produce inferences including treatment difference in LS means, 95% CI for the treatment difference, and the p-value. Point estimates (LS means and treatment difference) will be calculated as the average of the 100 complete-data estimates. A total variance estimate will be calculated as a weighted sum of within-imputation variance, which is the average of the complete-data variance estimates, and a between-imputation variance term. The sample code for performing the primary analysis is provide in Section 9.1.

5.9.3.2 Additional Analysis

When there is missing data at Month 3, the following sensitivity analyses will be conducted to assess the robustness of the primary analysis.

- Pattern mixture model (PMM) approach which accommodates situations where the missingness mechanism is missing not at random (MNAR). The details are described in Section 9.2.
- Completer analysis: the primary ANCOVA model will be applied to analyze the subset of $IFAS_{AIP}$ patients with non-missing Month 3 ALA value.

The upper limit normal (ULN) of the ALA level observed in the healthy volunteers is 1.5 mmol/mol Cr. The proportions of patients with Month 3 ALA levels below the thresholds of 1.5, 2, 3, and 4 times of the ULN in each treatment arm will be calculated. The difference between the proportions and the 95% exact confidence interval for the difference will be calculated. Patients with missing Month 3 ALA values will be included in the denominator.

Q-Q plot of the residual of the ANCOVA completer analysis will be provided for a visual examination of the normality assumption.

A non-parametric Wilcoxon rank sum test will be performed as a sensitivity analysis of the primary model.

5.9.3.3 Durability of ALA Lowering

The primary ANCOVA model (without missing data imputation) will be applied to evaluate the treatment effect at Week 2, Month 1 and Month 2. The LS mean ALA levels over time will be plotted for each treatment arm to assess the longitudinal treatment effect of Givosiran on ALA lowering. Spaghetti plots for individual patient will also be provided.

In the givosiran-treated arm, it is expected that there will be substantial ALA knockdown starting at Month 1 and maintained throughout the treatment duration. To assess the durability of treatment effect in ALA knockdown, the overall treatment effect over 3 post-baseline visits Month 1, Month 2 and Month 3 will be evaluated through a mixed effect model repeated measurement (MMRM). The MMRM model includes baseline ALA level as a continuous fixed covariate, stratification factors (prior hemin prophylaxis status and historical attack rates), visit and treatment as fixed factors, and patient as a random factor. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the unstructured covariance structure fails to converge, a compound symmetry covariance structure will be used. The overall LS mean treatment difference measuring the average treatment effect on ALA from Month 1 to 3 and associated p value will be estimated.

6 STATISTICAL ANALYSIS

6.1 Patient Disposition

Number and percentage of patients in the following categories will be summarized by treatment arm and overall as appropriate:

- Randomized
- Full Analysis Set (FAS)
- Full Analysis Set for AIP patients (FAS_{AIP})
- Safety Analysis Set (if different from FAS)
- Discontinued treatment and primary reasons for treatment discontinuation
- Withdrew from the study during the 6m-DB period and primary reasons for withdrawal
- Withdrew from the study and primary reasons for withdrawal.

The number and percent of patients enrolled by country and site will be summarized by randomized treatment arm and overall. The number and percent of patients in each randomization stratification factor recorded in IRS, and a comparison of the number and percent of patients in each randomization stratification factor in IRS versus the clinical database will be summarized by randomized treatment arm and overall.

6.2 Demographics and Baseline Characteristics

Demographic, background (e.g. medical history) and baseline disease characteristics will be summarized by treatment arm and overall for FAS and FAS_{AIP}.

Age, height, weight, and body mass index (BMI) will be summarized by descriptive statistics. Age group, sex, race, ethnicity, and country will be summarized by presenting the numbers and percentages of patients in each category.

The following baseline disease characteristics will also be summarized: age at diagnosis, time from diagnosis to randomization, prior hemin prophylaxis status (Y/N) at screening, number of attacks requiring hospitalization, urgent healthcare visits or IV hemin administration at home in the last 6 months prior to the study, prior chronic symptoms when not having attacks (Y/N) and prior chronic opioid medication usage (Y/N).

6.3 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to primary analysis and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the final protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol deviation and whether or not this deviation is classified as a major protocol deviation. This file will be finalized prior to study unblinding.

6.4 Drug Exposure

Exposure to study medication in days and the number of study drug SC administrations received will be summarized by treatment arm.

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

- Last dose date + 84 days
- Analysis cut-off date
- The date of withdrawal consent

Duration of exposure is defined as date of the last exposure – date of the first dose +1. The exposure during the 6m-DB period is right censored by the date of the first OLE dose, i.e. the last exposure day in the 6m-DB period is no later than the day before the first OLE dose. Similarly, the exposure during the OLE is left censored by the date of the first OLE dose, i.e. the Day 1 of the OLE is the day of the first OLE dose.

Dose interruptions and compliance are not taken into account for duration of exposure.

6.5 Efficacy Analysis

Porphyria Attacks

Porphyria patients experiencing acute attacks present with highly morbid and potentially life-threatening symptoms relating to dysfunction across the central, peripheral, and autonomic nervous system. The most commonly seen signs and symptoms of a porphyria attack include diffuse, severe neurovisceral pain mostly in the abdomen, back, or limbs, nausea and vomiting, fatigue, hypertension, tachycardia, motor weakness.

The porphyria attacks in the primary endpoint are those attacks requiring hospitalization, urgent healthcare visits or IV hemin administration at home. For data collection, each attack is reported with one associated outcome in the hierarchical order, e.g., if an attack requires both hospitalization and urgent healthcare, the attack will be reported as requiring hospitalization only.

The start time of a porphyria attack is defined as the time at which acute and sustained worsening of the patient's porphyria manifestations beyond normal day-to-day variability. The end of porphyria attacks is characterized by recovery back to within a patient's normal day-to-day variability. Attacks that occur on the same calendar day of the last treated attack will be considered a part of the original attack and will count as one attack in the calculation of the AAR. Any attacks that begin on the next day from the last treated attack ends will constitute a new attack.

6.5.1 Primary Endpoint

The primary endpoint of the study is the annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP

over the initial 6m-DB period. The analysis set for the primary endpoint is FAS_{AIP} (defined in Section 4).

The number of qualifying porphyria attacks is annualized for each patient using below formula:

$$\text{Annualized attack rate (AAR)} = \frac{\text{total number of porphyria attacks}}{\text{total number of days in the treatment period}} \times 365.25.$$

Let λ_G represent the mean AAR for givosiran arm and λ_O represent the mean AAR for the placebo arm. The hypotheses of the primary analysis are:

$$H_o: \frac{\lambda_G}{\lambda_O} = 1 \qquad H_A: \frac{\lambda_G}{\lambda_O} \neq 1$$

The statistical significance level for the primary analysis of the primary endpoint is 2-sided 0.049. An estimated AAR ratio : $\frac{\lambda_G}{\lambda_O} < 1$ and 2-sided p-value < 0.049 will lead to rejection of H_o.

6.5.1.1 Primary Analysis using Negative Binomial Regression Model

The primary analysis will be performed using a negative binomial regression model that include fixed effects of the treatment arms, the stratification factors for the AIP patients, which include the hemin prophylaxis regimen prior to the study (yes vs no) and historical AAR (high vs low). The logarithm of the amount of time (in the units of year) that each patient spends in the 6m-DB period will be included in the model as an offset variable. With the offset variable, the negative binomial regression model essentially models the AAR for each patient based on the length of time in the treatment period, and hence will be able to account for the different lengths of follow-up time.

For patients who discontinue the study treatment early (before the end of the 6m-DB period), best efforts will be made to continue the collection of attack data. The primary efficacy analysis will be based on all qualified attacks occurring in the 6m-DB period, including those that occur after treatment discontinuation. For patients who discontinue study treatment and subsequently receive hemin prophylaxis, the primary analysis will include all attack data collected prior to the start of the hemin prophylaxis regimen. No imputation of attack data is planned for the primary analysis.

In addition to p-value, an estimated ratio of mean AARs between the two treatment arms with the corresponding 95% confidence interval will be estimated from the negative binomial regression model. Descriptive statistics for the median and interquartile range of the annualized attack rate will also be presented by treatment arm.

6.5.1.2 Component Analysis

The annualized rate of porphyria attacks is a composite endpoint including three components: 1) attacks requiring hospitalization; 2) attacks requiring urgent healthcare visit, or 3) attacks requiring IV hemin administration at home. The AAR of each of the three components will be analyzed using the same negative binomial regression model and the estimated ratio of mean AARs between treatment arms with corresponding 95% confidence interval will be provided.

6.5.1.3 Sensitivity Analysis

All reported potential attacks (reported by eDiary or by investigators) will be analyzed using the primary analysis method as a sensitivity analysis.

The attack data collection is on calendar days, i.e. start and stop times by hours are not collected. To evaluate the impact of potential under- or over-counting of attacks due to the 1-day window, the following two sensitivity analyses will be performed:

- Count all discrete attacks even if they overlap during a day.
- Extend the attack counting windows to a 2-day window, i.e. attacks that occurs on the same calendar day, or are separated by one calendar day will be counted as one attack.

An Andersen-Gill model using the same fixed effects as the primary model will be performed treating the attacks as recurrent events.

If the number of zero attacks are high, a zero inflated NB model will be performed to provide further assurance of the results.

Attack data could be missing if a patient stops attack data collection before the end of the 6m-DB period, e.g. due to study discontinuation. The missing data period is defined as the time (days) between the end of attack data collection and Day 162 which is the earliest expected end of the 6m-DB period of a patient, inclusive. For patients who provide attack data through the end of 6m-DB period, no missing data imputation will be performed. In case of missing attack data, pattern mixture model (PMM) with MI will be performed to assess the robustness of primary analysis results to the possible violation of the MAR missingness assumption. The details of the PMM approach for attack data is described in Section 9.3.

6.5.2 Secondary Endpoints

The annualized-event-rate type of endpoints include the annualized rate of hemin administration in AIP patients (FAS_{AIP}) and the AAR requiring hospitalization, urgent care visits or IV hemin treatment at home in any AHP patients (FAS) over the 6m-DB period. The number of events for each patient is annualized in the same way as AAR defined for the primary endpoint, divided by treatment period (in days) and multiplied by a year (365.25 days). The annualized rate of hemin administration in FAS_{AIP} will be analyzed using a negative binomial regression model similar as the one used for the the primary endpoint. The AAR in FAS will be analyzed using a negative binomial regression model including treatment arm only since the stratification factors for AIP patients are not applicable to non-AIP patients.

The biomarker endpoints include the ALA levels in AIP patients (FAS_{AIP}) at 3 months and 6 months, the urinary PBG levels in AIP patients (FAS_{AIP}) at 6 months. Any postbaseline ALA/PBG values measured within 3 days after hemin use during the 6m-DB period will be treated as missing and excluded from analysis. For each biomarker secondary endpoint, the biomarker levels at the specified time point will be estimated and compared between two treatment arms using an MMRM model. The model will include baseline of the corresponding biomarker level as a continuous covariate, stratification factors (prior hemin prophylaxis status and historical attack rates), visit, treatment, and visit by treatment as fixed effects, and patient as a random effect. An unstructured covariance matrix will be used to model the within-subject error. If the fit of the unstructured covariance structure fails to converge, a compound symmetry covariance structure will be used. The least squares (LS) means and the corresponding SEM and 95% CIs for each treatment arm, along with the LS mean difference between two treatment arms at each post-baseline visit will be presented. The p-value for the treatment difference for each endpoint (ALA at Month 3 and Month 6; PBG at Month 6) will be presented. In addition, the

overall p values measuring the average treatment effect on ALA and PBG during the entire 6m-DB period will also be reported. For ALA at Month 3 and Month 6, sensitivity analyses will be performed using a PMM approach as described in Section 9.2.

For the Physical Component Summary (PCS) of SF-12 score, change from baseline at Month 6 in AIP patients (FAS_{AIP}) will be analyzed using an MMRM model with baseline score as a continuous covariate and fixed effect terms including treatment arms, stratification factors, visit (Month 3 or Month 6), and visit by treatment interaction. The endpoint will be derived according to the questionnaire manual.

Daily worst scores for pain measured by BPI-SF in Numeric Rating Scale (NRS), nausea measured by NRS and fatigue measured by BFI-SF in NRS are collected through eDiary. For each of the symptoms (pain, nausea, and fatigue), daily eDiary entries will be averaged into a weekly (i.e. 7 day) score. The AUC over 6 months in the respective score will be calculated for each patient based on weekly mean scores. For each of the scores, the mean AUC will be compared between treatment arms in FAS_{AIP} using ANCOVA model with fixed effects of treatment arms and stratification factors, and with the corresponding score at baseline as a covariate.

Symptoms are expected to be elevated during porphyria attacks. This AUC approach encapsulates the peaks of these symptoms during attacks as well as lingering chronicity of these symptoms throughout the entire treatment period. AUC approaches have been used in other episodic conditions to compare both the severity and duration of pain across groups.

The weekly mean score will be calculated using the following algorithm:

1. A completed week will be defined as a week where a patient completes 4 or more daily entries in a week, which is consistent with other diary-based PRO research. The available eDiary entries for a completed week will be averaged to determine the weekly symptom score.
2. A missing week will be defined as a week where a patient is missing 4 or more daily entries. In a missing week, if there is one or more recorded attack days during the week, the mean score will be derived based on the following rules:
   - If there are 1 or more completed attack day diary entries, the mean weekly score will be computed as an average of the daily entries for that week.
   - If there is no completed attack day diary entry, the mean weekly score will be imputed using the average of all non-missing mean weekly scores with 1 or more attack day from patients in the same treatment arm.

   Patients are expected to have elevated symptom scores during porphyria attacks, which can span over multiple days. This algorithm intends to preserve any completed eDiary entries collected during days associated with attacks.
3. In a missing week without any attack days, the mean weekly score will be imputed by the last observed non-missing week without any attack days. If there is no last observed non-missing week without any attack days, the imputation will be based on baseline observation carried forward (BOCF).

6.5.3 Exploratory Endpoints

The continuous exploratory endpoints in AIP patients for the 6m-DB period including EQ-5D-5L index score, Patient Global Impression of Change (PGIC), and Porphyria Patient Experience Questionnaire (PPEQ) will be analyzed using an MMRM model similar to the one employed for the secondary endpoint PCS SF-12.

For endpoints assessed in patients with any AHP (FAS) over the 6m-DB period, the analyses will be conducted using methods similar as those employed for the AIP patients (FAS_{AIP}), without including stratification factors which are not applicable to non-AIP patients.

For EQ-5D-5L, a categorical summary of the numbers and percentages of patients reporting each ordinal response within each EQ-5D domain will also be presented.

The endpoints for the OLE period will be summarized descriptively.

6.5.4 Subgroup Analysis

For the primary endpoint, subgroup analyses will be conducted to assess the consistency of treatment effect for the AIP patients during the 6m-DB period. A negative binomial regression model will be fit to estimate the treatment effect within each subgroup. The model includes treatment arms as a fixed effect and each patient's time on study as an offset variable. An estimated ratio of mean AARs between the two treatment arms with the corresponding 95% CI will be estimated for each subgroup. If the number of patients in either treatment arm of a subgroup is less than 10, only descriptive statistics will be presented.

- Age at Screening (<65 or ≥65)
- Race (White or Non-white)
- Sex (Female or Male)
- Baseline BMI (<25 or ≥25)
- Prior hemin prophylaxis status (Yes or No)
- Historical attack rates based on the hemin prophylaxis status prior to the study (high or low)

  For patients on a hemin prophylaxis regimen at the time of screening, if AAR ⩾7, the patient is considered having high attack rates prior to the study. For patients who were not on a hemin prophylaxis regimen at screening, AAR ≥12 is considered with high attack rates.
- Prior chronic opioid use (Yes or No)
- Prior chronic symptoms when not having attacks (Yes or No)

Other subgroups may be examined, if deemed appropriate. In addition, region- and/or country-specific analyses may be performed to support regulatory submission as needed.

The subgroup analyses may also be performed for other secondary endpoints.

The number of non-AIP patients (including HCP, VP, ADP, or any AHP without identified mutation in a porphyria-related gene) is anticipated to be only a few. Descriptive statistics on the primary and secondary endpoints will be provided by treatment arm for non-AIP patients.

6.6 Pharmacodynamic Analysis

Analyses of secondary endpoints relating to ALA and PBG levels are described in Section 6.5.2. In addition, ALA, PBG, and ALAS1 levels will be summarized descriptively at each scheduled visit.

Population PK/PD analyses will be described in a separate population PK/PD analysis plan.

6.7 Pharmacokinetic Analysis

Plasma concentrations of givosiran and its major metabolite, AS(N-1)3' givosiran, will be summarized by nominal sampling time.

In patients at East Asian study centers with intense PK sampling, plasma and urine PK parameters of givosiran and AS(N-1)3' givosiran will be determined using non-compartmental methods. PK parameters include maximum plasma concentration (Cmax), time to maximum plasma concentration (tmax), elimination half-life ($t_{½\beta}$), and area under the concentration-time curve (AUC). Other parameters may be calculated, if deemed necessary. PK parameters will be described using summary statistics.

Population PK analysis will be used to describe plasma PK of givosiran and AS(N-1)3' givosiran across all patients. The population PK analysis will be described in a separate modeling and simulation analysis plan.

6.8 Safety Analysis

An adverse event (AE) is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the AEs that first occurs or worsens after the first dose of study drug. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

No inferential safety analysis is planned.

6.8.1 Adverse Events

AEs will be classified by the MedDRA coding system (version 21.0) and displayed in tables and data listings using system organ class (SOC) and preferred term (PT).

AEs will be summarized by the numbers and percentages of patients reporting at least one AE, having at least one AE by primary SOC and PT. A patient with multiple occurrences of an AE will be counted only once in the respective AE category. Patients who report multiple occurrences of the same AE (PT) will be classified according to the most related or most severe occurrence, respectively.

Injection site reaction (ISR) events will be thoroughly summarized. The numbers and percentages of patients reporting at least one ISR event and the numbers and percentages of patients with different ISR symptoms will be summarized. Note that multiple symptoms of a single ISR will

not be counted as multiple ISRs. In addition, ISRs will be characterized by injection. The incidence rate of ISR, defined as the number of ISRs divided by total number of injections will also be summarized. If there are multiple ISRs that occur between two consecutive injections, these events are considered caused by the earlier injection and counted as one ISR.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment (possibly related or definitely related), any severe AE, any severe AE related to treatment, any serious AE (SAE), any SAE related to treatment, any AE/SAE of clinical interest; any AE/SAE leading to treatment discontinuation, any study drug related AE/SAE leading to treatment discontinuation, any AE/SAE leading to study withdrawal, any study drug related AE/SAE leading to study withdrawal, and any deaths.

Tabulations by SOC and PT may be produced for the following. The SOC and PT within each SOC will be presented alphabetically.

- All AEs;
- AEs by severity;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;
- AEs leading to temporary treatment interruption;
- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AEs leading to study withdrawal;
- SAEs leading to study withdrawal.

Tabulations by PT in decreasing order in frequency in the givosiran arm will be produced for the following.

- All AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;

AEs and SAEs will also be summarized by maximum relationship to study drug and by maximum severity.

AEs mapping to the MedDRA high level term (HLT) of injection site reaction will be summarized by SOC and preferred term. AEs mapping to the standard MedDRA query (SMQ) Drug Related Hepatic Disorder will be summarized by SOC and preferred term. Adverse event mapping to the Acute Pancreatitis SMQ will be summarized by SOC and preferred term (PT). AEs mapping to the Anaphylactic Reaction SMQ will be summarized by PT. AEs mapping to the SMQ Malignant or Unspecified Tumors will be summarized by high level term and preferred term. Other SMQs or AE groupings may be evaluated.

All AEs collected will be listed along with the information collected on those AEs, e.g. AE relationship to study drug, AE outcome etc. By-patient listings will also be provided for the following: all deaths, all SAEs, and all AEs leading to treatment discontinuation or study withdrawal, all AEs leading to death.

A listing of ISRs will be presented including descriptions, onset and resolution date, severity, treatment given, and event outcome.

6.8.2 Laboratory Data

Knowledge of several clinical laboratory tests, including ALA, PBG, and ALAS1, has the potential to unblind the reviewer. Procedures to protect the blind, including permissions to view these lab values, are described in the Data Integrity Plan (Amendment 3, 07Sep2018).

Clinical laboratory values will be expressed in Standard International (SI) units. Missing laboratory data will not be imputed.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, coagulation studies and thyroid and liver function tests). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

For each continuous laboratory parameter, results will be categorized as low, normal, or high based on the laboratory normal ranges used in this study.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category, where the "worst" post-baseline category will be based on the maximum difference (in absolute value) from the upper or lower limits of the normal range.

A listing will be produced for all patient with amylase and lipase >3×ULN and all patients with abnormal liver function tests defined as an ALT >3×ULN, AST >3×ULN, and/or total bilirubin >2×ULN at any time point.

A table will be produced to summarize the number and percentage of patients in each of below category at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALP > 1.5×ULN,
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN,
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN,

EDISH plots for ALT, AST, and total bilirubin will also be provided.

For hematology and blood chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the NCI CTCAE Version 4.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

All laboratory data will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.8.3 Vital Signs and Physical Examination

Descriptive statistics by visit and treatment arm will be provided for each variable.

Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

6.8.4 Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, and QTc interval. For post-baseline assessments where ECG is performed in triplicate, the average of the 3 (or all available) readings will be used for analysis. Observations with the following diagnosis or findings will be excluded from analysis: artificial pacemaker, atrial fibrillation, atrial flutter, left bundle branch block, and right bundle branch block.

Corrected QT interval (QTc), if not collected, will be calculated using both Fridericia's and Bazett's correction formula.

Bazett's square-root corrected QT: QTcB (ms) = QT (ms) $\times \sqrt{\frac{HR(bpm)}{60}}$

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) $\times \sqrt[3]{\frac{HR(bpm)}{60}}$.

PR, QRS, QT, QTc (ie, QTcB and QTcF) and RR intervals and their change from time-matched and pre-dose baseline will be summarized for each treatment group by scheduled visit. Subjects will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTc interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTc interval. The number and percentage of subjects in each category will be summarized for each treatment group.

All ECG data for each patient will be provided in a data listing.

6.8.5 Prior and Concomitant Medications

Prior medications are those medications taken prior to the first dose of study drug, regardless of when it ended. If the medication end date is before the date of first dose of study drug, the medication will be summarized as prior medication regardless of whether the start date is missing or not.

Concomitant medications are medications, other than study drug, taken at or after the first dose of study drug, as well as medications with a start date prior to first dose of study drug and are ongoing after first dose of study drug. If medication start date is on or after date of first dose of study drug, the medication will be summarized as concomitant medication regardless of whether the medication end date is missing or not. If the end date of a medication is missing or incomplete such that it cannot be determined whether it is before first study drug dose, it will be counted as a concomitant medication.

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2017 or later). Results will be tabulated by anatomical therapeutic class (ATC) and preferred term.

The subset of concomitant medications which may be used to treat porphyria pain, including analgesic medications taken at home (collected through eDiary) and taken at a healthcare facility (e.g. during an attack), will be summarized separately.

6.9 Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time during study as well as at each scheduled visit will be summarized. The titer results for patients with confirmed positive ADA results will also be summarized using descriptive statistics.

ADA data and patients with confirmed positive ADA results will be presented in data listings.

7 CHANGES FROM PREVIOUS VERSION(S)

The SAP Amendment 1 includes more details previously not discussed in the original SAP. Some changes to analysis methods are made following regulatory feedback and further assessments. The Amendment is signed off before the study is unblinded. The details of changes and rationales are listed in below table.

Summary of Changes	Rationale
In the protocol and original SAP, an interim sample size reassessment (SSR) was planned but was not conducted during study.	An SSR was originally planned to be performed at the time of the interim analysis to potentially increase the number of AIP patients in the study from approximately 70 to 90. Due to fast enrollment of the study leading to greater than expected numbers of patients randomized, an interim analysis for SSR was therefore no longer necessary.
For the interim analysis of ALA at Month 3, the missing data imputation was mLOCF in the original SAP; in this amendment, the imputation will be based on MI. Additional sensitivity analyses are added including a PMM and a non-parametric method.	Updates made following regulatory feedback.
For the analysis of ALA/PBG at the end of 6m-DB period, the primary analysis was based on ANCOVA/mLOCF in the original SAP; in this amendment, the primary analysis for ALA/PBG will be based on MMRM and a sensitivity analysis using PMM is added for ALA.	Both MMRM and MI/ANCOVA are based on MAR assumption and MMRM is often considered a more simple and powerful method. PMM is added to assess the robustness of the results when missingness mechanism is MNAR.
For the analysis of primary endpoint attack rate, PMM is added to address potential missing attack data and other sensitivity analyses are added (Section 6.5.1.3).	These sensitivity analyses are added to assess the robustness of the primary analysis results.
The median instead of the mean of valid pre-dose values is used to derive baseline ALA/PBG.	Due to the variability with PD measures, the median is less affected by extreme values than the mean.
For the secondary endpoint PCS SF-12, the primary analysis method is changed from ANCOVA to MMRM.	This endpoint is measured at Month 3 and Month 6. MMRM implicitly imputes missing data using data from both time points while ANCOVA does not address missing data.

For the secondary endpoints daily worst score of pain, nausea, and fatigue, the AUC over 6 months will be derived by first calculating the weekly mean scores (Section 6.5.2).	Updated to address regulatory feedback on missing data handling and justification of AUC method.

8 REFERENCES

Keene ON, Roger JH, Hartley BF, Kenward MG. Missing data sensitivity analysis for recurrent event data using controlled imputation. *Pharm Stat*. 2014;13(4):258-264. doi:10.1002/pst.1624

Rubin, D.B. (1987), Multiple Imputation for Nonresponse in Surveys, New York: John Wiley & Sons, Inc.

Rubin, D.B. (1996), "Multiple Imputation After 18+ Years," Journal of the American Statistical Association, 91, 473–489.

Siddiqui, O. (2011). MMRM versus MI in Dealing with Missing Data – A Comparison Based on 25 NDA Data Sets, Journal of Biopharmaceutical Statistics, 21: 423–436, 2011.

☒

9 APPENDICES

9.1 Sample SAS Code for Interim Analysis of ALA Data

At the interim analysis, ANCOVA/MI is specified as the primary analysis of ALA data. The analysis is performed in 3 steps with sample SAS code provided below.

Step 1. 100 datasets will be imputed. Negative imputed values will be replaced by 0.10.

```
PROC MI data=&dsin. seed=1234 nimpute=100 out=&method._out;
        by treatment;
        em maxiter=300 converge=1e-4 itprint outem=outem;
        var &cov. &base. &varlist. &resp.;
        mcmc chain=multiple initial=em;
run;
*Apply truncations to negative imputed values;
data &method._out;
        set &method._out;
        if m3 < 0 then m3 = 0.10;
        if m2 < 0 then m2 = 0.10;
        if m1 < 0 then m1 = 0.10;
        if m0_5 < 0 then m0_5 = 0.10;
        if m0 < 0 then m0 = 0.10;
run;
```

Notes:

dsin=alydsv: an input dataset with treatment group, subject id, covariates (2 stratification factors in 0/1 format), creatinine normalized ALA at baseline (m0), week 2(m0_5), Month 1 (m1), Month 2 (m2), and Month 3 (m3).
resp: m3
base: m0
trt: trt01p
cov: 2 stratification factors in 0/1 format
varlist: values at week 2, month 1 and 2 (m0_5, m1, m2)

Step 2. The imputed datasets will be analyzed using ANCOVA model.

```
PROC MIXED data=&method._out method=reml;
        by _Imputation_;
        class &trt. (ref='Placebo') &cov.;
        model &resp.= &trt. &base. &cov. /ddfm=kr;
        lsmeans &trt. / pdiff=control('Placebo') cl diff;
        ods output lsmeans = &method._lsm;
        ods output diffs = &method._diff;
run;
```

Step 3. Combine the analysis results using Rubin's formula through PROC MIANALYZE.

```
PROC MIANALYZE data=&method._lsm;
        by &trt.;
        modeleffects estimate;
```

```
        stderr stderr;
        ods output ParameterEstimates=&method._LSest;
run;
```

9.2 Details of the Pattern Mixture Model for ALA

For the IA analysis of ALA level at Month 3, if there are missing data at Month 3, the PMM sensitivity analysis will be performed. Patients with missing data at Month 3 will be categorized into 3 mutually exclusive patterns as described below:

1. Placebo patients: the missing Month 3 ALA value is considered MAR and will be imputed using MI estimated from placebo patients.
2. Givosiran patients who have taken at least one dose within 56 days of Month 3: due to the long PD effect observed in the AS1-001 study, treatment effect of givosiran is assumed to maintain for up to 56 days. Therefore, the missing Month 3 ALA value will be imputed using the distribution of observed givosiran data assuming MAR.
3. Givosiran patients who have not taken any dose within 56 days of Month 3: a conservative approach will be taken to assume the givosiran treatment effect is similar to that of placebo at Month 3. Therefore, the missing Month 3 ALA value will be imputed using the distribution of observed placebo data using copy reference (CR) approach.

Missing values will be imputed 100 times to generate 100 complete datasets. The same ANCOVA model applied to the ALA data at the interim analysis will be fit to each complete dataset for the ALA level at Month 3 (Section 5.9.3.1). The resulting estimates from the 100 analyses will be combined using Rubin's formulae.

The detailed steps of implementation are provided below with sample SAS code.

Step 0. Assign a missing pattern to each patient whose ALA at Month 3 is missing.

Step 1. One hundred datasets will be imputed. MCMC method will be used to impute missing data for Patterns 1 and 2 with MAR. FCS method will be applied to Pattern 3 with MNAR.

Step 1.1 For Patterns 1 and 2, MI with MAR assumption will be performed.

```
PROC MI data=DATAIN seed=1234 nimpute=100 out=DATA_STEP1_1;
        by treatment;
        em maxiter=300 converge=1e-4 itprint outem=outem;
        var &cov. &base. &varlist. &resp.;
        mcmc chain=multiple initial=em;
run;
```

Step 1.2 For Pattern 3 patients, replace the ALA in DATA_STEP1_1 with data from DATAIN and denote the updated data DATA_STEP1_2. Impute missing values for Pattern 3 using the copy reference (CR) method.

```
proc mi data=DATA_STEP1_2 seed=&seed. nimpute=1 out=DATA_STEP2;
        by _imputation_;
        class treatment &cov.;
        fcs nbiter=30 reg (&resp. = &cov. &base. &varlist.);
```

```
        mnar model (&resp. / modelobs= (treatment='Placebo'));
        var &cov. &base. &varlist. &resp.;
run;
```

Negative imputed values will be replaced by 0.10 following Section 9.1.

Steps 2 and 3. Follow Section 9.1.

At the final analysis, the PMM will also be performed as a sensitivity analysis to evaluate the treatment effect in ALA at Month 3 and Month 6, respectively. The implementation steps of PMM are similar as those described above.

9.3 Details of the Pattern Mixture Model for Attack Data

Patients with missing attack data will be categorized into one of the following missing patterns. MI approach for each missing pattern is described as below (Keene, 2014).

1. Patients treated with placebo during the 6m-DB period.

   The missing data are considered MAR and will be imputed based on the attack rate of the placebo arm.

2. Early withdrawals treated with givosiran during the 6m-DB period.

   Givosiran patients who withdraw on or before 56 days after the first dose of givosiran are considered as not having obtained positive treatment effect. Therefore, missing data will be imputed based on the attack rate of the placebo arm using copy reference (CR) approach.

3. Non-early withdrawals treated with givosiran during the 6m-DB period.

   Givosiran patients who withdraw more than 56 days after the first dose of givosiran are considered as having achieved some treatment effect during the on-treatment period (on or before 56 days after the last dose of givosiran), and the effect will be lost during the off-treatment period (more than 56 days after the last dose of givosiran). Missing data during the on-treatment period are considered MAR and will be imputed based on the attack rate of the givosiran arm. Missing data during the off-treatment period will be imputed based on the attack rate of the placebo arm using unconditional reference (UR) approach assuming this period represents a new episode for the patient.

The imputation model is the same as the primary negative binomial regression model. The primary model will be fitted to each complete dataset with imputed values. Rubin's rules will be applied to combine the estimates.

This sensitivity analysis can be implemented in the following steps.

Step 0. Assign a missing pattern to each patient whose duration of follow-up period (observed period before withdrawal) is less than 162 days and calculate the duration of missing period. For non-early withdrawals treated with givosiran (missing pattern 3), there are 2 missing periods, i.e. the on-treatment period and the off-treatment period. Duration of each missing period needs to be derived.

Let dur be the duration of the follow-up period. The duration of missing period for missing patterns 1 and 2 is 162 days-dur. The duration of missing periods for missing pattern 3 also

depends on the last day of on-treatment period (denoted as x), which is the last dosing day+56 days.

Scenarios	Duration of missing period on-treatment (days)	Duration of missing period off-treatment (days)	Total duration of missing period (days)
$x \leq dur$	0	$162 - dur$	$162 - dur$
$dur < x \leq 162$	$x - dur$	$162 - x$	$162 - dur$
$x > 162$	$162 - dur$	0	$162 - dur$

Step 1. Fit the imputation model, derive and sample from the posterior distribution for each coefficient and the over-dispersion parameter. The following example SAS code will be applied to generate 100 posterior draws.

```
PROC GENMOD data=data_attack;
        class treatment strat_1 strat_2;
        model attack_observed=treatment strat_1 strat_2 / link=log dist=negbin
        offset=logfollowup_observed;
        bayes outpost=data_out thinning=20 nmc=2000 nbi=1000;
run;
```

Step 2. For each draw (after thinning) the expected number of events is calculated for the follow-up period and the missing period(s) after withdrawal for each patient, based on the duration of the follow-up period and the missing period(s). The design matrices for an individual patient are modified to assign the withdrawal to the desired treatment arm for the follow-up and the missing period(s) depending on the imputation method, i.e. MAR, CR, and UR. The number of attacks from missing period(s) can be sampled based on the conditional/marginal distribution of the number attacks from missing period(s) given that from the follow-up period. The total number of numbers of attacks is the sum of the observed and the imputed number of attacks. (Keene, 2014)

Missing Pattern	Number of attacks observed during the follow-up period	Number of attacks imputed during the missing period(s)	Imputation method	Total number of attacks
1	y_1	$\widehat{y_2}$	MAR	$y_1 + \widehat{y_2}$
2	$y1$	$\widehat{y_2}$	CR	$y_1 + \widehat{y_2}$
3	$y1$	$\widehat{y_{21}}$ $\widehat{y_{22}}$	MAR UR	$y_1 + \widehat{y_{21}} + \widehat{y_{22}}$

Step 3. Fit the total number of attacks using the primary analysis model and 162 days being the total duration of the follow-up period for all patients with missing attack data. Derived parameters from the model will be combined across 100 imputation datasets using Rubin's formula as implemented in the MIANALYZE procedure. The estimated treatment difference and their confidence intervals will be transformed to the original scale.

```
PROC GENMOD data=data_attack;
        by _Imputation_;
        class treatment strat_1 strat_2;
        model attack_total=treatment strat_1 strat_2 / link=log dist=negbin
        offset=logfollowup_total;
run;
```

Signature Page for VV-CLIN-002504 v1.0

Approval	[redacted]-2018 16:42:17 GMT+0000

Approval	[redacted]-2018 18:44:07 GMT+0000

Signature Page for VV-CLIN-002504 v1.0

STATISTICAL ANALYSIS PLAN

ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias

Protocol Number:	ALN-AS1-003
Protocol Version and Date:	Original protocol: 06 September 2017
Investigational Drug:	ALN-AS1 (Givosiran)
Phase:	Phase 3
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Tel: [redacted] Fax: [redacted]
Sponsor Representative:	[redacted]
Analysis Plan Version and Date:	Original SAP: 05 December 2017

Confidentiality Statement

The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without expressed written authorization of Alnylam Pharmaceuticals, Inc.

APPROVAL SIGNATURE PAGE

ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias

Protocol Number:	ALN-AS1-003
Protocol Version and Date:	Original protocol: 06 September 2017
Analysis Plan Version and Date:	Original SAP: 05 December 2017

This Statistical Analysis Plan has been reviewed and approved by:

[redacted]

5 Dec 2017
Date

[redacted]

5 DEC 2017
Date

TABLE OF CONTENTS

1 Introduction 7
2 Study Overview 8
2.1 Synopsis of Study Design 8
2.2 Randomization Methodology 8
2.3 Blinding 9
2.4 Study Procedures 9
3 Objectives and Endpoints 10
3.1 Objectives 10
3.1.1 Primary Objective 10
3.1.2 Secondary Objectives 10
3.1.3 Exploratory Objectives 10
3.2 Endpoints 11
3.2.1 Primary Endpoint 11
3.2.2 Secondary Endpoints 11
3.2.3 Exploratory Endpoints 11
3.2.4 Safety Endpoints 12
4 Patient Population 13
5 General Statistical Methods 14
5.1 Determination of Sample Size 14
5.2 General Considerations 14
5.3 Computing Environment 15
5.4 Interim Analyses 15
5.4.1 Interim Analysis on ALA Data 15
5.4.2 Sample Size Reassessment 19
5.5 Background Characteristics 20
5.5.1 Patient Disposition 20
5.5.2 Demographics and Baseline characteristics 21
5.5.3 Prior and Concomitant Medications 21
5.6 Randomization Stratification Factors 21
5.7 Multiple Comparisons/Multiplicity 22
5.8 Protocol Deviations 22
5.9 Derived Analysis Visit Windows 22
6 Statistical Analysis 24

6.1 Efficacy Analysis 24
6.1.1 Primary Endpoint 24
6.1.2 Secondary Endpoints 25
6.1.3 Sensitivity Analysis 26
6.1.4 Subgroup Analysis 26
6.2 Pharmacodynamic Analysis 27
6.3 Pharmacokinetic Analysis 27
6.4 Safety Analyses 27
6.4.1 Extent of Exposure 28
6.4.2 Adverse Events 28
6.4.3 Laboratory Data 29
6.4.4 Vital Signs and Physical Examination 30
6.4.5 Electrocardiogram 30
6.5 Anti-Drug Antibody 31
7 Changes to Planned Analyses 32
8 References 33

LIST OF TABLES

Table 1: Planned Analyses at Interim and at 6-Month 17
Table 2: Power Calculations for Interim Analysis Based on ALA at 3 Months 18
Table 3: Summary of simulation results on chance (%) of sample size increase 20
Table 4: Summary of simulation results on power (%) 20
Table 5: Derived Analysis Visit Windows 23

LIST OF FIGURES

Figure 1: Study Design 8
Figure 2: ALA Levels over Time (in Days) for Individual AIP Paitents in the Givosiran Phase 1 Study (Cohort C) 18

ABBREVIATIONS

Abbreviation	Definition
6m-DB	6-month double-blind placebo-controlled
AAR	Annualized attack rate
ADP	ALA dehydratase deficient porphyria
AE	Adverse event
ALA	Aminolevulinic acid
ALAS1	Aminolevulinic acid synthase
ANCOVA	Analysis of covariance
ATC	Anatomic Therapeutic Class
AUC	Area under curve
BMI	Body mass index
CSR	Clinical study report
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form
HBMS	Hydroxymethylbilane Synthase
HCP	Hereditary Coproporphyria
HRQOL	Health-related quality of life
IA	Interim analysis
ICH	International Conference on Harmonisation
IRS	Interactive response system
ISR	Injection site reaction
LLN	Lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
mRNA	messenger RNA
OLE	Open label extension
PBG	Porphobilinogen
PD	Pharmacodynamics
PK	Pharmacokinetics
PT	Preferred Term
Q1	First quartile
Q3	Third quartile
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation

Abbreviation	Definition
SOC	System organ class
SSR	Sample size reassessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper Limit of Normal
VP	Variegate porphyria
WHO	World Health Organization

1 INTRODUCTION

Acute hepatic porphyrias (AHPs) are a family of rare, serious and life-threatening disorders characterized by acute and severe neurovisceral attacks, often requiring a hospitalization or an urgent healthcare visit, as well as by chronic debilitating symptoms. Patients are treated with intravenous hemin during acute attacks, but there is a high unmet need for safe and efficacious therapies to prevent attacks and decrease the chronic symptoms in between attacks. Givosiran is an investigational RNA interference (RNAi) agent in development for the treatment of AHPs in adult and adolescent patients. It acts to inhibit synthesis of liver aminolevulinic acid synthase (ALAS1) messenger RNA (mRNA) with consequent reductions in aminolevulinic acid (ALA) and porphobilinogen (PBG) levels, the neurotoxic intermediates that are causal in this disease. Givosiran is formulated for administration via subcutaneous (SC) injection.

The ENVISION Study (ALN-AS1-003) is a Phase 3 study designed to evaluate the efficacy and safety of SC-administered givosiran in patients with AHPs. This statistical analysis plan (SAP) has been developed based on the protocol of the ENVISION study (Original protocol dated 06 September 2017).

The analysis methods described in the protocol may be updated in the statistical analysis plan (SAP). Any change to the data analysis methods described in the protocol, as well as the justification for the change, will be described in the SAP and clinical study report (CSR). Additional exploratory analyses of the data may be conducted when deemed appropriate.

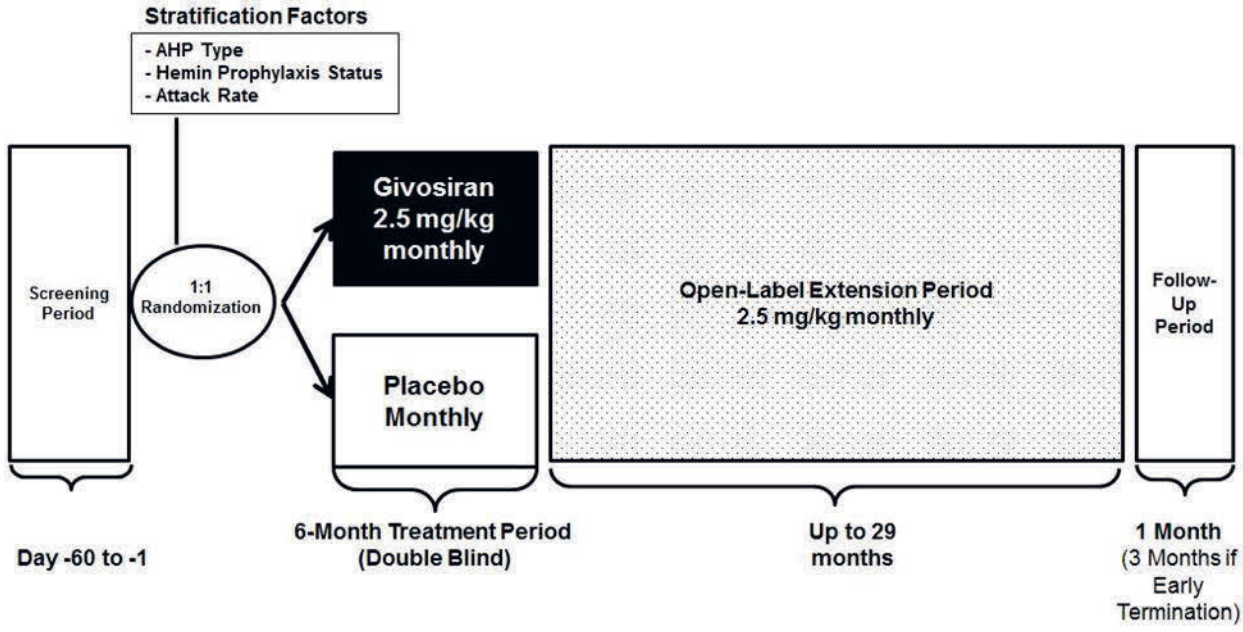
2 STUDY OVERVIEW

2.1 Synopsis of Study Design

The ENVISION Study (ALN-AS1-003) is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of givosiran in approximately 74 patients with AHPs; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 6 months, followed by an open-label extension (OLE) study of up to 30 months to evaluate the long-term safety and efficacy of givosiran.

The study design schema is presented in Figure 1.

Figure 1: Study Design



2.2 Randomization Methodology

Patients will be randomized 1:1 to the givosiran treatment arm and the placebo arm in a double-blinded manner. Treatment will be stratified at randomization by AHP type (acute intermittent porphyria (AIP) [with genetic evidence of mutation in the hydroxymethylbilane synthase *(HMBS)* gene] vs hereditary coproporphyria (HCP), variegate porphyria (VP), ALA dehydratase deficient porphyria (ADP), or any AHP without identified mutation in a porphyria-related gene).

Randomization for AIP patients will be further stratified by each patient's use of hemin prophylaxis regimen at the time of screening and by each patient's historical annualized attack rate. Patients on a hemin prophylaxis regimen prior to study entry will be stratified by their historical annualized attack rate: <7 attacks vs ≥7 attacks. Patients who were not on a hemin prophylaxis regimen prior to study entry will be stratified by their historical annualized attack rate: <12 attacks vs ≥12 attacks.

2.3 Blinding

Treatment assignments will be maintained by the interactive response system (IRS). Any unplanned unblinding occurring during the 6-month double-blind placebo-controlled treatment period (referred to as the 6m-DB period hereafter) will be documented and reported in the CSR.

The independent (external) data monitoring committee (DMC) and an independent (external) biostatistics group supporting the DMC will have access to subject level treatment assignments. An interim analysis based on creatinine-normalized urinary ALA (referred to as ALA or urinary ALA hereafter) data for regulatory filing will be performed by the independent biostatistics group (Section 5.4.1). If the ALA interim analysis results in a decision to submit a regulatory filing to support an accelerated approval or a conditional marketing authorization before completing the 6m-DB period, an Alnylam internal unblinded team will be formed to prepare the filing. The Alnylam internal unblinded team will not be involved in the further conduct of the study until the primary efficacy analysis of the study following the double-blind treatment period is conducted. A blinded study conduct team will be in place throughout the double-blind treatment period of the study regardless of the ALA interim analysis results. The blinded study conduct team will not have access to treatment assignment or any treatment revealing information (e.g. PK, ALA, PBG, and ALAS1) until the study is unblinded for the primary efficacy anlaysis. If the ALA interim analysis does not result in a decision of an earlier regulatory filing then the study team will stay blinded until the study is unblinded for the primary efficacy anlaysis.

A blinded sample size reassessment (Section 5.4.2) will be performed by an Alnylam internal team and the sample size reassessment will not utilize any unblinded information.

2.4 Study Procedures

The schedule of assessments is described in the study protocol (Table 1, Table 2, and Table 3).

3 OBJECTIVES AND ENDPOINTS

3.1 Objectives

3.1.1 Primary Objective

- Evaluate the effect of SC givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP

3.1.2 Secondary Objectives

- Evaluate the effects of givosiran, compared to placebo, on urinary ALA levels in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on urinary PBG levels in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on hemin usage in patients with AIP
- Evaluate the effects of givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP
- Evaluate the effects of givosiran compared to placebo in patients with AIP on the symptoms of pain, nausea, and fatigue
- Evaluate the effects of givosiran, compared to placebo, in patients with AIP on the Physical Component Summary (PCS) of the 12-item Short-Form Health Survey (SF-12)
- Evaluate the safety and tolerability of givosiran in patients with any AHP

3.1.3 Exploratory Objectives

- Evaluate the effects of givosiran, compared to placebo, in patients with AIP and in patients with any AHP over the 6m-DB period on:
  - Rate of all porphyria attacks (requiring hospitalization, urgent healthcare visit, IV hemin administration at home, or treated at home without IV hemin)
  - Urinary ALAS1 mRNA levels
  - Analgesic usage (opioid and non-opioid)
  - Additional quality of life (QOL) measures, including missed days of work/school
- Assess the within-patient treatment effect of givosiran over the OLE period in patients with AIP and in patients with any AHP who had previously been randomized to placebo treatment
- Assess the long-term treatment effect of givosiran in patients with AIP and in patients with any AHP
- Characterize the PK of and assess the antidrug antibodies (ADA) of givosiran in patients with any AHP

3.2 Endpoints

3.2.1 Primary Endpoint

Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6m-DB period.

3.2.2 Secondary Endpoints

- Urinary ALA levels in patients with AIP at 3 months
- Urinary ALA levels in patients with AIP at 6 months
- Urinary PBG levels in patients with AIP at 6 months
- Annualized rate of administered hemin doses in patients with AIP over the 6m-DB period
- Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP over the 6m-DB period
- Daily worst pain score as measured by Brief Pain Inventory-Short Form (BPI-SF) numeric rating scale (NRS) in patients with AIP over the 6m-DB period
- Daily worst nausea score as measured by NRS in patients with AIP over the 6m-DB period
- Daily worst fatigue score as measured by Brief Fatigue Inventory-Short Form (BFI-SF) NRS in patients with AIP over the 6m-DB period
- Change from baseline in the Physical Component Summary (PCS) of the 12-item Short-Form Health Survey (SF-12) in patients with AIP at 6 months

3.2.3 Exploratory Endpoints

Exploratory endpoints will be measured in patients with AIP and in patients with any AHP over the 6m-DB period or over the OLE period:

- Rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home
- Rate of all porphyria attacks
- Rate of administered hemin doses
- Urinary ALA and PBG levels
- Urinary ALAS1 mRNA levels
- Daily worst pain, daily worst nausea, and daily worst fatigue scores over 12 months
- PCS of the SF-12
- EQ-5D-5L index score
- Analgesic usage (opioid and non-opioid)
- Plasma PK parameters of givosiran

- Incidence and titer of ADA

3.2.4 Safety Endpoints

The primary safety parameter is the adverse events (AEs) that occurred on or after the time of the first dose of study drug is administered. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. The primary summaries of the safety of givosiran versus placebo will be based on safety parameters during the 6m-DB period. Since porphyria attacks will be recorded for efficacy assessment of the study drug, they will not be treated as AEs or SAEs.

4 PATIENT POPULATION

The populations (analysis sets) for the 6m-DB phase are defined as follows:

- Full Analysis Set (FAS): All randomized patients (AHP) who received at least one dose of study drug. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- Full Analysis Set in AIP patients (FAS_{AIP}): All randomized AIP patients (with identified mutation in the *HMBS* gene) who received at least one dose of study drug. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- Safety Analysis Set: All patients who received at least one dose of study drug, grouped according to the treatment actually received. Patients who received at least one dose of givosiran will be included in the givosiran arm.
- PK Analysis Set: All patients who received at least one dose of study drug and have evaluable PK data contributing to the estimation of PK parameter.
- PD Analysis Set: All patients who received at least one dose of study drug and who have at least one postdose urine sample for the determination of ALA or PBG will be included in the PD analyses.

The primary population used to evaluate efficacy will be the FAS_{AIP} for the primary endpoint and secondary endpoints in AIP patients, and FAS for the secondary endpoint of annualized attack rate in AHP patients. Safety will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively.

5 GENERAL STATISTICAL METHODS

5.1 Determination of Sample Size

The planned total enrollment for the study is approximately 74 patients, including approximately 70 AIP patients.

Seventy patients will yield at least 90% power to detect a 45% reduction in the annualized attack rate at a 2-sided 5% significance level assuming a mean annualized attack rate of 8, a standard deviation (SD) of 5 in the control arm, and a mean annualized attack rate of 4.4 with SD of 3 in the givosiran arm, using a negative binomial model. This study design will still have at least 80% power even if the dropout rate is as high as 15% under the same assumptions.

5.2 General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, SD, median, interquartile range (Q1, Q3), minimum value (min), and maximum value (max). 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 and median will be reported to 1 greater decimal place, and the SD will be reported to 2 additional decimal places. Any values that require transformation to standard units (metric or SI) will be converted with the appropriate corresponding precision.

An unblinded ALA interim analysis is planned; details are provided in Section 5.4.1. In addition, a blinded interim analysis for sample size reassessment is also planned (Section 5.4.2).

The details of planned analyses at the end of the 6m-DB period on the primary and secondary endpoints are described in section 6.1. For the exploratory endpoints in the double-blind and OLE periods, descriptive statistics will be provided. Additional exploratory analysis may be conducted when deemed appropriate. The efficacy of givosiran will be further evaluated on the OLE data of patients who are in the control arm during the double-blind period. Further details on statistical analysis of the OLE data will be provided in an amendment to this SAP.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

Baseline definitions

For ALA and PBG measurements, the baseline values are defined as the average of all available values measured during screening up to the first dose of study drug administered. During the screening period, a total of two urine samples are collected on two different days when patients are not experiencing an attack, and ≥4 days after receiving hemin for prophylaxis or for the treatment of an attack. During the Day 1 visit, before the first dose of study drug is administered, the third urine sample for ALA/PBG is scheduled to be collected. If a patient is having an attack or it has been less than 4 days after the last hemin dose on Day 1, the collection of the third urine sample will not be collected on Day 1 and will be postponed until at least 4 days after the last hemin dose. The baseline ALA/PBG values are calculated by taking the average of measurements taken on or prior to Day 1. Any ALA/PBG samples taken during an attack or within 3 days after receiving hemin on or prior to Day 1 will be excluded from the baseline ALA/PBG calculation.

Daily worst scores in pain, nausea and fatigue, as well as analgesic medication use at home, are collected using an electronic diary (eDiary) by patients or caregivers. During the Screening period, the eDiary variables are collected when patients are not experiencing a porphyria attack. For each eDiary variable collected in the Screening period, the average of a minimum of 4 days and a maximum of 7 days (consecutive days not required) is defined as the baseline value. If there are more than 7 days collected for any variable, the measurements closest to Day 1 will be used. All measurements used for the baseline value must be taken when a patient is not experiencing a porphyria attack.

For all other measures, baseline for both treatment groups will be defined as the last non missing value available up to the first dose of study drug (Day 1), unless otherwise specified.

5.3 Computing Environment

All statistical analyses will be performed using SAS statistical software Version 9.3 (or later), unless otherwise noted.

5.4 Interim Analyses

5.4.1 Interim Analysis on ALA Data

As noted in Section 2.3 an unblinded interim efficacy analysis based on ALA levels will be conducted for potential regulatory submission to achieve an accelerated approval/conditional marketing authorization. An interim analysis data cutoff will be made when approximately 30 AIP patients have finished the Month 3 visit. A snapshot of the study database (referred to as snapshot hereafter) will be taken to include all study data up to the cutoff date and the snapshot will be used for the interim analysis.

The analysis sets for the interim analysis are defined as follows:

- Interim Full Analysis Set (IFAS): All randomized patients (AHP) who received at least one dose of study drug on or before the cutoff date. Patients will be grouped by their randomly assigned treatment group (i.e. as randomized).
- Interim Full Analysis Set in AIP patients ($IFAS_{AIP}$): A randomized AIP patient will be included in the $IFAS_{AIP}$ if

1) the patient received the first dose of study drug more than 92 days before the cutoff date; OR,
2) the patient received the first dose of study drug between 78 and 92 days before the cutoff date and both criteria below are met
    a. the patient has finished the Month 3 visit on or before the cutoff date
    b. all AIP patients who have a first dose date before the patient are included in the $IFAS_{AIP}$

Patients will be grouped by their randomly assigned treatment group (i.e. as randomized) for the efficacy analyses and grouped according to the treatment actually received for safety analyses.

- Interim Safety Analysis Set (ISAS): All randomized patients who received at least one dose of study drug on or before the cutoff date, grouped according to the treatment actually received.

The efficacy endpoint for the interim analysis is the ALA level at the Month 3 visit. The primary efficacy analysis set for the ALA data is $IFAS_{AIP}$. The ALA data of the non-AIP patients who have finished the Month 3 visit in the IFAS will be summarized descriptively and listed. The safety analyses will be based on the ISAS. The safety analyses will also be performed for the $IFAS_{AIP}$ and non-AIP patients in the ISAS separately according to the treatment actually received. PK analysis at interim will be based on the patients who have evaluable PK data contributing to the estimation of PK parameters in each analysis set. PD analysis at the interim will be based on patients who have at least one postdose urine sample for the determination of ALA or PBG in each analysis set. Other analyses planned for the end of 6m-DB period, eg, patient disposition, demographic, ADA, and etc., will also be performed at the interim as detailed in Table 1 below, which lists the planned analyses at the interim and at the end of 6m-DB period.

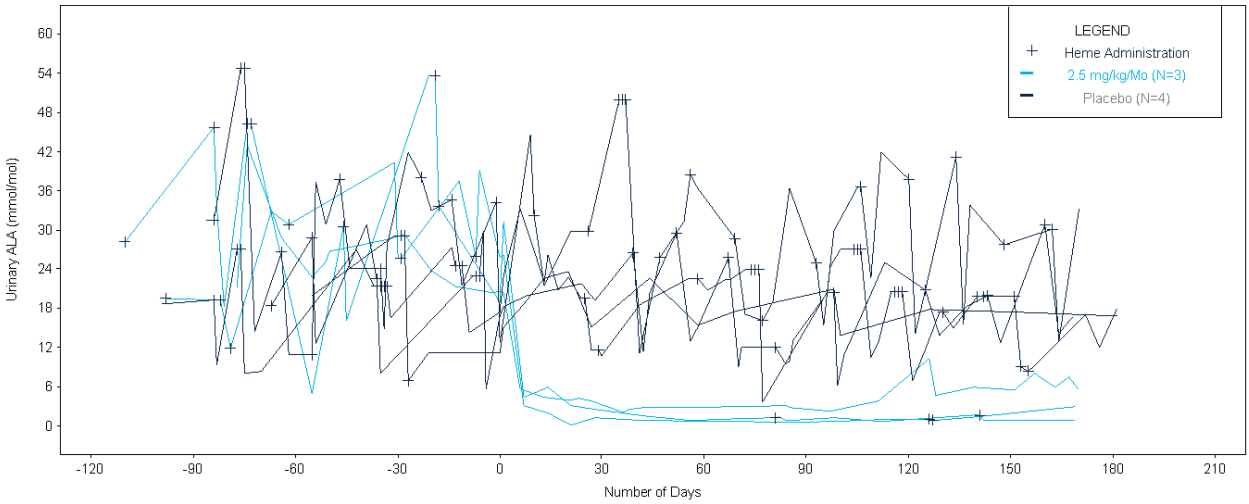
Table 1: Planned Analyses at Interim and at 6-Month

	Interim Analysis[a]		6m-DB Period Analysis
	Interim Full Analysis Set in AIP patients ($IFAS_{AIP}$)	**Interim Safety Analysis Set (ISAS)**	**Analysis sets specified in Section 4**
Patient disposition	Yes	Yes	Yes
Demographics	Yes	Yes	Yes
Extent of exposure	Yes	Yes	Yes
Background information (e.g. medical history)	Yes	Yes	Yes
Baseline disease characteristics	Yes	Yes	Yes
Prior and Concomitant medications	Yes	Yes	Yes
PK/PD/ADA	Yes	Yes	Yes
Efficacy analyses	ALA data only	No	Full efficacy analyses.
Safety	Yes	Yes	Yes

[a] ALA data of the non-AIP patients who have finished the Month 3 visit in the IFAS will be summarized descriptively and listed. The safety analyses will also be performed for the non-AIP patients according to the treatment actually received.

Three months of treatment is considered an appropriate time point for assessment of the efficacy of givosiran based on ALA levels seen in the givosiran Phase 1 study. In the Phase 1 study, there were 40 individual patients (5 patients were in more than one treatment group) treated with study drug, of which 38 received givosiran and 7 received placebo. One analysis looked at AIP patients with recurrent attacks, the patient population of the ENVISION study. Among them, 3 patients were treated with givosiran at the Phase 3 dose (2.5mg/kg monthly) for three months and 4 patients were treated with placebo. The ALA levels for both treatment (N=3) and placebo (N=4) patients were plotted over time (in days) in Figure 2 below. All three patients receiving givosiran experienced a rapid and robust decrease in ALA levels starting at Day 7 after the first dose, with plateau in ALA levels at Day 21 that was maintained throughout the entire dosing period (3 months).

Figure 2: ALA Levels over Time (in Days) for Individual AIP Paitents in the Givosiran Phase 1 Study (Cohort C)



The planned sample size for the interim analysis is based on the following power considerations. Assuming the mean ALA levels (SD) at 3 months in the placebo and givosiran arms are 20.0 (11.1) and 2.3 (1.1) mmol/mol (based on Phase 1 study data), 15 AIP patients in each arm will yield more than 90% power in the ALA comparison at α level of 0.001. Table 2 below shows the power of the ALA interim analysis under the above assumptions and when the ALA level at 3 months for the givosiran arm and/or its SD is higher than assumed.

Table 2: Power Calculations for Interim Analysis Based on ALA at 3 Months

Placebo ALA (SD=11.1) mmol/mol	20.0	20.0	20.0	20.0
Givosiran ALA (SD) mmol/mol	2.3 (1.1)	3.3 (1.1)	2.3 (2.1)	3.3 (2.1)
Power	95%	91%	94%	91%

The ALA level at 3 months will be compared between two treatment arms using an ANCOVA model with the corresponding baseline ALA as a covariate. The significance level for the comparison at the interim is 0.001 (2-sided). Any ALA values measured within 3 days after hemin use will be excluded from the ALA analysis and will be treated as missing.

All efforts will be made to obtain the ALA data at the Month 3 visit from the approximately 30 AIP patients evaluated. In the event that the Month 3 measurement is missing, the following imputation approaches will be used. A modified Last Observation Carried Forward (mLOCF) approach will be used if a patient has ALA measurement on or after Day 14: the ALA measurement taken closest to the target day of Month 3 visit will be used as Month 3 measurement in ANCOVA model for 3-month ALA inference analysis. The mLOCF approach can be justified based on Phase 1 data (Figure 2) which demonstrated rapid (within 7 days) and robust ALA level lowering that was sustained. In the unlikely event that a patient does not have any ALA data on Day 14 and beyond, the Month 3 value will be imputed using a multiple

imputation (MI) method (Section 6.1.2) based on the data set in which mLOCF imputation has been applied.

A sensitivity analysis will be performed using the same ANCOVA model above in the AIP patients who have non-missing Month 3 ALA value in the IFAS.

Another sensitivity analysis comparing the proportions of patients with ALA levels at 3 months approaching a level that is near normal, which is defined as less than 1.5 x ULN, between the treatment arms will be performed, 95% exact confidence interval for the difference between the two arms will be provided. Patients who have no Month 3 ALA value even after applying the mLOCF imputation will be treated as non-responders in this analysis.

Additionally, for the purpose of assessing the effect of givosiran on lowering and maintaining reduced ALA levels over time, the ALA levels at Month 1 and Month 2 for those patients who are included in the 3-month efficacy analysis will also be analyzed using the same ANCOVA model. Nominal p-values will be provided for the comparisons of ALA levels between the two arms at Month 1 and at Month 2.

Stopping the study for efficacy or futility is not planned.

5.4.2 Sample Size Reassessment

At the time of the interim analysis for ALA, the assumption of the original sample size estimates will also be re-evaluated in a blinded way to ensure the study is adequately powered for the 6m-DB period analysis on the annualized attack rate (AAR). The available attack data of patients in the $IFAS_{AIP}$ will be used to estimate the aggregated mean AAR (placebo and givosiran arm pooled without unblinding) of the study. The assumptions of the AAR for the givosiran and placebo arms along with their SDs for the originally planned sample size of the study are outlined in Section 5.1. However, if the estimated aggregated AAR is less than 4.5 (threshold for sample size increase), there is a concern that the AAR of the placebo arm is lower than the protocol assumption of a mean of 8 (with SD=5) given a 45% treatment effect, and therefore, approximately 20 additional AIP patients (approximately 10 in each arm) will be enrolled.

Simulation studies were carried out to help understand the chance of increasing the sample size and the power for different threshold values under 3 different scenarios. A 45% reduction in AAR in the treatment arm compared to the control arm is assumed. The threshold value of 4.5 for the sample size increase is based on the below simulation results. The 3 scenarios in the simulation study are:

1. The scenario in the original protocol: control arm with mean=8 (SD=5) vs. treatment arm with mean=4.4 (SD=3);
2. Mean=6 (SD=4) vs. mean=3.3 (SD=2)
3. Mean=4 (SD=2.8) vs. mean=2.2 (SD=1.5)

For different threshold values for a sample size increase, the chance of a sample size increase at the time of the interim analysis (each was estimated by the frequency out of 10,000 simulation runs) is summarized in Table 3.

- Under scenario #1, to compare the estimated mean AAR based on the AIP patients who have completed 3 months of treatment to a threshold value of 4.5, the chance of the estimated AAR being less than 4.5 is approximately 4.8%, i.e. for a threshold value of 4.5, the chance of a sample size increase is approximately 4.8%.

- Under scenario #2, the chance of a sample size increase for a threshold of 4.5 is approximately 42.9%.
- Under scenario #3, the chance of a sample size increase for a threshold of 4.5 is approximately 97.0%.

Table 3: Summary of simulation results on chance (%) of sample size increase

Scenarios	Threshold			
	3.5	**4.0**	**4.5**	**5.0**
#1. 8(SD=5) vs 4.4(3)	0.2	1.2	4.8	13.0
#2. 6(4) vs 3.3(2)	9.9	21.3	42.9	66.0
#3. 4(2.8) vs 2.2 (1.5)	75.0	88.7	97.0	99.5

The simulation results on power impact are summarized in Table 4.

Without a sample size reassessment at the interim, the power of the primary endpoint testing is approximately 91%, 88% and 78%, for the 3 scenarios, respectively.

If the threshold for a sample size increase is set at 4.5, the power for the 3 scenarios increases to approximately 92%, 91% and 87%, respectively.

Table 4: Summary of simulation results on power (%)

Scenarios	No SSR	Threshold			
		3.5	**4.0**	**4.5**	**5.0**
#1. 8(SD=5) vs 4.4(3)	91.3	91.8	91.8	91.7	91.9
#2. 6(4) vs 3.3(2)	88.2	89.1	89.5	91.0	92.8
#3. 4(2.8) vs 2.2 (1.5)	78.0	84.9	86.9	87.3	87.7

Therefore a threshold value of 4.5 will be used, as it balances the need to increase the same size when the AAR is smaller than anticipated with the preference to maintain the sample size when an increase is not needed, based on the simulation results.

5.5 Background Characteristics

Patient disposition, demographic and baseline characteristics, prior and concomitant medications, study drug exposure, and other disease background characteristics will be summarized. Additionally, all patient data will be presented in patient data listings. No statistical hypothesis testing will be performed.

5.5.1 Patient Disposition

Number and percentage of patients in the following categories will be summarized by treatment arm and overall as appropriate:

- Randomized
- Full Analysis Set (FAS)
- Full Analysis Set for AIP patients (FAS_{AIP})
- Safety Analysis Set (if different from FAS)
- Temporarily interrupted dose due to AEs
- Discontinued treatment and primary reasons for treatment discontinuation

- Withdrew from the study during the 6m-DB period and primary reasons for withdrawal
- Withdrew from the study and primary reasons for withdrawal.

Patients randomized by country, site and randomization stratification factor stratum will be summarized by randomized treatment arm and overall.

5.5.2 Demographics and Baseline characteristics

Demographic, background (e.g. medical history) and baseline disease characteristics will be summarized by treatment arm and by porphyria type and overall for FAS.

Age, height, weight, and body mass index (BMI) will be summarized by descriptive statistics. Age group, sex, race, ethnicity, and country will be summarized by presenting the numbers and percentages of patients in each category.

The following baseline disease characteristics will also be summarized: age at diagnosis, time from diagnosis to randomization, prior hemin prophylaxis status (Y/N) at screening, number of attacks requiring hospitalization, urgent healthcare visits or IV hemin administration at home in the last 6 months prior to the study, prior chronic symptoms when not having attacks (Y/N) and prior chronic analgesic medication usage (Y/N).

5.5.3 Prior and Concomitant Medications

Prior medications are those medications taken prior to the first dose of study drug, regardless of when it ended. If the medication end date is before the date of first dose of study drug, the medication will be summarized as prior medication regardless of whether the start date is missing or not.

Concomitant medications are medications, other than study drug, taken at or after the first dose of study drug, as well as medications with a start date prior to first dose of study drug and are ongoing after first dose of study drug. If medication start date is on or after date of first dose of study drug, the medication will be summarized as concomitant medication regardless of whether the medication end date is missing or not. If the end date of a medication is missing or incomplete such that it cannot be determined whether it is before first study drug dose, it will be counted as a concomitant medication.

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2017 or later). Results will be tabulated by anatomic therapeutic class (ATC) and preferred term.

Prior medications and concomitant medications collected through the 6m-DB period will be tabulated by treatment arm and by porphyria type and overall based on FAS.

The subset of concomitant medications which may be used to treat porphyria pain, including analgesic medications taken at home (collected through eDiary) and taken at a healthcare facility (e.g. during an attack), will be summarized separately.

5.6 Randomization Stratification Factors

Randomization for this study is first stratified by AHP type: AIP [with genetic evidence of a mutation in the *HMBS* gene] vs HCP, VP, ADP, or any AHP without an identified mutation in a porphyria-related gene).

The AIP patient stratum is further stratified by the use of hemin prophylaxis or not immediately prior to Screening, and on the historical AAR. Patients who had been on hemin prophylaxis will be stratified by their historical AAR of <7 vs ≥ 7. Patients who had not been on a hemin

prophylaxis will be stratified by their historical AAR: <12 vs ≥12. This historical AAR is calculated based on the medical record reviewed for inclusion criterion of at least 2 attacks requiring hospitalization, urgent healthcare visit or IV hemin administration at home within the 6 months prior to Screening.

As only a few patients in the stratum of HCP, VP, ADP, or any AHP without identified mutation in a porphyria-related gene are anticipated to be enrolled in the study, there is no further stratification within the stratum.

The actual stratum of a patient will be used in the statistical analysis in case that the actual stratum is different from the stratum used in randomization.

5.7 Multiple Comparisons/Multiplicity

At the time of the unblinded interim analysis for accelerated/conditional approval, only ALA levels will be assessed (the first secondary efficacy end point of the study); for this, the significance level of 0.001 will be used when comparing the ALA levels between the two treatment arms using an ANCOVA model.

In the analyses at the end of the 6m-DB period, a fixed-sequence testing strategy for the primary and secondary endpoints will be implemented to control the overall type I error rate. The significance level of 0.049 will be used at the analysis, reflecting a penalty of 0.001 for the unblinded interim analysis. The primary endpoint will be compared between treatment arms at the significance level of 0.049. If the primary analysis of the primary endpoint is statistically significant, then the secondary endpoints will each be tested at the same significance level of 0.049 in the order specified in the efficacy endpoint section (Section 3.2).

If the test of an endpoint in the sequence is not statistically significant, the testing of remaining endpoints in the sequence will stop and the null hypotheses for the subsequent tests will not be rejected.

There will be no adjustment for multiple comparisons for any other analyses.

5.8 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to primary analysis and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the final protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol deviation and whether or not this deviation is classified as a major protocol deviation. This file will be finalized prior to study unblinding.

5.9 Derived Analysis Visit Windows

Derived analysis visit window will apply to data collected on regular visit schedules (such as PD, clinical laboratory parameter, ECG, vital signs, etc.), except for PK, using study Day

(defined in Section 5.2). The visit windows for study visits during the 6m-DB period are defined in the Table 5 below.

If more than one visit falls within a window, the visit closest to the target day will be selected. If there are multiple visits with the same distance from the scheduled visit day, the last value will be selected.

Table 5: Derived Analysis Visit Windows

Study Visit	Target Day	Window
Screening	Day -60 to -1	≤ -1
Day 1	1	1
Day 15	15	2 – 22
Day 29	29	23 – 43
Day 57	57	44 – 71
Day 85	85	72 – 106
Day 113	113	107 – 127
Day 141	141	128 – 155
Day 169	169	156 - the earlier of (Day 176, and the day of the first dose of OLE)

6 STATISTICAL ANALYSIS

6.1 Efficacy Analysis

Porphyria Attacks

Porphyria patients experiencing acute attacks present with highly morbid and potentially life-threatening symptoms relating to dysfunction across the central, peripheral, and autonomic nervous system. The most commonly seen signs and symptoms of a porphyria attack include diffuse, severe neurovisceral pain mostly in the abdomen, back, or limbs, nausea and vomiting, fatigue, hypertension, tachycardia, motor weakness.

The porphyria attacks in the primary endpoint are those attacks requiring hospitalization, urgent healthcare visits or IV hemin administration at home.

The start time of a porphyria attack is defined as the time at which acute and sustained worsening of the patient's porphyria manifestations beyond normal day-to-day variability. The end of porphyria attacks is characterized by recovery back to within a patient's normal day-to-day variability. Attacks that occur within the same calendar day of the last treated attack will be considered a part of the original attack and will count as one attack in the calculation of the AAR. Any attacks that begin on the next day from the last treated attack ends will constitute a new attack.

6.1.1 Primary Endpoint

The primary endpoint of the study is the annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the initial 6m-DB period. The analysis set for the primary endpoint is FAS_{AIP} (defined in Section 4).

The number of qualifying porphyria attacks is annualized for each patient. The number of attacks is annualized by dividing by the total number of days treated, and multiplying by the number of days in a year (365.25 days).

$$\text{Annualized attack rate (AAR)} = \frac{\text{total number of porphyria attacks}}{\text{total number of days in the treatment period}} \times 365.25.$$

Let λ_G represent the mean AAR for givosiran arm and λ_O represent the mean AAR for control arm. The hypotheses of the primary analysis are:

$$H_o: \frac{\lambda_G}{\lambda_O} = 1 \qquad H_A: \frac{\lambda_G}{\lambda_O} \neq 1$$

The statistical significance level for the primary analysis of the primary endpoint is 2-sided 0.049. An estimated AAR ratio $: \frac{\lambda_G}{\lambda_O} < 1$ and 2-sided p-value < 0.049 from the negative binomial regression model will lead to rejection of H_o.

The mean AARs for the control and givosiran arms are compared using a negative binomial regression model that include fixed effects of the treatment arms, the stratification factors for the AIP patients, which include the hemin prophylaxis regimen prior to the study (yes or no) and historical AAR (high: ≥7 if on hemin prophylaxis regimen, ≥12 if not on hemin prophylaxis; vs low: <7 if on hemin prophylaxis, <12 if not). The logarithm of the amount of time (in the units of year) that each patient spends in the 6m-DB period will be included in the model as an offset

variable. With the offset variable, the negative binomial regression model essentially models the AAR for each patient based on the length of time in the treatment period, and hence would be able to account for the different lengths of follow-up time.

For patients who discontinue the study treatment early (before the end of the 6m-DB period), best efforts to continue collection of attack data will be made. The primary efficacy analysis will be based on all qualified attacks occurring in the 6m-DB period, including those that occur after treatment discontinuation until the end of the 6m-DB period for patients who discontinue treatment and have not withdrawn from the study. For patients who discontinue study treatment and subsequently receive hemin prophylaxis, the primary analysis will include all attack data collected up to the start of the hemin prophylaxis regimen. No imputation of attack data is planned for the primary analysis.

In addition to the primary analysis p-value, an estimated ratio of mean AARs between the two treatment arms with the corresponding 95% confidence interval will be estimated from the negative binomial regression model. Descriptive statistics for the median and interquartile range of the annualized attack rate will also be presented by treatment arm.

6.1.2 Secondary Endpoints

The annualized-event-rate type of endpoints includes annualized rate of hemin administration in AIP patients (FASAIP) and AAR requiring hospitalization, urgent care visits or IV hemin treatment at home in any AHP patients (FAS) over the 6m-DB period. The number of events for each patient is annualized in the same way as AAR defined for the primary endpoint, divided by treatment period (in days) and multiplied by a year (365.25 days). The negative binomial regression model for the primary analysis of the primary endpoint will be used to analyze endpoints of annualized event rates.

The biomarker endpoints include the ALA levels in AIP patients (FAS_{AIP}) at 3 months and 6 months, the urinary PBG levels in AIP patients (FAS_{AIP}) at 6 months. For each secondary endpoint, the biomarker level of the time point of the endpoint will be compared between the two treatment arms using an analysis of covariance (ANCOVA) model with fixed effects of the treatment arms, stratification factors for AIP patients (prior hemin prophylaxis status and historical attack rates), and with the corresponding biomarker levels at the baseline as a covariate. Any ALA/PBG values measured within 3 days after hemin use during the 6m-DB period will be excluded from the ALA/PBG analysis and will be treated as missing. Missing ALA/PBG values will be imputed using a similar mLOCF approach (Section 5.4.1) and MI method.

In the MI method, data that are missing even after mLOCF will be multiply imputed separately for each treatment arm using a regression procedure, with baseline value as covariate. After imputation, the complete dataset will be analyzed using the specified ANCOVA model above. One hundred imputed datasets (per treatment arm) will be generated from the MI regression procedure. Each of the imputed datasets will then be analyzed via the ANCOVA model and the resulting estimates (LS means and standard errors) combined using SAS PROC MIANALZYE to produce inferential results (difference in LS means, 95% CI for the difference, and the p-value from the test that the difference is zero). Point estimates (LS means and differences) will be calculated as the average of the 100 complete-data estimates. A total variance estimate will be calculated as a weighted sum of within-imputation variance, which is the average of the

complete-data variance estimates, and a between-imputation variance term. Complete details may be found in the SAS documentation for the MIANALYZE procedure (see Combining Inferences from Imputed Data Sets under Details):

http://support.sas.com/documentation/onlinedoc/stat/131/mianalyze.pdf.

Analyses will be conducted using PROC MI and PROC MIANALYZE in SAS 9.3 (or later).

Change from baseline in the Physical Component Summary (PCS) of SF-12 in AIP patients (FAS_{AIP}) at 6 months will be analyzed by an ANCOVA model with fixed effects of treatment arms and stratification factors, and with the PCS score at baseline as a covariate. Partial missing data at month 6 (i.e. missing item responses) will be adjusted according to the questionnaire manual. The analysis of this endpoint will be based on observed data.

Daily worst scores for pain (by BPI-SF in Numeric Rating Scale (NRS)), nausea (in NRS) and fatigue (by BFI-SF in NRS) in AIP patients (FAS_{AIP}) over the 6m-DB period are collected through eDiary. The AUC over 6 months in the respective score will be calculated for each patient. For each of the scores, the mean AUC will be compared between treatment arms using ANCOVA model with fixed effects of treatment arms and stratification factors, and with the corresponding score at baseline as a covariate. The analyses of the eDairy data will be based on observed data.

6.1.3 Sensitivity Analysis

The analyses of the primary and secondary endpoints based on the AAR, without adjustment for the length of follow-up in the treatment period, will be conducted as sensitivity analyses. These sensitivity analyses use the same negative binomial regression model as in the primary analysis, excluding the offset variable.

The attacks in the AAR for the primary endpoint (in AIP patients) and secondary endpoint (in all AHP patients) are those requiring hospitalization, or urgent healthcare visit, or IV hemin administration at home. The annualized attack rate of each of the three components will be calculated and analyzed using the same negative binomial regression model for the primary analysis of the primary endpoint. For AAR of each component, the estimated ratio of mean AARs between treatment arms and corresponding 95% confidence interval will be provided.

Since MI method will be used for handling missing data in the ANCOVA models for the ALA/PBG related secondary endpoints. Additional sensitivity analyses for the MAR assumption in the MI approach, such as tipping point analysis, may be performed if appropriate.

6.1.4 Subgroup Analysis

Population subgroups of interest will be displayed using descriptive summaries and figures for the primary and selected secondary endpoints for each treatment arm.

Efficacy analysis for the primary endpoint will be repeated for the following subgroups when deemed appropriate. Negative binomial regression analysis will be conducted only for subgroups that there are at least 5 patients in each treatment arm. If number of patients in either treatment arm of a subgroup is less than 5, only descriptive statistics will be presented.

- Baseline age group (<18 / 18-64 / ≥ 65 years)
- Race (White or Non-white)
- Sex (Female or Male)

- Baseline BMI (<median or ≥median)
- Prior hemin prophylaxis status (Yes or No)
- Historical attack rates based on the hemin prophylaxis status prior to the study (high or low)

  For patients on a hemin prophylaxis regimen at the time of screening, if AAR ≥7, the patient is considered having high attack rates prior to the study. For patients who were not on a hemin prophylaxis regimen at screening, AAR ≥12 is considered with high attack rates.
- Prior chronic analgesic use (Yes or No)
- Prior chronic symptoms when not having attacks (Yes or No)

Other subgroups may be examined, if deemed appropriate.

In addition, region- and/or country-specific analyses will be performed to support regulatory submission as needed and when deemed appropriate.

The subgroup analyses may also be performed for other secondary endpoints if deemed appropriate.

The number of patients in the stratum of non-AIP (i.e. HCP, VP, ADP, or any AHP without identified mutation in a porphyria-related gene) is anticipated to be only a few. Descriptive statistics on the primary and secondary endpoints will be provided by treatment within the stratum.

6.2 Pharmacodynamic Analysis

Analyses of secondary endpoints relating to ALA and PBG levels are described in Section 6.1. In addition, ALA, PBG, and ALAS1 levels will be summarized descriptively at each scheduled visit.

Population PK/PD analyses are planned for all patients in the study and will be described in a separate population PK/PD analysis plan.

6.3 Pharmacokinetic Analysis

PK analyses will be conducted using noncompartmental methods. PK parameters include maximum plasma concentration (Cmax), time to maximum plasma concentration (tmax), elimination half-life ($t_{½\beta}$), area under the concentration-time curve (AUC), apparent clearance (CL/F), and apparent volume of distribution (V/F). Other parameters may be calculated, if deemed necessary.

Population PK analysis is planned for all patients in the study and will be described in a separate population PK analysis plan.

6.4 Safety Analyses

An adverse event (AE) is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the AEs that first occurs or worsens after the first dose of study drug. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

6.4.1 Extent of Exposure

Exposure to study medication in days and the number of study drug SC administrations received will be summarized by treatment arm.

Duration of treatment exposure (days) = date of the last dose of study drug − the date of the first dose of study drug) + 28. The number 28 is the number of days between two scheduled doses of study drug. For example, if the last dose of study drug is taken on day 31, the treatment exposure would be calculated as (31-1) + 28 = 58 days.

Dose interruptions and compliance are not taken into account for duration of exposure.

6.4.2 Adverse Events

AEs will be classified by the MedDRA coding system (version 20.1 or later) and displayed in tables and data listings using system organ class (SOC) and preferred term (PT).

AEs will be summarized by the numbers and percentages of patients reporting at least one AE, having at least one AE by primary SOC and PT. A patient with multiple occurrences of an AE will be counted only once in the respective AE category. Patients who report multiple occurrences of the same AE (PT) will be classified according to the most related or most severe occurrence, respectively.

All AE summaries will be summarized (frequency counts and percentages) by SOC and/or PT, unless specified otherwise. The SOC will be presented alphabetically and the PT will be sorted within each SOC in decreasing order of frequency in the givosiran arm.

The following events are considered to be AEs of clinical interest (AECI):

- ALT elevations >3× ULN (or >3× the baseline ALT measurement if baseline is >ULN)
- Lipase or amylase >3×ULN (or >3× the baseline lipase or amylase measurement if baseline is >ULN)
- Severe or serious injection site reactions (ISRs), ISRs that are associated with a recall phenomenon (reaction at the site of a prior injection with subsequent injections) or, ISRs that lead to temporary dose interruption or permanent discontinuation of study drug.
- Hypersensitivity/Anaphylactic Reaction

The incidence of severe or serious ISRs will be also summarized. The incidence rate is defined as the number of severe or serious ISRs divided by total number of injections. If there are multiple ISRs that occur between two consecutive injections, these events are considered caused by the earlier injection and counted as one ISR.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment (possibly related or definitely related), any severe AE, any severe AE related to treatment, any serious AE (SAE), any SAE related to treatment, any AE/SAE of clinical interest; any AE/SAE leading to treatment discontinuation, any study drug related AE/SAE leading to treatment discontinuation, any AE/SAE leading to study withdrawal, any study drug related AE/SAE leading to study withdrawal, and any deaths.

No inferential safety analysis is planned.

Tabulations by SOC and PT will be produced for the following:

- All AEs;
- AEs by severity;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;
- AECIs;
- SAEs of clinical interest;
- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AECIs leading to treatment discontinuation;
- SAEs of clinical interest leading to treatment discontinuation;
- AEs leading to study withdrawal;
- SAEs leading to study withdrawal.

Tabulations by PT in decreasing order in frequency in the givosiran arm will be produced for the following:

- All AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;

AEs and SAEs will also be summarized by maximum relationship to study drug and by maximum severity.

All AEs collected will be listed along with the information collected on those AEs, e.g. AE relationship to study drug, AE outcome etc. By-patient listings will also be provided for the following: all deaths, all SAEs, and all AEs leading to treatment discontinuation or study withdrawal, all AEs leading to death.

A listing of ISRs will be presented including descriptions, onset and resolution date, severity, treatment given, and event outcome.

6.4.3 Laboratory Data

Knowledge of several clinical laboratory tests, including ALA, PBG, and ALAS1, has the potential to unblind the reviewer. Procedures to protect the blind, including permissions to view these lab values, will be described in a separate document.

Clinical laboratory values will be expressed in Standard International (SI) units. Laboratory data collected and recorded as below the limit of detection will be set equal to the lower limit of detection for the calculation of summary statistics.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, coagulation studies and thyroid and liver function tests). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

For each continuous laboratory parameter, results will be categorized as low, normal, or high based on the laboratory normal ranges used in this study.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category, where the "worst" post-baseline category will be based on the maximum difference (in absolute value) from the upper or lower limits of the normal range.

A listing will be produced for all patient with amylase and lipase >3×ULN and all patients with abnormal liver function tests defined as an ALT >3×ULN, AST >3×ULN, and/or total bilirubin >2×ULN at any time point.
A table will be produced to summarize the number and percentage of patients in each of below category at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN,
- ALP > 1.5×ULN,
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN,
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN,
- INR >1.2.

EDISH plots for ALT, AST, and total bilirubin will also be provided.

For hematology and blood chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the NCI CTCAE Version 4.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

All laboratory data will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.4.4 Vital Signs and Physical Examination

Descriptive statistics by visit and treatment arm will be provided for each variable.

Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

6.4.5 Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, and QTc interval. For post-baseline assessments where ECG is performed in triplicate, the average of the 3 (or all available) readings will be used for analysis. Observations with the following diagnosis or findings will be excluded from analysis: artificial pacemaker, atrial fibrillation, atrial flutter, left bundle branch block, and right bundle branch block.

Corrected QT interval (QTc), if not collected, will be calculated using both Fridericia's and Bazett's correction formula.

Bazett's square-root corrected QT: $QTcB\ (ms) = QT\ (ms) \times \sqrt{\frac{HR(bpm)}{60}}$

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) × $\sqrt[3]{\frac{HR(bpm)}{60}}$.

PR, QRS, QT, QTc (ie, QTcB and QTcF) and RR intervals and their change from time-matched and pre-dose baseline will be summarized for each treatment group by scheduled visit. Subjects will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTc interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTc interval. The number and percentage of subjects in each category will be summarized for each treatment group.
In addition, the following outputs will be generated:

1. Relationship between baseline QT (uncorrected) and RR interval will be explored graphically;
2. Exposure-response analysis: relationship between givosiran concentrations and QTcF, maximum givosiran concentrations and maximum post-baseline absolute QTcF, maximum givosiran concentrations and maximum change from baseline QTcF will be explored graphically with pooled data from all visits;
3. PD-response analysis: relationship between change from baseline in QTcF and ALA will be explored graphically;
4. Adverse event listings using Torsade de pointes/QT prolongation SMQ.

All ECG data for each patient will be provided in a data listing.

6.5 Anti-Drug Antibody

Number of patients testing positive for ADA pre-treatment (baseline positive) and post-treatment will be summarized by porphyria type and frequency of ADA positivity will be presented as a percent by porphyria type and across all patients. In addition, maximum ADA titer and range of titer values will be presented by porphyria type and across all patients. A listing of patients with ADA positivity with their AEs will be provided.

7 CHANGES TO PLANNED ANALYSES

Hypersensitivity/Anaphylactic Reaction has been added to the list of AECIs in this SAP (not in the original protocol), see Section 6.4.2.

8 REFERENCES

Not applicable