CLINICAL STUDY PROTOCOL

ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1 (givosiran)
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016 Protocol Amendment 3 03 February 2017 Protocol Amendment 4 02 August 2017 Protocol Amendment 5 03 May 2018 Protocol Amendment 6 28 May 2019 Protocol Amendment 7 29 April 2020 Protocol Amendment 8 29 March 2021
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD

[redacted]

30 MAR 2021

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1 (givosiran)

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

- Characterize the pharmacokinetic (PK) profile of ALN-AS1 and incidence of antidrug antibodies (ADA) over time
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19 pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen, including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

Reference Therapy, Dose, and Mode of Administration

Not applicable

Duration of Treatment and Study

The duration of treatment is up to 48 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 56 months.

Statistical Methods

Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation.

Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study.

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X	X[a]	X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1. On Day 1, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the

Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided

that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through Month 36 (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Physical Examination[b]				X				X				X
Body Weight, BMI, and Height[c]				X				X				X
Vital Signs[d]		X		X		X		X		X		X
Triplicate 12-Lead ECG[e]				X								
Clinical Laboratory Assessment[f]		X		X		X		X		X		X
Pregnancy Test[g]		X		X		X		X		X		X
Study Drug Administration[h]		X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[i]		X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[j]				X				X				X
Blood Sample for PK Analysis[k]				X								
Antidrug Antibodies[l]				X								X
EQ-5D-5L Questionnaire[m]				X				X				X

Table 2: Schedule of Assessments: Month 19 through Month 36 (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Diary Review (including BPI-SF)[n]	X											
Phone Contact[o]	X		X		X		X		X		X	
AEs	Continuous											
Concomitant Medications	Continuous											

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

c Height will be measured at Screening/Baseline only.

d Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

e Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients

receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.

f Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

g Urine pregnancy tests will be performed. Results must be available before dosing.

h ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

i Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

j On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

k Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

l On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

m In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

n Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

o Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

Table 3: Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen)

Study Visit (M)	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
	M37/ M38[a]	M39[a]	M40/ M41[a]	M42	M43/ M44[a]	M45[a]	M46/ M47[a]	M48/ EOT	M49	
Study Visit (D±Visit Window)	1111±7/1141±7	1171±10	1201±7/1231±7	1261±10	1291±7/1321±7	1351±10	1381±7/1411±7	1441±10	1471±10	
Physical Examination[d]				X				X	X	X
Body Weight, BMI, and Height[e]				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]				X					X	
Clinical Laboratory Assessment[h]	X	X	X	X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X	X	
Antidrug Antibodies[n]				X				X	X	
EQ-5D-5L Questionnaire[o]				X				X	X	

Table 3: Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen)

Study Visit (M)	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
	M37/ M38[a]	M39[a]	M40/ M41[a]	M42	M43/ M44[a]	M45[a]	M46/ M47[a]	M48/ EOT	M49	
Study Visit (D±Visit Window)	1111±7/1141±7	1171±10	1201±7/1231±7	1261±10	1291±7/1321±7	1351±10	1381±7/1411±7	1441±10	1471±10	
Diary Review (including BPI-SF)[p]	X									
Phone Contact[q]	X		X		X		X			
AEs	Continuous									
Concomitant Medications	Continuous									

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; ET = Early Termination; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled treatment with ALN-AS1 through Month 48 will return for an end of study (EOS) visit at Month 49. Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+7] days) after his/her last dose of ALN-AS1.

c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+7 days]) following the last dose of ALN-AS1.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA analysis (also including possible biomarkers and homocysteine levels) will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
COVID-19	Coronavirus disease 2019
CRF	Case report form
CYP	Cytochrome P450
DDI	Drug-drug interaction
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA

Abbreviation	Definition
NHP	Nonhuman primate
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.(1) The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.(2) In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.(3, 4)

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.(5) The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.(6)

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.(2) However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.(7-10) AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.(11) It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.(12) AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).(2) The initial diagnosis involves demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA

(typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.(6-8) Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.(7)

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.(13) In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.(14) With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.(15)

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.(16, 17) In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.(18-22) Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.(22-24) In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.(25) While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).(26) Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).(5) In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.(27) In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option. Given the significant morbidity and mortality, there remains a significant unmet need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent

attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.(28) Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.(29) The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 (givosiran) comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when

compared to patients with AIP who are ASHE.(30) For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related serious adverse events (SAEs), severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Clinical Data Summary

1.6.1. Clinical Pharmacodynamics

Dosing has been completed in Part A and Part B of the study. In both Parts A and B of the study, durable and dose-dependent reductions of ALAS1 mRNA (up to 66% in the 2.5 mg/kg dose group) as measured by cERD were observed after ALN-AS1 treatment. ALAS1 reductions were highly correlated with reductions in ALA (mean maximal 86%) PBG (mean maximal 95%) levels in a dose-dependent manner and were sustained for ≥180 days.

Dosing is currently ongoing in Part C of the study. The mean maximal reduction in ALAS1 mRNA-relative to baseline in ALN-AS1-treated patients in Cohort 1 (2.5 mg/kg Q3M) was 39% and in Cohort 2 (2.5 mg/kg Q1M) 66%. All patients treated with ALN-AS1 also had decreases in their peak ALA and PBG levels in the treatment period compared to the run-in period, along with reduced fluctuations in these levels in the treatment period (data not shown). No reductions were observed in the placebo-treated patients.

1.6.2. Clinical Activity

Clinical activity was not evaluated in Parts A and B of the study because ASHE patients included in this study were not actively experiencing attacks of porphyria.

Dosing is currently ongoing in Part C of the study. All patients in Cohorts 1 and 2 were enrolled in a run-in phase for 10-15 weeks that was followed by a treatment phase for up to 24 weeks. No investigational product was administered during the run-in phase. Therefore, each patient acted as their own control, allowing for individual ALA / PBG levels, and overall attack rate while on ALN-AS1 treatment to be compared to that observed in the run-in phase.

Figure 1 shows in Cohort 1 ALN-AS1-treated patients there was a 74% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 63-94% reduction), compared with a 23% decrease in the annualized attack rate for the placebo-treated patient. In ALN-AS1-treated patients this was accompanied by an approximate 76% mean decrease in annualized acute hemin usage (range 52-95%) and a 10.3 times increase in the maximal attack free interval compared to run-in period (range 4.2-16.3).

Figure 1: Part C Cohort 1 Summary of Treatment Period Clinical Efficacy Relative to Run-in

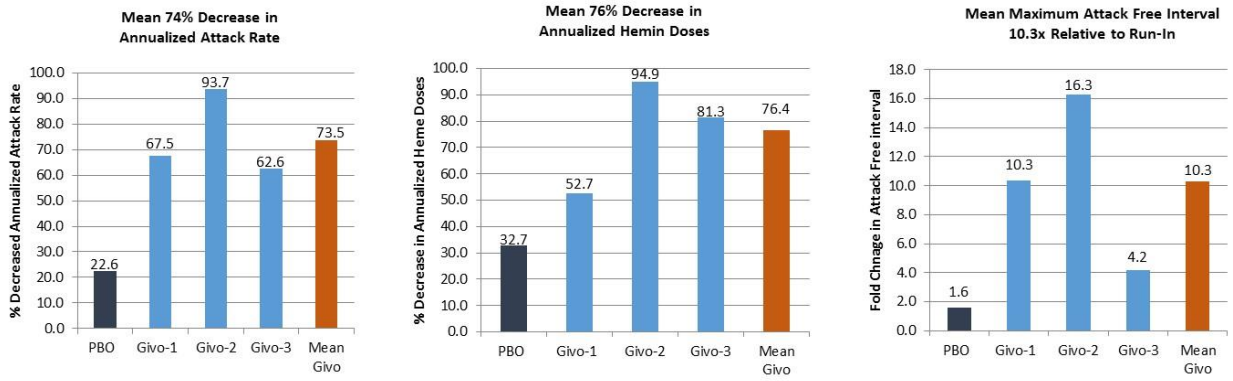
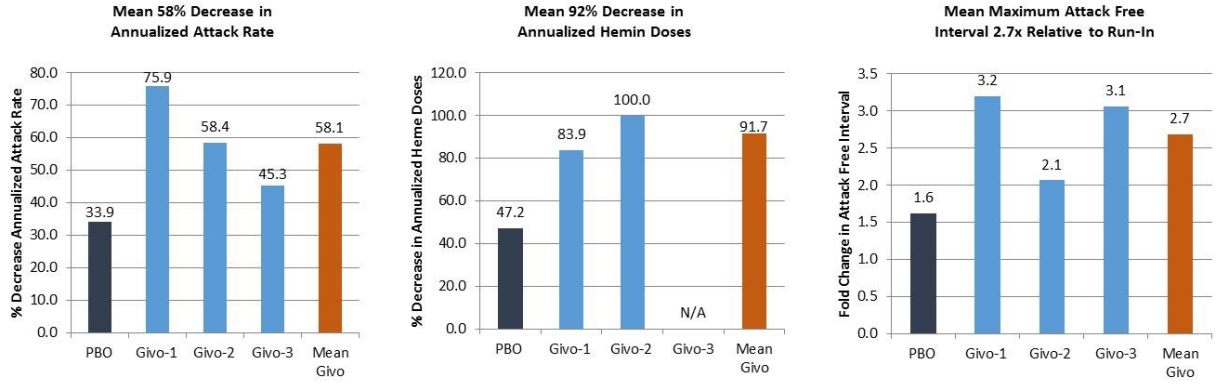


Figure 2 shows in Cohort 2 ALN-AS1-treated patients, there was a 58% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 45-76% reduction). This compares favorably with data from the placebo-treated patient who showed a 34% reduction in the annualized attack rate for the treatment period versus the run-in period. In ALN-AS1-treated patients, the reduction in attack rate was accompanied by a 92% mean decrease in annualized hemin usage (range 84-100%) and a 2.7x increase in the mean maximal attack free interval compared to run-in period (range 2.1-3.2).

Figure 2: Part C Cohort 2 Summary of Treatment Period Clinical Efficacy Relative to Run-in



1.6.3. Clinical Safety

In Part A and Part B of Study ALN-AS1-001, a total of 11 patients (11/13; 85.0%) who received ALN-AS1 reported at least 1 AE. All 5 placebo-treated patients (5/5; 100%) reported at least 1 AE. AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients (5/13; 38%): diarrhoea, dyspepsia, hematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (each AE reported only by an individual patient; 1/13; 8% each). There were 2 SAEs of abdominal pain: 1 patient each in the 0.035 mg/kg and 0.1 mg/kg ALN-AS1 dose groups. Both were considered to be not related and resolved without sequelae. There were no AEs leading to discontinuation. All AEs were mild or moderate in severity, except for 1 of the SAEs of abdominal pain (0.10 mg/kg dose group), which was considered severe. Adverse events related to abnormal laboratory values were seen in 1 patient in the 0.35 mg/kg ALN-AS1 dose group who had AEs of ALT increased and AST increased, which were moderate and considered by the Investigator to be unrelated to ALN-AS1. No clinically significant findings were observed with routine monitoring of CRP and a panel of 9 proinflammatory cytokines collected predose and up to 24 hours post-dose. There were no other clinically significant laboratory abnormalities related to study drug or changes in vital signs, or ECGs in patients who were administered ALN AS1 or placebo.

Dosing is ongoing in Part C of the study. All patients who received ALN-AS1 in cohort 1 and 2 reported at least 1 AE. AEs that were reported in at least 2 subjects were nausea in 3 patients (50%) and abdominal pain, vomiting, nasopharyngitis, headache, cough and oropharyngeal pain in 2 patients (33.3%) each. All AEs were mild or moderate in severity. AEs considered possibly or definitely related to ALN-AS1 were reported in 4 patients (66.6%), 1 each: renal impairment in Cohort 1 and injection site reaction, myalgia, and headache in Cohort 2. Both placebo-treated patients reported AEs. No AE was experienced in more than 1 patient.

In Part C, Cohort 1 or 2, there were no SAEs or AEs leading to discontinuation in patients administered ALN-AS1 or placebo. No clinically significant laboratory abnormalities related to study drug or changes in vital signs, ECG, or physical exam findings were observed in patients administered ALN-AS1 or placebo.

Part C Cohort 3 5.0 mg/kg SC monthly dosing is ongoing. In this cohort there was one fatal SAE of acute pancreatitis, which was determined to be unlikely related to study drug or placebo by the Investigator due to the presence of gall bladder sludge found at the time of her presentation. Contributors to this patient's adverse clinical course included: pre-existing chronic debilitation from recurrent AIP attacks requiring monthly hospitalization, porphyria-attributed quadriplegia requiring nursing home care, delay in hospital admission and complications from thromboembolism (multiple risk factors included pancreatitis, obesity, immobilisation from quadriparesis and recent hospitalization for infected portacath removal). Serum lipase was added to all scheduled laboratory assessments in November 2016 as part of additional safety monitoring. To date, all lipase results (available in 11 of 15 subjects) have been at or below the upper limit of normal with no trends seen with dosing of study drug or placebo.

Further information on the safety, efficacy, PK and PD of ALN-AS1 are available in the Investigator’s Brochure.

1.6.4. Drug-Drug Interactions

An open-label drug-drug interaction (DDI) study (ALN-AS1-004) was conducted in AIP patients who are ASHE to evaluate the effect of a single dose of 2.5 mg/kg ALN-AS1 administered SC on the pharmacokinetics of probe substrates for 5 major CYP enzymes that account for the metabolism of approximately 80% of prescribed drugs.(31, 32) Results from this study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in the metabolic activity of some of the 5 CYP enzymes studied, thereby leading to higher concentrations of some substrates and their metabolites. Treatment with ALN-AS1 resulted in an approximately 3-fold increase in exposure (as measured by AUC) of caffeine (a sensitive substrate for CYP1A2) and an approximately 2-fold increase in exposure of dextromethorphan (a sensitive substrate for CYP2D6). Exposure of midazolam (a sensitive substrate for CYP3A4) and omeprazole (a sensitive substrate for CYP2C19) increased less than 2-fold after treatment with ALN-AS1. There was no effect of ALN-AS1 treatment on losartan (a sensitive substrate for CYP2C9).

1.7. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical finding of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

The important potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Pancreatitis: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.
- Liver transaminase abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of liver function test (LFT) abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.

- Anaphylactic reactions: There is a potential risk of developing a severe allergic reaction with ALN-AS1 administration. In the present study, one patient in the 2.5 mg/kg monthly dose group with a history of asthma and multiple allergies experienced an SAE of anaphylactic reaction that was determined by the Investigator to be definitely related to study drug given the temporal relationship of ALN-AS1 treatment to the onset of the reaction (within minutes). The patient was treated and recovered and was discontinued from the study. Guidance on dose administration and monitoring for anaphylactic reactions has been included in this protocol.The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.
- Homocysteine elevations: Blood homocysteine levels may be increased in patients with AHP, vitamin deficiencies, or chronic kidney disease.(33-35) During treatment with ALN-AS1, increases in blood homocysteine levels have been observed compared to levels before treatment. The clinical relevance of the elevations in blood homocysteine during ALN-AS1 treatment is unknown. The protocol includes monitoring for changes in blood homocysteine levels during treatment with ALN-AS1. Vitamin B6 supplementation is recommended for patients with elevated homocysteine levels (see Section 6.3).

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels

- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7 in Appendix Section 11.2). If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19 pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. In such cases, the patient or caregiver must receive appropriate training on ALN-AS1 administration and the use of epinephrine (epi pen or

equivalent) prior to dosing. This measure is intended to remain in effect only during periods of time when the COVID-19 pandemic impedes the ability of patients to travel to the study site or healthcare professionals to go to patients' homes for dosing. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

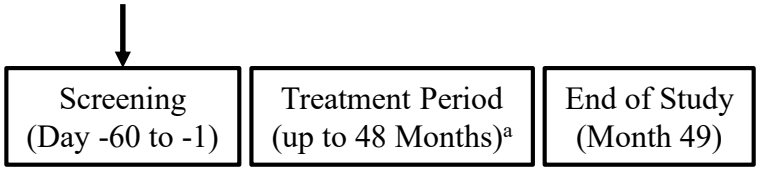
Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 49. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria (see Section 5.3) or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

All patients are asked to participate in a Posttreatment Follow-up visit after they have received their last dose of ALN-AS1.

Figure 3: Study Design



a Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+14] days) after their last dose of ALN-AS1.

4.2. Duration of Treatment and Study

The duration of treatment is up to 48 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 56 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening

3. Women of child-bearing potential (WOCBP) must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Discontinuation of Study Drug and/or Study

Patients or their legal guardians (in the case that the patient is a minor) are free to discontinue treatment and/or study or withdraw their consent at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or of the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient from the study at any time if this is considered to be in the patient's best interest.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively. If a patient stops participation from the study or withdraws consent from the study, he/she will not be able to re-enroll in the study.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). A positive urine pregnancy test should be confirmed by a serum pregnancy test prior to discontinuing the study drug.

If a patient discontinues dosing due to a serious adverse event (SAE), the SAE should be followed as described in Section 7.5.6. When a patient discontinues study drug dosing, the primary reason must be recorded in the electronic case report form (eCRF). Patients who discontinue study drug and remain on study may receive treatment consistent with local standard practice for their disease per Investigator judgement, as applicable.

Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+14] days) after their last dose of ALN-AS1.

5.3.2. Discontinuation from the Study

A patient or their legal guardian may decide to stop the patient's participation in the study at any time. Patients considering to stop the study should be informed that they can discontinue treatment and complete study assessments including follow-up, as per the SOA, or alternatively may complete any minimal assessments for which the patient consents. The Investigator may withdraw a patient at any time if this is considered to be in the patient's best interest.

However, study integrity and interpretation is best maintained if all randomized patients continue study assessments and follow-up. Stopping study participation could mean:

- If a patient discontinues from the study, he/she will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+14] days) after his/her last dose of ALN-AS1.
- A patient can stop taking the study drug and stop study-related visits, but allow the investigator and study team to review the patient's medical records, public records or be contacted in order to receive information about the patient's health

When a patient stops the study, the discontinuation and reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report the observations as thoroughly as possible. If a patient stops the study due to an adverse event (AE), including an SAE, the AE should be followed as described in Section 7.5.6.

If the patient wants to stop participation in the study, he/she should notify the study doctor in

writing or in any other form that may be locally required. The personal data already collected during the study, including patient's biological samples, will still be used together with the data collected on other patients in the study according to the informed consent and applicable laws.

In addition to stopping participation in the study, the patient could decide to withdraw his/her consent as explained in Section 5.3.3

5.3.3. Withdrawal of Consent to Collect and Process the Patient's Personal Data

The patient may decide to withdraw his/her consent informing the study doctor at any time in writing, or in any other form that may be locally required. This means that the patient wants to stop participation in the study and any further collection of his/her personal data.

- The sponsor will continue to keep and use a patient's study information (including any data resulting from the analysis of the patient's biological samples until time of withdrawal) according to applicable law. This is done to guarantee the validity of the study, determine the effects of the study treatment, and ensure completeness of study documentation.
- The patient can also request that collected samples be destroyed or returned (to the extent it is permitted by applicable law) at any time.
- Patients who withdraw their consent to collect and use personal data should understand that public records may be reviewed to determine the patient's survival status as allowed per local and national regulations.

In US and Japan, otherwise, samples not yet analyzed at the time of withdrawal may still be used for further testing/analysis in accordance with the terms of the protocol and of the informed consent form.

In EU and rest of world, in any event, samples not yet analyzed at the time of withdrawal will not be used any longer, unless permitted by applicable law. They will be stored or destroyed according to applicable legal requirements.

5.3.4. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1, Table 2, and Table 3). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 5, Table 6, and Table 7 in Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

The study drug should be injected into the abdomen or upper arms or thighs. Detailed instructions for study drug administration are presented in the Pharmacy Manual. As is consistent with good medical practice for subcutaneous drug administration, patients will be observed for a minimum of 20 minutes after each injection. Treatment for anaphylactic reactions should be readily available where patients are being dosed, and follow country and/or local hospital treatment guidelines as shown in Table 8.(36)

ALN-AS1 will be administered by a qualified and authorized health care professional trained in the recognition and management of anaphylactic reactions. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), study drug administration may be conducted at a location other than the study center by a home healthcare professional, where applicable country and local regulations and infrastructure allow, after consultation with the medical monitor, during particular study visits, as specified in the Schedules of Assessments. If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19 pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. In such cases, the patient or caregiver must receive appropriate training on ALN-AS1 administration and the use of epinephrine (epi pen or equivalent) prior to dosing. This measure is intended to remain in effect only during periods of time when the COVID-19 pandemic impedes the ability of patients to travel to the study site or healthcare professionals to go to patients' homes for dosing. However, study drug administration at the study center should be considered for patients who have ongoing study drug-related AEs or known risk factors for developing anaphylactic reaction, including but not limited to: a prior history of anaphylactic reaction to food, medications or due to unknown etiology, worsening injection site reactions with repeat dosing, or anyone in the opinion of the investigator that would

benefit from clinical observation following dosing. Patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual. In addition, instructions and procedures related to administration of ALN-AS1 by a patient or caregiver will be provided in the Patient/Caregiver Storage and Administration Instructions.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.3.2.1. Monitoring and Dosing Rules in Patients with Potential Cases of Anaphylactic Reaction

An anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). For reference see Section 11.3.(36)

Stop administering the study medication immediately if an anaphylactic reaction to the study medication is suspected. Study medication must be permanently discontinued in patients for

whom an anaphylactic reaction is assessed as related to the study medication.

Laboratory testing: Obtain blood sample for tryptase, total IgE, and ADA antidrug antibodies (ADA) ideally within 15 minutes to 3 hours after the onset of a suspected anaphylactic reaction; however, up to 6 hours is acceptable. An additional blood sample to assess tryptase, total IgE, and ADA should be obtained between 1 to 2 weeks from onset of event. Local laboratory may be used to analyze samples; however, parallel samples should be sent to the central laboratory for analysis. Sample collection and shipping instructions are included in the Laboratory Manual.

Reporting: The PI or designee must notify the sponsor or designee within 24 hours of the occurrence of a suspected case of anaphylactic reaction or being informed of the case as required for AEs of Clinical Interest (AECI) and SAEs, per AE reporting requirements (Section 7.5.6.1, Section 7.5.6.2 and 7.5.6.3).

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center, or the home healthcare professional, will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. In cases where ALN-AS1 is administered at home by a patient/caregiver, dosing may be prepared and administered by the patient/caregiver according to procedures detailed in the Patient/Caregiver Storage and Administration Instructions. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

Additional storage and preparation details are provided in the Pharmacy Manual and the Patient/Caregiver Storage and Administration Instructions

6.2.5. Packaging and Labeling

All packaging, labeling, and production of study drug will be in compliance with current Good Manufacturing Practice specifications, as well as applicable local regulations. Study drug labels and external packaging will include all appropriate information as per local labeling requirements.

Additional details will be available in the Pharmacy Manual.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.
Further instructions about drug accountability are detailed in the Pharmacy Manual.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

During treatment with ALN-AS1, blood homocysteine levels may show an increase compared to levels before treatment. Blood homocysteine levels will be assessed as indicated in the Schedule of Assessments (see Table 3 or Table 7). It is recommended that patients with increased blood homocysteine levels receive a supplement containing vitamin B6. All vitamin supplements should be recorded on the concomitant medications eCRF.

Patients with porphyria could have altered hepatic heme synthesis, and treatment with ALN-AS1 could also modulate this pathway and secondarily impact CYP enzyme activity. Results from a DDI study in AIP patients who are ASHE are presented in Section 1.6.4. The DDI study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in activity in some of the CYP enzymes resulting in corresponding low to moderate increase in the plasma levels of drugs that are metabolized by these CYP enzymes. Based on the moderate decrease in CYP2D6 or CYP1A2 activity, investigators will review all concomitant medications that are primarily metabolized by these enzymes and monitor the patient's clinical response to these medications during the study. Medications metabolized primarily by CYP2D6 and CYP1A2 with a narrow therapeutic index (ie that require regular laboratory monitoring) may need to be monitored more frequently to determine if a dose adjustment of the concomitant medication is required. For patients who require new medications while on study, selection of medications that are not primarily metabolized by CYP2D6 or CYP1A2 should be considered. Refer to the individual product's prescription information to determine if there is a need to monitor concomitant medications for differences in safety or efficacy of the medication based on reported DDIs with CYP2D6 or CYP1A2. For more detailed and up-to-date information on CYP substrates, see:
https://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm.

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies.

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration.

Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, transdermal hormonal, or intrauterine hormone-releasing system as methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]).
- If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- Sexual abstinence, when this is in line with the preferred and usual lifestyle of the patient, is considered an acceptable method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug (defined above). Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not considered sexual abstinence and do not meet criteria for an acceptable method of birth control. As determined by the investigator, the reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods if they start a heterosexual relationship during the study and continue to do so for the entire period of risk associated with the study drug (defined above).
- WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12

months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedules of study assessments are provided in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7.

Where applicable country and local regulations and infrastructure allow for home healthcare, healthcare may take place at a location other than the clinical trial site to perform study assessments including targeted physical exam/body system assessment, assessments for vital signs, and collection of blood and urine samples for safety laboratory assessments, and PD assessments, at all timepoints as specified in the Schedule of Assessments.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.(37) The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples

will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 4 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

Body weight measured on the dosing day will be used to calculate the dose of ALN-AS1 through M12. After M12, weight obtained within 6 months during a study center visit or offsite may be used for dosing calculations.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate standard 12-lead ECGs will be performed using central ECG equipment, with readings approximately 1 minute apart and recorded as specified in the Schedule of Assessments. Patients should be seated or supine for at least 5 minutes before each ECG is obtained. The electrophysiological parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB), and Fridericia corrected QT interval (QTcF).

When ECG and blood sample collection occur at the same time, ECGs should be performed before blood samples are drawn.

Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes.

In all patients receiving ≤2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 2-hour (±15 minutes) postdose readings, paired with PK timepoints (Table 4) as specified in the Schedule of Assessments.

Similarly, in all patients receiving >2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 4-hour (±20 minutes) postdose readings, paired with PK timepoints (Table 4), as specified in the Schedule of Assessments.

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

On dosing days up to the Month 18 visit, results from hematology, chemistry, and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed assessments must be reviewed to allow same day study drug administration and additional samples for central analysis must also be collected. On dosing days from Month 19 through Month 48, a review of laboratory results within 14 days is not required.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the

Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
GGT	
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Coagulation	
Prothrombin time	International Normalized Ratio
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	
Inflammation	
C-reactive protein	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; GGT=gamma-glutamyltransferase; WOCBP=women of child bearing potential.
a eGFR will be calculated using the Modification of Diet in Renal Disease formula.(38)

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Sample collection for patients who experience a potential anaphylactic reaction is discussed in Section 6.2.3.2.1.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg,

events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.
- Lipase >3×ULN (or >3× the baseline lipase measurement if baseline is >ULN)
- Anaphylactic Reactions. Anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). Symptoms of an anaphylactic reaction may include skin or mucosal tissue (e.g. generalized hives, pruritus, angioedema), respiratory compromise (e.g. wheezing, bronchospasm, hypoxia), reduced blood pressure or associated symptoms (e.g. syncope, hypotonia). See Section 11.3 (Table 8) for guidance on diagnosing anaphylactic reactions.(36)

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, the Sponsor or its designee should be notified within 24 hours using a supplemental AEs of clinical interest (AECI) eCRF. Additional clinical and laboratory information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance

electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers. Samples may also be used for testing homocysteine levels.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.(39) In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

7.8. COVID-19 Data Collection

Information on the coronavirus disease 2019 (COVID-19) infection status of the patient, if known, and other information on the impact of the COVID-19 pandemic on the patient's participation in the study will be collected.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria

attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

Additional data summaries to help understand any impact of the COVID-19 pandemic on efficacy, PK, PD, and safety assessments will be performed, as appropriate.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

The Investigator will inform the patient/legal guardian if new information becomes available that may be relevant to the patient's/legal guardian's willingness to continue participation in the study. Communication of this information should be documented.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all patient materials for the study (except those that support the need to remove an apparent immediate hazard to the patient). The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Serious Breach of Protocol

Investigators must notify the Medical Monitor within 24 hours of becoming aware of a potential serious breach of the protocol. A serious breach is a breach that is likely to affect to a significant degree the safety and rights of a study participant or the reliability and robustness of the data generated in the clinical trial.

9.1.4. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' confidentiality will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.5. End of the Study

The end of the study is defined as last patient last visit.

9.1.6. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study

must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010;375(9718):924-37.

6. Floderus Y. Variations in Porphobilinogen and 5-Aminolevulinic Acid Concentrations in Plasma and Urine from Asymptomatic Carriers of the Acute Intermittent Porphyria Gene with Increased Porphyrin Precursor Excretion. Clinical Chemistry. 2006;52(4):701-7.

7. Anderson K. Disorders of Heme Biosynthesis. 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transplantation. 2012;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach JC. Acute Intermittent Porphyria: From Clinical to Molecular Aspects. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF, British, et al. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. PanhematinPI. Panhematin Prescribing Information. 2017.

17. NormosangSmPC. Normosang Summary of Product Characteristics. 2017.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013;36(5):849-57.

26. Anderson KE, Egger NG, Goeger DE. Heme arginate for prevention of acute porphyric attacks. Acta Haematologica. 1997;98:120.

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002;48(11):1891-900.

31. Ryu JY, Song IS, Sunwoo YE, Shon JH, Liu KH, Cha IJ, et al. Development of the "Inje cocktail" for high-throughput evaluation of five human cytochrome P450 isoforms in vivo. Clin Pharmacol Ther. 2007;82(5):531-40.

32. Williams D, Tao X, Zhu L, Stonier M, Lutz JD, Masson E, et al. Use of a cocktail probe to assess potential drug interactions with cytochrome P450 after administration of belatacept, a costimulatory immunomodulator. Br J Clin Pharmacol. 2017;83(2):370-80.

33. Ventura P, Corradini E, Di Pierro E, Marchini S, Marcacci M, Cuoghi C, et al. Hyperhomocysteinemia in patients with acute porphyrias: A potentially dangerous metabolic crossroad? Eur J Intern Med. 2020;79:101-7.

34. To-Figueras J, Lopez RM, Deulofeu R, Herrero C. Preliminary report: hyperhomocysteinemia in patients with acute intermittent porphyria. Metabolism. 2010;59(12):1809-10.

35. Maron BA, Loscalzo J. The treatment of hyperhomocysteinemia. Annu Rev Med. 2009;60:39-54.

36. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. Ann Emerg Med. 2006;47(4):373-80.

37. Atkinson TM, Mendoza TR, Sit L, Passik S, Scher HI, Cleeland C, et al. The Brief Pain Inventory and its "pain at its worst in the last 24 hours" item: clinical trial endpoint considerations. Pain Med. 2010;11(3):337-46.

38. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

39. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 4 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 4: Pharmacokinetic Time Points

Study Day		Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days		Predose (within 60 minutes before dosing)	X
		02:00 (±15 minutes)[b]	X
		06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days		Anytime during visit	X
Month 1 (Day 31) ±7 days,[a]		Predose (within 60 minutes before dosing)	X
Month 3 (Day 91) ±7 days,		02:00 (±15 minutes)[b]	X
Month 6 (Day 181) ±7 days, Month 9 (Day 271) ±10 days[d], Month 15 (Day 451) ±10 days[d], Month 18 (Day 541) ±10 days Month 24 (Day 721) ±10 days		04:00 (±20 minutes)[c]	

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.
a At the Day 31, Month 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.
b Only in patients receiving ≤2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
c Only in patients receiving >2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
d Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

			Treatment Period (Screening/Baseline through Month 18)													
Study Visit (M)	**Screening/ Baseline[a]**		**M1**		**M2**	**M3**	**M4/ M5**	**M6**	**M7/ M8[b]**	**M9**	**M10/ M11[b]**	**M12**	**M13/ M14[b]**	**M15[b]**	**M16/ M17[b]**	**M18**
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
AEs		Continuous														
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1 ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days up to the Month 18 visit,results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed predose assessments may be used for review in order to allow same day study drug administration, and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1. After M12, weight measured at either the previous study center visit or on current study center visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 6: Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
	M19/ M20[a]	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36**
Study Visit (D±Visit Window)	**571±14/601±14**	**631±14**	**661±14/691±14**	**721±14**	**751±14/781±14**	**811±14**	**841±14/871±14**	**901±14**	**931±14/961±14**	**991±14**	**1021±14/1051±14**	**1081±14**
Physical Examination[b]				X				X				X
Body Weight, BMI, and Height[c]				X				X				X
Vital Signs[d]	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[e]												
Clinical Laboratory Assessment[f]		X		X		X		X		X		X
Pregnancy Test[g]	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[h]	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[i]		X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[j]				X				X				X
Blood Sample for PK Analysis[k]				X								
Antidrug Antibodies[l]				X								X

Table 6: Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
	M19/ M20[a]	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36**
Study Visit (D±Visit Window)	**571±14/601±14**	**631±14**	**661±14/691±14**	**721±14**	**751±14/781±14**	**811±14**	**841±14/871±14**	**901±14**	**931±14/961±14**	**991±14**	**1021±14/1051±14**	**1081±14**
EQ-5D-5L Questionnaire[m]				X				X				X
Diary Review (including BPI-SF)[n]	X											
AEs	Continuous											
Concomitant Medications	Continuous											

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.
- In situations where a study visit cannot be completed at the study center or offsite by a home healthcare professional visit, the study Investigator (or delegate) may verbally contact the patient within the study visit window to assess for any AEs, concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. Targeted physical examinations/body system assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow. See Section 7.5.3 for details on the physical examination.

c Height will be measured at Screening/Baseline only.

d Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Vital sign measurements may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

e Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details. With the implementation of Amendment 7, ECGs are no longer required.

f Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. Collection of blood and urine samples for safety laboratory assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

g Urine pregnancy tests will be performed. Results must be available before dosing.

h ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight obtained within 6 months during a study center visit or offsite will be used to calculate the weight-based dose of ALN-AS1.

i Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose. Collection of samples for PD assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

j On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

k Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

l On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose. Antidrug antibody assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit.

m In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible. In situations where a planned study visit cannot be completed at the study center, the patient will complete the EQ-5D-5L questionnaire at home.

n Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

Table 7: Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M37/ M38[a]**	**M39[a]**	**M40/ M41[a]**	**M42**	**M43/ M44[a]**	**M45[a]**	**M46/ M47[a]**	**M48/ EOT**	**M49**	
Study Visit (D±Visit Window)	**1111±14/1141±14**	**1171±14**	**1201±14/1231±14**	**1261±14**	**1291±14/1321±14**	**1351±14**	**1381±14/1411±14**	**1441±14**	**1471±14**	
Physical Examination[d]				X				X	X	X
Body Weight, BMI, and Height[e]				X				X	X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]										
Clinical Laboratory Assessment[h]		X		X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X	X	
Antidrug Antibodies[m]								X	X	

Table 7: Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M37/ M38[a]**	**M39[a]**	**M40/ M41[a]**	**M42**	**M43/ M44[a]**	**M45[a]**	**M46/ M47[a]**	**M48/ EOT**	**M49**	
Study Visit (D±Visit Window)	**1111±14/1141±14**	**1171±14**	**1201±14/1231±14**	**1261±14**	**1291±14/1321±14**	**1351±14**	**1381±14/1411±14**	**1441±14**	**1471±14**	
EQ-5D-5L Questionnaire[n]				X				X	X	
Diary Review (including BPI-SF)[o]	X									
AEs	Continuous									
Concomitant Medications	Continuous									

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS=End of Study; EOT=End of Treatment; ET=early termination; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.
- In situations where a study visit cannot be completed at the study center or offsite by a home healthcare professional visit, the study Investigator (or delegate) may verbally contact the patient within the study visit window to assess for any AEs, concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled doses ALN-AS1 through Month 48 will return for an end of study (EOS) visit at Month 49. Patients who discontinue treatment prior to Month 48 will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return 3 months (84 [+14] days) after their last dose of ALN-AS1 for a safety-follow-up visit.

c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+14 days]) following the last dose of ALN-AS1.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. Targeted physical examinations/body system assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Vital sign measurements may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details. With the implementation of Amendment 7, ECGs are no longer required.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. Collection of blood and urine samples for safety laboratory assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

i Urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight obtained within 6 months during a study center visit or offsite will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose. Collection of samples for PD assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

l On dosing days, blood and urine samples for ALAS1 mRNA analysis (also including possible biomarkers and homocysteine levels) will be collected will be collected within 1 hour predose.

m On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose. Antidrug antibody assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit.

n In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible. In situations where a planned study visit cannot be completed at the study center, the patient will complete the EQ-5D-5L questionnaire at home.

o Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. Anaphylactic Reactions

Table 8: Sampson Criteria for Anaphylactic Reactions

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)
   AND AT LEAST ONE OF THE FOLLOWING
   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):
   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):
   a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; *BP*, blood pressure.
*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 × age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.

Adapted from Sampson et al. 2006 (36)

ALN-AS1-002 PROTOCOL AMENDMENT 8
SUMMARY OF CHANGES DATED 29 MARCH 2021

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

1. RATIONALE FOR PROTOCOL AMENDMENT

The purpose of this protocol amendment is to recommend testing of blood homocysteine levels. In addition, it is recommended that patients with increased blood homocysteine levels receive a supplement containing vitamin B6.

These recommendations are being made because during ALN-AS1 treatment, increases in blood homocysteine levels have been observed compared to levels before ALN-AS1 treatment. Thus, monitoring for changes in blood homocysteine levels during treatment with ALN-AS1 has been incorporated into the protocol. Blood homocysteine levels may also be increased in patients with acute hepatic porphyria (AHP), vitamin deficiencies, or chronic kidney disease. The clinical relevance of the elevations in blood homocysteine during ALN-AS1 treatment is unknown.

Detailed reasons for each change are provided in Section 2. Corrections to typographical errors, punctuation, grammar, abbreviations, and formatting are not detailed.

2. PROTOCOL AMENDMENT 8 DETAILED SUMMARY OF CHANGES

The primary section(s) of the protocol affected by the changes in Protocol Amendment 8 are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~ and added text is indicated by **bold** font, relevant to each purpose described.

Purpose: Add measuring blood homocysteine levels as part of the exploratory biomarker assessment.

The primary change occurs in Section 7.6 Exploratory Biomarkers

Revised text: (in the first paragraph)

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers. **Samples may also be used for testing homocysteine levels.**

Section(s) also reflecting this change:

- Table 3, Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen) (footnote l)
- Table 7, Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen) (footnote l)

Purpose: To add increases in blood homocysteine levels to the benefit-risk assessment, noting the unknown clinical relevance, and to inform the Investigator about the possibility to supplement with vitamin B6 should increases in homocysteine levels occur.

The primary change occurs in Section 1.7 Benefit-Risk Assessment

Added text: (added 4th bullet point)

The important potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- **Homocysteine elevations: Blood homocysteine levels may be increased in patients with AHP, vitamin deficiencies, or chronic kidney disease.(33-35) During treatment with ALN-AS1, increases in blood homocysteine levels have been observed compared to levels before treatment. The clinical relevance of the elevations in blood homocysteine during ALN-AS1 treatment is unknown. The protocol includes monitoring for changes in blood homocysteine levels during treatment with ALN-AS1. Vitamin B6 supplementation is recommended for patients with elevated homocysteine levels (see Section 6.3).**

Purpose: To add that vitamin B6 supplementation is an accepted potential concomitant medication if increases in blood homocysteine levels are observed.

The changes include the revisions to Section 6.3 Concomitant Medications

Added text: (3rd paragraph)

During treatment with ALN-AS1, blood homocysteine levels may show an increase compared to levels before treatment. Blood homocysteine levels will be assessed as indicated in the Schedule of Assessments (see Table 3 or Table 7). It is recommended that patients with increased blood homocysteine levels receive a supplement containing Vitamin B6. All vitamin supplements should be recorded on the concomitant medications eCRF.

CLINICAL STUDY PROTOCOL

ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1 (givosiran)
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016 Protocol Amendment 3 03 February 2017 Protocol Amendment 4 02 August 2017 Protocol Amendment 5 03 May 2018 Protocol Amendment 6 28 May 2019 Protocol Amendment 7 29 April 2020
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD

30 Apr 2020

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1 (givosiran)

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

- Characterize the pharmacokinetic (PK) profile of ALN-AS1 and incidence of antidrug antibodies (ADA) over time
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19 pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen, including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

Reference Therapy, Dose, and Mode of Administration

Not applicable

Duration of Treatment and Study

The duration of treatment is up to 48 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 56 months.

Statistical Methods

Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation.

Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study.

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X	X[a]	X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1. On Day 1, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the

Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided

that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through Month 36 (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Physical Examination[b]				X				X				X
Body Weight, BMI, and Height[c]				X				X				X
Vital Signs[d]		X		X		X		X		X		X
Triplicate 12-Lead ECG[e]				X								
Clinical Laboratory Assessment[f]		X		X		X		X		X		X
Pregnancy Test[g]		X		X		X		X		X		X
Study Drug Administration[h]		X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[i]		X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[j]				X				X				X
Blood Sample for PK Analysis[k]				X								
Antidrug Antibodies[l]				X								X
EQ-5D-5L Questionnaire[m]				X				X				X

Table 2: Schedule of Assessments: Month 19 through Month 36 (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Diary Review (including BPI-SF)[n]	X											
Phone Contact[o]	X		X		X		X		X		X	
AEs	Continuous											
Concomitant Medications	Continuous											

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

c Height will be measured at Screening/Baseline only.

d Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

e Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients

receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.

f Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

g Urine pregnancy tests will be performed. Results must be available before dosing.

h ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

i Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

j On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

k Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

l On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

m In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

n Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

o Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

Table 3: Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen)

Study Visit (M)	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
	M37/ M38[a]	M39[a]	M40/ M41[a]	M42	M43/ M44[a]	M45[a]	M46/ M47[a]	M48/ EOT	M49	
Study Visit (D±Visit Window)	1111±7/1141±7	1171±10	1201±7/1231±7	1261±10	1291±7/1321±7	1351±10	1381±7/1411±7	1441±10	1471±10	
Physical Examination[d]				X				X	X	X
Body Weight, BMI, and Height[e]				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]				X					X	
Clinical Laboratory Assessment[h]	X	X	X	X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X	X	
Antidrug Antibodies[n]				X				X	X	
EQ-5D-5L Questionnaire[o]				X				X	X	

Table 3: Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen)

Study Visit (M)	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
	M37/ M38[a]	M39[a]	M40/ M41[a]	M42	M43/ M44[a]	M45[a]	M46/ M47[a]	M48/ EOT	M49	
Study Visit (D±Visit Window)	1111±7/1141±7	1171±10	1201±7/1231±7	1261±10	1291±7/1321±7	1351±10	1381±7/1411±7	1441±10	1471±10	
Diary Review (including BPI-SF)[p]	X									
Phone Contact[q]	X		X		X		X			
AEs	Continuous									
Concomitant Medications	Continuous									

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; ET = Early Termination; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled treatment with ALN-AS1 through Month 48 will return for an end of study (EOS) visit at Month 49. Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+7] days) after his/her last dose of ALN-AS1.

c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+7 days]) following the last dose of ALN-AS1.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
COVID-19	Coronavirus disease 2019
CRF	Case report form
CYP	Cytochrome P450
DDI	Drug-drug interaction
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA

Abbreviation	Definition
NHP	Nonhuman primate
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.(1) The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.(2) In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.(3, 4)

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.(5) The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.(6)

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.(2) However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.(7-10) AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.(11) It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.(12) AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).(2) The initial diagnosis involves demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA

(typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.(6-8) Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.(7)

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.(13) In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.(14) With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.(15)

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.(16, 17) In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.(18-22) Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.(22-24) In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.(25) While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).(26) Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).(5) In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.(27) In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option. Given the significant morbidity and mortality, there remains a significant unmet need for

an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.(28) Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.(29) The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 (givosiran) comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have

higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.(30) For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related serious adverse events (SAEs), severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Clinical Data Summary

1.6.1. Clinical Pharmacodynamics

Dosing has been completed in Part A and Part B of the study. In both Parts A and B of the study, durable and dose-dependent reductions of ALAS1 mRNA (up to 66% in the 2.5 mg/kg dose group) as measured by cERD were observed after ALN-AS1 treatment. ALAS1 reductions were highly correlated with reductions in ALA (mean maximal 86%) PBG (mean maximal 95%) levels in a dose-dependent manner and were sustained for ≥180 days.

Dosing is currently ongoing in Part C of the study. The mean maximal reduction in ALAS1 mRNA-relative to baseline in ALN-AS1-treated patients in Cohort 1 (2.5 mg/kg Q3M) was 39% and in Cohort 2 (2.5 mg/kg Q1M) 66%. All patients treated with ALN-AS1 also had decreases in their peak ALA and PBG levels in the treatment period compared to the run-in period, along with reduced fluctuations in these levels in the treatment period (data not shown). No reductions were observed in the placebo-treated patients.

1.6.2. Clinical Activity

Clinical activity was not evaluated in Parts A and B of the study because ASHE patients included in this study were not actively experiencing attacks of porphyria.

Dosing is currently ongoing in Part C of the study. All patients in Cohorts 1 and 2 were enrolled in a run-in phase for 10-15 weeks that was followed by a treatment phase for up to 24 weeks. No investigational product was administered during the run-in phase. Therefore, each patient acted as their own control, allowing for individual ALA / PBG levels, and overall attack rate while on ALN-AS1 treatment to be compared to that observed in the run-in phase.

Figure 1 shows in Cohort 1 ALN-AS1-treated patients there was a 74% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 63-94% reduction), compared with a 23% decrease in the annualized attack rate for the placebo-treated patient. In ALN-AS1-treated patients this was accompanied by an approximate 76% mean decrease in annualized acute hemin usage (range 52-95%) and a 10.3 times increase in the maximal attack free interval compared to run-in period (range 4.2-16.3).

Figure 1: Part C Cohort 1 Summary of Treatment Period Clinical Efficacy Relative to Run-in

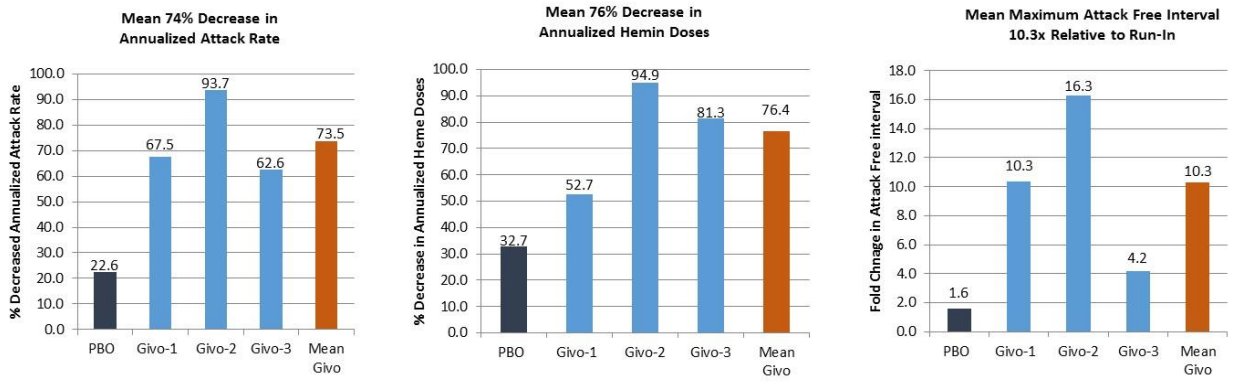
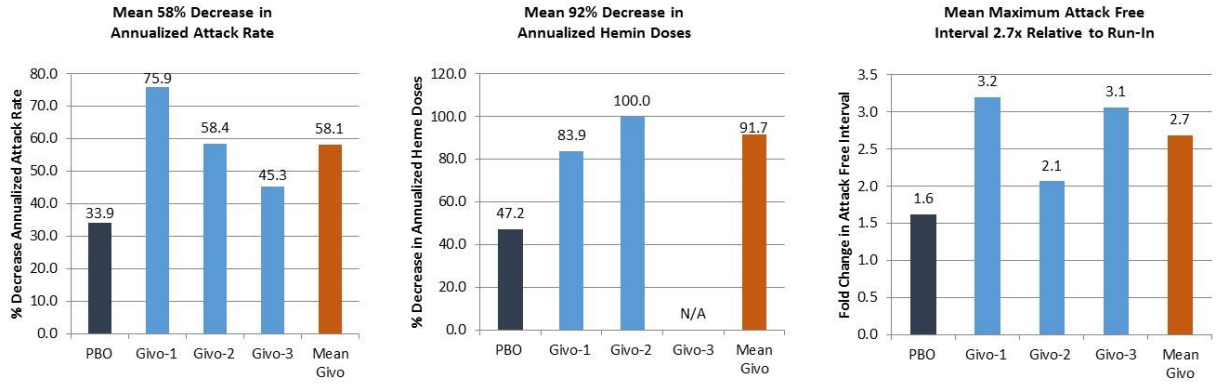


Figure 2 shows in Cohort 2 ALN-AS1-treated patients, there was a 58% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 45-76% reduction). This compares favorably with data from the placebo-treated patient who showed a 34% reduction in the annualized attack rate for the treatment period versus the run-in period. In ALN-AS1-treated patients, the reduction in attack rate was accompanied by a 92% mean decrease in annualized hemin usage (range 84-100%) and a 2.7x increase in the mean maximal attack free interval compared to run-in period (range 2.1-3.2).

Figure 2: Part C Cohort 2 Summary of Treatment Period Clinical Efficacy Relative to Run-in



1.6.3. Clinical Safety

In Part A and Part B of Study ALN-AS1-001, a total of 11 patients (11/13; 85.0%) who received ALN-AS1 reported at least 1 AE. All 5 placebo-treated patients (5/5; 100%) reported at least 1 AE. AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients (5/13; 38%): diarrhoea, dyspepsia, hematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (each AE reported only by an individual patient; 1/13; 8% each). There were 2 SAEs of abdominal pain: 1 patient each in the 0.035 mg/kg and 0.1 mg/kg ALN-AS1 dose groups. Both were considered to be not related and resolved without sequelae. There were no AEs leading to discontinuation. All AEs were mild or moderate in severity, except for 1 of the SAEs of abdominal pain (0.10 mg/kg dose group), which was considered severe. Adverse events related to abnormal laboratory values were seen in 1 patient in the 0.35 mg/kg ALN-AS1 dose group who had AEs of ALT increased and AST increased, which were moderate and considered by the Investigator to be unrelated to ALN-AS1. No clinically significant findings were observed with routine monitoring of CRP and a panel of 9 proinflammatory cytokines collected predose and up to 24 hours post-dose. There were no other clinically significant laboratory abnormalities related to study drug or changes in vital signs, or ECGs in patients who were administered ALN AS1 or placebo.

Dosing is ongoing in Part C of the study. All patients who received ALN-AS1 in cohort 1 and 2 reported at least 1 AE. AEs that were reported in at least 2 subjects were nausea in 3 patients (50%) and abdominal pain, vomiting, nasopharyngitis, headache, cough and oropharyngeal pain in 2 patients (33.3%) each. All AEs were mild or moderate in severity. AEs considered possibly or definitely related to ALN-AS1 were reported in 4 patients (66.6%), 1 each: renal impairment in Cohort 1 and injection site reaction, myalgia, and headache in Cohort 2. Both placebo-treated patients reported AEs. No AE was experienced in more than 1 patient.

In Part C, Cohort 1 or 2, there were no SAEs or AEs leading to discontinuation in patients administered ALN-AS1 or placebo. No clinically significant laboratory abnormalities related to study drug or changes in vital signs, ECG, or physical exam findings were observed in patients administered ALN-AS1 or placebo.

Part C Cohort 3 5.0 mg/kg SC monthly dosing is ongoing. In this cohort there was one fatal SAE of acute pancreatitis, which was determined to be unlikely related to study drug or placebo

by the Investigator due to the presence of gall bladder sludge found at the time of her presentation. Contributors to this patient's adverse clinical course included: pre-existing chronic debilitation from recurrent AIP attacks requiring monthly hospitalization, porphyria-attributed quadriplegia requiring nursing home care, delay in hospital admission and complications from thromboembolism (multiple risk factors included pancreatitis, obesity, immobilisation from quadriparesis and recent hospitalization for infected portacath removal). Serum lipase was added to all scheduled laboratory assessments in November 2016 as part of additional safety monitoring. To date, all lipase results (available in 11 of 15 subjects) have been at or below the upper limit of normal with no trends seen with dosing of study drug or placebo.

Further information on the safety, efficacy, PK and PD of ALN-AS1 are available in the Investigator's Brochure.

1.6.4. Drug-Drug Interactions

An open-label drug-drug interaction (DDI) study (ALN-AS1-004) was conducted in AIP patients who are ASHE to evaluate the effect of a single dose of 2.5 mg/kg ALN-AS1 administered SC on the pharmacokinetics of probe substrates for 5 major CYP enzymes that account for the metabolism of approximately 80% of prescribed drugs.(31, 32) Results from this study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in the metabolic activity of some of the 5 CYP enzymes studied, thereby leading to higher concentrations of some substrates and their metabolites. Treatment with ALN-AS1 resulted in an approximately 3-fold increase in exposure (as measured by AUC) of caffeine (a sensitive substrate for CYP1A2) and an approximately 2-fold increase in exposure of dextromethorphan (a sensitive substrate for CYP2D6). Exposure of midazolam (a sensitive substrate for CYP3A4) and omeprazole (a sensitive substrate for CYP2C19) increased less than 2-fold after treatment with ALN-AS1. There was no effect of ALN-AS1 treatment on losartan (a sensitive substrate for CYP2C9).

1.7. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical finding of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

The important potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and

close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.

- Pancreatitis: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.
- Liver transaminase abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of liver function test (LFT) abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Anaphylactic reactions: There is a potential risk of developing a severe allergic reaction with ALN-AS1 administration. In the present study, one patient in the 2.5 mg/kg monthly dose group with a history of asthma and multiple allergies experienced an SAE of anaphylactic reaction that was determined by the Investigator to be definitely related to study drug given the temporal relationship of ALN-AS1 treatment to the onset of the reaction (within minutes). The patient was treated and recovered and was discontinued from the study. Guidance on dose administration and monitoring for anaphylactic reactions has been included in this protocol.

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including

ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7 in Appendix Section 11.2). If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19 pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. In such cases, the patient or caregiver must receive appropriate training on ALN-AS1 administration and the use of epinephrine (epi pen or equivalent) prior to dosing. This measure is intended to remain in effect only during periods of time when the COVID-19 pandemic impedes the ability of patients to travel to the study site or healthcare professionals to go to patients' homes for dosing. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

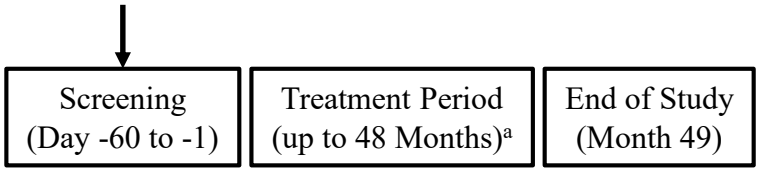
Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 49. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria (see Section 5.3) or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

All patients are asked to participate in a Posttreatment Follow-up visit after they have received their last dose of ALN-AS1.

Figure 3: Study Design



[a] Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+14] days) after their last dose of ALN-AS1.

4.2. Duration of Treatment and Study

The duration of treatment is up to 48 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 56 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening
3. Women of child-bearing potential (WOCBP) must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Discontinuation of Study Drug and/or Study

Patients or their legal guardians (in the case that the patient is a minor) are free to discontinue treatment and/or study or withdraw their consent at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or of the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the

Investigator. The Investigator may withdraw a patient from the study at any time if this is considered to be in the patient's best interest.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively. If a patient stops participation from the study or withdraws consent from the study, he/she will not be able to re-enroll in the study.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). A positive urine pregnancy test should be confirmed by a serum pregnancy test prior to discontinuing the study drug.

If a patient discontinues dosing due to a serious adverse event (SAE), the SAE should be followed as described in Section 7.5.6. When a patient discontinues study drug dosing, the primary reason must be recorded in the electronic case report form (eCRF). Patients who discontinue study drug and remain on study may receive treatment consistent with local standard practice for their disease per Investigator judgement, as applicable.

Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+14] days) after their last dose of ALN-AS1.

5.3.2. Discontinuation from the Study

A patient or their legal guardian may decide to stop the patient's participation in the study at any time. Patients considering to stop the study should be informed that they can discontinue treatment and complete study assessments including follow-up, as per the SOA, or alternatively may complete any minimal assessments for which the patient consents. The Investigator may withdraw a patient at any time if this is considered to be in the patient's best interest.

However, study integrity and interpretation is best maintained if all randomized patients continue study assessments and follow-up. Stopping study participation could mean:

- If a patient discontinues from the study, he/she will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+14] days) after his/her last dose of ALN-AS1.
- A patient can stop taking the study drug and stop study-related visits, but allow the investigator and study team to review the patient's medical records, public records or be contacted in order to receive information about the patient's health

When a patient stops the study, the discontinuation and reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report the observations as thoroughly as possible. If a patient stops the study due to an adverse event (AE), including an SAE, the AE should be followed as described in Section 7.5.6.

If the patient wants to stop participation in the study, he/she should notify the study doctor in writing or in any other form that may be locally required. The personal data already collected during the study, including patient's biological samples, will still be used together with the data collected on other patients in the study according to the informed consent and applicable laws.

In addition to stopping participation in the study, the patient could decide to withdraw his/her consent as explained in Section 5.3.3

5.3.3. Withdrawal of Consent to Collect and Process the Patient's Personal Data

The patient may decide to withdraw his/her consent informing the study doctor at any time in writing, or in any other form that may be locally required. This means that the patient wants to stop participation in the study and any further collection of his/her personal data.

- The sponsor will continue to keep and use a patient's study information (including any data resulting from the analysis of the patient's biological samples until time of withdrawal) according to applicable law. This is done to guarantee the validity of the study, determine the effects of the study treatment, and ensure completeness of study documentation.
- The patient can also request that collected samples be destroyed or returned (to the extent it is permitted by applicable law) at any time.
- Patients who withdraw their consent to collect and use personal data should understand that public records may be reviewed to determine the patient's survival status as allowed per local and national regulations.

In US and Japan, otherwise, samples not yet analyzed at the time of withdrawal may still be used for further testing/analysis in accordance with the terms of the protocol and of the informed consent form.

In EU and rest of world, in any event, samples not yet analyzed at the time of withdrawal will not be used any longer, unless permitted by applicable law. They will be stored or destroyed according to applicable legal requirements.

5.3.4. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1, Table 2, and Table 3). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 5, Table 6, and Table 7 in Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

The study drug should be injected into the abdomen or upper arms or thighs. Detailed instructions for study drug administration are presented in the Pharmacy Manual. As is consistent with good medical practice for subcutaneous drug administration, patients will be observed for a minimum of 20 minutes after each injection. Treatment for anaphylactic reactions should be readily available where patients are being dosed, and follow country and/or local hospital treatment guidelines as shown in Table 8.(33)

ALN-AS1 will be administered by a qualified and authorized health care professional trained in the recognition and management of anaphylactic reactions. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), study drug administration may be conducted at a location other than the study center by a home healthcare professional, where applicable country and local regulations and infrastructure allow, after consultation with the medical monitor, during particular study visits, as specified in the Schedules of Assessments. If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19

pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. In such cases, the patient or caregiver must receive appropriate training on ALN-AS1 administration and the use of epinephrine (epi pen or equivalent) prior to dosing. This measure is intended to remain in effect only during periods of time when the COVID-19 pandemic impedes the ability of patients to travel to the study site or healthcare professionals to go to patients' homes for dosing. However, study drug administration at the study center should be considered for patients who have ongoing study drug-related AEs or known risk factors for developing anaphylactic reaction, including but not limited to: a prior history of anaphylactic reaction to food, medications or due to unknown etiology, worsening injection site reactions with repeat dosing, or anyone in the opinion of the investigator that would benefit from clinical observation following dosing. Patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual. In addition, instructions and procedures related to administration of ALN-AS1 by a patient or caregiver will be provided in the Patient/Caregiver Storage and Administration Instructions.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a

reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.3.2.1. Monitoring and Dosing Rules in Patients with Potential Cases of Anaphylactic Reaction

An anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). For reference see Section 11.3.(33)

Stop administering the study medication immediately if an anaphylactic reaction to the study medication is suspected. Study medication must be permanently discontinued in patients for whom an anaphylactic reaction is assessed as related to the study medication.

Laboratory testing: Obtain blood sample for tryptase, total IgE, and ADA antidrug antibodies (ADA) ideally within 15 minutes to 3 hours after the onset of a suspected anaphylactic reaction; however, up to 6 hours is acceptable. An additional blood sample to assess tryptase, total IgE, and ADA should be obtained between 1 to 2 weeks from onset of event. Local laboratory may be used to analyze samples; however, parallel samples should be sent to the central laboratory for analysis. Sample collection and shipping instructions are included in the Laboratory Manual.

Reporting: The PI or designee must notify the sponsor or designee within 24 hours of the occurrence of a suspected case of anaphylactic reaction or being informed of the case as required for AEs of Clinical Interest (AECI) and SAEs, per AE reporting requirements (Section 7.5.6.1, Section 7.5.6.2 and 7.5.6.3).

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center, or the home healthcare professional, will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. In cases where ALN-AS1 is administered at home by a patient/caregiver, dosing may be prepared and administered by the patient/caregiver according to procedures detailed in the Patient/Caregiver Storage and Administration Instructions. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

Additional storage and preparation details are provided in the Pharmacy Manual and the Patient/Caregiver Storage and Administration Instructions

6.2.5. Packaging and Labeling

All packaging, labeling, and production of study drug will be in compliance with current Good Manufacturing Practice specifications, as well as applicable local regulations. Study drug labels and external packaging will include all appropriate information as per local labeling requirements.

Additional details will be available in the Pharmacy Manual.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.
Further instructions about drug accountability are detailed in the Pharmacy Manual.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

Patients with porphyria could have altered hepatic heme synthesis, and treatment with ALN-AS1 could also modulate this pathway and secondarily impact CYP enzyme activity. Results from a DDI study in AIP patients who are ASHE are presented in Section 1.6.4. The DDI study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in activity in some of the CYP enzymes resulting in corresponding low to moderate increase in the plasma levels of drugs that are metabolized by these CYP enzymes. Based on the moderate decrease in CYP2D6 or CYP1A2 activity, investigators will review all concomitant medications that are primarily metabolized by these enzymes and monitor the patient's clinical response to these medications during the study. Medications metabolized primarily by CYP2D6 and CYP1A2 with a narrow therapeutic index (ie that require regular laboratory monitoring) may need to be monitored more frequently to determine if a dose adjustment of the concomitant medication is required. For patients who require new medications while on study, selection of medications that are not primarily metabolized by CYP2D6 or CYP1A2 should be considered. Refer to the individual product's prescription information to determine if there is a need to monitor concomitant medications for differences in safety or efficacy of the medication based on reported DDIs with CYP2D6 or CYP1A2. For more detailed and up-to-date information on CYP substrates, see:
https://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm.

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study,

necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies.

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration.

Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, transdermal hormonal, or intrauterine hormone-releasing system as methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]).
- If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- Sexual abstinence, when this is in line with the preferred and usual lifestyle of the patient, is considered an acceptable method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug (defined above). Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not considered sexual abstinence and do not meet criteria for an acceptable method of birth control. As determined by the investigator, the reliability of sexual abstinence needs to be evaluated in relation to

the duration of the clinical trial and the preferred and usual lifestyle of the patient. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods if they start a heterosexual relationship during the study and continue to do so for the entire period of risk associated with the study drug (defined above).

- WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedules of study assessments are provided in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7.

Where applicable country and local regulations and infrastructure allow for home healthcare, healthcare may take place at a location other than the clinical trial site to perform study assessments including targeted physical exam/body system assessment, assessments for vital signs, and collection of blood and urine samples for safety laboratory assessments, and PD assessments, at all timepoints as specified in the Schedule of Assessments.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.(34) The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 4 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

Body weight measured on the dosing day will be used to calculate the dose of ALN-AS1 through M12. After M12, weight obtained within 6 months during a study center visit or offsite may be used for dosing calculations.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate standard 12-lead ECGs will be performed using central ECG equipment, with readings approximately 1 minute apart and recorded as specified in the Schedule of Assessments. Patients should be seated or supine for at least 5 minutes before each ECG is obtained. The electrophysiological parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB), and Fridericia corrected QT interval (QTcF).

When ECG and blood sample collection occur at the same time, ECGs should be performed before blood samples are drawn.

Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes.

In all patients receiving ≤2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 2-hour (±15 minutes) postdose readings, paired with PK timepoints (Table 4) as specified in the Schedule of Assessments.

Similarly, in all patients receiving >2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 4-hour (±20 minutes) postdose readings, paired with PK timepoints (Table 4), as specified in the Schedule of Assessments.

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

On dosing days up to the Month 18 visit, results from hematology, chemistry, and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed assessments must be reviewed to allow same day study drug administration and

additional samples for central analysis must also be collected. On dosing days from Month 19 through Month 48, a review of laboratory results within 14 days is not required.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
GGT	
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Coagulation	
Prothrombin time	International Normalized Ratio
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	
Inflammation	
C-reactive protein	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; GGT=gamma-glutamyltransferase; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.(35)

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Sample collection for patients who experience a potential anaphylactic reaction is discussed in Section 6.2.3.2.1.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg,

events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.
- Lipase >3×ULN (or >3× the baseline lipase measurement if baseline is >ULN)
- Anaphylactic Reactions. Anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). Symptoms of an anaphylactic reaction may include skin or mucosal tissue (e.g. generalized hives, pruritus, angioedema), respiratory compromise (e.g. wheezing, bronchospasm, hypoxia), reduced blood pressure or associated symptoms (e.g. syncope, hypotonia). See Section 11.3 (Table 8) for guidance on diagnosing anaphylactic reactions.(33)

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, the Sponsor or its designee should be notified within 24 hours using a supplemental AEs of clinical interest (AECI) eCRF. Additional clinical and laboratory information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance

electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.(36) In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

7.8. COVID-19 Data Collection

Information on the coronavirus disease 2019 (COVID-19) infection status of the patient, if known, and other information on the impact of the COVID-19 pandemic on the patient's participation in the study will be collected.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria

attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

Additional data summaries to help understand any impact of the COVID-19 pandemic on efficacy, PK, PD, and safety assessments will be performed, as appropriate.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

The Investigator will inform the patient/legal guardian if new information becomes available that may be relevant to the patient's/legal guardian's willingness to continue participation in the study. Communication of this information should be documented.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all patient materials for the study (except those that support the need to remove an apparent immediate hazard to the patient). The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Serious Breach of Protocol

Investigators must notify the Medical Monitor within 24 hours of becoming aware of a potential serious breach of the protocol. A serious breach is a breach that is likely to affect to a significant degree the safety and rights of a study participant or the reliability and robustness of the data generated in the clinical trial.

9.1.4. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' confidentiality will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.5. End of the Study

The end of the study is defined as last patient last visit.

9.1.6. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study

must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010;375(9718):924-37.

6. Floderus Y. Variations in Porphobilinogen and 5-Aminolevulinic Acid Concentrations in Plasma and Urine from Asymptomatic Carriers of the Acute Intermittent Porphyria Gene with Increased Porphyrin Precursor Excretion. Clinical Chemistry. 2006;52(4):701-7.

7. Anderson K. Disorders of Heme Biosynthesis. 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transplantation. 2012;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach JC. Acute Intermittent Porphyria: From Clinical to Molecular Aspects. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF, British, et al. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. PanhematinPI. Panhematin Prescribing Information. 2017.

17. NormosangSmPC. Normosang Summary of Product Characteristics. 2017.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013;36(5):849-57.

26. Anderson KE, Egger NG, Goeger DE. Heme arginate for prevention of acute porphyric attacks. Acta Haematologica. 1997;98:120.

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002;48(11):1891-900.

31. Ryu JY, Song IS, Sunwoo YE, Shon JH, Liu KH, Cha IJ, et al. Development of the "Inje cocktail" for high-throughput evaluation of five human cytochrome P450 isoforms in vivo. Clin Pharmacol Ther. 2007;82(5):531-40.

32. Williams D, Tao X, Zhu L, Stonier M, Lutz JD, Masson E, et al. Use of a cocktail probe to assess potential drug interactions with cytochrome P450 after administration of belatacept, a costimulatory immunomodulator. Br J Clin Pharmacol. 2017;83(2):370-80.

33. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. Ann Emerg Med. 2006;47(4):373-80.

34. Atkinson TM, Mendoza TR, Sit L, Passik S, Scher HI, Cleeland C, et al. The Brief Pain Inventory and its "pain at its worst in the last 24 hours" item: clinical trial endpoint considerations. Pain Med. 2010;11(3):337-46.

35. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

36. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 4 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 4: Pharmacokinetic Time Points

Study Day		Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days		Predose (within 60 minutes before dosing)	X
		02:00 (±15 minutes)[b]	X
		06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days		Anytime during visit	X
Month 1 (Day 31) ±7 days,[a]		Predose (within 60 minutes before dosing)	X
Month 3 (Day 91) ±7 days,		02:00 (±15 minutes)[b]	X
Month 6 (Day 181) ±7 days, Month 9 (Day 271) ±10 days[d], Month 15 (Day 451) ±10 days[d], Month 18 (Day 541) ±10 days Month 24 (Day 721) ±10 days		04:00 (±20 minutes)[c]	

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.
a At the Day 31, Month 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.
b Only in patients receiving ≤2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
c Only in patients receiving >2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
d Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

			Treatment Period (Screening/Baseline through Month 18)													
Study Visit (M)	**Screening/ Baseline[a]**		**M1**		**M2**	**M3**	**M4/ M5**	**M6**	**M7/ M8[b]**	**M9**	**M10/ M11[b]**	**M12**	**M13/ M14[b]**	**M15[b]**	**M16/ M17[b]**	**M18**
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
AEs		Continuous														

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D14 ±2	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1 ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days up to the Month 18 visit,results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed predose assessments may be used for review in order to allow same day study drug administration, and additional samples for central analysis must also be collected.
i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1. After M12, weight measured at either the previous study center visit or on current study center visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.
p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 6: Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
	M19/ M20[a]	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36**
Study Visit (D±Visit Window)	**571±14/601±14**	**631±14**	**661±14/691±14**	**721±14**	**751±14/781±14**	**811±14**	**841±14/871±14**	**901±14**	**931±14/961±14**	**991±14**	**1021±14/1051±14**	**1081±14**
Physical Examination[b]				X				X				X
Body Weight, BMI, and Height[c]				X				X				X
Vital Signs[d]	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[e]												
Clinical Laboratory Assessment[f]		X		X		X		X		X		X
Pregnancy Test[g]	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[h]	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[i]		X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[j]				X				X				X
Blood Sample for PK Analysis[k]				X								
Antidrug Antibodies[l]				X								X

Table 6: Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
	M19/ M20[a]	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36**
Study Visit (D±Visit Window)	**571±14/601±14**	**631±14**	**661±14/691±14**	**721±14**	**751±14/781±14**	**811±14**	**841±14/871±14**	**901±14**	**931±14/961±14**	**991±14**	**1021±14/1051±14**	**1081±14**
EQ-5D-5L Questionnaire[m]				X				X				X
Diary Review (including BPI-SF)[n]	X											
AEs	Continuous											
Concomitant Medications	Continuous											

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.
- In situations where a study visit cannot be completed at the study center or offsite by a home healthcare professional visit, the study Investigator (or delegate) may verbally contact the patient within the study visit window to assess for any AEs, concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. Targeted physical examinations/body system assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow. See Section 7.5.3 for details on the physical examination.
c Height will be measured at Screening/Baseline only.
d Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Vital sign measurements may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.
e Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details. With the implementation of Amendment 7, ECGs are no longer required.
f Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. Collection of blood and urine samples for safety laboratory assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.
g Urine pregnancy tests will be performed. Results must be available before dosing.
h ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight obtained within 6 months during a study center visit or offsite will be used to calculate the weight-based dose of ALN-AS1.
i Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose. Collection of samples for PD assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.
j On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
k Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
l On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose. Antidrug antibody assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit.
m In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible. In situations where a planned study visit cannot be completed at the study center, the patient will complete the EQ-5D-5L questionnaire at home.
n Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

Table 7: Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M37/ M38[a]**	**M39[a]**	**M40/ M41[a]**	**M42**	**M43/ M44[a]**	**M45[a]**	**M46/ M47[a]**	**M48/ EOT**	**M49**	
Study Visit (D±Visit Window)	**1111±14/1141±14**	**1171±14**	**1201±14/1231±14**	**1261±14**	**1291±14/1321±14**	**1351±14**	**1381±14/1411±14**	**1441±14**	**1471±14**	
Physical Examination[d]				X				X	X	X
Body Weight, BMI, and Height[e]				X				X	X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]										
Clinical Laboratory Assessment[h]		X		X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X	X	
Antidrug Antibodies[m]								X	X	

Table 7: Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M37/ M38[a]**	**M39[a]**	**M40/ M41[a]**	**M42**	**M43/ M44[a]**	**M45[a]**	**M46/ M47[a]**	**M48/ EOT**	**M49**	
Study Visit (D±Visit Window)	**1111±14/1141±14**	**1171±14**	**1201±14/1231±14**	**1261±14**	**1291±14/1321±14**	**1351±14**	**1381±14/1411±14**	**1441±14**	**1471±14**	
EQ-5D-5L Questionnaire[n]				X				X	X	
Diary Review (including BPI-SF)[o]	X									
AEs	Continuous									
Concomitant Medications	Continuous									

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS=End of Study; EOT=End of Treatment; ET=early termination; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.
- In situations where a study visit cannot be completed at the study center or offsite by a home healthcare professional visit, the study Investigator (or delegate) may verbally contact the patient within the study visit window to assess for any AEs, concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled doses ALN-AS1 through Month 48 will return for an end of study (EOS) visit at Month 49. Patients who discontinue treatment prior to Month 48 will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return 3 months (84 [+14] days) after their last dose of ALN-AS1 for a safety-follow-up visit.

c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+14 days]) following the last dose of ALN-AS1.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. Targeted physical examinations/body system assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Vital sign measurements may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details. With the implementation of Amendment 7, ECGs are no longer required.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. Collection of blood and urine samples for safety laboratory assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

i Urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight obtained within 6 months during a study center visit or offsite will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose. Collection of samples for PD assessments may be conducted offsite at all time points, where applicable country and local regulations and infrastructure allow.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose. Antidrug antibody assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit.

n In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible. In situations where a planned study visit cannot be completed at the study center, the patient will complete the EQ-5D-5L questionnaire at home.

o Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. Anaphylactic Reactions

Table 8: Sampson Criteria for Anaphylactic Reactions

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)
   AND AT LEAST ONE OF THE FOLLOWING
   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):
   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):
   a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; *BP*, blood pressure.
*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 × age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.

Adapted from Sampson et al. 2006 (33)

ALN-AS1-002 PROTOCOL AMENDMENT 7
SUMMARY OF CHANGES DATED 29 APRIL 2020

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

1. RATIONALE FOR PROTOCOL AMENDMENT

The purpose of this protocol amendment is to incorporate Urgent Safety Measures (USMs) that were communicated to investigators in a Dear Investigator Letter to assure the safety of study participants while minimizing risks to study integrity amid the COVID-19 pandemic. These changes are in line with guidance from both the European Medicines Agency and the United States Food and Drug Administration on the conduct of clinical trials during the COVID-19 pandemic.[EMA 2020; FDA 2020]

The USM modifications and new procedures are outlined below, and a detailed summary of the specific protocol changes is provided in Section 2. These changes should be adopted immediately per the Dear Investigator Letter dated 07 April 2020.

- **ALN-AS1 (givosiran) Dosing Outside the Study Center by Patient or Caregiver**

  Following appropriate training on ALN-AS1 administration and the use of epinephrine (epi pen or equivalent), dosing will be permitted at a location other than the study center (eg, at home) by the patient or caregiver at all time points under the oversight of the Investigator and following consultation with the medical monitor. This measure is intended to remain in effect only during periods of time when the COVID-19 pandemic impedes the ability of patients to travel to the study site or healthcare professionals to go to patients' homes for dosing.

  ALN-AS1-002 is a dedicated open-label extension study. One patient in the ALN-AS1-002 study had an anaphylactic reaction within minutes of receiving her 3rd dose of givosiran and was successfully treated with epinephrine. This patient discontinued from the study. To date, all current study patients have been stable on treatment with givosiran for a median duration of 36 months, with monthly dosing occurring both at the study site and offsite by home healthcare professionals. Detailed instructions and procedures related to administration of givosiran by a patient or caregiver will be provided in the Patient/Caregiver Storage and Administration Instructions.

- **Study Visit Window**

  Except for assessments with other specified timing requirements, study assessments and dosing are to be performed within a visit window of ±14 days (previously ±7 days). Study drug doses must be administered at least 14 days apart, and this is expected to be tolerated based on safety data from the Phase 1 study (ALN-AS1-001), which evaluated dosing at 5.0 mg/kg monthly (twice the therapeutic dose of 2.5◦mg/kg monthly).

- **Assessments of Adverse Events, Concomitant Medications (including hemin use), Hospitalizations/Procedures, and Porphyria Attacks**

  In situations where a study visit cannot be completed at the study center or offsite by a healthcare professional visit, the study Investigator (or delegate) may verbally contact the patient within the study visit window to assess for any adverse events (AEs), concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.

- **Time Period to Obtain Weight for Dose Determination**

  Dose will be based on weight obtained within 6 months (previously at the current or prior study visit) prior to dosing. Dosing weight may be collected during clinical study center visits or offsite.

  Given the expanded use of offsite administration, use of a prior weight permits accurate determination of dose and volume without manual calculations or verbal orders. All patients are adult; therefore, substantial fluctuations in body weight over a 6-month period are not expected. Prior analyses of the study data have shown mean body weight to be stable over time.

- **EQ-5D-5L Assessments**

  In situations where a planned study visit cannot be completed at the study center, the patient will complete the 5-level EQ-5D (EQ-5D-5L) questionnaire at home.

- **Assessment of ECG**

  Further assessment of ECG is no longer warranted, because no clinically significant ECG findings, including QT interval prolongation, have been observed following comprehensive analyses across ALN-AS1-002 and the Phase 3 study ALN-AS1-003 to date. To minimize exposure to COVID-19, decrease the burden on patients and trial sites, and align with the US and EU prescribing information, which do not require ECG assessment for dosing, ECGs are no longer required in this study. For further information, refer to the givosiran (ALN-AS1) Investigator's Brochure.

- **Assessments Required to be Performed at Study Center Visits**

  Where applicable country and local regulations and infrastructure allow for home healthcare, healthcare may take place at a location other than the clinical trial site to perform study assessments including targeted physical exam/body system assessment, assessments for vital signs, and collection of blood and urine samples for safety laboratory assessments, and PD assessments, at all timepoints as specified in the Schedule of Assessments.

- **Antidrug Antibody Assessments Required to be Performed at Study Center Visits**

  Given the expanded use of offsite dosing, antidrug antibody (ADA) assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit (previously at the clinical study center visit).

The incidence of ADAs due to ALN-AS1 has been very low across clinical studies of ALN-AS1. There have been no treatment-emergent ADAs in the current study. In the Phase 3 study ALN-AS1-003, there has been only 1 case of treatment-emergent ADAs due to ALN-AS1. The antibody titer was low and transient, with the patient subsequently testing negative. Based on the collective evidence to date, ALN-AS1 is considered to have a very low risk of eliciting an immune response.

- **Collection of Information Related to COVID-19**

  Information related to the impact of the COVID-19 pandemic on patient participation in the study will be collected for each patient. Additional information regarding collection of this information, including completion of a new case report form specific to COVID-19, will be provided separately.

  This change is implemented to enable analysis of the impact of the COVID-19 global pandemic on clinical trial data.

- **Updates to Study Administration**

  Text was updated to provide clarification on Investigator responsibilities regarding communication of new study information to patients and Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs).

References:

FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/fda-guidance-conduct-clinical-trials-medical-products-during-covid-19-pandemic

Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, Version 1.0 (20/03/2020). https://www.ema.europa.eu/en/news/guidance-sponsors-how-manage-clinical-trials-during-covid-19-pandemic

2. PROTOCOL AMENDMENT 7 DETAILED SUMMARY OF CHANGES

The primary section(s) of the protocol affected by the changes in Protocol Amendment 7 are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~ and added text is indicated by **bold** font, relevant to each purpose described.

Purpose: To expand the use of offsite administration to include ALN-AS1 dosing by the patient or caregiver.

The primary change occurs in Section 6.2.2, Dose and Administration

Revised text:

2nd paragraph

~~ALN-AS1 will be administered by a qualified and authorized health care professional trained in the recognition and management of anaphylactic reactions.~~ The study drug should be injected into the abdomen or upper arms or thighs. Detailed instructions for study drug administration are presented in the Pharmacy Manual. As is consistent with good medical practice for subcutaneous drug administration, patients will be observed for a minimum of 20 minutes after each injection. Treatment for anaphylactic reactions should be readily available where patients are being dosed, and follow country and/or local hospital treatment guidelines as shown in Table 8 (33).

3rd paragraph

ALN-AS1 will be administered by a qualified and authorized health care professional trained in the recognition and management of anaphylactic reactions. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), study drug administration may be conducted at a location other than the study center by a home healthcare professional, where applicable country and local regulations and infrastructure allow, after consultation with the medical monitor, during particular study visits, as specified in the Schedules of Assessments**. If the patient is unable to come to the study site, and a visit by a home healthcare professional is not possible due to circumstances related to the COVID-19 pandemic, ALN-AS1 may be administered by the patient or the caregiver under the oversight of the Investigator, and following consultation with the medical monitor, as allowed by applicable country and local regulations. In such cases, the patient or caregiver must receive appropriate training on ALN-AS1 administration and the use of epinephrine (epi pen or equivalent) prior to dosing. This measure is intended to remain in effect only during periods of time when the COVID-19 pandemic impedes the ability of patients to travel to the study site or healthcare professionals to go to patients' homes for dosing.** However, study drug administration at the study center should be considered for patients who have ongoing study drug-related AEs or known risk factors for developing anaphylactic reaction, including but not limited to: a prior history of anaphylactic reaction to food, medications or due to unknown etiology, worsening injection site reactions with repeat dosing, or anyone in the

opinion of the investigator that would benefit from clinical observation following dosing. Patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

Last paragraph:

Detailed instructions for study drug administration are found in the Pharmacy Manual**. In addition, instructions and procedures related to administration of ALN-AS1 by a patient or caregiver will be provided in the Patient/Caregiver Storage and Administration Instructions.**

Section(s) also reflecting this change:

- Synopsis
- Section 4.1
- Section 6.2.4

Purpose: To expand the study visit windows.

The primary changes occur in Table 6, Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen), and Table 7, Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen).

Revised text: In Tables 6 and 7, the visit window was changed to **±14 days** for all visits (previously either ±7 or ±10 days).

Section(s) also reflecting this change:

- Section 4.1
- Section 5.3.1
- Section 5.3.2

Purpose: To allow the study Investigator (or delegate) to verbally contact the patient within the study visit window to assess for any AEs, concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.

The change is added as a third bullet point following Table 6, Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen), and Table 7, Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen).

Added text:

- **In situations where a study visit cannot be completed at the study center or offsite by a home healthcare professional visit, the study Investigator (or delegate) may verbally contact the patient within the study visit window to assess for any AEs, concomitant medications (including hemin use), hospitalizations/procedures, and porphyria attacks.**

Purpose: To increase the window for obtaining body weight for dose determination.

The change primarily occurs in Section 7.5.2, Weight and Height, 2nd paragraph

Revised text: Body weight measured on the dosing day will be used to calculate the dose of ALN-AS1 through M12. After M12, weight **obtained within 6 months during a** ~~measured at either the previous study center visit or current~~ study center visit **or offsite** may be used for dosing calculations.

Section(s) also reflecting this change:

- Table 6, footnote h
- Table 7, footnote j

Purpose: To allow for remote completion of the EQ-5D-5L questionnaires.

The changes occur in Table 6, Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen), and Table 7, Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen).

Revised text:

Table 6, footnote m

[m] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible. **In situations where a planned study visit cannot be completed at the study center, the patient will complete the EQ-5D-5L questionnaire at home.**

Table 7, footnote n

[n] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible. **In situations where a planned study visit cannot be completed at the study center, the patient will complete the EQ-5D-5L questionnaire at home.**

Purpose: To remove the requirement for ECGs.

The changes occur in Table 6, Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen), and Table 7, Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen).

Revised text:

Table 6, footnote e

[e] Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details. **With the implementation of Amendment 7, ECGs are no longer required**.

Table 7, footnote g

[g] Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details. **With the implementation of Amendment 7, ECGs are no longer required**.

Purpose: To allow certain study visit assessments to be performed offsite by a health care professional and provide a window for safety laboratory assessments prior to dosing by the patient or caregiver.

The change occurs in Section 7, Study Assessments.

Revised text: The schedules of study assessments are provided in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7.

Where applicable country and local regulations and infrastructure allow for home healthcare, healthcare may take place at a location other than the clinical trial site to perform study assessments including targeted physical exam/body system assessment, assessments for vital signs, and collection of blood and urine samples for safety laboratory assessments, and PD assessments, at all timepoints as specified in the Schedule of Assessments.

Other section(s) also reflecting these changes:

- Table 6, footnotes b, d, f, and i
- Table 7, footnotes d, f, h, and k

- Section 7.5.5

Purpose: To broaden the window for ADA assessments required to be performed at study center visits.

The changes occur in Table 6, Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen), and Table 7, Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen).

Revised text:

Table 6, footnote l

[l] On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose. **Antidrug antibody assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit.**

Table 7, footnote m

[m] On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose. **Antidrug antibody assessments that cannot be collected in the intended visit window may be completed within 6 months at the next study center visit.**

Purpose: To collect information related to the impact of the COVID-19 pandemic on patient participation in the study.

The primary change occurs in a newly added Section 7.8, COVID-19 Data Collection.

Added text:

7.8 COVID-19 Data Collection

Information on the coronavirus disease 2019 (COVID-19) infection status of the patient, if known, and other information on the impact of the COVID-19 pandemic on the patient's participation in the study will be collected.

Section(s) also reflecting this change:

- Section 8.2.9

Purpose: To provide clarification on Investigator responsibilities regarding communication of new study information to patients and IRB/IECs.

These changes occur in Section 9.1.1, Informed Consent, and Section 9.1.2, Ethical Review.

Added text:

Section 9.1.1, 4th paragraph

The Investigator will inform the patient/legal guardian if new information becomes available that may be relevant to the patient's/legal guardian's willingness to continue participation in the study. Communication of this information should be documented.

Revised text:

Section 9.1.2, 2nd paragraph

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all ~~advertising used to recruit patients~~ **patient materials** for the study **(except those that support the need to remove an apparent immediate hazard to the patient)**. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1 (givosiran)
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016 Protocol Amendment 3 03 February 2017 Protocol Amendment 4 02 August 2017 Protocol Amendment 5 03 May 2018 Protocol Amendment 6 28 May 2019
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD [redacted]

29 MAY 2019

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1 (givosiran)

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

- Characterize the pharmacokinetic (PK) profile of ALN-AS1 and incidence of antidrug antibodies (ADA) over time
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen, including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

Reference Therapy, Dose, and Mode of Administration

Not applicable

Duration of Treatment and Study

The duration of treatment is up to 48 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 56 months.

Statistical Methods

Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation.

Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study.

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X	X[a]	X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1. On Day 1, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the

Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided

that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through Month 36 (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Physical Examination[b]				X				X				X
Body Weight, BMI, and Height[c]				X				X				X
Vital Signs[d]		X		X		X		X		X		X
Triplicate 12-Lead ECG[e]				X								
Clinical Laboratory Assessment[f]		X		X		X		X		X		X
Pregnancy Test[g]		X		X		X		X		X		X
Study Drug Administration[h]		X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[i]		X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[j]				X				X				X
Blood Sample for PK Analysis[k]				X								
Antidrug Antibodies[l]				X								X
EQ-5D-5L Questionnaire[m]				X				X				X

Table 2: Schedule of Assessments: Month 19 through Month 36 (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Diary Review (including BPI-SF)[n]	X											
Phone Contact[o]	X		X		X		X		X		X	
AEs	Continuous											
Concomitant Medications	Continuous											

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

c Height will be measured at Screening/Baseline only.

d Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

e Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients

receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.

f Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

g Urine pregnancy tests will be performed. Results must be available before dosing.

h ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

i Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

j On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

k Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

l On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

m In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

n Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

o Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

Table 3: Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen)

Study Visit (M)	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
	M37/ M38[a]	M39[a]	M40/ M41[a]	M42	M43/ M44[a]	M45[a]	M46/ M47[a]	M48/ EOT	M49	
Study Visit (D±Visit Window)	1111±7/1141±7	1171±10	1201±7/1231±7	1261±10	1291±7/1321±7	1351±10	1381±7/1411±7	1441±10	1471±10	
Physical Examination[d]				X				X	X	X
Body Weight, BMI, and Height[e]				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]				X					X	
Clinical Laboratory Assessment[h]	X	X	X	X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X	X	
Antidrug Antibodies[n]				X				X	X	
EQ-5D-5L Questionnaire[o]				X				X	X	

Table 3: Schedule of Assessments: Month 37 through End of Study (Every 3 Months Dosing Regimen)

Study Visit (M)	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
	M37/ M38[a]	M39[a]	M40/ M41[a]	M42	M43/ M44[a]	M45[a]	M46/ M47[a]	M48/ EOT	M49	
Study Visit (D±Visit Window)	1111±7/1141±7	1171±10	1201±7/1231±7	1261±10	1291±7/1321±7	1351±10	1381±7/1411±7	1441±10	1471±10	
Diary Review (including BPI-SF)[p]	X									
Phone Contact[q]	X		X		X		X			
AEs	Continuous									
Concomitant Medications	Continuous									

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; ET = Early Termination; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled treatment with ALN-AS1 through Month 48 will return for an end of study (EOS) visit at Month 49. Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+7] days) after his/her last dose of ALN-AS1.

c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+7 days]) following the last dose of ALN-AS1.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
DDI	Drug-drug interaction
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate

Abbreviation	Definition
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.(1) The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.(2) In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.(3, 4)

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.(5) The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.(6)

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.(2) However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.(7-10) AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.(11) It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.(12) AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).(2) The initial diagnosis involves demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA

(typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.(6-8) Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.(7)

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.(13) In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.(14) With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.(15)

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.(16, 17) In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.(18-22) Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.(22-24) In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.(25) While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).(26) Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).(5) In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.(27) In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option. Given the significant morbidity and mortality, there remains a significant unmet need for

an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.(28) Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.(29) The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 (givosiran) comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have

higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.(30) For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related serious adverse events (SAEs), severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Clinical Data Summary

1.6.1. Clinical Pharmacodynamics

Dosing has been completed in Part A and Part B of the study. In both Parts A and B of the study, durable and dose-dependent reductions of ALAS1 mRNA (up to 66% in the 2.5 mg/kg dose group) as measured by cERD were observed after ALN-AS1 treatment. ALAS1 reductions were highly correlated with reductions in ALA (mean maximal 86%) PBG (mean maximal 95%) levels in a dose-dependent manner and were sustained for ≥180 days.

Dosing is currently ongoing in Part C of the study. The mean maximal reduction in ALAS1 mRNA-relative to baseline in ALN-AS1-treated patients in Cohort 1 (2.5 mg/kg Q3M) was 39% and in Cohort 2 (2.5 mg/kg Q1M) 66%. All patients treated with ALN-AS1 also had decreases in their peak ALA and PBG levels in the treatment period compared to the run-in period, along with reduced fluctuations in these levels in the treatment period (data not shown). No reductions were observed in the placebo-treated patients.

1.6.2. Clinical Activity

Clinical activity was not evaluated in Parts A and B of the study because ASHE patients included in this study were not actively experiencing attacks of porphyria.

Dosing is currently ongoing in Part C of the study. All patients in Cohorts 1 and 2 were enrolled in a run-in phase for 10-15 weeks that was followed by a treatment phase for up to 24 weeks. No investigational product was administered during the run-in phase. Therefore, each patient acted as their own control, allowing for individual ALA / PBG levels, and overall attack rate while on ALN-AS1 treatment to be compared to that observed in the run-in phase.

Figure 1 shows in Cohort 1 ALN-AS1-treated patients there was a 74% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 63-94% reduction), compared with a 23% decrease in the annualized attack rate for the placebo-treated patient. In ALN-AS1-treated patients this was accompanied by an approximate 76% mean decrease in annualized acute hemin usage (range 52-95%) and a 10.3 times increase in the maximal attack free interval compared to run-in period (range 4.2-16.3).

Figure 1: Part C Cohort 1 Summary of Treatment Period Clinical Efficacy Relative to Run-in

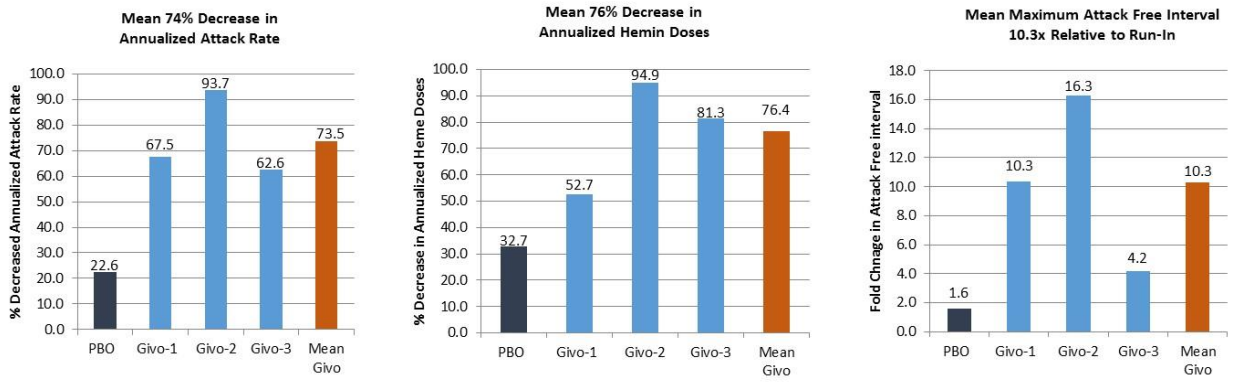
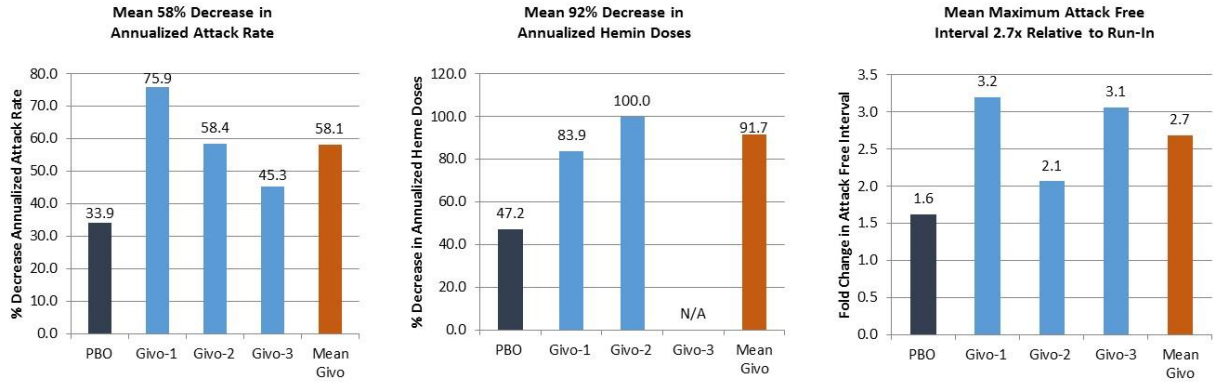


Figure 2 shows in Cohort 2 ALN-AS1-treated patients, there was a 58% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 45-76% reduction). This compares favorably with data from the placebo-treated patient who showed a 34% reduction in the annualized attack rate for the treatment period versus the run-in period. In ALN-AS1-treated patients, the reduction in attack rate was accompanied by a 92% mean decrease in annualized hemin usage (range 84-100%) and a 2.7x increase in the mean maximal attack free interval compared to run-in period (range 2.1-3.2).

Figure 2: Part C Cohort 2 Summary of Treatment Period Clinical Efficacy Relative to Run-in



1.6.3. Clinical Safety

In Part A and Part B of Study ALN-AS1-001, a total of 11 patients (11/13; 85.0%) who received ALN-AS1 reported at least 1 AE. All 5 placebo-treated patients (5/5; 100%) reported at least 1 AE. AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients (5/13; 38%): diarrhoea, dyspepsia, hematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (each AE reported only by an individual patient; 1/13; 8% each). There were 2 SAEs of abdominal pain: 1 patient each in the 0.035 mg/kg and 0.1 mg/kg ALN-AS1 dose groups. Both were considered to be not related and resolved without sequelae. There were no AEs leading to discontinuation. All AEs were mild or moderate in severity, except for 1 of the SAEs of abdominal pain (0.10 mg/kg dose group), which was considered severe. Adverse events related to abnormal laboratory values were seen in 1 patient in the 0.35 mg/kg ALN-AS1 dose group who had AEs of ALT increased and AST increased, which were moderate and considered by the Investigator to be unrelated to ALN-AS1. No clinically significant findings were observed with routine monitoring of CRP and a panel of 9 proinflammatory cytokines collected predose and up to 24 hours post-dose. There were no other clinically significant laboratory abnormalities related to study drug or changes in vital signs, or ECGs in patients who were administered ALN AS1 or placebo.

Dosing is ongoing in Part C of the study. All patients who received ALN-AS1 in cohort 1 and 2 reported at least 1 AE. AEs that were reported in at least 2 subjects were nausea in 3 patients (50%) and abdominal pain, vomiting, nasopharyngitis, headache, cough and oropharyngeal pain in 2 patients (33.3%) each. All AEs were mild or moderate in severity. AEs considered possibly or definitely related to ALN-AS1 were reported in 4 patients (66.6%), 1 each: renal impairment in Cohort 1 and injection site reaction, myalgia, and headache in Cohort 2. Both placebo-treated patients reported AEs. No AE was experienced in more than 1 patient.

In Part C, Cohort 1 or 2, there were no SAEs or AEs leading to discontinuation in patients administered ALN-AS1 or placebo. No clinically significant laboratory abnormalities related to study drug or changes in vital signs, ECG, or physical exam findings were observed in patients administered ALN-AS1 or placebo.

Part C Cohort 3 5.0 mg/kg SC monthly dosing is ongoing. In this cohort there was one fatal SAE of acute pancreatitis, which was determined to be unlikely related to study drug or placebo

by the Investigator due to the presence of gall bladder sludge found at the time of her presentation. Contributors to this patient's adverse clinical course included: pre-existing chronic debilitation from recurrent AIP attacks requiring monthly hospitalization, porphyria-attributed quadriplegia requiring nursing home care, delay in hospital admission and complications from thromboembolism (multiple risk factors included pancreatitis, obesity, immobilisation from quadriparesis and recent hospitalization for infected portacath removal). Serum lipase was added to all scheduled laboratory assessments in November 2016 as part of additional safety monitoring. To date, all lipase results (available in 11 of 15 subjects) have been at or below the upper limit of normal with no trends seen with dosing of study drug or placebo.

Further information on the safety, efficacy, PK and PD of ALN-AS1 are available in the Investigator's Brochure.

1.6.4. Drug-Drug Interactions

An open-label drug-drug interaction (DDI) study (ALN-AS1-004) was conducted in AIP patients who are ASHE to evaluate the effect of a single dose of 2.5 mg/kg ALN-AS1 administered SC on the pharmacokinetics of probe substrates for 5 major CYP enzymes that account for the metabolism of approximately 80% of prescribed drugs.(31, 32) Results from this study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in the metabolic activity of some of the 5 CYP enzymes studied, thereby leading to higher concentrations of some substrates and their metabolites. Treatment with ALN-AS1 resulted in an approximately 3-fold increase in exposure (as measured by AUC) of caffeine (a sensitive substrate for CYP1A2) and an approximately 2-fold increase in exposure of dextromethorphan (a sensitive substrate for CYP2D6). Exposure of midazolam (a sensitive substrate for CYP3A4) and omeprazole (a sensitive substrate for CYP2C19) increased less than 2-fold after treatment with ALN-AS1. There was no effect of ALN-AS1 treatment on losartan (a sensitive substrate for CYP2C9).

1.7. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical finding of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

The important potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and

close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.

- Pancreatitis: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.
- Liver transaminase abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of liver function test (LFT) abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Anaphylactic reactions: There is a potential risk of developing a severe allergic reaction with ALN-AS1 administration. In the present study, one patient in the 2.5 mg/kg monthly dose group with a history of asthma and multiple allergies experienced an SAE of anaphylactic reaction that was determined by the Investigator to be definitely related to study drug given the temporal relationship of ALN-AS1 treatment to the onset of the reaction (within minutes). The patient was treated and recovered and was discontinued from the study. Guidance on dose administration and monitoring for anaphylactic reactions has been included in this protocol.

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including

ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7 in Appendix Section 11.2). Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

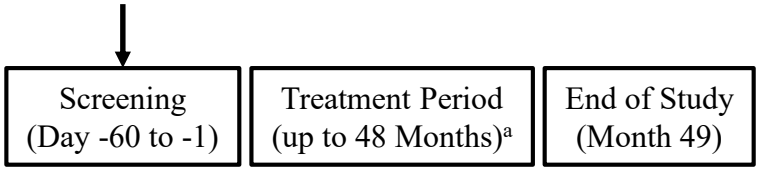
Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 49. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria (see Section 5.3) or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

All patients are asked to participate in a Posttreatment Follow-up visit after they have received their last dose of ALN-AS1.

Figure 3: Study Design



[a] Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+ 7] days) after their last dose of ALN-AS1.

4.2. Duration of Treatment and Study

The duration of treatment is up to 48 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 56 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening

3. Women of child-bearing potential (WOCBP) must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Discontinuation of Study Drug and/or Study

Patients or their legal guardians (in the case that the patient is a minor) are free to discontinue treatment and/or study or withdraw their consent at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or of the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient from the study at any time if this is considered to be in the patient's best interest.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively. If a patient stops participation from the study or withdraws consent from the study, he/she will not be able to re-enroll in the study.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). A positive urine pregnancy test should be confirmed by a serum pregnancy test prior to discontinuing the study drug.

If a patient discontinues dosing due to a serious adverse event (SAE), the SAE should be followed as described in Section 7.5.6. When a patient discontinues study drug dosing, the primary reason must be recorded in the electronic case report form (eCRF). Patients who discontinue study drug and remain on study may receive treatment consistent with local standard practice for their disease per Investigator judgement, as applicable.

Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+ 7] days) after their last dose of ALN-AS1.

5.3.2. Discontinuation from the Study

A patient or their legal guardian may decide to stop the patient's participation in the study at any time. Patients considering to stop the study should be informed that they can discontinue treatment and complete study assessments including follow-up, as per the SOA, or alternatively may complete any minimal assessments for which the patient consents. The Investigator may withdraw a patient at any time if this is considered to be in the patient's best interest.

However, study integrity and interpretation is best maintained if all randomized patients continue study assessments and follow-up. Stopping study participation could mean:

- If a patient discontinues from the study, he/she will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+7] days) after his/her last dose of ALN-AS1.
- A patient can stop taking the study drug and stop study-related visits, but allow the investigator and study team to review the patient's medical records, public records or be contacted in order to receive information about the patient's health

When a patient stops the study, the discontinuation and reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report the observations as thoroughly as possible. If a patient stops the study due to an adverse event (AE), including an SAE, the AE should be followed as described in Section 7.5.6.

If the patient wants to stop participation in the study, he/she should notify the study doctor in

writing or in any other form that may be locally required. The personal data already collected during the study, including patient's biological samples, will still be used together with the data collected on other patients in the study according to the informed consent and applicable laws.

In addition to stopping participation in the study, the patient could decide to withdraw his/her consent as explained in Section 5.3.3

5.3.3. Withdrawal of Consent to Collect and Process the Patient's Personal Data

The patient may decide to withdraw his/her consent informing the study doctor at any time in writing, or in any other form that may be locally required. This means that the patient wants to stop participation in the study and any further collection of his/her personal data.

- The sponsor will continue to keep and use a patient's study information (including any data resulting from the analysis of the patient's biological samples until time of withdrawal) according to applicable law. This is done to guarantee the validity of the study, determine the effects of the study treatment, and ensure completeness of study documentation.
- The patient can also request that collected samples be destroyed or returned (to the extent it is permitted by applicable law) at any time.
- Patients who withdraw their consent to collect and use personal data should understand that public records may be reviewed to determine the patient's survival status as allowed per local and national regulations.

In US and Japan, otherwise, samples not yet analyzed at the time of withdrawal may still be used for further testing/analysis in accordance with the terms of the protocol and of the informed consent form.

In EU and rest of world, in any event, samples not yet analyzed at the time of withdrawal will not be used any longer, unless permitted by applicable law. They will be stored or destroyed according to applicable legal requirements.

5.3.4. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1, Table 2, and Table 3). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 5, Table 6, and Table 7 in Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

ALN-AS1 will be administered by a qualified and authorized health care professional trained in the recognition and management of anaphylactic reactions. The study drug should be injected into the abdomen or upper arms or thighs. Detailed instructions for study drug administration are presented in the Pharmacy Manual. As is consistent with good medical practice for subcutaneous drug administration, patients will be observed for a minimum of 20 minutes after each injection. Treatment for anaphylactic reactions should be readily available where patients are being dosed, and follow country and/or local hospital treatment guidelines as shown in Table 8.(33)

After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), study drug administration may be conducted at a location other than the study center by a home healthcare professional, where applicable country and local regulations and infrastructure allow, after consultation with the medical monitor, during particular study visits, as specified in the Schedules of Assessments. However, study drug administration at the study center should be considered for patients who have ongoing study drug-related AEs or known risk factors for developing anaphylactic reaction, including but not limited to: a prior history of anaphylactic reaction to food, medications or due to unknown etiology, worsening injection site reactions with repeat dosing, or anyone in the opinion of the investigator that would benefit from clinical observation following dosing. Patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.3.2.1. Monitoring and Dosing Rules in Patients with Potential Cases of Anaphylactic Reaction

An anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). For reference see Section 11.3.(33)

Stop administering the study medication immediately if an anaphylactic reaction to the study medication is suspected. Study medication must be permanently discontinued in patients for whom an anaphylactic reaction is assessed as related to the study medication.

Laboratory testing: Obtain blood sample for tryptase, total IgE, and ADA antidrug antibodies (ADA) ideally within 15 minutes to 3 hours after the onset of a suspected anaphylactic reaction; however, up to 6 hours is acceptable. An additional blood sample to assess tryptase, total IgE, and ADA should be obtained between 1 to 2 weeks from onset of event. Local laboratory may be used to analyze samples; however, parallel samples should be sent to the central laboratory for analysis. Sample collection and shipping instructions are included in the Laboratory Manual.

Reporting: The PI or designee must notify the sponsor or designee within 24 hours of the occurrence of a suspected case of anaphylactic reaction or being informed of the case as required

for AEs of Clinical Interest (AECI) and SAEs, per AE reporting requirements (Section 7.5.6.1, Section 7.5.6.2 and 7.5.6.3).

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

All packaging, labeling, and production of study drug will be in compliance with current Good Manufacturing Practice specifications, as well as applicable local regulations. Study drug labels and external packaging will include all appropriate information as per local labeling requirements.

Additional details will be available in the Pharmacy Manual.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.
Further instructions about drug accountability are detailed in the Pharmacy Manual.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

Patients with porphyria could have altered hepatic heme synthesis, and treatment with ALN-AS1

could also modulate this pathway and secondarily impact CYP enzyme activity. Results from a DDI study in AIP patients who are ASHE are presented in Section 1.6.4. The DDI study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in activity in some of the CYP enzymes resulting in corresponding low to moderate increase in the plasma levels of drugs that are metabolized by these CYP enzymes. Based on the moderate decrease in CYP2D6 or CYP1A2 activity, investigators will review all concomitant medications that are primarily metabolized by these enzymes and monitor the patient's clinical response to these medications during the study. Medications metabolized primarily by CYP2D6 and CYP1A2 with a narrow therapeutic index (ie that require regular laboratory monitoring) may need to be monitored more frequently to determine if a dose adjustment of the concomitant medication is required. For patients who require new medications while on study, selection of medications that are not primarily metabolized by CYP2D6 or CYP1A2 should be considered. Refer to the individual product's prescription information to determine if there is a need to monitor concomitant medications for differences in safety or efficacy of the medication based on reported DDIs with CYP2D6 or CYP1A2. For more detailed and up-to-date information on CYP substrates, see: https://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm.

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies.

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration.

Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, transdermal hormonal, or intrauterine hormone-releasing system as methods of contraception. WOCBP using hormonal

methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]).

- If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- Sexual abstinence, when this is in line with the preferred and usual lifestyle of the patient, is considered an acceptable method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug (defined above). Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not considered sexual abstinence and do not meet criteria for an acceptable method of birth control. As determined by the investigator, the reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods if they start a heterosexual relationship during the study and continue to do so for the entire period of risk associated with the study drug (defined above).
- WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedules of study assessments are provided in Table 1, Table 2, and Table 3; and Table 5, Table 6, and Table 7.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study

(ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.(34) The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 4 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

Body weight measured on the dosing day will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured at either the previous study center visit or current study center visit may be used for dosing calculations.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate standard 12-lead ECGs will be performed using central ECG equipment, with readings approximately 1 minute apart and recorded as specified in the Schedule of Assessments. Patients should be seated or supine for at least 5 minutes before each ECG is obtained. The electrophysiological parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB), and Fridericia corrected QT interval (QTcF).

When ECG and blood sample collection occur at the same time, ECGs should be performed before blood samples are drawn.

Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes.

In all patients receiving ≤2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 2-hour (±15 minutes) postdose readings, paired with PK timepoints (Table 4) as specified in the Schedule of Assessments.

Similarly, in all patients receiving >2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 4-hour (±20 minutes) postdose readings, paired with PK timepoints (Table 4), as specified in the Schedule of Assessments.

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

On dosing days up to the Month 18 visit, results from hematology, chemistry, and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed assessments must be reviewed to allow same day study drug administration and additional samples for central analysis must also be collected. On dosing days from Month 19 through Month 48, a review of laboratory results within 14 days is not required.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
GGT	
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Coagulation	
Prothrombin time	International Normalized Ratio
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	
Inflammation	
C-reactive protein	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; GGT=gamma-glutamyltransferase; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.(35)

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Sample collection for patients who experience a potential anaphylactic reaction is discussed in Section 6.2.3.2.1.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg,

events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.
- Lipase >3×ULN (or >3× the baseline lipase measurement if baseline is >ULN)
- Anaphylactic Reactions. Anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). Symptoms of an anaphylactic reaction may include skin or mucosal tissue (e.g. generalized hives, pruritus, angioedema), respiratory compromise (e.g. wheezing, bronchospasm, hypoxia), reduced blood pressure or associated symptoms (e.g. syncope, hypotonia). See Section 11.3 (Table 8) for guidance on diagnosing anaphylactic reactions.(33)

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, the Sponsor or its designee should be notified within 24 hours using a supplemental AEs of clinical interest (AECI) eCRF. Additional clinical and laboratory information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the

protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.(36) In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Serious Breach of Protocol

Investigators must notify the Medical Monitor within 24 hours of becoming aware of a potential serious breach of the protocol. A serious breach is a breach that is likely to affect to a significant

degree the safety and rights of a study participant or the reliability and robustness of the data generated in the clinical trial.

9.1.4. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' confidentiality will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.5. End of the Study

The end of the study is defined as last patient last visit.

9.1.6. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered

into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken A-M, Poblete-Gutiérrez P, Frank J. The acute hepatic porphyrias: Current status and future challenges. Best Practice & Research Clinical Gastroenterology. 2010;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010;375(9718):924-37.

6. Floderus Y. Variations in Porphobilinogen and 5-Aminolevulinic Acid Concentrations in Plasma and Urine from Asymptomatic Carriers of the Acute Intermittent Porphyria Gene with Increased Porphyrin Precursor Excretion. Clinical Chemistry. 2006;52(4):701-7.

7. Anderson K. Disorders of Heme Biosynthesis. 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transplantation. 2012;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach JC. Acute Intermittent Porphyria: From Clinical to Molecular Aspects. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjö I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: A population-based study. Scandinavian Journal of Clinical and Laboratory Investigation. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clinics in Liver Disease. 2004;8(4):807-38.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Annals of Clinical Biochemistry. 2013;50(3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. PanhematinPI. Panhematin Prescribing Information. 2017.

17. NormosangSmPC. Normosang Summary of Product Characteristics. 2017.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013;36(5):849-57.

26. Anderson K, Egger N, Goeger D. Heme arginate for prevention of acute porphyric attacks. Acta Haematologica. 1997;98:120.

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002;48(11):1891-900.

31. Ryu JY, Song IS, Sunwoo YE, Shon JH, Liu KH, Cha IJ, et al. Development of the "Inje

cocktail" for high-throughput evaluation of five human cytochrome P450 isoforms in vivo. Clin Pharmacol Ther. 2007;82(5):531-40.

32. Williams D, Tao X, Zhu L, Stonier M, Lutz JD, Masson E, et al. Use of a cocktail probe to assess potential drug interactions with cytochrome P450 after administration of belatacept, a costimulatory immunomodulator. Br J Clin Pharmacol. 2017;83(2):370-80.

33. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. Ann Emerg Med. 2006;47(4):373-80.

34. Atkinson TM, Mendoza TR, Sit L. The Brief Pain Inventory and its “pain at its worst in the last 24 hours” item: Clinical trial endpoint considerations. Pain Medicine. 2010;11(3):337-46.

35. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

36. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 4 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 4: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±15 minutes)[b]	X
	06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days	Anytime during visit	X
Month 1 (Day 31) ±7 days,[a] Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 9 (Day 271) ±10 days[d], Month 15 (Day 451) ±10 days[d], Month 18 (Day 541) ±10 days Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±15 minutes)[b]	X
	04:00 (±20 minutes)[c]	

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

a At the Day 31, Month 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.
b Only in patients receiving ≤2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
c Only in patients receiving >2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
d. Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D14 ±2	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D14 ±2	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
AEs		Continuous														
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

			Treatment Period (Screening/Baseline through Month 18)													
Study Visit (M)	**Screening/ Baseline[a]**		**M1**		**M2**	**M3**	**M4/ M5**	**M6**	**M7/ M8[b]**	**M9**	**M10/ M11[b]**	**M12**	**M13/ M14[b]**	**M15[b]**	**M16/ M17[b]**	**M18**
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**

- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1 ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3,

Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days up to the Month 18 visit,results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed predose assessments may be used for review in order to allow same day study drug administration, and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1. After M12, weight measured at either the previous study center visit or on current study center visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 6: Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
	M19/ M20[a]	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**
Physical Examination[b]				X				X				X
Body Weight, BMI, and Height[c]				X				X				X
Vital Signs[d]	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[e]				X								
Clinical Laboratory Assessment[f]		X		X		X		X		X		X
Pregnancy Test[g]	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[h]	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[i]		X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[j]				X				X				X
Blood Sample for PK Analysis[k]				X								
Antidrug Antibodies[l]				X								X
EQ-5D-5L Questionnaire[m]				X				X				X
Diary Review (including BPI-SF)[n]	X											
AEs	Continuous											
Concomitant Medications	Continuous											

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Table 6: Schedule of Assessments: Month 19 through Month 36 (Every Month Dosing Regimen)

	Treatment Period (Month 19 through Month 36)											
	M19/ M20[a]	M21[a]	M22/ M23[a]	M24	M25/ M26[a]	M27[a]	M28/ M29[a]	M30	M31/ M32[a]	M33[a]	M34/ M35[a]	M36
Study Visit (D±Visit Window)	571±7/601±7	631±10	661±7/691±7	721±10	751±7/781±7	811±10	841±7/871±7	901±10	931±7/961±7	991±10	1021±7/1051±7	1081±10

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

c Height will be measured at Screening/Baseline only.

d Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

e Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.

f Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

g Urine pregnancy tests will be performed. Results must be available before dosing.

h ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the at the previous study center visit or current study center visit will be used to calculate the weight-based dose of ALN-AS1.
i Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
j On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
k Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
l On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
m In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
n Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

Table 7: Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M37/ M38[a]**	**M39[a]**	**M40/ M41[a]**	**M42**	**M43/ M44[a]**	**M45[a]**	**M46/ M47[a]**	**M48/ EOT**	**M49**	
Study Visit (D±Visit Window)	**1111±7/1141±7**	**1171±10**	**1201±7/1231±7**	**1261±10**	**1291±7/1321±7**	**1351±10**	**1381±7/1411±7**	**1441±10**	**1471±10**	
Physical Examination[d]				X				X	X	X
Body Weight, BMI, and Height[e]				X				X	X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]				X					X	
Clinical Laboratory Assessment[h]		X		X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X	X	
Antidrug Antibodies[m]								X	X	

Table 7: Schedule of Assessments: Month 37 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 37 through EOS)								EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M37/ M38[a]**	**M39[a]**	**M40/ M41[a]**	**M42**	**M43/ M44[a]**	**M45[a]**	**M46/ M47[a]**	**M48/ EOT**	**M49**	
Study Visit (D±Visit Window)	**1111±7/1141±7**	**1171±10**	**1201±7/1231±7**	**1261±10**	**1291±7/1321±7**	**1351±10**	**1381±7/1411±7**	**1441±10**	**1471±10**	
EQ-5D-5L Questionnaire[n]				X				X	X	
Diary Review (including BPI-SF)[o]	X									
AEs	Continuous									
Concomitant Medications	Continuous									

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS=End of Study; EOT=End of Treatment; ET=early termination; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled doses ALN-AS1 through Month 48 will return for an end of study (EOS) visit at Month 49. Patients who discontinue treatment prior to Month 48 will be asked to complete an early termination (ET) visit at which all Month 49 visit procedures will be performed; they must also return 3 months (84 [+ 7] days) after their last dose of ALN-AS1 for a safety-follow-up visit.

c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+7 days]) following the last dose of ALN-AS1.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the at the previous study center visit or current study center visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
n In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
o Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. Anaphylactic Reactions

Table 8: Sampson Criteria for Anaphylactic Reactions

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)
   AND AT LEAST ONE OF THE FOLLOWING
   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):
   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):
   a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; *BP*, blood pressure.
*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 × age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.

Adapted from Sampson et al. 2006 (33)

ALN-AS1-002 PROTOCOL AMENDMENT 6
SUMMARY OF CHANGES DATED 28 MAY 2019

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

Rationale for Protocol Amendment

The primary purpose for this protocol amendment is to provide updated information from a recently completed drug-drug interaction study (ALN-AS1-004) performed in AIP patients who are asymptomatic high excreters in the concomitant medications section (Section 6.3). The results of the study indicated that ALN-AS1 treatment resulted in moderate reduction in CYP1A2 and CYP2D6 activity, weak reduction in CYP3A4 and CYP2C19 activity, and no change in the activity of CYP2C9.

This amendment also extends the treatment period to 48 months to continue the study until ALN-AS1 is anticipated to be commercially available in the countries where the study sites are located.

Additional updates are being implemented as noted below: clarification that patients may continue to receive ALN-AS1 until it is commercially available in the patient's territory, addition of guidance for serious breaches of protocol, and deletion of Section 11.3, List of Sensitive CYP3A substrates and those with a Narrow Therapeutic Range.

A detailed summary of changes is provided in Table 1. Corrections to typographical errors, punctuation, grammar, abbreviations, and formatting (including administrative changes between protocol amendments 5 and 6) are not detailed.

Table 1: Protocol Amendment 6 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in Protocol Amendment 6 are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: Updated Section 1.6, Clinical Data Summary, with results of the open-label drug-drug interaction study (ALN-AS1-004) to support changes in Section 6.3, Concomitant Medications.

The primary change is the addition of Section 1.2.2.3, Drug-drug Interaction Study ALN-AS1-004

Added text:

1.6.4 Drug-drug Interaction Study ALN-AS1-004

An open-label drug-drug interaction (DDI) study (ALN-AS1-004) was conducted in AIP patients who are ASHE to evaluate the effect of a single dose of 2.5 mg/kg ALN-AS1 administered SC on the pharmacokinetics of probe substrates for 5 major CYP enzymes that account for the metabolism of approximately 80% of prescribed drugs.(31, 32) Results from this study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in the metabolic activity of some of the 5 CYP enzymes studied, thereby leading to higher concentrations of some substrates and their metabolites. Treatment with ALN-AS1 resulted in an approximately 3-fold increase in exposure (as measured by AUC) of caffeine (a sensitive substrate for CYP1A2) and an approximately 2-fold increase in exposure of dextromethorphan (a sensitive substrate for CYP2D6). Exposure of midazolam (a sensitive substrate for CYP3A4) and omeprazole (a sensitive substrate for CYP2C19) increased less than 2-fold after treatment with ALN-AS1. There was no effect of ALN-AS1 treatment on losartan (a sensitive substrate for CYP2C9).

Purpose: Clarify that subjects may continue until givosiran is commercially available or the givosiran development program is discontinued.

The primary change is the addition of 2 sentences to Section 4.1, Summary of Study Design.

Added text:

Patients may receive ALN-AS1 as long as they do not fulfill any of the study discontinuation criteria (see Section 5.3) or until ALN-AS1 is commercially available in the patient's territory or the ALN-AS1 development program is discontinued (whichever comes first).

All patients are asked to participate in a Posttreatment Follow-up visit after they have received their last dose of ALN-AS1.

Section(s) also containing this change:

- *Synopsis, Study Design*

Purpose: Updated concomitant medications to indicate that givosiran treatment could affect drugs that are metabolized primarily by CYP2D6 and CYP1A2.

The primary change occurs in Section 6.3, Concomitant Medications

Formerly read:

Drug-drug interaction studies in NHP, could not exclude the potential for ALN-AS1 to alter the clearance of drugs metabolized by the CYP3A enzymes. Therefore, patients on medications metabolized by CYP3A, especially those with narrow therapeutic ranges, may require monitoring for their drug levels or drug response. ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 6 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]

Now reads:

~~Drug-drug interaction studies in NHP, could not exclude the potential for ALN-AS1 to alter the clearance of drugs metabolized by the CYP3A enzymes. Therefore, patients on medications metabolized by CYP3A, especially those with narrow therapeutic ranges, may require monitoring for their drug levels or drug response. ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 6 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]~~

Patients with porphyria could have altered hepatic heme synthesis, and treatment with ALN-AS1 could also modulate this pathway and secondarily impact CYP enzyme activity. Results from a DDI study in AIP patients who are ASHE are presented in Section 1.6.4. The DDI study demonstrated that ALN-AS1 treatment resulted in weak to moderate reduction in activity in some of the CYP enzymes resulting in corresponding low to moderate increase in the plasma levels of drugs that are metabolized by these CYP enzymes. Based on the moderate decrease in CYP2D6 or CYP1A2 activity, investigators will review all concomitant medications that are primarily metabolized by these enzymes and monitor the patient's clinical response to these medications during the study. Medications metabolized primarily by CYP2D6 and CYP1A2 with a narrow therapeutic index (ie that require regular laboratory monitoring) may need to be monitored more frequently to determine if a dose adjustment of the concomitant medication is required. For patients who require new medications while on study, selection of medications that are not primarily metabolized by CYP2D6 or CYP1A2 should be considered. Refer to the individual product's prescription information to determine if there is a need to monitor concomitant medications for differences in safety or efficacy of the medication based on reported DDIs with CYP2D6 or CYP1A2. For more detailed and up-to-date information on CYP substrates, see: https://www.fda.gov/drugs/developmentapprovalprocess/developmentresources/druginteractionslabeling/ucm093664.htm.

Purpose: Extend treatment duration so that patients may continue on treatment for up to 48 months.

The primary change occurs in Section 4.2, Duration of Treatment and Study.

Formerly read: The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

Now reads: The duration of treatment is up to **48**~~36~~ months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to ~~44~~**56** months.

Section(s) also containing this change:

- *Synopsis, Duration of Treatment and Study*
- *Synopsis, Table 2 (Table 3 added)*
- *Section 4.1, Summary of Study design and Figure 3*
- *Section 5.3.1 Discontinuation of Study Drug*
- *Section 5.3.2 Discontination from the Study*

- *Section 6.2.2, Dose and Administration*
- *Section 6.3, Concomitant Medications*
- *Section 7, Study Assessments*
- *Section 7.5.5, Clinical Laboratory Assessments*
- *Section 11.2, Table 5 (Table 6 added)*

Purpose: Section 9.1.3, Serious Breach of Protocol, was added to align with the process for the reporting potential serious breaches of protocol.

The primary change is the addition of Section 9.1.3, *Serious Breach of Protocol*

Added text:

9.1.3. Serious Breach of Protocol

Investigators must notify the Medical Monitor within 24 hours of becoming aware of a potential serious breach of the protocol. A serious breach is a breach that is likely to affect to a significant degree the safety and rights of a study participant or the reliability and robustness of the data generated in the clinical trial.

Purpose: Because data from the human drug-drug interaction study showed that givosiran treatment resulted in a moderate (>2-fold) reduction of CYP2D6 and CYP1A2 metabolic activity, the guidance for investigators in Section 6.3 was updated to recommend that medications metabolized primarily by CYP2D6 and CYP1A2 with a narrow therapeutic index may need to be monitored more frequently to determine if a dose adjustment of the concomitant medication is required. In the absence of comprehensive lists of products metabolized by CYP2D6 and CYP1A2, Section 6.3 now directs the Investigator to the product information of any concomitant medication required and to the Table of Substrates for CYP enzymes on the FDA Website to determine if concomitant medications metabolized primarily by CYP2D6 and CYP1A2 need to be monitored more frequently. Section 11.2, List of Sensitive CYP3A Substrates and Those with a Narrow Therapeutic Range, was deleted because , based on the results from the human drug-drug interaction study, givosiran treatment results in only a weak (<2-fold) reduction of CYP3A4 metabolic activity, and the recommendation to monitor the drug response in patients on medications primarily metabolized by CYP3A4 was not considered necessary.

The primary change occurs in Section 11.3, List of Sensitive CYP3A Substrates and Those with a Narrow Therapeutic Range.

Section 11.3 was deleted.

Purpose: Correct typographical errors, punctuation, grammar, abbreviations, and formatting

These changes are not listed individually.

CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1 (givosiran)
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016 Protocol Amendment 3 03 February 2017 Protocol Amendment 4 02 August 2017 Protocol Amendment 5 03 May 2018
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD

4 MAY 2018

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1 (givosiran)

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

- Characterize the pharmacokinetic (PK) profile of ALN-AS1 and incidence of antidrug antibodies (ADA) over time
- Assess changes in health-related quality of life (QOL)
- Characterize analytes related to targeting the heme biosynthesis pathway

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen,

including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

Reference Therapy, Dose, and Mode of Administration

Not applicable

Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

Statistical Methods

Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation.

Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study.

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X	X[a]	X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1. On Day 1, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the

Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided

that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M37**	
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	X
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]		X		X		X		X		X		X	X	X
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	X
Pregnancy Test[i]		X		X		X		X		X		X	X	X
Study Drug Administration[j]		X		X		X		X		X		X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M37**	
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Blood Sample for PK Analysis[m]				X										
Antidrug Antibodies[n]				X								X	X	
EQ-5D-5L Questionnaire[o]				X				X				X	X	
Diary Review (including BPI-SF)[p]	X													
Phone Contact[q]	X		X		X		X		X		X			
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; ET = Early Termination; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.
b Patients who complete all scheduled treatment with ALN-AS1 through Month 36 will return for an end of study (EOS) visit at Month 37. Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+7] days) after his/her last dose of ALN-AS1.
c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+7 days]) following the last dose of ALN-AS1.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the previous study center visit or the current study center visit may be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate
NOAEL	No observed adverse effect level

Abbreviation	Definition
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.[1] The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.[2] In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.[3, 4]

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.[5] The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.[6]

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.[2] However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.[7-10] AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.[11] It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.[12] AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).[2] The initial diagnosis involves demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA

(typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.[6-8] Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.[7]

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.[13] In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.[14] With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.[15]

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.[16, 17] In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.[18-22] Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.[22-24] In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.[25] While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).[26] Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).[5] In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.[27] In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option [34]. Given the significant morbidity and mortality, there remains a significant unmet

need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.[28] Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.[29] The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 (givosiran) comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have

higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.[30] For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related serious adverse events (SAEs), severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Clinical Data Summary

1.6.1. Clinical Pharmacodynamics

Dosing has been completed in Part A and Part B of the study. In both Parts A and B of the study, durable and dose-dependent reductions of ALAS1 mRNA (up to 66% in the 2.5 mg/kg dose group) as measured by cERD were observed after ALN-AS1 treatment. ALAS1 reductions were highly correlated with reductions in ALA (mean maximal 86%) PBG (mean maximal 95%) levels in a dose-dependent manner and were sustained for ≥180 days.

Dosing is currently ongoing in Part C of the study. The mean maximal reduction in ALAS1 mRNA-relative to baseline in ALN-AS1-treated patients in Cohort 1 (2.5 mg/kg Q3M) was 39% and in Cohort 2 (2.5 mg/kg Q1M) 66%. All patients treated with ALN-AS1 also had decreases in their peak ALA and PBG levels in the treatment period compared to the run-in period, along with reduced fluctuations in these levels in the treatment period (data not shown). No reductions were observed in the placebo-treated patients.

1.6.2. Clinical Activity

Clinical activity was not evaluated in Parts A and B of the study because ASHE patients included in this study were not actively experiencing attacks of porphyria.

Dosing is currently ongoing in Part C of the study. All patients in Cohorts 1 and 2 were enrolled in a run-in phase for 10-15 weeks that was followed by a treatment phase for up to 24 weeks. No investigational product was administered during the run-in phase. Therefore, each patient acted as their own control, allowing for individual ALA / PBG levels, and overall attack rate while on ALN-AS1 treatment to be compared to that observed in the run-in phase.

Figure 1 shows in Cohort 1 ALN-AS1-treated patients there was a 74% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 63-94% reduction), compared with a 23% decrease in the annualized attack rate for the placebo-treated patient. In ALN-AS1-treated patients this was accompanied by an approximate 76% mean decrease in annualized acute hemin usage (range 52-95%) and a 10.3 times increase in the maximal attack free interval compared to run-in period (range 4.2-16.3).

Figure 1: Part C Cohort 1 Summary of Treatment Period Clinical Efficacy Relative to Run-in

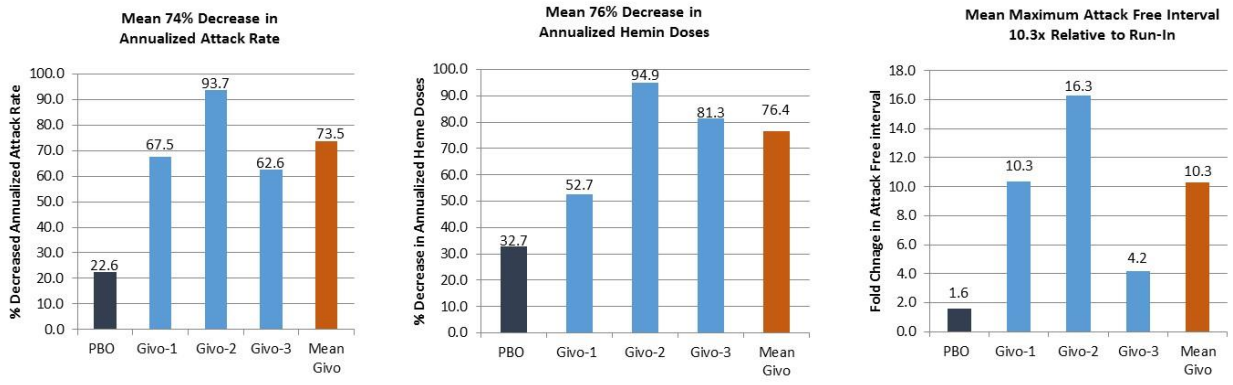
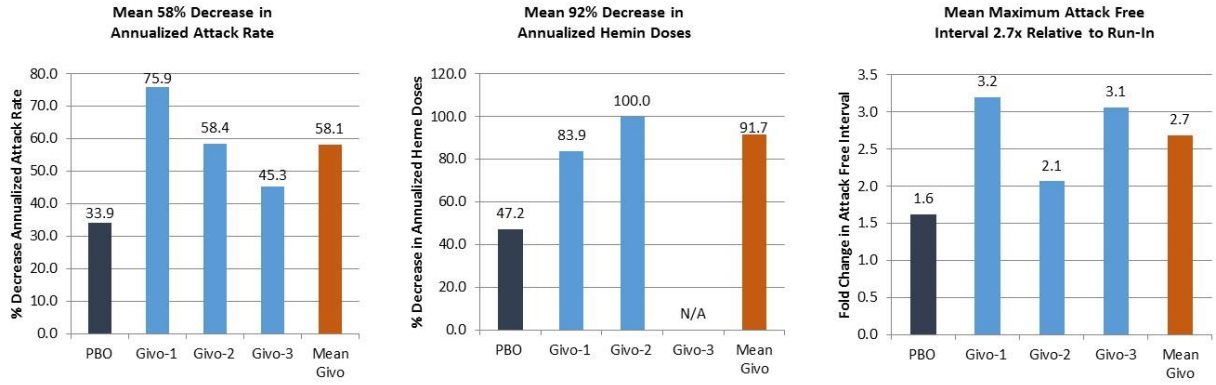


Figure 2 shows in Cohort 2 ALN-AS1-treated patients, there was a 58% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 45-76% reduction). This compares favorably with data from the placebo-treated patient who showed a 34% reduction in the annualized attack rate for the treatment period versus the run-in period. In ALN-AS1-treated patients, the reduction in attack rate was accompanied by a 92% mean decrease in annualized hemin usage (range 84-100%) and a 2.7x increase in the mean maximal attack free interval compared to run-in period (range 2.1-3.2).

Figure 2: Part C Cohort 2 Summary of Treatment Period Clinical Efficacy Relative to Run-in



1.6.3. Clinical Safety

In Part A and Part B of Study ALN-AS1-001, a total of 11 patients (11/13; 85.0%) who received ALN-AS1 reported at least 1 AE. All 5 placebo-treated patients (5/5; 100%) reported at least 1 AE. AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients (5/13; 38%): diarrhoea, dyspepsia, hematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (each AE reported only by an individual patient; 1/13; 8% each). There were 2 SAEs of abdominal pain: 1 patient each in the 0.035 mg/kg and 0.1 mg/kg ALN-AS1 dose groups. Both were considered to be not related and resolved without sequelae. There were no AEs leading to discontinuation. All AEs were mild or moderate in severity, except for 1 of the SAEs of abdominal pain (0.10 mg/kg dose group), which was considered severe. Adverse events related to abnormal laboratory values were seen in 1 patient in the 0.35 mg/kg ALN-AS1 dose group who had AEs of ALT increased and AST increased, which were moderate and considered by the Investigator to be unrelated to ALN-AS1. No clinically significant findings were observed with routine monitoring of CRP and a panel of 9 proinflammatory cytokines collected predose and up to 24 hours post-dose. There were no other clinically significant laboratory abnormalities related to study drug or changes in vital signs, or ECGs in patients who were administered ALN AS1 or placebo.

Dosing is ongoing in Part C of the study. All patients who received ALN-AS1 in cohort 1 and 2 reported at least 1 AE. AEs that were reported in at least 2 subjects were nausea in 3 patients (50%) and abdominal pain, vomiting, nasopharyngitis, headache, cough and oropharyngeal pain in 2 patients (33.3%) each. All AEs were mild or moderate in severity. AEs considered possibly or definitely related to ALN-AS1 were reported in 4 patients (66.6%), 1 each: renal impairment in Cohort 1 and injection site reaction, myalgia, and headache in Cohort 2. Both placebo-treated patients reported AEs. No AE was experienced in more than 1 patient.

In Part C, Cohort 1 or 2, there were no SAEs or AEs leading to discontinuation in patients administered ALN-AS1 or placebo. No clinically significant laboratory abnormalities related to study drug or changes in vital signs, ECG, or physical exam findings were observed in patients administered ALN-AS1 or placebo.

Part C Cohort 3 5.0 mg/kg SC monthly dosing is ongoing. In this cohort there was one fatal SAE of acute pancreatitis, which was determined to be unlikely related to study drug or placebo

by the Investigator due to the presence of gall bladder sludge found at the time of her presentation. Contributors to this patient's adverse clinical course included: pre-existing chronic debilitation from recurrent AIP attacks requiring monthly hospitalization, porphyria-attributed quadriplegia requiring nursing home care, delay in hospital admission and complications from thromboembolism (multiple risk factors included pancreatitis, obesity, immobilisation from quadriparesis and recent hospitalization for infected portacath removal). Serum lipase was added to all scheduled laboratory assessments in November 2016 as part of additional safety monitoring. To date, all lipase results (available in 11 of 15 subjects) have been at or below the upper limit of normal with no trends seen with dosing of study drug or placebo.

Further information on the safety, efficacy, PK and PD of ALN-AS1 are available in the Investigator's Brochure.

1.7. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical finding of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

The important potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Pancreatitis: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.
- Liver transaminase abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of liver function test (LFT) abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Anaphylactic reactions: There is a potential risk of developing a severe allergic reaction with ALN-AS1 administration. In the present study, one patient in the

2.5 mg/kg monthly dose group with a history of asthma and multiple allergies experienced an SAE of anaphylactic reaction that was determined by the Investigator to be definitely related to study drug given the temporal relationship of ALN-AS1 treatment to the onset of the reaction (within minutes). The patient was treated and recovered and was discontinued from the study. Guidance on dose administration and monitoring for anaphylactic reactions has been included in this protocol.

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and ADA
- Duration and treatment of porphyria attacks

- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

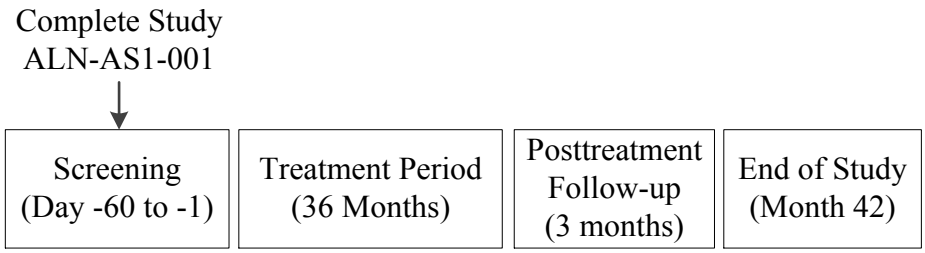
The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2 ; and Table 4 and Table 5 in Appendix Section 11.2). Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 37. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria

attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Figure 3: Study Design



4.2. Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6

months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening
3. Women of child-bearing potential (WOCBP) must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration

5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Discontinuation of Study Drug and/or Study

Patients or their legal guardians (in the case that the patient is a minor) are free to discontinue treatment and/or study or withdraw their consent at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or of the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient from the study at any time if this is considered to be in the patient's best interest.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively. If a patient stops participation from the study or withdraws consent from the study, he/she will not be able to re-enroll in the study.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). A positive urine pregnancy test should be confirmed by a serum pregnancy test prior to discontinuing the study drug.

If a patient discontinues dosing due to a serious adverse event (SAE), the SAE should be followed as described in Section 7.5.6. When a patient discontinues study drug dosing, the primary reason must be recorded in the electronic case report form (eCRF). Patients who discontinue study drug and remain on study may receive treatment consistent with local standard practice for their disease per Investigator judgement, as applicable.

Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; they must also return for a safety-follow-up visit 3 months (84 [+ 7] days) after their last dose of ALN-AS1.

5.3.2. Discontinuation from the Study

A patient or their legal guardian may decide to stop the patient's participation in the study at any time. Patients considering to stop the study should be informed that they can discontinue treatment and complete study assessments including follow-up, as per the SOA, or alternatively may complete any minimal assessments for which the patient consents. The Investigator may withdraw a patient at any time if this is considered to be in the patient's best interest.

However, study integrity and interpretation is best maintained if all randomized patients continue study assessments and follow-up. Stopping study participation could mean:

- If a patient discontinues from the study, he/she will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; the patient must also return for a safety-follow-up visit at 3 months (84 [+7] days) after his/her last dose of ALN-AS1.
- A patient can stop taking the study drug and stop study-related visits, but allow the investigator and study team to review the patient's medical records, public records or be contacted in order to receive information about the patient's health

When a patient stops the study, the discontinuation and reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report the observations as thoroughly as possible. If a patient stops the study due to an adverse event (AE), including an SAE, the AE should be followed as described in Section 7.5.6.

If the patient wants to stop participation in the study, he/she should notify the study doctor in writing or in any other form that may be locally required. The personal data already collected during the study, including patient's biological samples, will still be used together with the data collected on other patients in the study according to the informed consent and applicable laws.

In addition to stopping participation in the study, the patient could decide to withdraw his/her consent as explained in Section 5.3.3

5.3.3. Withdrawal of Consent to Collect and Process the Patient's Personal Data

The patient may decide to withdraw his/her consent informing the study doctor at any time in writing, or in any other form that may be locally required. This means that the patient wants to stop participation in the study and any further collection of his/her personal data.

- The sponsor will continue to keep and use a patient's study information (including any data resulting from the analysis of the patient's biological samples until time of withdrawal) according to applicable law. This is done to guarantee the validity of the study, determine the effects of the study treatment, and ensure completeness of study documentation.
- The patient can also request that collected samples be destroyed or returned (to the extent it is permitted by applicable law) at any time.
- Patients who withdraw their consent to collect and use personal data should understand that public records may be reviewed to determine the patient's survival status as allowed per local and national regulations.

In US and Japan, otherwise, samples not yet analyzed at the time of withdrawal may still be used for further testing/analysis in accordance with the terms of the protocol and of the informed consent form.

In EU and rest of world, in any event, samples not yet analyzed at the time of withdrawal will not be used any longer, unless permitted by applicable law. They will be stored or destroyed according to applicable legal requirements.

5.3.4. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1 and Table 2). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 4 and Table 5 in Appendix Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

ALN-AS1 will be administered by a qualified and authorized health care professional trained in the recognition and management of anaphylactic reactions. The study drug should be injected into the abdomen or upper arms or thighs. Detailed instructions for study drug administration are presented in the Pharmacy Manual. As is consistent with good medical practice for subcutaneous drug administration, patients will be observed for a minimum of 20 minutes after each injection. Treatment for anaphylactic reactions should be readily available where patients are being dosed, and follow country and/or local hospital treatment guidelines as shown in Table 7.[35]

After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), study drug administration may be conducted at a location other than the study center by a home healthcare professional, where applicable country and local

regulations and infrastructure allow, after consultation with the medical monitor, during particular study visits, as specified in the Schedules of Assessments. However, study drug administration at the study center should be considered for patients who have ongoing study drug-related AEs or known risk factors for developing anaphylactic reaction, including but not limited to: a prior history of anaphylactic reaction to food, medications or due to unknown etiology, worsening injection site reactions with repeat dosing, or anyone in the opinion of the investigator that would benefit from clinical observation following dosing. Patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.3.2.1. Monitoring and Dosing Rules in Patients with Potential Cases of Anaphylactic

Reaction

An anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). For reference see Section 11.4.[35]

Stop administering the study medication immediately if an anaphylactic reaction to the study medication is suspected. Study medication must be permanently discontinued in patients for whom an anaphylactic reaction is assessed as related to the study medication.

Laboratory testing: Obtain blood sample for tryptase, total IgE, and ADA antidrug antibodies (ADA) ideally within 15 minutes to 3 hours after the onset of a suspected anaphylactic reaction; however, up to 6 hours is acceptable. An additional blood sample to assess tryptase, total IgE, and ADA should be obtained between 1 to 2 weeks from onset of event. Local laboratory may be used to analyze samples; however, parallel samples should be sent to the central laboratory for analysis. Sample collection and shipping instructions are included in the Laboratory Manual.

Reporting: The PI or designee must notify the sponsor or designee within 24 hours of the occurrence of a suspected case of anaphylactic reaction or being informed of the case as required for AEs of Clinical Interest (AECI) and SAEs, per AE reporting requirements (Section 7.5.6.1, Section 7.5.6.2 and 7.5.6.3).

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

All packaging, labeling, and production of study drug will be in compliance with current Good Manufacturing Practice specifications, as well as applicable local regulations. Study drug labels and external packaging will include all appropriate information as per local labeling requirements.

Additional details will be available in the Pharmacy Manual.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed

at the clinical study center according to applicable regulations.
Further instructions about drug accountability are detailed in the Pharmacy Manual.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

Drug-drug interaction studies in NHP, could not exclude the potential for ALN-AS1 to alter the clearance of drugs metabolized by the CYP3A enzymes. Therefore, patients on medications metabolized by CYP3A, especially those with narrow therapeutic ranges, may require monitoring for their drug levels or drug response. ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 6 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies.

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration.

Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, transdermal hormonal, or intrauterine hormone-releasing system as methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]).
- If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- Sexual abstinence, when this is in line with the preferred and usual lifestyle of the patient, is considered an acceptable method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug (defined above). Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not considered sexual abstinence and do not meet criteria for an acceptable method of birth control. As determined by the investigator, the reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods if they start a heterosexual relationship during the study and continue to do so for the entire period of risk associated with the study drug (defined above).
- WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedule of study assessments is provided in Table 1.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.[32] The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 3 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for at least 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

Body weight measured on the dosing day will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured at either the previous study center visit or current study center visit may be used for dosing calculations.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate standard 12-lead ECGs will be performed using central ECG equipment, with readings approximately 1 minute apart and recorded as specified in the Schedule of Assessments (Table 1). Patients should be seated or supine for at least 5 minutes before each ECG is obtained. The electrophysiological parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB), and Fridericia corrected QT interval (QTcF).

When ECG and blood sample collection occur at the same time, ECGs should be performed before blood samples are drawn.

Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes.

In all patients receiving ≤2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 2-hour (±15 minutes) postdose readings, paired with PK timepoints (Table 3) as specified in the Schedule of Assessments (Table 1).

Similarly, in all patients receiving >2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 4-hour (±20 minutes) postdose readings, paired with PK timepoints (Table 3), as specified in the Schedule of Assessments (Table 1).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

On dosing days up to the Month 18 visit, results from hematology, chemistry, and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed assessments must be reviewed to allow same day study drug administration and additional samples for central analysis must also be collected. On dosing days from Month 19 through Month 36, a review of laboratory results within 14 days is not required.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
GGT	
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Coagulation	
Prothrombin time	International Normalized Ratio
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	
Inflammation	
C-reactive protein	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; GGT=gamma-glutamyltransferase; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.[33]

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Sample collection for patients who experience a potential anaphylactic reaction is discussed in Section 6.2.3.2.1.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg,

events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.
- Lipase >3×ULN (or >3× the baseline lipase measurement if baseline is >ULN)
- Anaphylactic Reactions. Anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). Symptoms of an anaphylactic reaction may include skin or mucosal tissue (e.g. generalized hives, pruritus, angioedema), respiratory compromise (e.g. wheezing, bronchospasm, hypoxia), reduced blood pressure or associated symptoms (e.g. syncope, hypotonia). See Section 11.4 (Table 7) for guidance on diagnosing anaphylactic reactions.[35]

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, the Sponsor or its designee should be notified within 24 hours using a supplemental AEs of clinical interest (AECI) eCRF. Additional clinical and laboratory information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the

protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.[34] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to

the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' confidentiality will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.4. End of the Study

The end of the study is defined as last patient last visit.

9.1.5. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical

study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005 Mar 15;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010 Oct;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006 May-Jun;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012 Nov 29;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010 Mar 13;375(9718):924-37.

6. Floderus Y, Sardh E, Moller C, Andersson C, Rejkjaer L, Andersson DE, et al. Variations in porphobilinogen and 5-aminolevulinic acid concentrations in plasma and urine from asymptomatic carriers of the acute intermittent porphyria gene with increased porphyrin precursor excretion. Clin Chem. 2006 Apr;52(4):701-7.

7. Anderson K. Disorders of heme biosynthesis. 8th ed. CR S, editor: McGraw-Hill, New York 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transpl. 2012 Feb;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach J-C, Puy H. 86 - Acute Intermittent Porphyria: From Clinical to Molecular Aspects. In: Guilard KMKMS, editor. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000 Jul;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004 Nov;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013 May;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. Panhematin. (hemin for injection). 2013.

17. Normosang. (human hemin). 2007.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977 May;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993 Sep 13;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991 Sep;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989 Jun 10;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988 Feb;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990 Feb;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001 Feb 15;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013 Sep;36(5):849-57.

26. Anderson K. Heme arginate for prevention of acute porphyric attacks (abstract). Acta Haematologica. 1997;98((suppl 1):120).

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013 Jan;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002 Nov;48(11):1891-900.

31. Division of Clinical Pharmacology, Indiana University, P450 Drug Interaction Table. 2013 [Accessed 06 February 2015]; Available from: http://medicine.iupui.edu/clinpharm/ddis/main-table/.

32. Atkinson TM M, TR, Sit L, Passik P, Scher, HI, Cleeland C, and Basch E. The Brief Pain Inventory and its “Pain at its Worst in the last 24 Hours” Item: Clinical Trial Endpoint Considerations. Pain Med. 2010 March;11(3):337–46.

33. Levey AS SL, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

34. Herdman M GC, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

35. Sampson HA, Muñoz-Furlong A, Campbell RL, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol 2006; 117:391.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 3 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 3: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±15 minutes)[b]	X
	06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days	Anytime during visit	X
Month 1 (Day 31) ±7 days,[a] Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 9 (Day 271) ±10 days[d], Month 15 (Day 451) ±10 days[d], Month 18 (Day 541) ±10 days Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±15 minutes)[b]	X
	04:00 (±20 minutes)[c]	

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

a At the Day 31, Month 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.
b Only in patients receiving ≤2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
c Only in patients receiving >2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
d. Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]		X														
AEs		Continuous														
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D14 ±2	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10

- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1 ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose at a minimum of 2 of the following visits: D1, Month 3, Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose at a minimum of two of the following visits: D1, Month 3,

Month 6, Month 9, Month 12, Month 15 (only if visit is done at clinic), or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days up to the Month 18 visit,results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed predose assessments may be used for review in order to allow same day study drug administration, and additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured at the current study center visit (dosing day) will be used to calculate the dose of ALN-AS1. After M12, weight measured at either the previous study center visit or on current study center visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M37, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M37**	
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	X
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]	X	X	X	X	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK Analysis[m]				X										

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												EOS/ ET[b]	Safety Follow-up[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M37**	
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Antidrug Antibodies[n]				X								X	X	
EQ-5D-5L Questionnaire[o]				X				X				X	X	
Diary Review (including BPI-SF)[PQ]	X													
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS=End of Study; EOT=End of Treatment; ET=early termination; M=month; PBG=porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who complete all scheduled doses ALN-AS1 through Month 36 will return for an end of study (EOS) visit at Month 37. Patients who discontinue treatment prior to Month 36 will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; they must also return 3 months (84 [+ 7] days) after their last dose of ALN-AS1 for a safety-follow-up visit.
c The safety follow-up visit is only required for patients who discontinue prior to study completion. The visit should be conducted 3 months (84 [+7 days]) following the last dose of ALN-AS1.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for at least 5 minutes. For patients receiving ≤2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose . At Month 24, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose, when paired with PK timepoints, otherwise ECGs will be collected predose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured at either the at the previous study center visit or current study center visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

A list of medications that are sensitive CYP3A substrates and those with a narrow therapeutic range is in Table 6.

Table 6: List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

Sensitive CYP3A Substrates			CYP3A Substrates With A Narrow Therapeutic Range
alfentanil	fluticasone		Alfentanil
aprepitant	lopinavir		Astemizole
budesonide	lovastatin		Cisapride
buspirone	lurasidone		Cyclosporine
conivaptan	maraviroc		Diergotamine
darifenacin	midazolam		Dihydroergotamine
darunavir	nisoldipine		Ergotamine
dasatinib	quetiapine		Fentanyl
dronedarone	saquinavir		Irinotecan
eletriptan	sildenafil		Pimozide
eplerenone	simvastatin		Quinidine
everolimus	sirolimus		Sirolimus
felodipine	tolvaptan		Tacrolimus
imatinib	tipranavir		Terfenadine
indinavir	triazolam		
	vardenafil		

Note: This is not an exhaustive list and availability of medications may differ between countries. For more information about clinically relevant drugs that are CYP3A substrates, see the Indiana University Division of Pharmacology website for reference: http://medicine.iupui.edu/clinpharm/ddis/clinical-table/.

11.4. Anaphylactic Reactions

Table 7: Sampson Criteria for Anaphylactic Reactions

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)
   AND AT LEAST ONE OF THE FOLLOWING
   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):
   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):
   a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; *BP*, blood pressure.
*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 × age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.

Adapted from Sampson et al. 2006 [35]

ALN-AS1-002 Protocol Amendment 5

Summary of Changes (dated 03 May 2018) compared to Protocol Amendment 4 (dated 02 August 2017)

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

Rationale for Protocol Amendment

The purpose of the amendment is to:

- Include clinical data on a single case of anaphylactic reaction, information regarding the potential risk for anaphylactic reactions, and provide updated guidance for dosing and monitoring. The event of anaphylactic reaction was previously reported to applicable regulatory authorities and Institutional Review Boards/Ethics Committees.
- Benefit-Risk Assessment (Section 1.7) modified to align with potential risks in the Investigator's Brochure. Information on reproductive health moved to Contraceptive Requirements (Section 6.4) and CYP inhibition moved to Concomitant Medications (Section 6 3).
- Update guidance and procedures on patient withdrawal from study.
- Schedule of Assessments footnotes were updated to:
    - Update end of study visit, early termination visit, and safety follow-up visit timing and assessments.
    - Clarify clinical laboratory testing required prior to dosing
    - Clarify timing of ECG assessments for patients administered ≤ 2.5 mg/kg and >2.5 mg/kg ALN-AS1
- provide the following clarifications:
    - patient withdrawal details regarding subsequent visits and data collection
    - definition of sexual abstinence
    - contraception with an intrauterine hormone-releasing system also requires use of a barrier method

A detailed summary of the changes is provided in Table 1. Corrections to typographical errors and formatting are not detailed.

Table 1: Protocol Amendment 5 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in the protocol amendment are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: Provide details of an anaphylactic reaction reported in Study ALN-AS1-002 and add anaphylactic reaction to potential risk

The primary change occurs in Section 1.7 Benefit-Risk Assessment

Added text: Anaphylactic reactions: There is a potential risk of developing a severe allergic reaction with givosiran administration. In the present study, one patient in the 2.5 mg/kg monthly dose group with a history of asthma and multiple allergies experienced an SAE of anaphylactic reaction that was determined by the Investigator to be definitely related to study drug given the temporal relationship of ALN-AS1 treatment to the onset of the reaction (within minutes). The patient was treated and recovered and was discontinued from the study.Guidance on dose administration and monitoring for anaphylactic reactions has been included in this protocol.

Section(s) also containing similar or related changes

- Section 6.2.2 Dose Administration
- Section 6.2.3.2 Indivdual Dose Modifications

Purpose: Updated labels of two potential risks to align with ALN-AS1 Invesigator's Brochure

The primary change occurs in Section 1.7 Benefit-Risk Assessment

Now reads: ~~Serum chemistry and coagulation change~~ **Pancreatitis:**
~~Liver function test abnormalities~~ **Liver transaminase abnormalities:**

Purpose: Clarified that only important potential risks are listed in Section 1.7. To align with the Investigator's Brochure, removed discussion of Reproductive Health and CYP Inhibition from important risks and, instead included details in respective sections in the protocol (reproductive health information moved to Section 6.4, Contraception Requirements and CYP inhibition information moved to Section 6.3, Concomitant Medication.

The primary change occurs in Section 1.7 Benefit-Risk Assessment

Now reads:

The **important** potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

Removed text:

- ~~Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. Women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use acceptable methods of contraception during studies with ALN-AS1.~~
- ~~CYP inhibition: A study in NHP demonstrated that ALN-AS1 dosing could potentially increase the clearance of drugs metabolized by the CYP3A isoform. As contraceptive hormones are metabolized via CYP3A, WOCBP must use a barrier method of contraception, in addition to hormonal contraception, during study participation. Additionally, Investigators will review all patient medications at study start and monitor responses to these medications during the study. A list of medications that are sensitive CYP3A substrates is in Table 7 in Appendix Section 11.3.In addition, a list of medications that are sensitive CYP3A substrates is available at the Indiana University, Division of Clinical Pharmacology, website.[31]~~

Section(s) also containing similar or related changes

- Section 6.3 Concomitant Medications (moved information from Section 1.7 that was not already included in Section 6.3)
- Section 6.4 Contraceptive Requirements (moved information from Section 1.7 that was not already included in Section 6.3)

Purpose: Updated exploratory PK objective to reflect corresponding exploratory endpoint (added "incidence of ADA")

The primary change occurs in Section 2.3 – Exploratory Objectives

Now reads: Characterize the PK profile of ALN-AS1 **and incidence of ADA** over time

Section(s) also containing this change:

Protocol synopsis

Purpose: Updated section title and wording to reflect current company language

The primary change occurs in Section 5.3 – Discontinuation of Study Drug and/or Study (new title)

Now reads:

Patients **or their legal guardians (in the case that the patient is a minor)** are free to discontinue ~~study drug~~ treatment **and/or study** or withdraw ~~from the study~~ **their consent** at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or ~~withdrawal from~~ **of** the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient **from the study** at any time if this is considered to be in the **patient's** best interest ~~of the patient~~.

~~Procedures for the discontinuation~~**Discontinuation** of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2 ~~, respectively.~~ **, respectively. If a patient stops participation from the study or withdraws consent from the study, he/she will not be able to re-enroll in the study.**

Purpose: Updated to reflect complete instructions to align with current Company language

The primary change occurs in Section 5.3.1 – Discontinuation of Study Drug

Now reads:

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section7.5.6.6 for reporting and follow-up of pregnancy). ~~Patients who discontinue treatment will be asked to complete the Month 39 visit assessments.~~ **A positive urine pregnancy test should be confirmed by a serum pregnancy test prior to discontinuing the study drug.**

If a patient discontinues dosing due to a serious adverse event (SAE), the SAE should be followed as described in Section 7.5.6. When a patient discontinues study drug dosing, the primary reason must be recorded in the electronic case report form (eCRF). Patients who discontinue study drug and remain on study may receive treatment consistent with local standard practice for their disease per Investigator judgement, as applicable.

Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; they must also return for a safety-follow-up visit at 3 months (84 [+7] days) after their last dose of ALN-AS1.

Purpose: Current section was replaced with more detailed information regarding premature discontinuation study drug and to align protocol text with sample Informed Consent Form.

The primary change occurs in Section 5.3.2 – Discontinuation from the Study

Now reads:

A patient or their legal guardian may decide to stop the patient's participation in the study at any time. Patients considering to stop the study should be informed that they can discontinue treatment and complete study assessments including follow-up, as per the SOA, or alternatively may complete any minimal assessments for which the patient consents. The Investigator may withdraw a patient at any time if this is considered to be in the patient's best interest.

However, study integrity and interpretation is best maintained if all randomized patients continue study assessments and follow-up. Stopping study participation could mean:

- **If a patient discontinues from the study, he/she will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed; the patient must also return for a safety-follow-up visit 3 months (84 [+7] days) after his/her last dose of ALN-AS1.**
- **A patient can stop taking the study drug and stop study-related visits, but allow the investigator and study team to review the patient's medical records, public records or be contacted in order to receive information about the patient's health**

When a patient ~~withdraws from~~**stops** the study, the **discontinuation and** reason for ~~study withdrawal~~**discontinuation** must be recorded in the appropriate section of the **electronic case report form (eCRF)** and all efforts **will be** made to complete and report the observations as thoroughly as possible. If a patient ~~withdraws~~**stops the study** due **to an adverse event (AE), including an** SAE, the ~~SAE~~**AE** should be followed as described in Section 7.5.6.

If the patient wants to stop participation in the study, he/she should notify the study doctor in writing or in any other form that may be locally required. The personal data already collected during the study, including patient's biological samples, will still be used together with the data collected on other patients in the study according to the informed consent and applicable laws.

In addition to stopping participation in the study, the patient could decide to withdraw his/her consent as explained in Section 5.3.3.

Purpose: New section added to discuss withdrawal of consent

The primary change occurs in Section 5.3.3 (new section)– Withdrawal of Consent to Collect and Process the Patient's Personal Data

New text:

The patient may decide to withdraw his/her consent informing the study doctor at any time in writing, or in any other form that may be locally required. This means that the patient wants to stop participation in the study and any further collection of his/her personal data.

- The sponsor will continue to keep and use a patient's study information (including any data resulting from the analysis of the patient's biological samples until time of withdrawal) according to applicable law. This is done to guarantee the validity of the study, determine the effects of the study treatment, and ensure completeness of study documentation.
- The patient can also request that collected samples be destroyed or returned (to the extent it is permitted by applicable law) at any time.
- Patients who withdraw their consent to collect and use Personal Data should understand that public records may be reviewed to determine the patient's survival status as allowed per local and national regulations.

In US and Japan, otherwise, samples not yet analyzed at the time of withdrawal may still be used for further testing/analysis in accordance with the terms of the protocol and of the informed consent form.

In EU and rest of world, in any event, samples not yet analyzed at the time of withdrawal will not be used any longer, unless permitted by applicable law. They will be stored or destroyed according to applicable legal requirements.

Purpose: Revise details of requirements for study drug administration due to potential risk of anaphylactic reactions

The primary change occurs in Section 6.2.2 Dose and Administration

Now reads:

ALN-AS1 will be administered by a qualified and authorized ~~staff at the study center. In the event that a patient is unable to come to the study center for a scheduled clinical study visit~~, **health care professional trained in the recognition and management of anaphylactic reactions. The study drug should be injected into the abdomen or upper arms or thighs. Detailed instructions for study drug administration are presented in the Pharmacy Manual. As is consistent with good medical practice for subcutaneous drug administration, patients will be observed for a minimum of 20 minutes after each injection. Treatment for anaphylactic reactions should be readily available where patients are being dosed, and follow country and/or local hospital treatment** guidelines.[36]**.**

After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), study drug administration may be conducted at a location other than the study center by a home healthcare professional, where applicable country and local regulations and infrastructure allow, after consultation with the medical monitor~~. For,~~ **during** particular **study** visits, as specified in the Schedules of Assessments), ~~dosing may also be performed~~. **However, study drug administration** at ~~a location other than~~the study center ~~by a qualified home healthcare professional where applicable country and local regulations and infrastructure allow. The study drug~~ should be ~~injected into~~**considered for patients who have ongoing study drug-related AEs or known risk factors for developing anaphylactic reaction, including but not limited to: a prior history of an anaphylactic reaction to food, medications or unknown etiology, worsening injection site reactions with repeat dosing, or anyone in** the ~~abdomen or upper arms or thighs. Patients must demonstrate that they can tolerate dose~~opinion of the **investigator that would benefit from clinical observation following** dosing. ~~at a location other than the study center is permitte~~ Patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

Purpose: Add instructions regarding management of potential cases of anaphylactic reaction

The primary change occurs in Section 6.2.3.2.1 Monitoring and Dosing Rules in Patients with Potential Cases of Anaphylactic Reactions

New text:

An anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). For reference see Section 11.4.[35]

Stop administering the study medication immediately if an anaphylactic reaction to the study medication is suspected. Study medication must be permanently discontinued in patients for whom anaphylactic reactions is assessed as related to the study medication.

Laboratory testing: Obtain blood sample for tryptase, total IgE, and ADA antidrug antibodies (ADA) ideally within 15 minutes to 3 hours after the onset of a suspected anaphylactic reaction; however, up to 6 hours is acceptable. An additional blood sample to assess tryptase, total IgE, and ADA should be obtained between 1 to 2 weeks from onset of event. Local laboratory may be used to analyze samples; however, parallel samples should be sent to the central laboratory for analysis. Sample collection and shipping instructions are included in the Laboratory Manual.

Reporting: The PI or designee must notify the sponsor or designee within 24 hours of the occurrence of a suspected case of anaphylactic reaction or being informed of the case as required for AEs of Clinical Interest (AECI) and SAEs, per AE reporting requirements (Section **Error! Reference source not found.**, Section **Error! Reference source not found.** and 7.5.6.3).

Purpose: To make the language on packaging and labeling more general and provide a reference to the Pharmacy Manual, where such language is easier to update and maintain.

The primary change occurs in Section 6.2.5- Packaging and Labeling

Now reads:

~~ALN-AS1 Solution for Injection (SC use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon faced bromobutyl 13 mm stopper and a flip-off Truedge aluminum seal~~

All packaging, labeling, and production of study drug will be in compliance with current Good Manufacturing Practice specifications, as well as applicable local regulations. Study drug labels and external packaging will include all appropriate information as per local labeling requirements.

Additional details will be available in the Pharmacy Manual.

Purpose: Revised definition of sexual abstinence to align with current standards and clarified that intrauterine hormone releasing system is a method requiring a barrier method

Benefit-Risk Assessment (Section 1.7) modified to align with potential risks in the Investigator's Brochure. Information on reproductive health moved to Contraceptive Requirements (Section 6.4) and CYP inhibition moved to Concomitant Medications (Section 6.3)

The primary change occurs in Section 6.4– Contraceptive Requirements

Now reads	Sexual abstinence, when **this is** in line with the preferred and usual lifestyle of the patient**, is considered an acceptable method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug (defined above).** Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not ~~acceptable methods of contraception.~~ **considered sexual abstinence and do not meet criteria for an acceptable method of birth control. As determined by the investigator, the reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient**. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods if they start ~~sexual relationships~~ **a heterosexual relationship** during the study and **continue to do so** for ~~3 months after their last dose administration.~~**the entire period of risk associated with the study drug (defined above).**

Purpose: Revised definition of sexual abstinence to align with current standards and clarified that intrauterine hormone releasing system is a method requiring a barrier method

The primary change occurs in Section 6.4 – Contraceptive Requirements

Now reads	Sexual abstinence, when **this is** in line with the preferred and usual lifestyle of the patient**, is considered an acceptable method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug (defined above).** Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not ~~acceptable methods of contraception.~~ **considered sexual abstinence and do not meet criteria for an acceptable method of birth control. As determined by the investigator, the reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient**. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods if they start ~~sexual relationships~~ **a heterosexual relationship** during the study and **continue to do so** for ~~3 months after their last dose administration.~~**the entire period of risk associated with the study drug (defined above).**
Now reads	Established use of oral, implantable, injectable, ~~or~~ transdermal hormonal**, or intrauterine hormone-releasing system as** methods of contraception: Women of child-bearing potential using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]).

Purpose: For consistency across documents, elevated lipase (see below) was added to the list of adverse events of clinical interest.

The primary change occurs in Section7.5.6.1 - Definitions

Added text:	Lipase >3×ULN (or >3× the baseline lipase measurement if baseline is >ULN)

Purpose: Update instructions for Pharmacokinetic Assessments at Month 9 and Month 15

The primary change occurs in Section 11.1, Table 4: Pharmacokinetic Time Points Footnotes

New text: **d. Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.**

Section(s) also containing similar or related changes

- Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)
- Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

Purpose: Added anaphylactic reactions to list of Adverse Events of Clinical Interest

The primary change occurs in Section 7.5.6.1 – Definitions

Added text: Anaphylactic Reactions. Anaphylactic reaction is a severe, potentially fatal, systemic allergic reaction with acute onset (minutes to hours). Symptoms of an anaphylactic reaction may include skin or mucosal tissue (e.g. generalized hives, pruritus, angioedema), respiratory compromise (e.g. wheezing, bronchospasm, hypoxia), reduced blood pressure or associated symptoms (e.g. syncope, hypotonia). See Section 11.4 for guidance on diagnosing anaphylactic reactions.[35]

Purpose: Clarify clinical laboratory testing required prior to dosing

The primary change occurs in Table 1, Schedule of Assessments

Now reads: [a] Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1. **On Day 1, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration and additional samples for central analysis must also be collected.**

Purpose: Clarify timing of ECG assessments for patients administered ≤ 2.5 mg/kg and >2.5 mg/kg ALN-AS1

The primary change occurs in Table 1 and Table 2, Schedule of Assessments

Now reads: [g] Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for **at least** 5 minutes. ~~On at least two of the following visits~~ **For patients receiving ≤2.5 mg/kg ALN-AS1,** ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose **at a minimum of 2 of the following visits**: D1, Month 3, Month 6, Month 9, Month 12, Month 15 **(only if visit is done at clinic),** or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose ~~on~~ at ~~least~~ **a minimum of** two of the following visits: D1, Month 3, Month 6, **Month 9,** Month 12, Month 15 **(only if visit is done at clinic),** or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

Purpose: to update the guidance on weight collection for dosing calculations

The Primary change occurs in Table 1, Schedule of Assessments

Now reads: [j] ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured ~~on the dosing day~~**at the current study center visit (dosing day)** will be used to calculate the dose of ALN-AS1 through M12. After M12, weight measured **at either the previous study center visit or the current study center visit** ~~on the nearest previous clinic visit~~may be used to calculate the weight-based dose of ALN-AS1.

Section(s) also containing similar or related changes

Table 1, Schedule of Assessments

Section 7.5.2 – Weight and Height

Table 4 – Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Table 5- Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

Purpose: Update end of study visit, early termination visit, and safety follow-up visit timing and assessments

The primary change occurs in Table 2 and Table 5

Now reads

~~Posttreatment Follow-up~~**EOS/ ET**[b]	~~ALA/PBG~~ **Monitoring/EOS**[c] **Safety Follow-up**[c]
~~M39~~[b] **M37**	~~M42~~

Now Reads

b P**atients who complete all scheduled treatment with ALN-AS1 through Month 36 will return for an end of study (EOS) visit at Month 37. Patients who discontinue treatment will be asked to complete an early termination (ET) visit at which all Month 37 visit procedures will be performed**; the ~~M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct the assessments performed at the. See Section~~ for ~~details on removal from treatment or assessment.~~

~~c The ALA/PBG Monitoring/EOS~~**a safety-follow-up** visit ~~will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.~~**; the patient must also return for a safety follow-up visit 3 months (84 [+7] days) after his/her last dose of ALN-AS1.**

Section(s) also containing similar or related changes

- Section 4.1 : Summary of Study Design
- Section 5.3.1: Discontinuation of Study Drug

Purpose: Add guidance for diagnosing anaphylactic reactions

The primary change occurs in Section 11.4 (new section) – Anaphylactic Reactions

Added text:

Table 2 Sampson Criteria for Anaphylactic Reactions

Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, flushing, swollen lips-tongue-uvula)
   AND AT LEAST ONE OF THE FOLLOWING
   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   b. Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours)
   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):
   a. Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; *BP*, blood pressure.
*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 × age]) from 1 to and less than 90 mm Hg from 11 to 17 years.

Adapted from Sampson et al. 2006 [35]

CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016 Protocol Amendment 3 03 February 2017 Protocol Amendment 4 02 August 2017
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD

09 June 2017

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

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

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen,

including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

Reference Therapy, Dose, and Mode of Administration

Not applicable

Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

Statistical Methods

Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation.

Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study.

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X		X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]			X													
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 5 minutes. On at least two of the following visits, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose: D1, Month 3, Month 6, Month 9, Month 12, Month 15, or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose on at least two of the following visits: D1, Month 3, Month 6, Month 12, Month 15, or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.
q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.
r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.
s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]		X		X		X		X		X		X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]		X		X		X		X		X		X	X	
Study Drug Administration[j]		X		X		X		X		X		X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK				X										

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Analysis[m]														
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
Phone Contact[q]	X		X		X		X		X		X			X
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability;

however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.

c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 5 minutes. ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose. See Section 7.5.4 for ECG assessment details.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.

i Urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate

Abbreviation	Definition
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.[1] The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.[2] In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.[3, 4]

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.[5] The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.[6]

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.[2] However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.[7-10] AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.[11] It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.[12] AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).[2] The initial diagnosis involves

demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA (typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.[6-8] Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.[7]

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.[13] In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.[14] With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.[15]

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.[16, 17] In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.[18-22] Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.[22-24] In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.[25] While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).[26] Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).[5] In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.[27] In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option [34]. Given the significant morbidity and mortality, there

remains a significant unmet need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.[28] Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.[29] The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have

higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.[30] For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related SAEs, severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Clinical Data Summary

Table 3 shows the current status of the 2 ongoing ALN-AS1 studies, as of 07 November 2016. Clinical data presented in this section are as of 07 November 2016, unless otherwise noted.

Table 3: Status of ALN-AS1 Clinical Studies as of 07 November 2016

Study Number	Study Description	Dose, Route, and Regimen	Patients Treated[a]
ALN-AS1-001	Phase 1, randomized (3:1 [ALN-AS1: placebo]) single-blind SAD and MAD, and double-blind multiple-dose safety, tolerability, PK, and PD study	**Part A:** 0.035-2.5 mg/kg; SC; single dose **Part B:** 0.35-1.0 mg/kg; SC; 2 doses 28 days apart **Part C:** 2.5 or 5.0 mg/kg; SC; either 2 doses quarterly or 4	**Part A in ASHE:** 15 ALN-AS1; 5 placebo **Part B in ASHE:** 6 ALN-AS1; 2 placebo **Part C in AIP patients with recurrent attacks:**

Study Number	Study Description	Dose, Route, and Regimen	Patients Treated[a]
		doses monthly	11 ALN-AS1; 4 placebo
ALN-AS1-002	OLE study for subjects completing Part C of study ALN-AS1-001	2.5 or 5.0 mg/kg; SC; monthly or quarterly dosing	A total of 2 patients have been dosed at 5 mg/kg quarterly x 1 dose

[a] 5 subjects had >1 treatment assignment: 2 subjects repeated Part A; 3 subjects enrolled in Part A and Part B

1.6.1. Clinical Pharmacodynamics

Dosing has been completed in Part A and Part B of the study. In both Parts A and B of the study, durable and dose-dependent reductions of ALAS1 mRNA (up to 66% in the 2.5 mg/kg dose group) as measured by cERD were observed after ALN-AS1 treatment. ALAS1 reductions were highly correlated with reductions in ALA (mean maximal 86%) PBG (mean maximal 95%) levels in a dose-dependent manner and were sustained for ≥180 days.

Dosing is currently ongoing in Part C of the study. The mean maximal reduction in ALAS1 mRNA-relative to baseline in ALN-AS1-treated patients in Cohort 1 (2.5 mg/kg Q3M) was 39% and in Cohort 2 (2.5 mg/kg Q1M) 66%. All patients treated with ALN-AS1 also had decreases in their peak ALA and PBG levels in the treatment period compared to the run-in period, along with reduced fluctuations in these levels in the treatment period (data not shown). No reductions were observed in the placebo-treated patients.

1.6.2. Clinical Activity

Clinical activity was not evaluated in Parts A and B of the study because ASHE patients included in this study were not actively experiencing attacks of porphyria.

Dosing is currently ongoing in Part C of the study. All patients in Cohorts 1 and 2 were enrolled in a run-in phase for 10-15 weeks that was followed by a treatment phase for up to 24 weeks. No investigational product was administered during the run-in phase. Therefore, each patient acted as their own control, allowing for individual ALA / PBG levels, and overall attack rate while on ALN-AS1 treatment to be compared to that observed in the run-in phase.

Figure 1 shows in Cohort 1 ALN-AS1-treated patients there was a 74% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 63-94% reduction), compared with a 23% decrease in the annualized attack rate for the placebo-treated patient. In ALN-AS1-treated patients this was accompanied by an approximate 76% mean decrease in annualized acute hemin usage (range 52-95%) and a 10.3 times increase in the maximal attack free interval compared to run-in period (range 4.2-16.3).

Figure 1: Part C Cohort 1 Summary of Treatment Period Clinical Efficacy Relative to Run-in

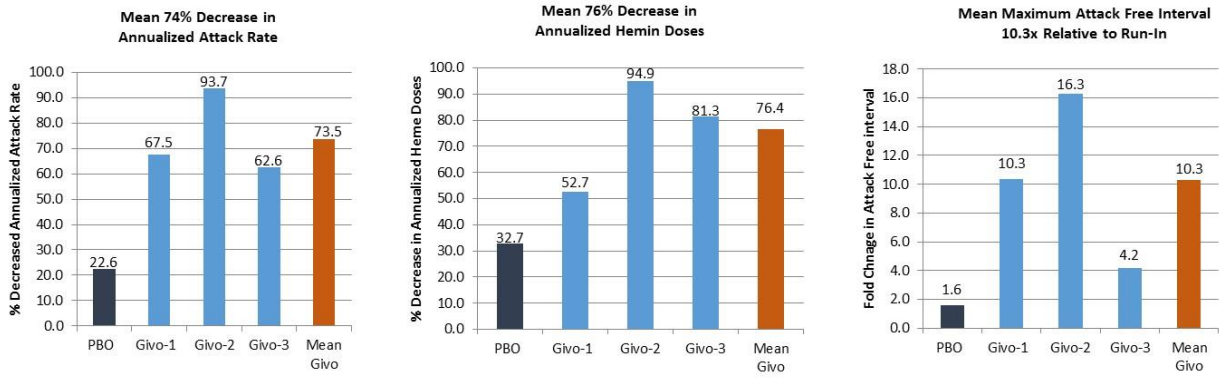
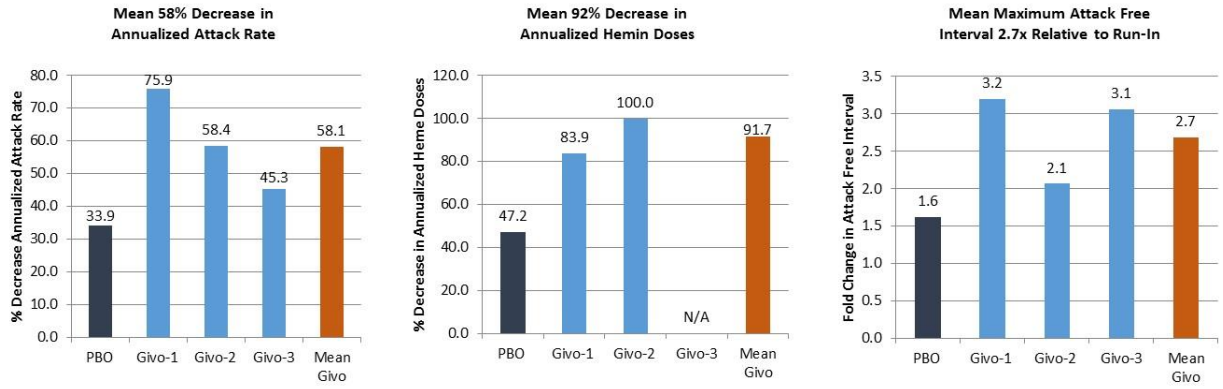


Figure 2 shows in Cohort 2 ALN-AS1-treated patients, there was a 58% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 45-76% reduction). This compares favorably with data from the placebo-treated patient who showed a 34% reduction in the annualized attack rate for the treatment period versus the run-in period. In ALN-AS1-treated patients, the reduction in attack rate was accompanied by a 92% mean decrease in annualized hemin usage (range 84-100%) and a 2.7x increase in the mean maximal attack free interval compared to run-in period (range 2.1-3.2).

Figure 2: Part C Cohort 2 Summary of Treatment Period Clinical Efficacy Relative to Run-in



1.6.3. Clinical Safety

In Part A and Part B of the study, a total of 11 patients (11/13; 85.0%) who received ALN-AS1 reported at least 1 AE. All 5 placebo-treated patients (5/5; 100%) reported at least 1 AE. AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients (5/13; 38%): diarrhoea, dyspepsia, hematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (each AE reported only by an individual patient; 1/13; 8% each). There were 2 SAEs of abdominal pain: 1 patient each in the 0.035 mg/kg and 0.1 mg/kg ALN-AS1 dose groups. Both were considered to be not related and resolved without sequelae. There were no AEs leading to discontinuation. All AEs were mild or moderate in severity, except for 1 of the SAEs of abdominal pain (0.10 mg/kg dose group), which was considered severe. Adverse events related to abnormal laboratory values were seen in

1 patient in the 0.35 mg/kg ALN-AS1 dose group who had AEs of ALT increased and AST increased, which were moderate and considered by the Investigator to be unrelated to ALN-AS1. No clinically significant findings were observed with routine monitoring of CRP and a panel of 9 proinflammatory cytokines collected predose and up to 24 hours post-dose. There were no other clinically significant laboratory abnormalities related to study drug or changes in vital signs, or ECGs in patients who were administered ALN AS1 or placebo.

Dosing is ongoing in Part C of the study. All patients who received ALN-AS1 in cohort 1 and 2 reported at least 1 AE. AEs that were reported in at least 2 subjects were nausea in 3 patients (50%) and abdominal pain, vomiting, nasopharyngitis, headache, cough and oropharyngeal pain in 2 patients (33.3%) each. All AEs were mild or moderate in severity. AEs considered possibly or definitely related to ALN-AS1 were reported in 4 patients (66.6%), 1 each: renal impairment in Cohort 1 and injection site reaction, myalgia, and headache in Cohort 2. Both placebo-treated patients reported AEs. No AE was experienced in more than 1 patient.

In Part C, Cohort 1 or 2, there were no SAEs or AEs leading to discontinuation in patients administered ALN-AS1 or placebo. No clinically significant laboratory abnormalities related to study drug or changes in vital signs, ECG, or physical exam findings were observed in patients administered ALN-AS1 or placebo.

Part C Cohort 3 5.0 mg/kg SC monthly dosing is ongoing. In this cohort there was one fatal SAE of acute pancreatitis, which was determined to be unlikely related to study drug or placebo by the Investigator due to the presence of gall bladder sludge found at the time of her presentation. Contributors to this patient's adverse clinical course included: pre-existing chronic debilitation from recurrent AIP attacks requiring monthly hospitalization, porphyria-attributed quadriplegia requiring nursing home care, delay in hospital admission and complications from thromboembolism (multiple risk factors included pancreatitis, obesity, immobilisation from quadriparesis and recent hospitalization for infected portacath removal). Serum lipase was added to all scheduled laboratory assessments in November 2016 as part of additional safety monitoring. To date, all lipase results (available in 11 of 15 subjects) have been at or below the upper limit of normal with no trends seen with dosing of study drug or placebo.

1.7. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical finding of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

The potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Serum chemistry and coagulation changes: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.
- Liver function test abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of LFT abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. Women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use acceptable methods of contraception during studies with ALN-AS1.
- CYP inhibition: A study in NHP demonstrated that ALN-AS1 dosing could potentially increase the clearance of drugs metabolized by the CYP3A isoform. As contraceptive hormones are metabolized via CYP3A, WOCBP must use a barrier method of contraception, in addition to hormonal contraception, during study participation. Additionally, Investigators will review all patient medications at study start and monitor responses to these medications during the study. A list of medications that are sensitive CYP3A substrates is in Table 7 in Appendix Section 11.3. In addition, a list of medications that are sensitive CYP3A substrates is available at the Indiana University, Division of Clinical Pharmacology, website.[31]

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

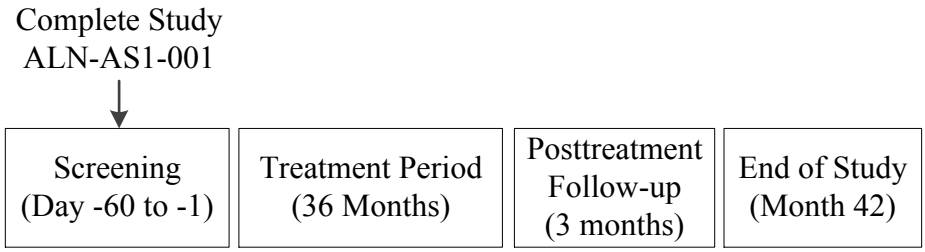
The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous

study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2; and Table 5 and Table 6 in Appendix Section 11.2). Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 39. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Figure 3: Study Design



4.2. Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening

3. WOCBP must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Removal from Treatment or Assessment

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or withdrawal from the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient at any time if this is considered to be in the best interest of the patient.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant

- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). Patients who discontinue treatment will be asked to complete the Month 39 visit assessments.

5.3.2. Withdrawal from Study

A patient may withdraw from the study at any time. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the Month 39 visit. When a patient withdraws from the study, the primary reason for study withdrawal must be recorded in the appropriate section of the eCRF and all efforts made to complete and report the observations as thoroughly as possible. If a patient withdraws due to a SAE, the SAE should be followed as described in Section 7.5.6.

5.3.3. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1 and Table 2). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 5 and Table 6 in Appendix Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in

accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

The study drug will be administered under the supervision of a healthcare professional. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

ALN-AS1 Solution for Injection (SC use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon faced bromobutyl 13 mm stopper and a flip-off Truedge aluminum seal.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1 and Table 2; and Table 5 and Table 6 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 7 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana

University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, or transdermal hormonal methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]). If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Placement of an intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- True sexual abstinence, when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods, if they start sexual relationships during the study and for 90 days after last dose administration.

WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedule of study assessments is provided in Table 1.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.[32] The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 4 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate standard 12-lead ECGs will be performed using central ECG equipment, with readings approximately 1 minute apart and recorded as specified in the Schedule of Assessments

(Table 1). Patients should be seated or supine for at least 5 minutes before each ECG is obtained. The electrophysiological parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB), and Fridericia corrected QT interval (QTcF).

When ECG and blood sample collection occur at the same time, ECGs should be performed before blood samples are drawn.

In all patients receiving ≤2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 2-hour (±15 minutes) postdose readings, paired with PK timepoints (Table 4) as specified in the Schedule of Assessments (Table 1).

Similarly, in all patients receiving >2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 4-hour (±20 minutes) postdose readings, paired with PK timepoints (Table 4), as specified in the Schedule of Assessments (Table 1).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

On dosing days up to the Month 18 visit, results from hematology, chemistry, and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed assessments may be reviewed to allow same day study drug administration, but additional samples for central analysis must also be collected.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
GGT	
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Coagulation	
Prothrombin time	International Normalized Ratio
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	
Inflammation	
C-reactive protein	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; GGT=gamma-glutamyltransferase; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.[33]

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg, events include allergic bronchospasm requiring intensive treatment in an emergency

room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, additional clinical information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.[34] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to

the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.4. End of the Study

The end of the study is defined as last patient last visit.

9.1.5. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical

study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005 Mar 15;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010 Oct;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006 May-Jun;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012 Nov 29;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010 Mar 13;375(9718):924-37.

6. Floderus Y, Sardh E, Moller C, Andersson C, Rejkjaer L, Andersson DE, et al. Variations in porphobilinogen and 5-aminolevulinic acid concentrations in plasma and urine from asymptomatic carriers of the acute intermittent porphyria gene with increased porphyrin precursor excretion. Clin Chem. 2006 Apr;52(4):701-7.

7. Anderson K. Disorders of heme biosynthesis. 8th ed. CR S, editor: McGraw-Hill, New York 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transpl. 2012 Feb;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach J-C, Puy H. 86 - Acute Intermittent Porphyria: From Clinical to Molecular Aspects. In: Guilard KMKMS, editor. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000 Jul;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004 Nov;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013 May;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. Panhematin. (hemin for injection). 2013.

17. Normosang. (human hemin). 2007.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977 May;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993 Sep 13;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991 Sep;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989 Jun 10;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988 Feb;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990 Feb;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001 Feb 15;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013 Sep;36(5):849-57.

26. Anderson K. Heme arginate for prevention of acute porphyric attacks (abstract). Acta Haematologica. 1997;98((suppl 1):120).

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013 Jan;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002 Nov;48(11):1891-900.

31. Division of Clinical Pharmacology, Indiana University, P450 Drug Interaction Table. 2013 [Accessed 06 February 2015]; Available from: http://medicine.iupui.edu/clinpharm/ddis/main-table/.

32. Atkinson TM M, TR, Sit L, Passik P, Scher, HI, Cleeland C, and Basch E. The Brief Pain Inventory and its “Pain at its Worst in the last 24 Hours” Item: Clinical Trial Endpoint Considerations. Pain Med. 2010 March;11(3):337–46.

33. Levey AS SL, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

34. Herdman M GC, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 4 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 4: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±15 minutes)[b]	X
	06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days	Anytime during visit	X
Month 1 (Day 31) ±7 days,[a] Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 9 (Day 271) ±10 days, Month 15 (Day 451) ±10 days, Month 18 (Day 541) ±10 days Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±15 minutes)[b]	X
	04:00 (±20 minutes)[c]	

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

a At the Day 31 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.
b Only in patients receiving ≤2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).
c Only in patients receiving >2.5 mg/kg ALN-AS1; paired with ECG (for ECG details, see Section 7.5.4).

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D14 ±2	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X		X		X		X		X		X
Clinical Laboratory Assessment[h]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for		X		X	X	X		X		X		X		X		X

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Exploratory Biomarker Analysis[l]																
Blood Sample for PK Analysis[m]		X	X	X		X		X		X		X		X		X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]			X													
AEs		Continuous														
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 5 minutes. On at least two of the following visits, ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose: D1, Month 3, Month 6, Month 9, Month 12, Month 15, or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose on at least two of the following visits: D1, Month 3, Month 6, Month 12, Month 15, or Month 18. On all other dosing days, ECGs will be collected within 1 hour predose (see Section 7.5.4 for ECG assessment details).

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days up to the Month 18 visit,results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit (at visits from D1 to Month 18), locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration[j]	X	X	X	X	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK Analysis[m]				X										

Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.
c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. Urine samples should not be collected during a porphyria attack.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Using central ECG equipment, triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 5 minutes. ECGs will be performed within 1 hour predose and 2 hours (±15 minutes) postdose. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose (see Section 7.5.4 for ECG assessment details).
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

A list of medications that are sensitive CYP3A substrates and those with a narrow therapeutic range is in Table 7.

Table 7: List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

Sensitive CYP3A Substrates			CYP3A Substrates With A Narrow Therapeutic Range
alfentanil	fluticasone		Alfentanil
aprepitant	lopinavir		Astemizole
budesonide	lovastatin		Cisapride
buspirone	lurasidone		Cyclosporine
conivaptan	maraviroc		Diergotamine
darifenacin	midazolam		Dihydroergotamine
darunavir	nisoldipine		Ergotamine
dasatinib	quetiapine		Fentanyl
dronedarone	saquinavir		Irinotecan
eletriptan	sildenafil		Pimozide
eplerenone	simvastatin		Quinidine
everolimus	sirolimus		Sirolimus
felodipine	tolvaptan		Tacrolimus
imatinib	tipranavir		Terfenadine
indinavir	triazolam		
	vardenafil		

Note: This is not an exhaustive list and availability of medications may differ between countries. For more information about clinically relevant drugs that are CYP3A substrates, see the Indiana University Division of Pharmacology website for reference: http://medicine.iupui.edu/clinpharm/ddis/clinical-table/.

ALN-AS1-002 Protocol Amendment 4

Summary of Changes (dated 02 August 2017) compared to Protocol Amendment 3 (dated 03 February 2017)

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

Rationale for Protocol Amendment

This protocol is being amended to update the ECG assessments to obtain triplicate 12-lead ECGs using central equipment and paired with plasma PK at times corresponding to nominal maximum concentration (C_{max}).

Also, Schedule of Assessments footnotes and related text were updated to define the visit range in which previously noted predose interpretation of hematology, coagulation, and chemistry test results are required.

A detailed summary of the changes is provided in Table 1. Corrections to typographical errors and formatting are not detailed.

Table 1: Protocol Amendment 4 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in the protocol amendment are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: To update text to require that same day predose and postdose ECG readings will be obtained from at least 2 separate clinic visits.

The primary change occurs in Section 7.5.4 Electrocardiogram

Added text:

Triplicate **standard** 12-lead ~~ECG~~**ECGs will be performed using central ECG equipment**, with readings approximately ~~2 minutes apart, will be recorded. Additional ECGs may be collected at the discretion of the Investigator. Standard computerized 12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Recordings will be obtained, after the patient has rested comfortably for approximately 10 minutes~~**1 minute apart and recorded as specified in the Schedule of Assessments (Table 1). Patients should be seated or supine for at least 5 minutes before each ECG is obtained.** The electrophysiological parameters assessed ~~include~~**will be** rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB)**,** and Fridericia~~-~~ corrected QT interval (QTcF).

When ECG and blood sample collection occur at the same time, ECGs should be performed before blood samples are drawn.

In all patients receiving ≤2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 2-hour (±15 minutes) postdose readings, paired with PK timepoints (Table 4) as specified in the Schedule of Assessments (Table 1).

Similarly, in all patients receiving >2.5 mg/kg ALN-AS1, it is required that ECGs will be obtained on at least 2 separate clinic visits (D1, and Months 3, 6, 9, 12, 15, or 18), each of which will include same day predose and 4-hour (±20 minutes) postdose readings, paired with PK timepoints (Table 4), as specified in the Schedule of Assessments (Table 1).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

Section(s) also containing similar or related changes

- Footnote to Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)
- Footnote to Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)
- Footnote to Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)
- Footnote to Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)
- Table 4: Pharmacokinetic Timepoints; revision applied to table and table footnotes.

Purpose: To revise Schedule of Assessments table, Pharmacokinetics table, and related footnotes to create additional timepoints for performing ECGs, and also to pair with PK timepoints.

The primary change occurs in footnote to Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Now reads:

Revised footnotes:

g **Using central ECG equipment, ~~T~~t**riplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for ~~10~~**5** minutes. On ~~D1,~~**at least two of the following visits,** ECGs will be performed within 1 hour predose and 2 hours (~~±10 minutes) postdose.~~**15 minutes) postdose: D1, Month 3, Month 6, Month 9, Month 12, Month 15, or Month 18. In patients receiving >2.5 mg/kg ALN-AS1, ECGs will be performed within 1 hour predose and 4 hours (±20 minutes) postdose on at least two of the following visits: D1, Month 3, Month 6, Month 12, Month 15, or Month 18.** On all other dosing days, ECGs will be collected within 1 hour predose **(see Section 7.5.4 for ECG assessment details).**

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1). **Blood sample collection for PK analysis at Month 9 and Month 15 is only necessary if ECG will be performed at same visit.**

Section(s) also containing similar or related changes

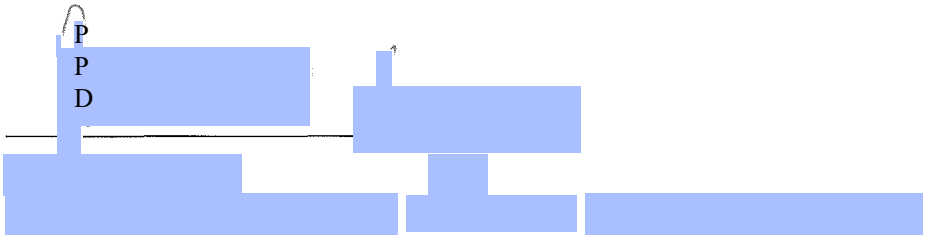
- Footnote to Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)
- Footnote to Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)
- Footnote to Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)
- Section 7.5.4 Electrocardiogram
- Table 4: Pharmacokinetic Timepoints; revision applied to table and table footnotes.

CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016 Protocol Amendment 3 03 February 2017
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.



03 Feb 2017

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

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

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

• Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein
Study Design This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.
Number of Planned Patients Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).
Diagnosis and Main Eligibility Criteria Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.
Investigational Product, Dose, and Mode of Administration ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer. The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen,

including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.
Reference Therapy, Dose, and Mode of Administration Not applicable
Duration of Treatment and Study The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.
Statistical Methods Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study. Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized. PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points. The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided. Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D14±2	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X						X				
Clinical Laboratory Assessment[h]	X		X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X
Blood Sample for PK		X	X	X		X		X				X				X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Analysis[m]																
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]			X													
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the

patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]		X		X		X		X		X		X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]		X		X		X		X		X		X	X	
Study Drug Administration[j]		X		X		X		X		X		X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK				X										

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Analysis[m]														
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
Phone Contact[q]	X		X		X		X		X		X			X
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability;

however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.

c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. . On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.

i Urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate

Abbreviation	Definition
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.[1] The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.[2] In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.[3, 4]

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.[5] The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.[6]

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.[2] However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.[7-10] AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.[11] It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.[12] AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).[2] The initial diagnosis involves

demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA (typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.[6-8] Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.[7]

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.[13] In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.[14] With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.[15]

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.[16, 17] In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.[18-22] Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.[22-24] In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.[25] While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).[26] Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).[5] In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.[27] In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option [34]. Given the significant morbidity and mortality, there

remains a significant unmet need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.[28] Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.[29] The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have

higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.[30] For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related SAEs, severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Clinical Data Summary

Table 3 shows the current status of the 2 ongoing ALN-AS1 studies, as of 07 November 2016. Clinical data presented in this section are as of 07 November 2016, unless otherwise noted.

Table 3: Status of ALN-AS1 Clinical Studies as of 07 November 2016

Study Number	Study Description	Dose, Route, and Regimen	Patients Treated[a]
ALN-AS1-001	Phase 1, randomized (3:1 [ALN-AS1: placebo]) single-blind SAD and MAD, and double-blind multiple-dose safety, tolerability, PK, and PD study	**Part A:** 0.035-2.5 mg/kg; SC; single dose **Part B:** 0.35-1.0 mg/kg; SC; 2 doses 28 days apart **Part C:** 2.5 or 5.0 mg/kg; SC; either 2 doses quarterly or 4	**Part A in ASHE:** 15 ALN-AS1; 5 placebo **Part B in ASHE:** 6 ALN-AS1; 2 placebo **Part C in AIP patients with recurrent attacks:**

Study Number	Study Description	Dose, Route, and Regimen	Patients Treated[a]
		doses monthly	11 ALN-AS1; 4 placebo
ALN-AS1-002	OLE study for subjects completing Part C of study ALN-AS1-001	2.5 or 5.0 mg/kg; SC; monthly or quarterly dosing	A total of 2 patients have been dosed at 5 mg/kg quarterly x 1 dose

[a] 5 subjects had >1 treatment assignment: 2 subjects repeated Part A; 3 subjects enrolled in Part A and Part B

1.6.1. Clinical Pharmacodynamics

Dosing has been completed in Part A and Part B of the study. In both Parts A and B of the study, durable and dose-dependent reductions of ALAS1 mRNA (up to 66% in the 2.5 mg/kg dose group) as measured by cERD were observed after ALN-AS1 treatment. ALAS1 reductions were highly correlated with reductions in ALA (mean maximal 86%) PBG (mean maximal 95%) levels in a dose-dependent manner and were sustained for ≥180 days.

Dosing is currently ongoing in Part C of the study. The mean maximal reduction in ALAS1 mRNA-relative to baseline in ALN-AS1-treated patients in Cohort 1 (2.5 mg/kg Q3M) was 39% and in Cohort 2 (2.5 mg/kg Q1M) 66%. All patients treated with ALN-AS1 also had decreases in their peak ALA and PBG levels in the treatment period compared to the run-in period, along with reduced fluctuations in these levels in the treatment period. No reductions were observed in the placebo-treated patients.

1.6.2. Clinical Activity

Clinical activity was not evaluated in Parts A and B of the study because ASHE patients included in this study were not actively experiencing attacks of porphyria.

Dosing is currently ongoing in Part C of the study. All patients in Cohorts 1 and 2 were enrolled in a run-in phase for 10-15 weeks that was followed by a treatment phase for up to 24 weeks. No investigational product was administered during the run-in phase. Therefore, each patient acted as their own control, allowing for individual ALA / PBG levels, and overall attack rate while on ALN-AS1 treatment to be compared to that observed in the run-in phase.

Figure 1 shows in Cohort 1 ALN-AS1-treated patients there was a 74% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 63-94% reduction), compared with a 23% decrease in the annualized attack rate for the placebo-treated patient. In ALN-AS1-treated patients this was accompanied by an approximate 76% mean decrease in annualized acute hemin usage (range 52-95%) and a 10.3 times increase in the maximal attack free interval compared to run-in period (range 4.2-16.3).

Figure 1: Part C Cohort 1 Summary of Treatment Period Clinical Efficacy Relative to Run-in

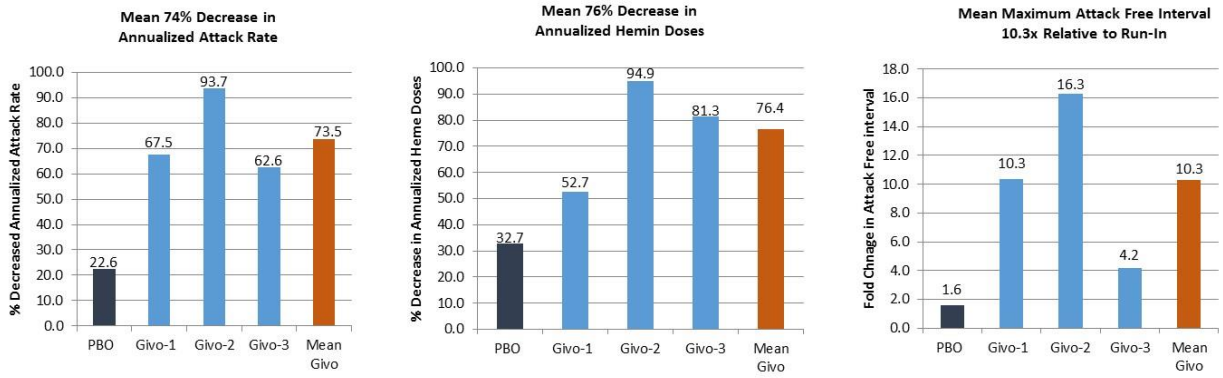
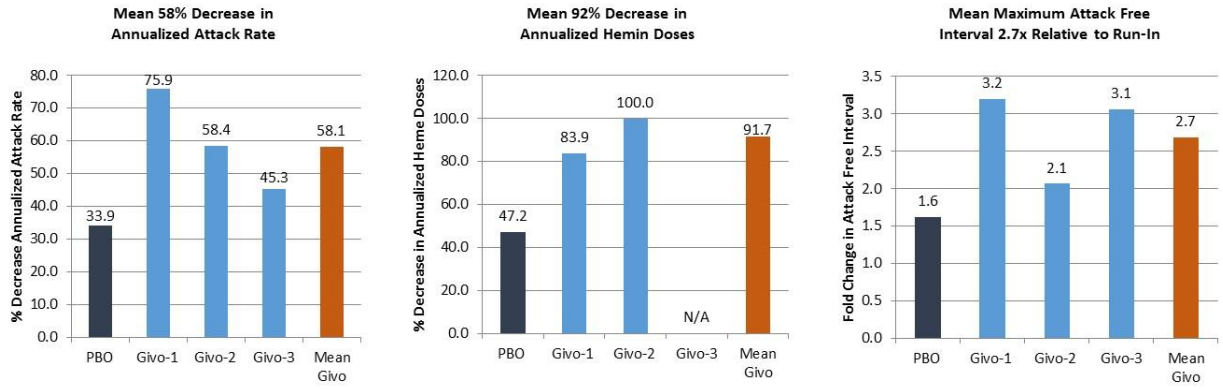


Figure 2 shows in Cohort 2 ALN-AS1-treated patients, there was a 58% mean decrease in the annualized attack rate for the treatment period versus the run-in period (range: 45-76% reduction). This compares favorably with data from the placebo-treated patient who showed a 34% reduction in the annualized attack rate for the treatment period versus the run-in period. In ALN-AS1-treated patients, the reduction in attack rate was accompanied by a 92% mean decrease in annualized hemin usage (range 84-100%) and a 2.7x increase in the mean maximal attack free interval compared to run-in period (range 2.1-3.2).

Figure 2: Part C Cohort 2 Summary of Treatment Period Clinical Efficacy Relative to Run-in



1.6.3. Clinical Safety

In Part A and Part B of the study, a total of 11 patients (11/13; 85.0%) who received ALN-AS1 reported at least 1 AE. All 5 placebo-treated patients (5/5; 100%) reported at least 1 AE. AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients (5/13; 38%): diarrhoea, dyspepsia, hematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (each AE reported only by an individual patient; 1/13; 8% each). There were 2 SAEs of abdominal pain: 1 patient each in the 0.035 mg/kg and 0.1 mg/kg ALN-AS1 dose groups. Both were considered to be not related and resolved without sequelae. There were no AEs leading to discontinuation. All AEs were mild or moderate in severity, except for 1 of the SAEs of abdominal pain (0.10 mg/kg dose group), which was considered severe. Adverse events related to abnormal laboratory values were seen in

1 patient in the 0.35 mg/kg ALN-AS1 dose group who had AEs of ALT increased and AST increased, which were moderate and considered by the Investigator to be unrelated to ALN-AS1. No clinically significant findings were observed with routine monitoring of CRP and a panel of 9 proinflammatory cytokines collected predose and up to 24 hours post-dose. There were no other clinically significant laboratory abnormalities related to study drug or changes in vital signs, or ECGs in patients who were administered ALN AS1 or placebo.

Dosing is ongoing in Part C of the study. All patients who received ALN-AS1 in cohort 1 and 2 reported at least 1 AE. AEs that were reported in at least 2 subjects were nausea in 3 patients (50%) and abdominal pain, vomiting, nasopharyngitis, headache, cough and oropharyngeal pain in 2 patients (33.3%) each. All AEs were mild or moderate in severity. AEs considered possibly or definitely related to ALN-AS1 were reported in 4 patients (66.6%), 1 each: renal impairment in Cohort 1 and injection site reaction, myalgia, and headache in Cohort 2. Both placebo-treated patients reported AEs. No AE was experienced in more than 1 patient.

In Part C, Cohort 1 or 2, there were no SAEs or AEs leading to discontinuation in patients administered ALN-AS1 or placebo. No clinically significant laboratory abnormalities related to study drug or changes in vital signs, ECG, or physical exam findings were observed in patients administered ALN-AS1 or placebo.

Part C Cohort 3 5.0 mg/kg SC monthly dosing is ongoing. In this cohort there was one fatal SAE of acute pancreatitis, which was determined to be unlikely related to study drug or placebo by the Investigator due to the presence of gall bladder sludge found at the time of her presentation. Contributors to this patient's adverse clinical course included: pre-existing chronic debilitation from recurrent AIP attacks requiring monthly hospitalization, porphyria-attributed quadriplegia requiring nursing home care, delay in hospital admission and complications from thromboembolism (multiple risk factors included pancreatitis, obesity, immobilisation from quadriparesis and recent hospitalization for infected portacath removal). Serum lipase was added to all scheduled laboratory assessments in November 2016 as part of additional safety monitoring. To date, all lipase results (available in 11 of 15 subjects) have been at or below the upper limit of normal with no trends seen with dosing of study drug or placebo.

1.7. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical finding of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

The potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Serum chemistry and coagulation changes: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.
- Liver function test abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of LFT abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. Women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use acceptable methods of contraception during studies with ALN-AS1.
- CYP inhibition: A study in NHP demonstrated that ALN-AS1 dosing could potentially increase the clearance of drugs metabolized by the CYP3A isoform. As contraceptive hormones are metabolized via CYP3A, WOCBP must use a barrier method of contraception, in addition to hormonal contraception, during study participation. Additionally, Investigators will review all patient medications at study start and monitor responses to these medications during the study. A list of medications that are sensitive CYP3A substrates is in Table 7 in Appendix Section 11.3. In addition, a list of medications that are sensitive CYP3A substrates is available at the Indiana University, Division of Clinical Pharmacology, website.[31]

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

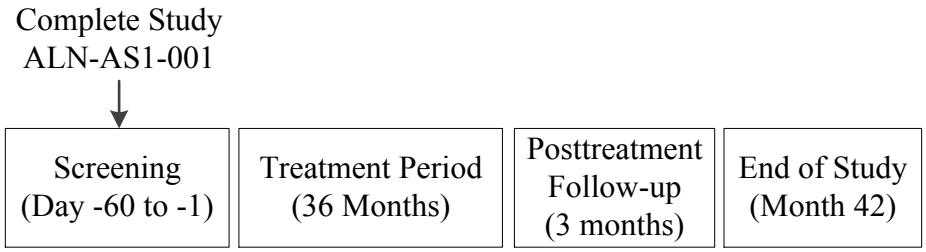
The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous

study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2; and Table 5 and Table 6 in Appendix Section 11.2). Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 39. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Figure 3: Study Design



4.2. Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening

3. WOCBP must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Removal from Treatment or Assessment

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or withdrawal from the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient at any time if this is considered to be in the best interest of the patient.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant

- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). Patients who discontinue treatment will be asked to complete the Month 39 visit assessments.

5.3.2. Withdrawal from Study

A patient may withdraw from the study at any time. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the Month 39 visit. When a patient withdraws from the study, the primary reason for study withdrawal must be recorded in the appropriate section of the eCRF and all efforts made to complete and report the observations as thoroughly as possible. If a patient withdraws due to a SAE, the SAE should be followed as described in Section 7.5.6.

5.3.3. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1 and Table 2). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 5 and Table 6 in Appendix Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in

accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

The study drug will be administered under the supervision of a healthcare professional. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

ALN-AS1 Solution for Injection (SC use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon faced bromobutyl 13 mm stopper and a flip-off Truedge aluminum seal.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1 and Table 2; and Table 5 and Table 6 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 7 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana

University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, or transdermal hormonal methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]). If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Placement of an intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- True sexual abstinence, when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods, if they start sexual relationships during the study and for 90 days after last dose administration.

WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedule of study assessments is provided in Table 1.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.[32] The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 4 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate 12-lead ECG, with readings approximately 2 minutes apart, will be recorded. Additional ECGs may be collected at the discretion of the Investigator. Standard computerized

12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Recordings will be obtained, after the patient has rested comfortably for approximately 10 minutes. The electrophysiological parameters assessed include rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB) and Fridericia-corrected QT interval (QTcF).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

On dosing days, results from hematology, chemistry, and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit, locally analyzed assessments may be reviewed to allow same day study drug administration, but additional samples for central analysis must also be collected.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
GGT	
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Coagulation	
Prothrombin time	International Normalized Ratio
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	
Inflammation	
C-reactive protein	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; GGT=gamma-glutamyltransferase; WOCBP=women of child bearing potential.

[a]eGFR will be calculated using the Modification of Diet in Renal Disease formula.[33]

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg, events include allergic bronchospasm requiring intensive treatment in an emergency

room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, additional clinical information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.[34] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to

the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.4. End of the Study

The end of the study is defined as last patient last visit.

9.1.5. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical

study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005 Mar 15;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010 Oct;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006 May-Jun;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012 Nov 29;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010 Mar 13;375(9718):924-37.

6. Floderus Y, Sardh E, Moller C, Andersson C, Rejkjaer L, Andersson DE, et al. Variations in porphobilinogen and 5-aminolevulinic acid concentrations in plasma and urine from asymptomatic carriers of the acute intermittent porphyria gene with increased porphyrin precursor excretion. Clin Chem. 2006 Apr;52(4):701-7.

7. Anderson K. Disorders of heme biosynthesis. 8th ed. CR S, editor: McGraw-Hill, New York 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transpl. 2012 Feb;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach J-C, Puy H. 86 - Acute Intermittent Porphyria: From Clinical to Molecular Aspects. In: Guilard KMKMS, editor. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000 Jul;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004 Nov;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013 May;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. Panhematin. (hemin for injection). 2013.

17. Normosang. (human hemin). 2007.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977 May;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993 Sep 13;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991 Sep;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989 Jun 10;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988 Feb;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990 Feb;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001 Feb 15;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013 Sep;36(5):849-57.

26. Anderson K. Heme arginate for prevention of acute porphyric attacks (abstract). Acta Haematologica. 1997;98((suppl 1):120).

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013 Jan;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002 Nov;48(11):1891-900.

31. Division of Clinical Pharmacology, Indiana University, P450 Drug Interaction Table. 2013 [Accessed 06 February 2015]; Available from: http://medicine.iupui.edu/clinpharm/ddis/main-table/.

32. Atkinson TM M, TR, Sit L, Passik P, Scher, HI, Cleeland C, and Basch E. The Brief Pain Inventory and its “Pain at its Worst in the last 24 Hours” Item: Clinical Trial Endpoint Considerations. Pain Med. 2010 March;11(3):337–46.

33. Levey AS SL, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

34. Herdman M GC, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 4 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 4: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X
	06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days	Anytime during visit	X
Month 1 (Day 31) ±7 days,[a] Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 18 (Day 541) ±10, and Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

a At the Day 31 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X						X				
Clinical Laboratory Assessment[h]	X		X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for		X		X	X	X		X		X		X		X		X

Table 5: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Exploratory Biomarker Analysis[l]																
Blood Sample for PK Analysis[m]		X	X	X		X		X				X				X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]			X													
AEs		Continuous														
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration[j]	X	X	X	X	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK Analysis[m]				X										

Table 6: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study procedures, including ALN-AS1 administration, may take place at the patient's home by a healthcare professional. Study procedures may occur at the patient's home at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M. Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.
c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. Urine samples should not be collected during a porphyria attack.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected predose. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit. On dosing days, results from hematology, chemistry (including LFTs), and coagulation tests collected within the prior 14 days must be reviewed by the Investigator before study drug administration. If results from within the prior 14 days are not available at a dosing visit, locally analyzed predose assessments may be used for review in order to allow same day study drug administration, but additional samples for central analysis must also be collected.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 4 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

A list of medications that are sensitive CYP3A substrates and those with a narrow therapeutic range is in Table 7.

Table 7: List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

Sensitive CYP3A Substrates			CYP3A Substrates With A Narrow Therapeutic Range
alfentanil	fluticasone		Alfentanil
aprepitant	lopinavir		Astemizole
budesonide	lovastatin		Cisapride
buspirone	lurasidone		Cyclosporine
conivaptan	maraviroc		Diergotamine
darifenacin	midazolam		Dihydroergotamine
darunavir	nisoldipine		Ergotamine
dasatinib	quetiapine		Fentanyl
dronedarone	saquinavir		Irinotecan
eletriptan	sildenafil		Pimozide
eplerenone	simvastatin		Quinidine
everolimus	sirolimus		Sirolimus
felodipine	tolvaptan		Tacrolimus
imatinib	tipranavir		Terfenadine
indinavir	triazolam		
	vardenafil		

Note: This is not an exhaustive list and availability of medications may differ between countries. For more information about clinically relevant drugs that are CYP3A substrates, see the Indiana University Division of Pharmacology website for reference: http://medicine.iupui.edu/clinpharm/ddis/clinical-table/.

ALN-AS1-002 Protocol Amendment 3

Summary of Changes (dated 03 February 2017) compared to Protocol Amendment 2 (dated 01 December 2016)

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

Rationale for Protocol Amendment

The purpose of this amendment is to update the risk-benefit assessment of the study protocol to align with the current Investigator's Brochure, to add regular monitoring of prothrombin time (PT), International Normalized Ratio (INR), and c-reactive protein (CRP), and to clarify the timing of the review of clinical laboratory assessments prior to scheduled dosing.

This amendment includes the following changes:

- Updated dose rationale and risk-benefit sections to align with current IB including preliminary clinical results from Part C of Study ALN-AS1-001 and nonclinical findings from the rat and NHP chronic toxicology studies
- Added assessments of PT, INR and CRP to regularly scheduled clinical laboratory draws
- Clarified that hematology, chemistry (including LFTs), and coagulation results must be reviewed prior to each dose, and that the interval between the blood sample collection and dosing should not exceed 14 days

A detailed summary of the changes is provided in Table 1. Corrections to typographical errors and formatting are not detailed.

Table 1: Protocol Amendment 3 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in the protocol amendment are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: To update the benefit-risk assessment with recent nonclinical and clinical data

The primary change occurs in Section 1.7 Benefit-Risk Assessment

Added text:

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication. **As presented in Section 1.6, emerging data demonstrate that patients given ALN-AS1 experienced reduced ALA/PBG levels, decreased attack frequency and reduced heme usage in the 24-week treatment period compared to the 12-week run-in period in Study ALN-AS1-001**

As detailed in Section 1.6, a fatal SAE of hemorrhagic pancreatitis was reported but deemed to be unlikely related to study drug. However, the potentially non-adverse nonclinical findings of angiectasis in the islets of Langerhans (endocrine, not exocrine region) of the rat pancreas (see Investigator's Brochure), do not exclude a potential association between ALN-AS1 and the SAE of hemorrhagic pancreatitis.

Purpose: To clarify procedures on predose clinical laboratory review

The primary change occurs in Section 7.5.5 Clinical Laboratory Assessments

Now reads:

~~Results~~**On dosing days, results** from ~~clinical~~ ~~**laboratory**~~**hematology, chemistry, and coagulation** tests collected ~~on dosing days are not required before study drug administration; however if results for clinical laboratory tests from~~ **within** the ~~previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments~~ **prior 14 days** must be ~~repeated and the results must be deemed acceptable~~ **reviewed** by the Investigator before study drug administration. **These results can be analyzed centrally or at a local laboratory. If results from within the prior 14 days are not available at a dosing visit, locally analyzed assessments may be reviewed to allow same day study drug administration, but additional samples for central analysis must also be collected.**

Section(s) also containing this change or similar changes:

- Table 1 Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen), footnote h
- Table 2 Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen), footnote h
- Table 5 Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen), footnote h
- Table 6 Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen), footnote h

Purpose: To add summary of ALN-AS1 clinical data to align with current version of the Investigator's Brochure

The primary change occurs in Section 1.6 Clinical Data Summary

Added text: Section 1.6 has been added, which summarizes the results to date on the safety, pharmacodynamics, and clinical activity of ALN-AS1.

Purpose: To clarify the details for visits taking place at a patient's home

The primary change occurs in Section 4.1 Summary of Study Design

Now reads: After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study ~~visits and~~ **procedures, including** ALN-AS1 administration, may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2; and Table 5 and Table 6 in Appendix Section 11.2). ~~Home visits~~**Study procedures** may occur **at the patient's home** at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. **Additional visits to collect clinical laboratory samples may occur at home at any point during the treatment period, within 14 days prior to scheduled dose administration.**

Section(s) also containing this change or similar changes:

- Synopsis, Study Design
- Table 1 Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen), footnote b
- Table 2 Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen), footnote a
- Section 6.2.2 Dose and Administration
- Table 5 Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen), footnote b
- Table 6 Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen), footnote a

Purpose: To add serum chemistry and coagulation changes as a potential risk in the benefit-risk analysis

The primary change occurs in Section 1.7 Benefit-Risk Assessment

Added text:

- **Serum chemistry and coagulation changes: Since an association between ALN-AS1 and the fatal SAE of hemorrhagic pancreatitis, complicated by a pulmonary embolism, can not be ruled out, patients will undergo routine monitoring of systemic and pancreatic inflammation and coagulation throughout the study. Hematology, chemistry and coagulation results are required to be available prior to each dose.**

Purpose: To expand clinical laboratory assessment to include coagulation tests and c-reactive protein evaluation

The primary change occurs in Section 7.5.5 Clinical Laboratory Assessments	
Added text:	Added rows to the clinical laboratory assessment table for coagulation tests (prothrombin time and International Normalized Ratio), and inflammation (c-reactive protein).

Purpose: To add GGT to the battery of liver function tests, per clarification letter dated 01 December 2016.

The primary change occurs in Section 7.5.5 Clinical Laboratory Assessments	
Added text:	Added row to the clinical laboratory assessment table for gamma-glutamyltransferase (GGT)

CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016 Protocol Amendment 2 01 December 2016
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD

1 DEC 2016

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

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

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 6 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen, including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to

be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.
Reference Therapy, Dose, and Mode of Administration Not applicable
Duration of Treatment and Study The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.
Statistical Methods Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study. Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized. PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points. The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided. Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X				X		X		X		X				X
Vital Signs[f]	X	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X				X						X				
Clinical Laboratory Assessment[h]	X		X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X				X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X		X	X	X		X		X		X		X		X
Blood Sample for PK		X	X	X		X		X				X				X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)														
			M1		M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Analysis[m]																
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]			X													
Phone Contact[r]							X		X		X		X		X	
AEs		Continuous														
Concomitant Medications[s]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.
c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.
d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose. Results from clinical laboratory tests collected on dosing days are not required before study drug administration; however, if results for clinical laboratory tests from the previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.
p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.
q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.
r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]		X		X		X		X		X		X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]		X		X		X		X		X		X	X	
Study Drug Administration[j]		X		X		X		X		X		X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK				X										

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Analysis[m]														
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
Phone Contact[q]	X		X		X		X		X		X			X
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.
b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.
c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose. Results from clinical laboratory tests collected on dosing days are not required before study drug administration; however, if results for clinical laboratory tests from the previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate

Abbreviation	Definition
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.[1] The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.[2] In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.[3, 4]

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.[5] The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.[6]

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.[2] However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.[7-10] AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.[11] It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.[12] AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).[2] The initial diagnosis involves

demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA (typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.[6-8] Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.[7]

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.[13] In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.[14] With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.[15]

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.[16, 17] In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.[18-22] Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.[22-24] In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.[25] While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).[26] Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).[5] In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.[27] In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option [34]. Given the significant morbidity and mortality, there

remains a significant unmet need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.[28] Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.[29] The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have

higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.[30] For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related SAEs, severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication.

The potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Liver function test abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of LFT abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.

- Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. Based on preliminary data from rat fertility/embryofetal development and rabbit embryofetal development studies, it is unlikely that ALN-AS1 administration to pregnant women poses a risk to embryofetal development. In a rat fertility/embryofetal development study at daily dose levels up to 16.5 mg/kg, no test article-related effects on female fertility or developmental endpoints were observed. In a rabbit embryofetal development study, maternal toxicity was observed at daily doses of >3 mg/kg (7.8-fold higher than the clinical monthly dose of 5.0 mg/kg). No terata were observed in this range finding embryo fetal development study. Women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use acceptable methods of contraception during studies with ALN-AS1.
- CYP inhibition: A study in NHP demonstrated that ALN-AS1 dosing could potentially increase the clearance of drugs metabolized by the CYP3A isoform. As contraceptive hormones are metabolized via CYP3A, WOCBP must use a barrier method of contraception, in addition to hormonal contraception, during study participation. Additionally, Investigators will review all patient medications at study start and monitor responses to these medications during the study. A list of medications that are sensitive CYP3A substrates is in Table 6 in Appendix Section 11.3. In addition, a list of medications that are sensitive CYP3A substrates is available at the Indiana University, Division of Clinical Pharmacology, website.[31]

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

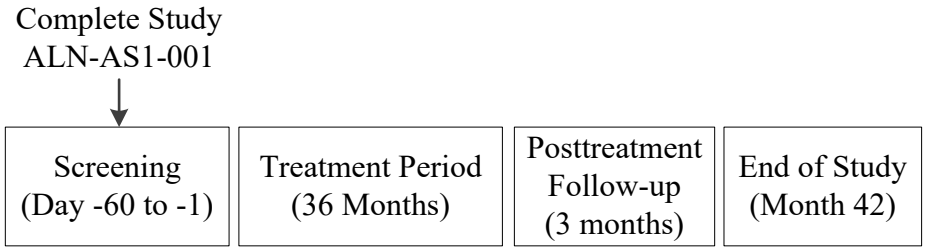
The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1

administration may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 39. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Figure 1: Study Design



4.2. Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening
3. WOCBP must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Removal from Treatment or Assessment

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or withdrawal from the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient at any time if this is considered to be in the best interest of the patient.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). Patients who discontinue treatment will be asked to complete the Month 39 visit assessments.

5.3.2. Withdrawal from Study

A patient may withdraw from the study at any time. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the Month 39 visit. When a patient withdraws from the study, the primary reason for study withdrawal must be

recorded in the appropriate section of the eCRF and all efforts made to complete and report the observations as thoroughly as possible. If a patient withdraws due to a SAE, the SAE should be followed as described in Section 7.5.6.

5.3.3. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1 and Table 2). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 4 and Table 5 in Appendix Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

The study drug will be administered under the supervision of a healthcare professional. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical

study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

ALN-AS1 Solution for Injection (SC use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon faced bromobutyl 13 mm stopper and a flip-off Truedge aluminum seal.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 6 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, or transdermal hormonal methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]). If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Placement of an intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- True sexual abstinence, when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods, if they start sexual relationships during the study and for 90 days after last dose administration.

WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedule of study assessments is provided in Table 1.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.[32] The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 3 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate 12-lead ECG, with readings approximately 2 minutes apart, will be recorded. Additional ECGs may be collected at the discretion of the Investigator. Standard computerized 12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Recordings will be obtained, after the patient has rested comfortably for approximately 10 minutes. The electrophysiological parameters assessed include rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB) and Fridericia-corrected QT interval (QTcF).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or

arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory.

Results from clinical laboratory tests collected on dosing days are not required before study drug administration; however, if results for clinical laboratory tests from the previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.

Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Lipase	Bicarbonate
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.[33]

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg, events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs

- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, additional clinical information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.[34] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to

the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.4. End of the Study

The end of the study is defined as last patient last visit.

9.1.5. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical

study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005 Mar 15;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010 Oct;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006 May-Jun;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012 Nov 29;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010 Mar 13;375(9718):924-37.

6. Floderus Y, Sardh E, Moller C, Andersson C, Rejkjaer L, Andersson DE, et al. Variations in porphobilinogen and 5-aminolevulinic acid concentrations in plasma and urine from asymptomatic carriers of the acute intermittent porphyria gene with increased porphyrin precursor excretion. Clin Chem. 2006 Apr;52(4):701-7.

7. Anderson K. Disorders of heme biosynthesis. 8th ed. CR S, editor: McGraw-Hill, New York 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transpl. 2012 Feb;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach J-C, Puy H. 86 - Acute Intermittent Porphyria: From Clinical to Molecular Aspects. In: Guilard KMKMS, editor. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000 Jul;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004 Nov;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013 May;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. Panhematin. (hemin for injection). 2013.

17. Normosang. (human hemin). 2007.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977 May;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993 Sep 13;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991 Sep;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989 Jun 10;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988 Feb;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990 Feb;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001 Feb 15;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013 Sep;36(5):849-57.

26. Anderson K. Heme arginate for prevention of acute porphyric attacks (abstract). Acta Haematologica. 1997;98((suppl 1):120).

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013 Jan;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002 Nov;48(11):1891-900.

31. Division of Clinical Pharmacology, Indiana University, P450 Drug Interaction Table. 2013 [Accessed 06 February 2015]; Available from: http://medicine.iupui.edu/clinpharm/ddis/main-table/.

32. Atkinson TM M, TR, Sit L, Passik P, Scher, HI, Cleeland C, and Basch E. The Brief Pain Inventory and its “Pain at its Worst in the last 24 Hours” Item: Clinical Trial Endpoint Considerations. Pain Med. 2010 March;11(3):337–46.

33. Levey AS SL, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

34. Herdman M GC, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 3 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 3: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X
	06:00 (±20 minutes)	X
Month 1 (Day 14) ±2 days	Anytime during visit	X
Month 1 (Day 31) ±7 days,[a] Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 18 (Day 541) ±10, and Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

a At the Day 31 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X															
Medical History/Disease History[c]	X	X														
Demographics	X															
Inclusion/ Exclusion Criteria	X	X														
Physical Examination[d]	X	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X		X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X				X						X				
Clinical Laboratory Assessment[h]	X		X	X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for		X		X	X	X		X		X		X		X		X

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]		Treatment Period (Screening/Baseline through Month 18)													
			M1		M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D14 ±2**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Exploratory Biomarker Analysis[l]																
Blood Sample for PK Analysis[m]		X	X	X		X		X				X				X
Antidrug Antibodies[n]		X		X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X															
EQ-5D-5L Questionnaire[p]	X							X				X				X
Diary Review (including BPI-SF)[q]			X													
AEs		Continuous														
Concomitant Medications[r]		Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available and deemed acceptable before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose. Results from clinical laboratory tests collected on dosing days are not required before study drug administration; however, if results for clinical laboratory tests from the previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration[j]	X	X	X	X	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK Analysis[m]				X										

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.

c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. Urine samples should not be collected during a porphyria attack.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose Results from clinical laboratory tests collected on dosing days are not required before study drug administration; however, if results for clinical laboratory tests from the previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

A list of medications that are sensitive CYP3A substrates and those with a narrow therapeutic range is in Table 6.

Table 6: List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

Sensitive CYP3A Substrates			CYP3A Substrates With A Narrow Therapeutic Range
alfentanil	fluticasone		Alfentanil
aprepitant	lopinavir		Astemizole
budesonide	lovastatin		Cisapride
buspirone	lurasidone		Cyclosporine
conivaptan	maraviroc		Diergotamine
darifenacin	midazolam		Dihydroergotamine
darunavir	nisoldipine		Ergotamine
dasatinib	quetiapine		Fentanyl
dronedarone	saquinavir		Irinotecan
eletriptan	sildenafil		Pimozide
eplerenone	simvastatin		Quinidine
everolimus	sirolimus		Sirolimus
felodipine	tolvaptan		Tacrolimus
imatinib	tipranavir		Terfenadine
indinavir	triazolam		
	vardenafil		

Note: This is not an exhaustive list and availability of medications may differ between countries. For more information about clinically relevant drugs that are CYP3A substrates, see the Indiana University Division of Pharmacology website for reference: http://medicine.iupui.edu/clinpharm/ddis/clinical-table/.

ALN-AS1-002 Protocol Amendment 2

Summary of Changes (dated 01 December 2016) compared to Protocol Amendment 1 (dated 10 November 2016)

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

Rationale for Protocol Amendment

The primary purpose of this amendment is to implement additional safety monitoring; specifically to require lipase monitoring and review of recent clinical laboratory results prior to dosing. These changes are being made in accordance with the study's Safety Review Committee recommendation pursuant to an unlikely related SAE of hemorrhagic pancreatitis with fatal outcome. This amendment includes the following changes:

- A Day 14 clinic visit has been added to allow safety assessment approximately 2 weeks following the first study drug dose.
- Lipase has been added to the battery of serum chemistry parameters regularly collected throughout the study
- Laboratory results from the previous clinical visit are now required to be available prior to each dose. If results from the previous visit are clinically significant or are not available, assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration
- Lipase testing is now required, and imaging should be considered by the Investigator, when a patient experiences uncharacteristic signs and symptoms during a porphyria attack.
- Imaging is now required following a clinical laboratory test result of serum lipase ≥3× the upper limit of normal.

Additionally, out of date text describing the nonclinical and clinical experience with ALN-AS1 has been removed and replaced with a reference to the current Investigator's Brochure, which has been updated to include a description of the aforementioned unlikely related fatal SAE.

A detailed summary of the changes is provided in Table 1. Corrections to typographical errors and formatting are not detailed.

Table 1: Protocol Amendment 2 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in the protocol amendment are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: To add a clinic visit approximately 2 weeks following the first dose of study drug

The primary change occurs in Table 1, Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Added text: A column has been added to the schedule of assessments reflecting a Day 14 visit during which safety assessments will be collected.

Section(s) also containing this change or similar changes:

- Table 3, Pharmacokinetic Time Points
- Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Purpose: To clarify that safety assessments must be available and deemed acceptable prior to administration of the first dose of study drug.

The primary change occurs in Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen), Footnote a

Now reads: a. Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available **and deemed acceptable** before the administration of the first dose of ALN-AS1.

Section(s) also containing this change or similar changes:

- Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Purpose: To require review of clinical laboratory tests from previous visit prior to each study drug administration.

The primary change occurs in Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen), Footnote h

Now reads: h. Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose~~; results are not required before dosing~~. **Results from clinical laboratory tests collected on dosing days are not required before study drug administration; however, if results for clinical laboratory tests from the previous study visit are not available or are deemed clinically significant, then clinical laboratory assessments must be repeated and the results must be deemed acceptable by the Investigator before study drug administration.** On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

Section(s) also containing this change or similar changes:

- Table 2, Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)
- Section 7.5.5 Clinical Laboratory Assessments
- Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)
- Table 5, Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

Purpose: To streamline the protocol introduction by removing out of date text describing the nonclinical and clinical experience with ALN-AS1.

The primary change occurs in Section 1 INTRODUCTION

Now reads:

Section 1.3.1 Nonclinical Summary and Section 1.3.2 Clinical Summary have been removed. Subsequent sections have been renumbered appropriately. Section 1.3 now reads:

ALN-AS1 comprises a synthetic siRNA covalently linked to a GalNAc containing ligand (ALN60519), specific for ALAS1 and formulated for administration via subcutaneous (SC) injection. ALN-60519 has a target region between nucleotide 918 to 937 of the ALAS1 mRNA. Attachment to a triantennary GalNAc ligand to the siRNA enables its distribution to the liver (which is the primary site of ALAS1 synthesis), followed by intracellular delivery to hepatocytes via ASGPR, resulting in engagement of the RNAi pathway and suppression of hepatic ALAS1 protein synthesis.

ALN-AS1 is currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

Further information on ALN-AS1, including the clinical and nonclinical experience to date, is available in in the current version of the Investigator's Brochure (IB).

Purpose: To require clinical laboratory tests and consider imaging when a patient experiences uncharacteristic signs and symptoms during a porphyria attack.

The primary change occurs in Section 7.5.5 Clinical Laboratory Assessments

Added text:	**At any point during the study, and based on Investigator judgment, if a patient experiences uncharacteristic signs and symptoms during a porphyria attack, lipase testing should be performed and imaging evaluations (eg, right upper quadrant ultrasound and/or other testing, based on Investigator judgment) should be considered by the Investigator. Signs and symptoms include, but are not limited to, uncharacteristic abdominal pain and worsening nausea and vomiting.**

Purpose: To require imaging following a result of serum lipase ≥3× the upper limit of normal

The primary change occurs in Section 7.5.5 Clinical Laboratory Assessments

Added text:	**Imaging is required for any serum lipase result of ≥3× the upper limit of normal, or as clinically indicated.**

Purpose: To add lipase to the serum chemistry battery as well as inadvertently omitted bicarbonate.

The primary change occurs in Section 7.5.5 Clinical Laboratory Assessments

Added text:	Lipase and bicarbonate have been added to the list of serum chemistry parameters to be collected as part of clinical laboratory tests.

Purpose: Correct typographical and formatting.

These changes are not listed individually.

CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original Protocol 19 July 2016 Protocol Amendment 0.1 (Sweden) 20 September 2016 Protocol Amendment 1 10 November 2016
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD

10 NOV 2016.

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

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

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

• Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein
Study Design This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 6 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.
Number of Planned Patients Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).
Diagnosis and Main Eligibility Criteria Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.
Investigational Product, Dose, and Mode of Administration ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer. The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the dosing regimen, including decreases in the dose level and changes in the dosing frequency. However any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to

be safe and well-tolerated in study ALN-AS1-001, SRC, Health Authority and Ethics Committee approval must be sought prior to dose escalation above 5.0 mg/kg in accordance with local Regulatory requirements.
Reference Therapy, Dose, and Mode of Administration Not applicable
Duration of Treatment and Study The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.
Statistical Methods Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study. Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized. PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points. The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided. Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X														
Medical History/Disease History[c]	X	X													
Demographics	X														
Inclusion/ Exclusion Criteria	X	X													
Physical Examination[d]	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X			X		X		X		X				X
Vital Signs[f]	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X			X						X				
Clinical Laboratory Assessment[h]	X		X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X			X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X	X	X	X		X		X		X		X		X
Blood Sample for PK Analysis[m]		X	X		X		X				X				X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Antidrug Antibodies[n]		X	X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X														
EQ-5D-5L Questionnaire[p]	X						X				X				X
Diary Review (including BPI-SF)[q]		X													
Phone Contact[r]						X		X		X		X		X	
AEs		Continuous													
Concomitant Medications[s]	Continuous														

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available before the administration of the first dose of ALN-AS1.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]		X		X		X		X		X		X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]		X		X		X		X		X		X	X	
Study Drug Administration[j]		X		X		X		X		X		X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK				X										

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Analysis[m]														
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
Phone Contact[q]	X		X		X		X		X		X			X
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.
b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.
c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate

Abbreviation	Definition
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.[1] The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.[2] In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.[3, 4]

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.[5] The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.[6]

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.[2] However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.[7-10] AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.[11] It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.[12] AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).[2] The initial diagnosis involves

demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA (typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.[6-8] Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.[7]

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.[13] In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.[14] With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.[15]

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.[16, 17] In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.[18-22] Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.[22-24] In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.[25] While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).[26] Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).[5] In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.[27] In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option [34]. Given the significant morbidity and mortality, there

remains a significant unmet need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.[28] Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.[29] The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 is a synthetic RNAi therapeutic currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

1.3.1. Nonclinical Summary

The pharmacology, safety pharmacology, drug metabolism and pharmacokinetics and toxicology of ALN-AS1 were evaluated in a series of in vitro and in vivo nonclinical studies.

ALN-AS1 is pharmacologically active in rodents and nonhuman primates (NHP) due to conservation within the siRNA target sequence. Transfection assays in human liver carcinoma cell line-G2 cells showed dose-dependent inhibition of endogenous ALAS1 mRNA levels with an half-maximal inhibitory concentration of approximately 26 pM of ALN-AS1. In multiple studies in the mouse, rat, and NHP, potent and dose-dependent pharmacologic activity has been demonstrated with subcutaneous (SC) administration of ALN-AS1, which results in consistent reduction of ALAS1 mRNA in liver. After single dose administration, ALAS1 mRNA suppression in liver is durable depending on the dose administered (1-4 weeks) and correlates with the extent of ALAS1 mRNA suppression observed in serum or urine. Dose dependent, steady-state ALAS1 mRNA reduction has also been demonstrated with repeat-dose regimens. Studies in rodent AIP models have confirmed that ALAS1 mRNA reduction with ALN-AS1 treatment correlates with decreases in ALA and PBG heme intermediates.

A Good Laboratory Practice (GLP)-compliant safety pharmacology study conducted in NHPs showed no functional cardiovascular or respiratory effects, with a no observed effect level (NOEL) of 150 mg/kg. Neurological assessments were conducted as part of a 13-week repeat dose GLP toxicity study in NHPs (weekly dosing); no ALN-AS1-related neurobehavioral observations occurred, with a NOEL for neurological effects of 150 mg/kg (highest dose tested). Genetic toxicity studies (bacterial reverse mutation, human peripheral blood lymphocyte chromosomal aberrations, and rat bone marrow micronucleus) assays were all negative at International Conference on Harmonization (ICH) S2 (R1) limit doses.

Two to 4-week exploratory (non-GLP) dose-range finding toxicology studies were conducted with ALN-AS1 in mice, rats, and NHPs. In all 3 species, no dose-limiting toxicity was observed up to the highest dose evaluated (300 mg/kg weekly SC dose), with the rat appearing to be the most sensitive species. These studies were followed by 13-week GLP toxicology studies in rats and NHPs where ALN-AS1 was administered daily (100 mg/kg) and weekly at doses of 0, 3, 10, and 30 mg/kg in the rat and weekly at doses of 0, 15, 50, and 150 mg/kg in the NHP. The no observed adverse effect levels (NOAELs) were 30 mg/kg in the rat and 150 mg/kg in the NHP (highest dose tested in each species).

Preliminary results from 26-week and 39-week GLP chronic toxicity studies conducted in rats and NHPs, respectively, support long-term clinical dosing. The repeat-dose toxicity study in rats included assessment of male fertility and early embryonic development. ALN-AS1 was administered weekly at doses of 0, 3, 10, or 30 mg/kg for up to 26 weeks. Weekly doses of 30 mg/kg resulted in adverse hepatocellular single cell necrosis and vacuolation in the liver, associated with mild increases in liver function tests (LFTs) at the conclusion of the dosing period. Angiectasis was observed in the islets of Langerhans, possibly a secondary change to hepatic changes, which is not considered adverse. Combined male fertility assessments did not indicate adverse reproductive or developmental findings up to the highest dose evaluated (30 mg/kg weekly SC dose). In the 39-week repeat-dose toxicity study in NHP, ALN-AS1 was administered weekly at doses of 0, 10, 30, or 100 mg/kg for up to 39 weeks. Weekly doses of 100 mg/kg were the lowest-observed-adverse-effect level based on single cell necrosis combined with increased alanine transaminase (approximately 2-fold above control). The NOAELs were 10 mg/kg in the rat and 30 mg/kg in the NHP. The 13-week recovery data in the rat and NHP studies is pending.

1.3.2. Clinical Summary

ALN-AS1 is being investigated in an ongoing, Phase 1 study (ALN-AS1-001) to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ALN-AS1 administered subcutaneously in patients with AIP. The study is being conducted in 3 parts. Part A and Part B are single-blind, placebo-controlled portions of the study in patients with AIP who are ASHE. Patients are randomized in a 3:1 ratio to receive either a single dose (Part A) or multiple doses (Part B; 2 doses 28 days apart) of ALN-AS1 or placebo. Patients who participate in Part A and Part B are not eligible to participate in this study. In Part C, patients with AIP who have recurrent porphyria attacks will receive multiple doses of ALN-AS1 dosing over a 12-week period in a randomized, double-blind, placebo-controlled part of the study. This study will enroll patients who completed Part C of study ALN-AS1-001.

Dosing has been completed in Part A (0.035-2.5 mg/kg) and Part B of the study (0.35-1.0 mg/kg). A total of 23 unique patients with AIP who are ASHE have received at least 1 dose of study drug (20 patients received at least 1 dose of ALN-AS1 and 3 patients received placebo only). ALN-AS1 has been generally well-tolerated. Adverse Events (AEs) in patients administered ALN-AS1, which were reported in 2 patients each, were abdominal pain, diarrhea, and hypoaesthesia in Part A; and nasopharyngitis, pruritus, and rash in Part B. In Part A, AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients: diarrhea, dyspepsia, haematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (1 patient each). In Part B, AEs considered possibly or definitely related to ALN-AS1 were reported in 3 patients: pruritus and

rash (2 patients each); and rash macular and rash pruritic (1 patient each). There were 2 SAEs of abdominal pain in Part A, 1 patient each in the 0.035 mg/kg and 0.10 mg/kg dose groups, which were not considered to be related to study drug or due to AIP. Across Part A and Part B, all AEs were mild or moderate in severity, except for 1 serious adverse event (SAE) of abdominal pain (0.10 mg/kg dose group), which was considered severe. There were no dose-related trends in AEs and no AEs leading to discontinuation.

Overall, a dose-dependent reduction of urinary ALA and PBG as well as ALAS1 mRNA was observed following single and multiple doses of ALN-AS1. Preliminary data demonstrate that administration of a single dose of ALN-AS1 at ≥1.0 mg/kg lowered ALA and PBG levels ≥80% and ALAS1 mRNA levels ≥50% when compared to baseline levels in patients with AIP who are ASHE. Similarly, administration of multiple doses of ALN-AS1 at ≥0.35 mg/kg lowered ALA and PBG levels ≥80%. ALAS1 mRNA levels were reduced ≥50% in the 1.0 mg/kg dose group when compared to baseline levels in patients with AIP who are ASHE. ALA and PBG levels in the 1.0 mg/kg dose group in Part A were sustained for ≥180 days and in both dose groups in Part B for ≥160 days. Specifically, the maximum mean reduction from baseline of urinary ALA and PBG reached 93% in the 2.5 mg/kg dose group, which were sustained for approximately 14 weeks. The maximum mean reduction in ALAS1 mRNA relative to baseline of 66% was demonstrated in the 2.5 mg/kg dose group which was maintained beyond 42 days.

Cumulatively, nonclinical and clinical data to date support long-term administration of ALN-AS1 in patients with AIP.

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.[30] For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing

frequency. However, any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN-AS1-001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN-AS1 has been generally well-tolerated. There have been no study drug-related SAEs, severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN-AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1.

1.6. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication.

The potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Liver function test abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of LFT abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. Based on preliminary data from rat fertility/embryofetal development and rabbit embryofetal development studies, it is unlikely that ALN-AS1 administration to pregnant women poses a risk to embryofetal development. In a rat fertility/embryofetal development study at daily dose levels up to 16.5 mg/kg, no test article-related effects on female fertility or developmental endpoints were observed. In a rabbit embryofetal development study, maternal toxicity was observed at daily

doses of >3 mg/kg (7.8-fold higher than the clinical monthly dose of 5.0 mg/kg). No terata were observed in this range finding embryo fetal development study. Women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use acceptable methods of contraception during studies with ALN-AS1.

- CYP inhibition: A study in NHP demonstrated that ALN-AS1 dosing could potentially increase the clearance of drugs metabolized by the CYP3A isoform. As contraceptive hormones are metabolized via CYP3A, WOCBP must use a barrier method of contraception, in addition to hormonal contraception, during study participation. Additionally, Investigators will review all patient medications at study start and monitor responses to these medications during the study. A list of medications that are sensitive CYP3A substrates is in Table 6 in Appendix Section 11.3. In addition, a list of medications that are sensitive CYP3A substrates is available at the Indiana University, Division of Clinical Pharmacology, website.[31]

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks

- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

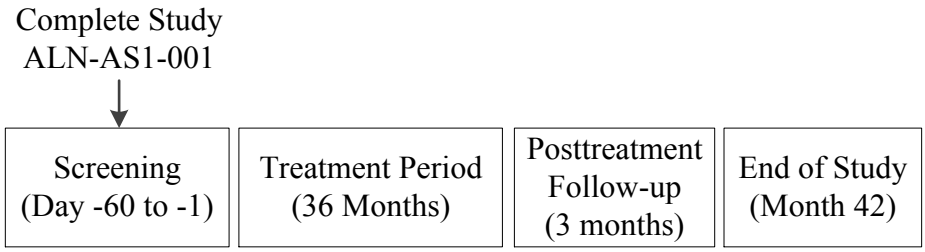
This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 39. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Figure 1: Study Design



4.2. Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in this study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening
3. WOCBP must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in this study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)

4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Removal from Treatment or Assessment

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or withdrawal from the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient at any time if this is considered to be in the best interest of the patient.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). Patients who discontinue treatment will be asked to complete the Month 39 visit assessments.

5.3.2. Withdrawal from Study

A patient may withdraw from the study at any time. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the Month 39 visit. When a patient withdraws from the study, the primary reason for study withdrawal must be recorded in the appropriate section of the eCRF and all efforts made to complete and report the observations as thoroughly as possible. If a patient withdraws due to a SAE, the SAE should be followed as described in Section 7.5.6.

5.3.3. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The study starting dose of ALN-AS1 is 5.0 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1 and Table 2). Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency (Table 4 and Table 5 in Appendix Section 11.2). However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements. See Section 6.2.3 for details on dose modifications.

The study drug will be administered under the supervision of a healthcare professional. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has a study drug related AE of a recurrent ISR, severe ISR, or clinically significant LFT abnormality and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months is permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

ALN-AS1 Solution for Injection (SC use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon faced bromobutyl 13 mm stopper and a flip-off Truedge aluminum seal.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is included in Table 6 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

WOCBP must be willing to use acceptable methods of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Birth control methods which may be considered as acceptable include:

- Established use of oral, implantable, injectable, or transdermal hormonal methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]). If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Placement of an intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- True sexual abstinence, when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods, if they start sexual relationships during the study and for 90 days after last dose administration.

WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedule of study assessments is provided in Table 1.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.[32] The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 3 in Appendix Section 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. Body temperature will be obtained in degrees Celsius,

heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate 12-lead ECG, with readings approximately 2 minutes apart, will be recorded. Additional ECGs may be collected at the discretion of the Investigator. Standard computerized 12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Recordings will be obtained, after the patient has rested comfortably for approximately 10 minutes. The electrophysiological parameters assessed include rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB) and Fridericia-corrected QT interval (QTcF).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory. In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.[33]

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg, events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs

- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, additional clinical information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.[34] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to

the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.4. End of the Study

The end of the study is defined as last patient last visit.

9.1.5. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical

study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005 Mar 15;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010 Oct;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006 May-Jun;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012 Nov 29;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010 Mar 13;375(9718):924-37.

6. Floderus Y, Sardh E, Moller C, Andersson C, Rejkjaer L, Andersson DE, et al. Variations in porphobilinogen and 5-aminolevulinic acid concentrations in plasma and urine from asymptomatic carriers of the acute intermittent porphyria gene with increased porphyrin precursor excretion. Clin Chem. 2006 Apr;52(4):701-7.

7. Anderson K. Disorders of heme biosynthesis. 8th ed. CR S, editor: McGraw-Hill, New York 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transpl. 2012 Feb;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach J-C, Puy H. 86 - Acute Intermittent Porphyria: From Clinical to Molecular Aspects. In: Guilard KMKMS, editor. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000 Jul;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004 Nov;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013 May;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. Panhematin. (hemin for injection). 2013.

17. Normosang. (human hemin). 2007.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977 May;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993 Sep 13;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991 Sep;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989 Jun 10;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988 Feb;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990 Feb;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001 Feb 15;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013 Sep;36(5):849-57.

26. Anderson K. Heme arginate for prevention of acute porphyric attacks (abstract). Acta Haematologica. 1997;98((suppl 1):120).

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013 Jan;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002 Nov;48(11):1891-900.

31. Division of Clinical Pharmacology, Indiana University, P450 Drug Interaction Table. 2013 [Accessed 06 February 2015]; Available from: http://medicine.iupui.edu/clinpharm/ddis/main-table/.

32. Atkinson TM M, TR, Sit L, Passik P, Scher, HI, Cleeland C, and Basch E. The Brief Pain Inventory and its “Pain at its Worst in the last 24 Hours” Item: Clinical Trial Endpoint Considerations. Pain Med. 2010 March;11(3):337–46.

33. Levey AS SL, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

34. Herdman M GC, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 3 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 3: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X
	06:00 (±20 minutes)	X
Month 1 (Day 31) ±7 days,[a] Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 18 (Day 541) ±10, and Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

a At the Month 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Informed Consent	X														
Medical History/Disease History[c]	X	X													
Demographics	X														
Inclusion/ Exclusion Criteria	X	X													
Physical Examination[d]	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X	X	X	X	X	X	X	X	X	X				X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X			X						X				
Clinical Laboratory Assessment[h]	X		X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X	X	X	X		X		X		X		X		X

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X		X		X				X				X
Antidrug Antibodies[n]		X	X		X		X				X				X
Porphyria Attack Kit Dispensation[o]	X														
EQ-5D-5L Questionnaire[p]	X						X				X				X
Diary Review (including BPI-SF)[q]		X													
AEs		Continuous													
Concomitant Medications[r]		Continuous													

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.
d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, urine samples should be collected within 24 hours of the start of clinical intervention (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits should be used for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.
p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.
q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.
r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration[j]	X	X	X	X	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK Analysis[m]				X										

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.

c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. Urine samples should not be collected during a porphyria attack.

d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

i Urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.

11.3. List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

A list of medications that are sensitive CYP3A substrates and those with a narrow therapeutic range is in Table 6.

Table 6: List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range

Sensitive CYP3A Substrates			CYP3A Substrates With A Narrow Therapeutic Range
alfentanil	fluticasone		Alfentanil
aprepitant	lopinavir		Astemizole
budesonide	lovastatin		Cisapride
buspirone	lurasidone		Cyclosporine
conivaptan	maraviroc		Diergotamine
darifenacin	midazolam		Dihydroergotamine
darunavir	nisoldipine		Ergotamine
dasatinib	quetiapine		Fentanyl
dronedarone	saquinavir		Irinotecan
eletriptan	sildenafil		Pimozide
eplerenone	simvastatin		Quinidine
everolimus	sirolimus		Sirolimus
felodipine	tolvaptan		Tacrolimus
imatinib	tipranavir		Terfenadine
indinavir	triazolam		
	vardenafil		
Note: This is not an exhaustive list and availability of medications may differ between countries. For more information about clinically relevant drugs that are CYP3A substrates, see the Indiana University Division of Pharmacology website for reference: http://medicine.iupui.edu/clinpharm/ddis/clinical-table/.			

ALN-AS1-002 Protocol Amendment 1

Summary of Changes (dated 10 November 2016) compared to Original Protocol (dated 19 July 2016)

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

Rationale for Protocol Amendment

The primary purpose of this protocol amendment is to define the starting dose in study ALN-AS1-002, and to clarify criteria for determining individual dose modifications.

The following changes are being implemented as outlined:

- Based on review of data from the ALN-AS1-001 study, the SRC has determined the study starting dose and dosing regimen for administration in ALN-AS1-002 to be 5.0 mg/kg administered every 3 months.
- If a patient has a study drug-related adverse event (AE) of a recurrent injection site reaction (ISR), severe ISR, or clinically significant liver function test (LFT) abnormality and the Investigator and Medical Monitor have concerns regarding further ALN-AS1 administration, a dose reduction to half the previously administered dose or a reduction in dosing frequency will be permitted. Subsequent ALN-AS1 administration at the previous dose may be considered based on the judgment of the Investigator and Medical Monitor.
- The benefit-risk profile remains unchanged; however, the potential risks associated with administration of ALN-AS1 in patients with acute intermittent porphyria (AIP) on reproductive health were updated to include preliminary nonclinical data. Based on these data, ALN-AS1 administration to pregnant women is unlikely to pose a risk to embryofetal development.
- While contraceptive requirements for patients enrolling in study ALN-AS1-002 remain unchanged from those in study ALN-AS1-001, wording of the inclusion criterion for WOCBP was clarified such that if hormonal methods of contraception are medically contraindicated, a double-barrier method will be considered an acceptable method of contraception.
- Since ALN-AS1 could increase the clearance of drugs metabolized by the CYP3A isoform, a list of medications that are CYP3A substrates is now included in the protocol.
- To support dosing for up to 36-months, preliminary nonclinical data from 26-week and 39-week Good Laboratory Practice (GLP) chronic toxicity studies conducted in rats and nonhuman primates (NHPs) have been updated.
- Clarified that a single blood sample for pharmacokinetic (PK) analysis will be collected at the Month 1 visit for patients with an every 3 month dosing regimen

- Made minor changes to schedules of assessment to increase consistency and clarity

A detailed summary of changes is provided in Table 1. Corrections to typographical errors, punctuation, grammar, abbreviations, and formatting, as well as those indicated in administrative change letter 1 (dated 18 August 2016), are not detailed.

Table 1: Protocol Amendment 1 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in the protocol amendment are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: Updated preliminary nonclinical data from 26-week and 39-week GLP chronic toxicity studies conducted in rats and NHPs

The primary change occurs in Section 1.3.1, Nonclinical Summary

Added text: **Preliminary results from 26-week and 39-week GLP chronic toxicity studies conducted in rats and NHPs, respectively, support long-term clinical dosing. The repeat-dose toxicity study in rats included assessment of male fertility and early embryonic development. ALN-AS1 was administered weekly at doses of 0, 3, 10, or 30 mg/kg for up to 26 weeks. Weekly doses of 30 mg/kg resulted in adverse hepatocellular single cell necrosis and vacuolation in the liver, associated with mild increases in liver function tests (LFTs) at the conclusion of the dosing period. Angiectasis was observed in the islets of Langerhans, possibly a secondary change to hepatic changes, which is not considered adverse. Combined male fertility assessments did not indicate adverse reproductive or developmental findings up to the highest dose evaluated (30 mg/kg weekly SC dose). In the 39-week repeat-dose toxicity study in NHP, ALN-AS1 was administered weekly at doses of 0, 10, 30, or 100 mg/kg for up to 39 weeks. Weekly doses of 100 mg/kg were the lowest-observed-adverse-effect level based on single cell necrosis combined with increased alanine transaminase (approximately 2-fold above control). The NOAELs were 10 mg/kg in the rat and 30 mg/kg in the NHP. The 13-week recovery data in the rat and NHP studies is pending.**

Section(s) also containing this change:

- Section 1.5, Dose Rationale

Purpose: Specified the starting dose level and frequency for administration in study ALN-AS1-002 as 5.0 mg/kg every 3 months and added preliminary clinical data from study ALN-AS1-001 supporting this starting dose level and frequency

The primary change occurs in Section 1.5, Dose Rationale

Added text **The study starting dose and regimen of ALN-AS1 is 5.0 mg/kg as an SC injection administered every 3 months. Based on emerging clinical data, the SRC may approve changes to the ALN-AS1 dosing regimen, including decreases in the dose level and changes in dosing frequency. However, any dose and dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in study ALN-AS1-001. SRC, Health Authority and Ethics Committee approval must be sought prior to dose level escalation above 5.0 mg/kg in accordance with local Regulatory requirements.**

The study starting dose and regimen was selected based on the benefit:risk profile of ALN-AS1 in Part C of the ongoing ALN AS1 001 study. Preliminary data to date indicate that administration of 5.0 mg/kg ALN AS1 has been generally well-tolerated. There have been no study drug related SAEs, severe AEs, or clinically significant changes in safety parameters. While a single 2.5 mg/kg dose of ALN-AS1 has also been generally well-tolerated

when administered to patients in Part A and Part C of study ALN-AS1-001, this dose has not resulted in near normalization of ALA and PBG levels. In addition, while emerging data from Part C indicate that an every 3 months dose of 2.5 mg/kg ALN-AS1 demonstrated some clinical activity (eg, decrease in attack frequency and decrease in heme use), some patients continued to experience breakthrough porphyria attacks during the treatment period, indicating that a higher dose may be required.

Section(s) also containing this change:

- Synopsis
- Section 6.2.2, Dose and Administration

Purpose: Updated potential risks associated with administration of ALN-AS1 on reproductive health to include preliminary nonclinical data

The primary change occurs in Section 1.6, Benefit-Risk Assessment

Now reads: Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. **Based on preliminary data from rat fertility/embryofetal development and rabbit embryofetal development studies, it is** unlikely **that** ALN-AS1 administration to pregnant women or animals poses a risk to embryofetal development~~; however, in the absence of data from embryofetal developmental toxicity studies with ALN-AS1,~~. **In a rat fertility/embryofetal development study at daily dose levels up to 16.5 mg/kg, no test article-related effects on female fertility or developmental endpoints were observed. In a rabbit embryofetal development study, maternal toxicity was observed at daily doses of >3 mg/kg (7.8 fold higher than the clinical monthly dose of 5.0 mg/kg). No terata were observed in this range-finding embryofetal development study.** Women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use acceptable method**s** of contraception during studies with ALN-AS1.

Purpose: Clarified the inclusion criterion for WOCBP when hormonal methods of contraception are medically contraindicated and removed reference to low-dose gestagens

The primary change occurs in Section 5.1, Inclusion Criteria

Now reads: 3. WOCBP must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use ~~1 highly effective method~~ **acceptable methods** of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. **Acceptable methods include hormonal methods along with an additional barrier method (eg, condom, diaphragm, or cervical cap), or a double barrier method of contraception for those WOCBP for whom a hormonal method of contraception is medically contraindicated due to underlying disease (see Section 6.4 for acceptable methods of contraception).**

Section(s) also containing this change:

- Section 6.4, Contraceptive Requirements

Purpose: Clarified the criteria for individual dose modifications based on study drug-related AEs

The primary change occurs in Section 6.2.3.2, Individual Dose Modifications

Now reads: Dose modifications are permitted for individual patients. If a patient has ~~an AE that is related to~~ **a** study drug ~~(eg,~~**related AE of a** recurrent **ISR,** ~~or~~ severe ~~or~~ISR~~s~~**,** or clinically significant LFT ~~abnormalities)~~**abnormality** and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and Investigator will review all available safety data for the patient. Based on this review, ~~it may be determined that the patient is a candidate for a dose reduction. Dose reduction to a dose, which was found to be safe and well-tolerated by the SRC,~~**a dose reduction to half the previously administered dose (eg, a 5.0 mg/kg dose would be reduced to a 2.5 mg/kg dose) or a reduction in dosing frequency from monthly to every 3 months** is permitted. Subsequent ALN-AS1 administration at the previous~~, or higher,~~ dose may be considered based on the judgment of the Investigator and Medical Monitor.

Purpose: While it was previously listed in the protocol in the Benefit -Risk Assessment that ALN-AS1 could increase clearance of drugs metabolized by the CYP3A isoform, it has been added to the concomitant medications section for clarification.

The primary change occurs in Section 6.3, Concomitant Medications

Added text: **ALN-AS1 could potentially increase the clearance of drugs metabolized by the CYP3A isoform. Investigators will review all medications at study start and monitor response to these medications during the study. For patients who require new medications while on study, selection of medications that are not mainly metabolized by the CYP3A isoform is recommended. A list of medications that are sensitive CYP3A substrates is in Table 6 Appendix Section 11.3. In addition, a more detailed and current list of sensitive CYP3A-metabolized medications and CYP3A-metabolized medications with a narrow therapeutic range is available at the Indiana University, Division of Clinical Pharmacology, website (http://medicine.iupui.edu/clinpharm/ddis/clinical-table/).[31]**

Section(s) also containing this change:

- Section 1.6, Benefit-Risk Assessment
- Table 6, List of Sensitive CYP3A Substrates and those with a Narrow Therapeutic Range, in Appendix Section 11.3

Purpose: Clarified that a single blood sample for pharmacokinetic (PK) analysis will be collected at the Month 1 visit for patients with an every 3 month dosing regimen

The primary change occurs in Table 3, Pharmacokinetic Time Points, in Appendix Section 11.1, Pharmacokinetic Assessment Time Points

Added text: **Footnote 'a': At the Month 1 visit, if the dosing regimen is every 3 months, ALN-AS1 is not administered and a single time point blood sample for PK analysis should be collected.**

Purpose: Made minor changes to schedules of assessment to increase consistency in procedures across the protocol

The changes occur in Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen) and Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Now reads: Adjustments to Table 1 and 4 have been made to indicate that at the Month 15 visit, Body Weight, BMI, and height will not be collected but Blood and Urine Sample for Exploratory Biomarker Analysis will be collected.

Purpose: To clarify the procedures for dispensing and using porphyria sample attack kits

The changes occur in Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen) and Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Now reads: **Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39, U**urine samples should be collected within 24 hours of the start of clinical intervention ~~for a porphyria attack~~(eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention ~~for a porphyria attack through M39~~. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

Purpose: To allow flexibility in method of collecting body temperature

The primary change occurs in Section 7.5.1, Vital Signs

Deleted text: Vital sign measurements include blood pressure, heart rate, ~~oral~~ body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. ~~Oral b~~**B**ody temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

Section(s) also containing this change:

- Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)
- Table 2, Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)
- Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)
- Table 5, Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

Purpose: Correct typographical errors, punctuation, grammar, abbreviations, and formatting, and incorporated administrative change letter 1 (dated 18 August 2016)

These changes are not listed individually.

ALN-AS1-002 Protocol Amendment 1

Summary of Changes (dated 10 November 2016) Compared to Amendment 0.1 (Sweden) dated 20 September 2016

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

Rationale for Protocol Amendment

The primary purpose for this protocol amendment is to clarify that based on review of data from the ongoing ALN-AS1-001 study, the Safety Review Committee (SRC) has determined the study starting dose and dosing regimen for administration in study ALN-AS1-002 is to be 5.0 mg/kg administered every 3 months.

Additionally, minor changes were made to schedules of assessment to increase consistency and clarity.

A detailed summary of changes is provided in Table 1. Corrections to typographical errors, punctuation, grammar, abbreviations, and formatting are not detailed.

Table 1: Protocol Amendment 1 Detailed Summary of Changes

The primary section(s) of the protocol affected by the changes in the protocol amendment are indicated. The corresponding text has been revised throughout the protocol. Deleted text is indicated by ~~strikeout~~; added text is indicated by **bold** font.

Purpose: Specified the starting dose and dosing regimen for administration in study ALN-AS1-002.

The primary change occurs in Section 1.5, Dose Rationale

Now reads: The **study** starting dose of ALN-AS1 is 5.0 mg/kg ~~ALN-AS1~~ as an SC injection administered every ~~month~~ **3 months** (Table 1 and Table 2). Based on emerging ~~study~~ **clinical** data, the SRC may approve ~~implementation of a lower~~ changes to the ALN-AS1 dosing~~, less frequent regimen~~ **regimen,** ~~or a fixed dose equivalent of ALN-AS1~~ including decreases in the dose level and changes in dosing frequency. However, **any dosing regimen will not exceed a previously explored dose level or frequency that was determined to be safe and well-tolerated in Study ALN-AS1-001. SRC, Health Authority**~~Regulatory~~ and Ethics Committee approval must be sought prior to dose **level** escalation **above 5.0 mg/kg in accordance with local Regulatory requirements.**~~or implementation of a more frequent dosing regimen~~.

Section(s) also containing this change:

- Synopsis
- Section 6.2.2, Dose and Administration

Purpose: Made minor changes to schedules of assessment to increase consistency in procedures across the protocol

The changes occur in Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen) and Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Now reads: Adjustments to Table 1 and 4 have been made to indicate that at the Month 15 visit, Body Weight, BMI, and height will not be collected but Blood and Urine Sample for Exploratory Biomarker Analysis will be collected.

Purpose: To clarify the procedures for dispensing and using porphyria sample attack kits

The changes occur in Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen) and Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Now reads: **Porphyria attack laboratory sample collection kits should be dispensed at the screening visit along with instructions for use. For any attack that occurs through M39,** ~~U~~**u**rine samples should be collected within 24 hours of the start of clinical intervention ~~for a porphyria attack~~(eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention ~~for a porphyria attack through M39~~. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

Purpose: To allow flexibility in method of collecting body temperature

The primary change occurs in Section 7.5.1, Vital Signs

Deleted text: Vital sign measurements include blood pressure, heart rate, ~~oral~~ body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. ~~Oral b~~**B**ody temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

Section(s) also containing this change:

- Table 1, Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)
- Table 2, Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)
- Table 4, Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)
- Table 5, Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

Purpose: Correct typographical errors, punctuation, grammar, abbreviations, and formatting

These changes are not listed individually.

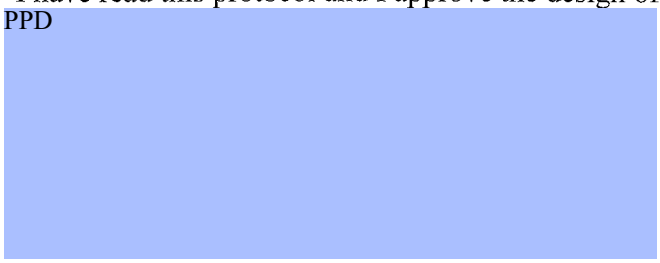
CLINICAL STUDY PROTOCOL
ALN-AS1-002

Protocol Title:	A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered ALN-AS1 in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1
Investigational Drug:	ALN-AS1
EudraCT Number:	2016-002638-54
IND Number:	126094
Protocol Date:	Original protocol 19 July 2016
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: PPD
Sponsor Contact:	PPD

SPONSOR PROTOCOL APPROVAL

I have read this protocol and I approve the design of this study.

PPD



19 July 2016

Date

INVESTIGATOR'S AGREEMENT

I have read the ALN-AS1-002 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROTOCOL SYNOPSIS

Protocol Title

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

Product Name

ALN-AS1

Indication

Patients with acute intermittent porphyria (AIP) who experience recurrent porphyria attacks

Phase

1/2

Study center(s)

The study will be conducted at up to 8 clinical study centers worldwide.

Objectives

Primary

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

Secondary

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

Exploratory

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

Endpoints

Primary

- Patient incidence of adverse events (AEs)

Secondary

- Change in urine delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

Exploratory

- Change in circulating 5-aminolevulinic acid synthase 1 (ALAS1) mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary

- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

Study Design

This is a multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months). After a patient has completed 6 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Patients will also be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center.

A Safety Review Committee will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study.

Number of Planned Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

Diagnosis and Main Eligibility Criteria

Patients with AIP, who have completed Part C of study ALN-AS1-001, will be eligible for screening in this study. Eligible patients will not be on a scheduled regimen of hemin to prevent porphyria attacks at Screening. Patients with alanine transaminase ≥2.0× upper limit of normal, total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome), or estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula) at Screening are not eligible for this study.

Investigational Product, Dose, and Mode of Administration

ALN-AS1 Solution for Injection (subcutaneous use [SC]) is comprised of a synthetic, small interfering RNA targeting ALAS1 and bearing a triantennary N-acetyl galactosamine ligand conjugated to the sense strand, formulated in phosphate buffer.

The planned starting dose of ALN-AS1 is 2.5 mg/kg ALN-AS1 as an SC injection administered every 3 months. Before initiating dosing in this study, the actual dose and dosing regimen administered will be determined based on SRC review of emerging data in Part C of study ALN-AS1-001. Based on SRC review, a higher dose of ALN-AS1 may be administered and/or the dosing regimen adjusted to

every month. Either fixed or weight-based doses may be administered. The dose and dosing regimen of ALN-AS1 administered in this study will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Further, the dose administered in this study will not exceed the maximum dose permitted in Part C of study ALN-AS1-001.

Reference Therapy, Dose, and Mode of Administration

Not applicable

Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

Statistical Methods

Statistical analyses will be primarily descriptive. Incidence of AEs will be summarized by maximum severity and relationship to ALN-AS1. Incidence of and serious adverse events (SAEs) and AEs leading to discontinuation will also be tabulated. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation.

Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and electrocardiogram data will be presented in by-patient listings. Descriptive statistics will be provided for electrocardiogram interval data and presented as both actual values and changes from baseline relative to each on study evaluation and to the last evaluation on-study.

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care. Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

PD analysis will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

The PK concentration of ALN-AS1 will be determined. Antidrug antibody results will be tabulated overall and by previous treatment in the previous study (ALN-AS1-001). A by-patient listing will also be provided.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway. Quality of life scores will also be summarized.

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Informed Consent	X														
Medical History/Disease History[c]	X	X													
Demographics	X														
Inclusion/ Exclusion Criteria	X	X													
Physical Examination[d]	X	X	X	X	X		X		X		X				X
Body Weight, BMI, and Height[e]	X	X			X		X		X		X		X		X
Vital Signs[f]	X	X	X	X	X		X		X		X		X		X
Triplicate 12-Lead ECG[g]	X	X			X						X				
Clinical Laboratory Assessment[h]	X		X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X		X		X		X		X		X
Study Drug Administration[j]		X			X		X		X		X		X		X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X		X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X	X	X	X		X		X		X		X		X
Blood Sample for PK Analysis[m]		X	X		X		X				X				X

Table 1: Schedule of Assessments: Screening/Baseline through Month 18 (Every 3 Months Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8	M9	M10/ M11	M12	M13/ M14	M15[b]	M16/ M17	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Antidrug Antibodies[n]		X	X		X		X				X				X
Porphyria Attack Laboratory Sample Collection Kit and Instructions[o]	X														
EQ-5D-5L Questionnaire[p]	X						X				X				X
Diary Review (including BPI-SF)[q]		X													
Phone Contact[r]						X		X		X		X		X	
AEs		Continuous													
Concomitant Medications[s]		Continuous													

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available before the administration of the first dose of ALN-AS1.

b After a patient has completed 12 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.
d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, oral body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o Urine samples should be collected within 24 hours of the start of clinical intervention for a porphyria attack (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention for a porphyria attack through M39. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.
p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.
q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.
r Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.
s Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]		X		X		X		X		X		X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]		X		X		X		X		X		X	X	
Study Drug Administration[j]		X		X		X		X		X		X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK				X										

Table 2: Schedule of Assessments: Month 19 through End of Study (Every 3 Months Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20**	**M21[a]**	**M22/ M23**	**M24**	**M25/ M26**	**M27[a]**	**M28/ M29**	**M30**	**M31/ M32**	**M33[a]**	**M34/ M35**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Analysis[m]														
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
Phone Contact[q]	X		X		X		X		X		X			X
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.
b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.
c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. Urine samples should not be collected during a porphyria attack.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, oral body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.
q Patients will be contacted by phone to collect AEs and concomitant medications. Patients will also be reminded to collect and send urine samples to the clinical study center, if possible, if they are unable to report to the clinical study center during a porphyria attack.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Delta-aminolevulinic acid
ALAS1	5-aminolevulinic acid synthase 1
ALP	Alkaline phosphatase
ASGPR	Asialoglycoprotein receptor
ASHE	Asymptomatic high excreters
BPI-SF	Brief Pain Inventory-Short Form
cERD	Circulating extracellular RNA detection assay
CRF	Case report form
CYP	Cytochrome P450
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated Glomerular Filtration Rate
EDC	Electronic data capture
EOS	End of study
EU	European Union
GalNAc	N-acetyl galactosamine
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISR	Injection site reaction
IV	Intravenous
LFT	Liver function test
mRNA	Messenger RNA
NHP	Nonhuman primate

Abbreviation	Definition
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OTC	Over the counter
PBG	Porphobilinogen
PBGD	Porphobilinogen deaminase
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
QOL	Quality of life
RNA	Ribonucleic acid
RNAi	RNA interference
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
siRNA	Small interfering RNA
SRC	Safety Review Committee
SUSAR	Suspected unexpected serious adverse reaction
ULN	Upper limit of normal
US	United States
WOCBP	Woman of child-bearing potential

1. INTRODUCTION

1.1. Disease Overview

The porphyrias are a family of rare metabolic disorders predominately caused by a genetic mutation in 1 of the 8 enzymes responsible for heme synthesis.[1] The 2 major categories are erythropoietic and hepatic, depending on the major site of expression of the underlying enzyme deficiency.[2] In addition, the porphyrias are classified as acute if patients present with neurovisceral symptoms (eg, abdominal pain and neurologic symptoms) or cutaneous if they present with dermal blistering or photosensitivity.[3, 4]

This study will include patients with acute intermittent porphyria (AIP), the most common acute hepatic porphyria (AHP). AIP occurs as a result of an autosomal dominant mutation leading to partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthesis pathway.[5] The rate limiting step in heme synthesis is the upstream enzyme 5-aminolevulinic acid synthase 1 (ALAS1), which is controlled by feedback repression via the end-product heme. In response to a decrease in endogenous heme in the liver, as can occur with fasting, hormonal alterations, or with cytochrome P450 (CYP) inducing drugs, ALAS1 is induced, resulting in increased flux through the heme synthesis pathway. In patients with AIP, this can result in the marked accumulation of the toxic heme intermediates porphobilinogen (PBG) and delta aminolevulinic acid (ALA) in the blood and urine due to the deficiency in the downstream PBGD. Clinically, this manifests as acute attacks characterized by severe abdominal pain, cardiovascular as well as neurologic and psychiatric dysfunction.[6]

1.1.1. Epidemiology

Over 370 mutations have been identified in the PBGD gene, which in most instances result in its reduction by approximately 50%. The exact prevalence of AIP is unknown due to under diagnosis, variation by geographic region, and the differences in penetrance observed for the different mutations.[2] However, it is estimated that the prevalence of AIP is 5 to 10 per 100,000 people in Western countries, while in Sweden it occurs in 1 in 1,000 people due to a founder effect associated with the resultant G593A mutation.[7-10] AIP shows incomplete penetrance and on average only 10% to 50% of carriers with a mutation present with clinical symptoms.[11] It has been proposed that unknown inherited co-factors, in addition to PBGD mutations, may explain why a small percentage of patients present with much more severe disease while the majority remain asymptomatic.

Patients with AIP present with acute attacks characterized by neurovisceral symptoms and signs including severe abdominal pain, nausea, vomiting, constipation, or less commonly diarrhea, hypertension, tachycardia, fever, muscle weakness, as well as neurological (eg, neuropathy, seizures) and psychiatric (eg, anxiety and confusion) manifestations.[12] AIP attacks typically start after puberty and can be triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, exogenous hormones, nutritional status, and infection. Clinically active disease is more common in women, where attacks often occur around the menstrual cycle and are likely precipitated by hormonal changes.

Many AIP patients remain undiagnosed given that the disease is rare and characterized by non-specific symptoms (eg, abdominal pain and nausea).[2] The initial diagnosis involves

demonstrating elevated urinary PBG (typically 20-50 × normal reference range) and ALA (typically 5-20 × normal reference range) when the patient is having acute attack symptoms. Once a test is positive for elevated ALA and PBG and porphyria is suspected, genetic testing for a PBGD mutation is performed.

There are 4 main clinical phenotypes of AIP. Latent gene carriers have never had an acute attack and are biochemically inactive (normal urinary ALA and PBG). Asymptomatic high excreters (ASHE) do not have current acute attacks, but are biochemically active (increased urinary ALA and PBG). The increased levels of ALA and PBG in ASHE patients are lower than those in AIP patients during an acute attack, but are still significantly higher than normal reference values.[6-8] Sporadic attack patients have infrequent acute attacks. Recurrent attack patients have ≥4 attacks per year requiring hospitalization or treatment by a medical professional.[7]

1.1.2. Current Management and Unmet Medical Need

Management of acute attacks often requires hospitalization. Patients are initially treated with supportive care and carbohydrate loading, analgesics and anti-emetics, and removal of known precipitating factors.[13] In patients with moderate to severe attacks, or who fail to respond to supportive measures, treatment with intravenous (IV) hemin (daily for 3-5 days) is commenced. Symptoms typically begin to improve after 2 to 5 days of hemin treatment, accompanied by a lowering of urinary ALA and PBG levels to below or at the upper limit of normal (ULN) reference value; however, in some patients attacks can last several weeks, requiring prolonged hemin use and hospitalization.[14] With prolonged and severe attacks, cranial nerve involvement can occur, leading to bulbar paralysis, respiratory failure, and death.[15]

Hemin, a blood derived therapy, was approved as Normosang® (heme arginate) in the European Union (EU) and as Panhematin® (lyophilised hematin) in the United States (US) in 1999 and 1983, respectively.[16, 17] In the EU, heme arginate is indicated for the treatment of acute attacks of hepatic porphyria (eg, AIP, variegate porphyria, and hereditary coproporphyria); whereas, in the US lyophilized hemin is limited to the amelioration of recurrent attacks of AIP temporally related to the menstrual cycle. Approval of both products was based on biochemical efficacy (ie, lowering of ALA and PBG) as well as data from uncontrolled case series and case reports.[18-22] Hemin side effects include thrombophlebitis, transient anticoagulation, and in rare cases, anaphylaxis or renal failure.[22-24] In addition, hemin administration requires a large vein for administration, often necessitating central venous access.

While the majority of patients who experience attacks have them sporadically, it is estimated that up to 1,000 AIP patients experience recurrent attacks (typically defined as ≥4 per year) in the US and EU combined.[25] While hemin is currently only approved to ameliorate acute attacks, it is administered prophylactically in some patients with recurrent attacks (eg, administered every 1-2 weeks).[26] Potential problems associated with chronic hemin administration include infusion site phlebitis, iron overload, and the need for chronic IV access and associated complications (eg, risk of infection or occlusion).[5] In addition, there have been reports of a decrease in efficacy with chronic hemin administration, as evidenced by the need to increase the frequency or dosage of hemin prophylaxis over time in attempt to maintain a clinical effect.[27] In patients with very severe recurrent attacks, or in whom hemin treatment is not effective, liver transplantation can be curative; however, organ transplantation is a high-risk procedure and is rarely used as a treatment option [34]. Given the significant morbidity and mortality, there

remains a significant unmet need for an efficacious, simple, fast-acting and better tolerated treatment for acute or prevent recurrent attacks in patients with AIP.

1.2. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism mediated by small interfering RNAs (siRNAs) for regulating gene expression. Typically, synthetic siRNAs are 19 to 25 base pair double stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at both or 1 of the 3' ends.[28] Such siRNAs can be designed to target an endogenous messenger RNA (mRNA) transcript of a given gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary mRNA sequence, cleavage of this target mRNA, and reduction of synthesis of the target protein, resulting in a decrease of the target protein levels.[29] The ability to selectively and potently degrade the mRNA encoding the ALAS1 protein (thereby decreasing accumulation of the toxic intermediates ALA and PBG) using an siRNA is a novel approach for the prevention and treatment of acute attacks in patients with AIP.

1.3. ALN-AS1

ALN-AS1 is a synthetic RNAi therapeutic currently in development for treatment of acute attacks in patients with AIP, the most common AHP.

1.3.1. Nonclinical Summary

The pharmacology, safety pharmacology, drug metabolism and pharmacokinetics and toxicology of ALN-AS1 were evaluated in a series of in vitro and in vivo nonclinical studies.

ALN-AS1 is pharmacologically active in rodents and nonhuman primates (NHP) due to conservation within the siRNA target sequence. Transfection assays in human liver carcinoma cell line-G2 cells showed dose-dependent inhibition of endogenous ALAS1 mRNA levels with an half-maximal inhibitory concentration of approximately 26 pM of ALN-AS1. In multiple studies in the mouse, rat, and NHP, potent and dose-dependent pharmacologic activity has been demonstrated with subcutaneous (SC) administration of ALN-AS1, which results in consistent reduction of ALAS1 mRNA in liver. After single dose administration, ALAS1 mRNA suppression in liver is durable depending on the dose administered (1-4 weeks) and correlates with the extent of ALAS1 mRNA suppression observed in serum or urine. Dose dependent, steady-state ALAS1 mRNA reduction has also been demonstrated with repeat-dose regimens. Studies in rodent AIP models have confirmed that ALAS1 mRNA reduction with ALN-AS1 treatment correlates with decreases in ALA and PBG heme intermediates.

A Good Laboratory Practice (GLP)-compliant safety pharmacology study conducted in NHPs showed no functional cardiovascular or respiratory effects, with a no observed effect level (NOEL) of 150 mg/kg. Neurological assessments were conducted as part of a 13-week repeat dose GLP toxicity study in NHPs (weekly dosing); no ALN-AS1-related neurobehavioral observations occurred, with a NOEL for neurological effects of 150 mg/kg (highest dose tested). Genetic toxicity studies (bacterial reverse mutation, human peripheral blood lymphocyte chromosomal aberrations, and rat bone marrow micronucleus) assays were all negative at International Conference on Harmonization (ICH) S2 (R1) limit doses.

Two to 4-week exploratory (non-GLP) dose-range finding toxicology studies were conducted with ALN-AS1 in mice, rats, and NHPs. In all 3 species, no dose-limiting toxicity was observed up to the highest dose evaluated (300 mg/kg weekly SC dose), with the rat appearing to be the most sensitive species. These studies were followed by 13-week GLP toxicology studies in rats and NHPs where ALN-AS1 was administered daily (100 mg/kg) and weekly at doses of 0, 3, 10, and 30 mg/kg in the rat and weekly at doses of 0, 15, 50, and 150 mg/kg in the NHP. The no observed adverse effect levels were 30 mg/kg in the rat and 150 mg/kg in the NHP (highest dose tested in each species). GLP chronic toxicity studies (26 weeks in the rat and 39 weeks in the NHP) have been conducted and are currently in the reporting phase. Results of these studies will be available to support the clinical dosing.

1.3.2. Clinical Summary

ALN-AS1 is being investigated in an ongoing, Phase 1 study (ALN-AS1-001) to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of ALN-AS1 administered subcutaneously in patients with AIP. The study is being conducted in 3 parts. Part A and Part B are single-blind, placebo-controlled portions of the study in patients with AIP who are ASHE. Patients are randomized in a 3:1 ratio to receive either a single dose (Part A) or multiple doses (Part B; 2 doses 28 days apart) of ALN-AS1 or placebo. Patients who participate in Part A and Part B are not eligible to participate in this study. In Part C, patients with AIP who have recurrent attacks will receive multiple doses of ALN-AS1 dosing over a 12-week period in a randomized, double-blind, placebo-controlled part of the study. This study will enroll patients who completed Part C of study ALN-AS1-001.

Dosing has been completed in Part A (0.035-2.5 mg/kg) and Part B of the study (0.35-1.0 mg/kg). A total of 23 unique patients with AIP who are ASHE have received at least 1 dose of study drug (20 patients received at least 1 dose of ALN-AS1 and 3 patients received placebo only). ALN-AS1 has been generally well-tolerated. Adverse Events (AEs) in patients administered ALN-AS1, which were reported in 2 patients each, were abdominal pain, diarrhea, and hypoaesthesia in Part A; and nasopharyngitis, pruritus, and rash in Part B. In Part A, AEs considered possibly or definitely related to ALN-AS1 were reported in 5 patients: diarrhea, dyspepsia, haematochezia, injection site erythema, injection site pain, blood creatinine increased, glomerular filtration rate decreased, and hypoesthesia (1 patient each). In Part B, AEs considered possibly or definitely related to ALN-AS1 were reported in 3 patients: pruritus and rash (2 patients each); and rash macular and rash pruritic (1 patient each). There were 2 SAEs of abdominal pain in Part A, 1 patient each in the 0.035 mg/kg and 0.10 mg/kg dose groups, which were not considered to be related to study drug or due to AIP. Across Part A and Part B, all AEs were mild or moderate in severity, except for 1 serious adverse event (SAE) of abdominal pain (0.10 mg/kg dose group), which was considered severe. There were no dose-related trends in AEs and no AEs leading to discontinuation.

Overall, a dose-dependent reduction of urinary ALA and PBG as well as ALAS1 mRNA was observed following single and multiple doses of ALN-AS1. Preliminary data demonstrate that administration of a single dose of ALN-AS1 at ≥1.0 mg/kg lowered ALA and PBG levels ≥80% and ALAS1 mRNA levels ≥50% when compared to baseline levels in patients with AIP who are ASHE. Similarly, administration of multiple doses of ALN-AS1 at ≥0.35 mg/kg lowered ALA and PBG levels ≥80%. ALAS1 mRNA levels were reduced ≥50% in the 1.0 mg/kg dose group when compared to baseline levels in patients with AIP who are ASHE. ALA and PBG levels in

the 1.0 mg/kg dose group in Part A were sustained for ≥180 days and in both dose groups in Part B for ≥160 days. Specifically, the maximum mean reduction from baseline of urinary ALA and PBG reached 93% in the 2.5 mg/kg dose group, which were sustained for approximately 14 weeks. The maximum mean reduction in ALAS1 mRNA relative to baseline of 66% was demonstrated in the 2.5 mg/kg dose group which was maintained beyond 42 days.

Cumulatively, nonclinical and clinical data to date support long-term administration of ALN-AS1 in patients with AIP.

1.4. Study Design Rationale

This is a multicenter, open-label extension study designed to evaluate the long-term safety and clinical activity of subcutaneously administered ALN-AS1 in patients with AIP who have completed clinical study ALN-AS1-001. The primary endpoint for the study is the incidence of AEs.

1.5. Dose Rationale

Clinical data from Part A and Part B of the ongoing study ALN-AS1-001 demonstrate that single (0.035-2.5 mg/kg) and multiple (0.35-1.0 mg/kg) doses of ALN-AS1 have been generally well-tolerated in patients with AIP who are ASHE. Additionally, data from this study indicate that a single dose of 2.5 mg/kg ALN-AS1 results in near normalization of urine ALA and PBG levels, which is sustained for approximately 14 weeks.

Patients in Part C of the ongoing study ALN-AS1-001 who are eligible for participation in this study will likely require a higher dose of ALN-AS1 to normalize ALA and PBG as they have higher levels of ALAS1 upregulation and higher baseline levels of ALA and PBG, when compared to patients with AIP who are ASHE.[30] For example, based on preliminary data in Part A and Part B of the ALN-AS1-001 study, the mean PBG level of patients who are ASHE is 21.9 mmol/mol Cr (N=28); whereas, in Part C of this study in patients with AHP who have recurrent porphyria attacks, the mean PBG level is approximately 49.3 mmol/mol Cr (N=12).

The planned starting dose of ALN-AS1 is 2.5 mg/kg ALN-AS1 as an SC injection administered every 3 months, which is also the initial dose administered to patients with AIP in Part C of study ALN-AS1-001. Before initiating dosing in this study, the actual dose and dosing regimen administered will be determined based on SRC review of emerging data in Part C of study ALN-AS1-001. Based on SRC review, a higher dose of ALN-AS1 may be administered and/or the dosing regimen adjusted to every month. Either fixed or weight-based doses may be administered. The dose and dosing regimen of ALN-AS1 administered in this study will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Further, the dose administered in this study will not exceed the maximum dose permitted in Part C of study ALN-AS1-001.

The duration of dosing in this study is supported by the overall favorable safety profile of ALN-AS1 in the ongoing clinical study ALN AS1-001 and the absence of new findings in nonclinical studies with ALN-AS1. GLP chronic toxicity studies (26 weeks in the rat and 39 weeks in the NHP) have been conducted and are currently in the reporting phase. Results of these studies will be available to support the clinical dosing.

1.6. Benefit-Risk Assessment

Patients participating in this study may experience a reduced number or severity of porphyria attacks, reduced treatment with IV hemin, and/or reduced requirement for pain medication.

The potential risks associated with administration of ALN-AS1 in patients with AIP are as follows:

- Injection site reactions (ISRs): ALN-AS1 is administered by SC injection. In the ongoing ALN-AS1 clinical study, AEs of ISRs have been infrequently reported and have been mild to moderate in severity with single dosing. Injection site rotation and close monitoring have been implemented in ALN-AS1 clinical studies to reduce the potential for ISRs.
- Liver function test (LFT) abnormalities: As ALN-AS1 is targeted for delivery to the liver, and reversible hepatic changes were observed in some animal species, there is a potential for development of LFT abnormalities. To minimize the potential for LFT abnormalities, patients are required to have adequate liver function at study entry and LFTs are monitored during the study.
- Reproductive health: No data are available on the use of ALN-AS1 in pregnancy; however, there is no suspicion of human teratogenicity based on class effects or genotoxic potential. ALN-AS1 was neither genotoxic nor clastogenic in in vitro and in vivo studies. It is unlikely ALN-AS1 administration to pregnant women or animals poses a risk to embryofetal development; however, in the absence of data from embryofetal developmental toxicity studies with ALN-AS1, women of child bearing potential (WOCBP) must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use an acceptable method of contraception during studies with ALN-AS1.
- CYP inhibition: A study in NHP demonstrated that ALN-AS1 dosing could potentially increase the clearance of drugs metabolized by the CYP3A isoform. As contraceptive hormones are metabolized via CYP3A, WOCBP must use a barrier method of contraception, in addition to hormonal contraception, during study participation. Additionally, Investigators will review all patient medications at study start and monitor responses to these medications during the study. A list of medications that may be CYP3A inhibitors and inducers is available at the Indiana University, Division of Clinical Pharmacology, website.[31]

The complete summary of the clinical and nonclinical data relevant to the investigational drug and its study in human subjects is provided in the Investigator's Brochure.

2. OBJECTIVES

2.1. Primary Objective

- Evaluate the long-term safety and tolerability of ALN-AS1 in patients with AIP

2.2. Secondary Objectives

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

2.3. Exploratory Objectives

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

3. ENDPOINTS

3.1. Primary Endpoint

- Patient incidence of AEs

3.2. Secondary Endpoints

- Change in urine ALA and PBG levels
- Frequency and characteristics of porphyria attacks
- Change in hemin administration

3.3. Exploratory Endpoints

- Change in circulating ALAS1 mRNA levels
- Concentrations of ALN-AS1 and antidrug antibodies
- Duration and treatment of porphyria attacks
- Number and duration of visits to a health care facility for acute porphyria care
- EQ-5D-5L questionnaire scores
- Pain assessment and narcotic use as recorded in a patient diary
- Exploratory biomarkers related to the target pathway, such as urine porphyrins, vitamin D binding protein

4. INVESTIGATIONAL PLAN

4.1. Summary of Study Design

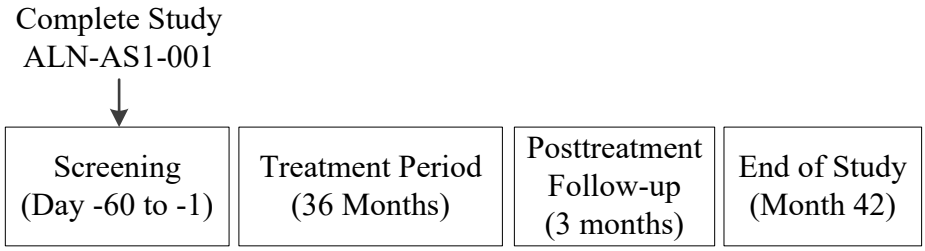
This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. The study will be conducted at up to 8 clinical study centers worldwide.

The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, safety assessments (eg, ECG and clinical laboratory tests) will be repeated before administration of ALN-AS1. It is anticipated that patients will begin ALN-AS1 administration in this study within 6 months after the last dose of study drug in the previous study. Eligibility will be confirmed during Screening/Baseline and before administration of the first dose of ALN-AS1 (Day 1). Patients will undergo assessments at the clinical study center every month for the first 3 months. Then, through Month 6, and according to the dosing regimen selected, assessments will take place at the clinical study center or via a phone contact (for an every month dosing regimen, visits will be every month; for an every 3 months dosing regimen, visits will be every 3 months; see Section 6.2.2). After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional (as indicated in the Schedules of Assessments in Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period. Patients will return to the clinical study center for a posttreatment follow-up visit after administration of the last dose of ALN-AS1. An ALA/PBG Monitoring/EOS visit will be conducted either at the clinical study center or via phone contact, unless treatment continues with ALN-AS1.

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use. Additionally, patients will be encouraged to report to the clinical study center if they experience a porphyria attack through Month 39. In the event that patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits containing instructions for collecting and sending urine samples to the clinical study center, if possible, will be provided. Patients will be contacted by phone after a porphyria attack to record attack details. If patients are experiencing signs and symptoms of a porphyria attack when ALN-AS1 administration is scheduled, the Investigator will determine whether administration of ALN-AS1 is appropriate. If patients are treated for a porphyria attack at a health care facility (eg, hospital, emergency department, infusion center, or outpatient clinic) other than the clinical study center, copies of medical records, including discharge summaries, will be requested.

A Safety Review Committee (SRC) will review safety and tolerability data collected during the study with the primary purpose of protecting the safety of patients participating in the study (see Section 4.6).

Figure 1: Study Design



4.2. Duration of Treatment and Study

The duration of treatment is up to 36 months. The estimated total time on study, inclusive of Screening/Baseline, for each patient is up to 44 months.

4.3. Number of Patients

Up to 24 patients are planned for enrollment in this study (including optional cohorts in ALN-AS1-001).

4.4. Method of Assigning Patients to Treatment Groups

This is an open-label study; all patients will receive ALN-AS1. A combination of the clinical study center number and screening number will create the unique patient identifier. The clinical study center number will be assigned by the Sponsor. Upon signing the Informed Consent Form (ICF), the patient will be assigned a screening number by the clinical study site, which will be the same unique patient identifier assigned in the previous study (ALN-AS1-001). The Investigator, or delegate, will confirm that the patient fulfills all the inclusion criteria and none of the exclusion criteria.

4.5. Blinding

This is an open-label study, and all patients will receive ALN-AS1.

4.6. Safety Review Committee

An SRC will be involved in the conduct of this study. The SRC has the responsibility for monitoring the safety of the study participants. The SRC will perform periodic reviews of safety and tolerability data during the course of the clinical study at least every 3 months for the first 6 months of the study and thereafter at least every 6 months. The SRC may also meet on an ad hoc basis for review of emergent safety and tolerability data, as defined in the SRC Charter for this clinical study. The SRC will not stop the study for efficacy.

The SRC will be comprised of the following members: Principal Investigator(s), or designee(s), the Sponsor Medical Monitor, and the Study Medical Monitor.

The membership of the SRC and reporting structure are further defined in the SRC Charter.

5. SELECTION AND WITHDRAWAL OF PATIENTS

5.1. Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be eligible for enrollment in the study:

1. Completed and, in the opinion of the Investigator, tolerated study drug dosing in Part C of study ALN-AS1-001
2. Not on a scheduled regimen of hemin to prevent porphyria attacks at Screening
3. WOCBP must have a negative serum pregnancy test, cannot be breast feeding, and must be willing to use 1 highly effective method of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration
4. Willing and able to comply with the study requirements and to provide written informed consent

5.2. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in the study:

1. Alanine transaminase ≥2.0×ULN or total bilirubin ≥2 mg/dL (unless bilirubin elevation is due to Gilbert's syndrome)
2. Estimated glomerular filtration rate ≤30 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease formula)
3. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetyl galactosamine ligand (GalNAc)
4. Received an investigational agent, other than ALN-AS1, or who are in follow-up of another clinical study of an investigational agent within 90 days before study drug administration
5. Other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation

5.3. Removal from Treatment or Assessment

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care. Any discontinuation of treatment or withdrawal from the study must be fully documented in the electronic case report form (eCRF), and should be followed up by the Investigator. The Investigator may withdraw a patient at any time if this is considered to be in the best interest of the patient.

Discontinuation of study drug and withdrawal from the study are described in Section 5.3.1 and Section 5.3.2, respectively.

5.3.1. Discontinuation of Study Drug

The Investigator or designee may discontinue dosing in a patient if the patient:

- Is in violation of the protocol
- Experiences a SAE considered to be possibly or definitely related to the study drug or an intolerable AE
- Becomes pregnant
- Is found to be considerably noncompliant with the protocol-requirements

Patients who are pregnant will be discontinued from study drug dosing immediately (see Section 7.5.6.6 for reporting and follow-up of pregnancy). Patients who discontinue treatment will be asked to complete the Month 39 visit assessments.

5.3.2. Withdrawal from Study

A patient may withdraw from the study at any time. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the Month 39 visit. When a patient withdraws from the study, the primary reason for study withdrawal must be recorded in the appropriate section of the eCRF and all efforts made to complete and report the observations as thoroughly as possible. If a patient withdraws due to a SAE, the SAE should be followed as described in Section 7.5.6.

5.3.3. Replacement of Patients

Patients discontinuing from study drug or withdrawing from the study will not be replaced.

6. TREATMENTS

6.1. Treatments Administered

ALN-AS1 Solution for Injection (SC use) is a clear, colorless to pale yellow solution essentially free of particulates. ALN-AS1 will be supplied by the Sponsor as a sterile solution for SC injection.

Study drug supplied for this study must not be used for any purpose other than the present study and must not be administered to any person not enrolled in the study. Study drug that has been dispensed to a patient and returned unused must not be re-dispensed to a different patient.

6.2. Investigational Study Drug

Detailed information describing the preparation, handling, and storage, packaging and labeling, as well as study drug accountability for ALN-AS1 is provided in the Pharmacy Manual.

6.2.1. Description

ALN-AS1 Solution for Injection (SC use) is comprised of a synthetic, siRNA targeting ALAS1 and bearing a triantennary GalNAc ligand conjugated to the sense strand, formulated in phosphate buffer.

6.2.2. Dose and Administration

The planned starting dose of ALN-AS1 is 2.5 mg/kg ALN-AS1 as an SC injection administered every 3 months (Table 1 and Table 2). Before initiating dosing in this study, the actual dose and dosing regimen administered will be determined based on SRC review of emerging data in Part C of study ALN-AS1-001. Based on SRC review, a higher dose of ALN-AS1 may be administered and/or the dosing regimen adjusted to every month (Table 4 and Table 5 in Appendix Section 11.2). Either fixed or weight-based doses may be administered. The dose and dosing regimen of ALN-AS1 administered in this study will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Further, the dose administered in this study will not exceed the maximum dose permitted in Part C of study ALN-AS1-001. See Section 6.2.3 for details on dose modifications.

The study drug will be administered under the supervision of a healthcare professional. After a patient has completed 12 months of ALN-AS1 administration in this study (6 months in an every month dosing regimen), and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center every 3 months for the first year, and thereafter every 6 months through the end of the treatment period.

If a patient does not receive a dose of ALN-AS1 within the specified dosing window, the Investigator should contact the Medical Monitor. After such consultation, the dose may be administered or considered missed and not administered.

Patients will be permitted to miss an occasional dose of study drug; however, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Detailed instructions for study drug administration are found in the Pharmacy Manual.

6.2.3. Dose Modifications

6.2.3.1. Study Population Dose Modifications

During the study, dose modifications are permitted for the study population, or based on the dose cohort into which patients were originally randomized in study ALN-AS1-001. Following SRC review of emerging safety and tolerability data from Part C of study ALN-AS1-001, or from this study (ALN-AS1-002), the dose and dosing regimen may be modified. However, the dose and dosing regimen of ALN-AS1 will not exceed a previously explored dose and dosing regimen that was determined to be safe and well-tolerated in study ALN-AS1-001. Patients enrolling in this study after a dose modification decision has been implemented may start at the newly identified dose and regimen.

6.2.3.2. Individual Dose Modifications

Dose modifications are permitted for individual patients. If a patient has an AE that is related to study drug (eg, recurrent or severe ISRs, or clinically significant LFT abnormalities) and the Investigator has concerns regarding further ALN-AS1 administration, the Medical Monitor and

Investigator will review all available safety data for the patient. Based on this review, it may be determined that the patient is a candidate for a dose reduction. Dose reduction to a dose, which was found to be safe and well-tolerated by the SRC, is permitted. Subsequent ALN-AS1 administration at the previous, or higher, dose may be considered based on the judgment of the Investigator and Medical Monitor.

6.2.4. Preparation, Handling, and Storage

Staff at each clinical study center or the home healthcare professional will be responsible for preparation of ALN-AS1 doses, according to procedures detailed in the Pharmacy Manual. No special procedures for the safe handling of study drug are required.

Study drug will be stored upright and refrigerated at approximately 5±3°C. Any deviation from the recommended storage conditions should be reported to the Sponsor and use of the study drug halted until authorization for its continued use has been provided by the Sponsor or designee.

A Sponsor representative or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

6.2.5. Packaging and Labeling

ALN-AS1 Solution for Injection (SC use) is packaged in 2 mL glass vials with a fill volume of no less than 0.55 mL. The container closure system consists of a Type I glass vial, a Teflon faced bromobutyl 13 mm stopper and a flip-off Truedge aluminum seal.

6.2.6. Accountability

The Investigator or designee will maintain accurate records of receipt and the condition of the study drug supplied for this study, including dates of receipt. In addition, accurate records will be kept of when and how much study drug is dispensed and administered to each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

At the completion of the study, there will be a final reconciliation of all study drugs. All used, partially used, and unused study drug will be returned to the Sponsor (or designee) or destroyed at the clinical study center according to applicable regulations.

6.3. Concomitant Medications

Use of concomitant medications will be recorded on the patient's case report form (CRF) as indicated in the Schedules of Assessments (Table 1 and Table 2; and Table 4 and Table 5 in Appendix Section 11.2). This includes all prescription medications, herbal preparations, over the counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication that is required for the patient's welfare may be administered by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded on the eCRF. Concomitant medication will be coded using an internationally recognized and accepted coding dictionary.

Patients are not permitted to be on hemin prophylaxis at Screening; however, patients are permitted to receive concomitant hemin treatment for the management of acute porphyria

attacks. Patients experiencing significant breakthrough attacks while enrolled in this study, necessitating frequent hemin treatment, may receive hemin prophylaxis if in the Investigator's judgment this is clinically beneficial to the patient. The Investigator should contact with the Sponsor and Medical Monitor before prophylactic hemin treatment. The dose, regimen, and dates of administration will be recorded on the patient's eCRF.

Pain medication (eg, opiates, opioids, narcotic analgesics) and glucose administration are permitted for the management of porphyria and for acute porphyria attacks.

If patients use nonsteroidal anti-inflammatory medications intermittently or chronically, they must have been able to tolerate them with no previous side effects (eg, gastric distress or bleeding).

Standard vitamins and topical medications are permitted; however, topical steroids must not be applied anywhere near the injection site(s) unless medically indicated.

6.4. Contraceptive Requirements

WOCBP must be willing to use a highly effective method of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration. Highly effective methods of birth control result in a low failure rate (ie, less than 1% per year). Birth control methods which may be considered as highly effective include:

- Established use of oral (except low-dose gestagens [eg, lynestrenol and norethisterone]), implantable, injectable, or transdermal hormonal methods of contraception. WOCBP using hormonal methods of contraception must also use a barrier method (condom or occlusive cap [diaphragm or cervical/vault cap] in conjunction with spermicide [eg, foam, gel, film, cream, or suppository]). If hormonal methods of contraception are medically contraindicated for WOCBP due to underlying disease, a double-barrier method (combination of male condom with either cap, diaphragm, or sponge, in conjunction with spermicide) is also considered an acceptable method of contraception.
- Placement of an intrauterine device
- Placement of an intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient)
- True sexual abstinence, when in line with the preferred and usual lifestyle of the patient. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Abstinent patients must agree to use 1 of the abovementioned contraceptive methods, if they start sexual relationships during the study and for 90 days after last dose administration.

WOCBP includes any female patient who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, bilateral tubal occlusion, hysterectomy, or

bilateral salpingectomy). Postmenopausal state is defined as no menses for 12 months without an alternative medical cause, confirmed by follicle stimulating hormone level within the postmenopausal range.

6.5. Treatment Compliance

Compliance with study drug administration will be verified through observation by study personnel or trained home healthcare professionals.

7. STUDY ASSESSMENTS

The schedule of study assessments is provided in Table 1.

7.1. Screening/Baseline Assessments

Informed consent, patient demographic data, and eligibility criteria will be confirmed at Screening/Baseline. An interval medical history/disease history will be obtained at Screening/Baseline. The last assessments performed, determining previous study (ALN-AS1-001) completion, will serve as the Screening/Baseline assessments in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1.

7.2. Clinical Activity Assessments

The clinical activity of ALN-AS1 will be assessed by the frequency and characteristics of porphyria attacks including, but not limited to, duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care (eg, hospital, emergency department, infusion center, or outpatient clinic). Concomitant hemin administration will also be recorded.

7.2.1. Patient Diary

Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use.

7.2.2. Brief Pain Inventory-Short Form Questionnaire

The Brief Pain Inventory-Short Form (BPI-SF) questionnaire will be used to evaluate pain.[32] The BPI will be completed as part of the patient diary.

7.3. Pharmacodynamic Assessments

Blood and urine samples will be collected for assessment of ALN-AS1 PD parameters. The PD effect of ALN-AS1 will be evaluated by urine ALA and PBG levels. Blood and urine samples will be collected for assessment of circulating ALAS1 mRNA levels in serum and in urine. Analysis for blood and urine PD parameters will take place at a central laboratory.

Details regarding the processing and aliquoting of samples for storage and analyses will be provided in the Laboratory Manual.

7.4. Pharmacokinetic Assessments

Blood samples will be collected to evaluate the PK profile of ALN-AS1 at the time points in the Schedule of Assessments. A detailed schedule of time points for the collection of blood samples for PK analysis is in Table 3 in Appendix 11.1).

The concentration of ALN-AS1 will be determined using a validated assay. Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5. Safety Assessments

The assessment of safety during the course of the study will consist of the surveillance and recording of AEs including SAEs, recording of concomitant medications and measurements of vital signs, physical examination, and ECG findings and clinical laboratory evaluations.

Safety will be monitored over the course of the study by an SRC as described in Section 4.6.

7.5.1. Vital Signs

Vital sign measurements include blood pressure, heart rate, oral body temperature, and respiratory rate. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes. Blood pressure should be taken using the same arm throughout the study. Oral body temperature will be obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respiration rate will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital sign assessments may be added at the discretion of the Investigator.

7.5.2. Weight and Height

Height will be measured in centimeters at the Screening/Baseline visit only. Body weight will be measured in kilograms. Body mass index will be calculated in the database.

7.5.3. Physical Examination

Complete physical examinations will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Targeted physical examinations will include the examination of the following: general appearance, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, and neurological/psychiatric.

7.5.4. Electrocardiogram

Triplicate 12-lead ECG, with readings approximately 2 minutes apart, will be recorded. Additional ECGs may be collected at the discretion of the Investigator. Standard computerized 12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Recordings will be obtained, after the patient has rested comfortably for

approximately 10 minutes. The electrophysiological parameters assessed include rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, Bazett-corrected QT interval (QTcB) and Fridericia-corrected QT interval (QTcF).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results have changed since the Screening/Baseline visit and to determine the clinical relevance of the results. These assessments will be recorded on the eCRF. For any clinically relevant ECG changes from the Screening/Baseline visit (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

7.5.5. Clinical Laboratory Assessments

The following clinical laboratory tests will be evaluated by a central laboratory. In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

Hematology	
Complete blood count with differential	
Serum Chemistry	
Sodium	Potassium
BUN	Phosphate
Creatinine and eGFR[a]	Albumin
Uric acid	Calcium
Total protein	Carbon dioxide
Glucose	Chloride
Liver Function Tests	
AST	ALP
ALT	Bilirubin (total and direct)
Urinalysis	
Visual inspection for appearance and color	Bilirubin
pH (dipstick)	Nitrite
Specific gravity	RBCs
Ketones	Urobilinogen
Albumin	Leukocytes
Glucose	Microscopy (if clinically indicated)
Protein	
Immunogenicity	
Antidrug antibodies	
Pregnancy Testing (WOCBP only)	
β-human chorionic gonadotropin	

Abbreviations: AST=aspartate transaminase; ALP=alkaline phosphatase; ALT=alanine transaminase; BUN=blood urea nitrogen; eGFR=estimated glomerular filtration rate; WOCBP=women of child bearing potential.

[a] eGFR will be calculated using the Modification of Diet in Renal Disease formula.[33]

7.5.5.1. Immunogenicity

Blood samples will be collected to evaluate antidrug antibodies. On days when ALN-AS1 is administered, blood samples for antidrug antibody testing must be collected before ALN-AS1 is administered.

Details regarding the processing, shipping, and analysis of the samples will be provided in the Laboratory Manual.

7.5.5.2. Pregnancy Testing

A pregnancy test will be performed for WOCBP only. A serum pregnancy test will be performed at Screening/Baseline and urine pregnancy tests will be performed thereafter per the Schedule of Assessments and any time pregnancy is suspected. Urine pregnancy tests may also be performed more frequently (eg, monthly) based on local country requirements. The results of the pregnancy test must be known before study drug administration. Patients who are pregnant are not eligible for study participation. Any woman with a positive pregnancy test during the study will be discontinued from study drug, but will continue to be followed for safety. Patients determined to be pregnant while on study will be followed until the pregnancy outcome is known (see Section 7.5.6.6 for follow-up instructions).

7.5.6. Adverse Events

7.5.6.1. Definitions

Adverse Event

According to the ICH E2A guideline Definitions and Standards for Expedited Reporting, and 21 Code of Federal Regulations 312.32, Investigational New Drug Safety Reporting, an AE is any untoward medical occurrence in a patient or clinical investigational subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (An event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (eg, events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

Adverse Event Severity

Adverse events are to be graded according to the categories detailed below:

Mild:	Mild events are those which are easily tolerated with no disruption of normal daily activity.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal daily activities.
Severe:	Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. AEs characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates)

AE severity and seriousness are assessed independently. 'Severity' characterizes the intensity of an AE. 'Serious' is a regulatory definition and serves as a guide to the Sponsor for defining regulatory reporting obligations (see definition for Serious Adverse Event).

Relationship of the Adverse Event to Study Treatment

The relationship of each AE to study treatment should be evaluated by the Investigator using the following criteria:

Definitely related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not related:	A clinical event, including laboratory test abnormality that has no temporal relationship to the medication or has more likely alternative etiology.

Adverse Events of Clinical Interest

The following events are considered to be adverse events of clinical interest:

- ISRs

- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator.

7.5.6.2. Eliciting and Recording Adverse Events

Eliciting Adverse Events

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC). In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings that are relevant to patient safety.

Recording Adverse Events

The Investigator is responsible for recording non-serious AEs that are observed or reported by the patient after administration of the first dose of study drug regardless of their relationship to study drug through the end of study. Non-serious AEs will be followed until the end of study.

The Investigator is responsible for recording SAEs that are observed or reported by the patient after the time when the informed consent is signed regardless of their relationship to study drug through the end of study. SAEs will be followed until satisfactory resolution, until baseline level is reached, or until the SAE is considered by the Investigator to be chronic or the patient is stable, as appropriate.

All AEs must be recorded in the source records for the clinical study center and in the eCRF for the patient, whether or not they are considered to be drug-related. Each AE must be described in detail: onset time and date, description of event, severity, relationship to investigational drug, action taken, and outcome (including time and date of resolution, if applicable).

For SAEs, record the event(s) on the eCRF. If the electronic data capture (EDC) system is unavailable, complete the backup SAE form.

For AEs that are considered AEs of clinical interest, additional clinical information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator or delegate should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed according to local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5.6.3. Serious Adverse Events Require Immediate Reporting to Sponsor/Designee

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the SAE criteria in Section 7.5.6.1 must be reported to the Sponsor or designee within 24 hours from the time that clinical study center personnel first learns of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number
- Description and date of onset of the event
- Criterion for serious
- Preliminary assignment of relationship to study drug
- Investigator/clinical study center information

To report the SAE, complete the eCRF. If the EDC system is unavailable, complete the backup SAE form. Within 24 hours of receipt of follow-up information, the Investigator must update the report. SAEs must be reported using the contact information provided in the Study Manual.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

7.5.6.4. Sponsor Safety Reporting to Regulatory Authorities

The Sponsor or its representative is required to report certain study events in an expedited manner to the Food and Drug Administration, the European Medicines Agency's EudraVigilance electronic system according to Directive 2001/20/EC, and to all country Regulatory Authorities where the study is being conducted, according to local applicable regulations.

The following describes the safety reporting timeline requirements for suspected unexpected serious adverse reactions (SUSARs) and other reportable events:

Immediately and within 7 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and fatal or life threatening. Follow-up information must be reported in the following 8 days.

Immediately and within 15 calendar days

- Any suspected adverse reaction that is associated with the use of the study drug, unexpected, and serious, but not fatal or life threatening, and there is evidence to suggest a causal relationship between the study drug and the reaction.
- Any finding from tests in laboratory animals that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any event in connection with the conduct of the study or the development of the study drug that may affect the safety of the trial patients.

In addition, periodic safety reporting to regulatory authorities will be performed by the Sponsor or its representative according to national and local regulations.

7.5.6.5. Serious Adverse Event Notification to the Institutional Review Board/Independent Ethics Committee

SUSARs will be reported to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) per their institutional policy by the Investigator or Sponsor (or Sponsor designee) according to country requirements. Copies of each report and documentation of IRB/IEC notification and acknowledgement of receipt will be kept in the Investigator's study file.

7.5.6.6. Pregnancy Reporting

If a female patient becomes pregnant during the course of this study through 90 days following the last dose of study drug, the Investigator must report the pregnancy to the Sponsor or designee within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy results in a postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly, then the Investigator should follow the procedures for reporting an SAE as outlined in Section 7.5.6.3.

7.5.6.7. Overdose Reporting

An overdose is defined as any dose administered to or taken by a patient (accidentally or intentionally) that exceeds the highest daily dose, or is at a higher frequency, than included in the protocol. It is up to the investigator to decide whether a dose is to be considered an overdose, in consultation with the Sponsor. Overdose must be recorded in the eCRF.

All reports of overdose (with or without an AE) must be reported within 24 hours to the Sponsor or designee.

7.6. Exploratory Assessments

Blood and urine samples will be collected for exploratory analyses. Aliquots of samples will be obtained and frozen, to permit future analysis of the effect of ALN-AS1 on exploratory biomarkers.

Where local regulations allow, biologic samples will be retained on behalf of the Sponsor for a maximum of 15 years following the last patient's last visit in the study. Details regarding the collection, processing, storage, and shipping of samples will be in the Laboratory Manual.

7.7. Other Assessments

7.7.1. EQ-5D-5L Questionnaire

QOL will be assessed through the use of the 5-level EQ-5D (EQ-5D-5L), a standardized questionnaire measuring health outcomes.[34] In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.

8. STATISTICS

A detailed Statistical Analysis Plan (SAP) will be written after finalizing the protocol and before database lock. The plan will detail the implementation of all the statistical analyses in accordance with the principle features stated in the protocol.

8.1. Determination of Sample Size

This is a Phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of ALN-AS1 in patients with AIP who completed study ALN-AS1-001. Safety, tolerability, PK, and PD of ALN-AS1 will be investigated. The sample size was not determined based on statistical considerations. Up to 24 patients are planned for this study (including optional cohorts in ALN-AS1-001).

8.2. Statistical Methodology

The statistical and analytical plans presented below summarize the more complete plans to be detailed in the SAP. Any changes to the methods described in the final SAP will be described and justified as needed in the clinical study report.

Statistical analyses will be primarily descriptive in nature. Descriptive statistics (eg, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be presented for categorical and ordinal variables. Analyses will be performed using SAS® (Version 9.2, or higher).

8.2.1. Populations to be Analyzed

The populations (analysis sets) are defined as follows:

Safety Analysis Set:	All patients who received any amount of study drug.
Clinical Activity Analysis Set:	All patients who received any amount of study drug and have both a baseline and at least 1 postdose assessment.
PK Analysis Set:	All patients who received any amount of study drug and have at least 1 postdose blood sample for PK and who have evaluable PK data.
PD Analysis Set:	All patients who received any amount of study drug and who have at least 1 postdose blood sample for the determination of ALN-AS1 will be included in the PD analyses.

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. The population used to evaluate clinical activity will be the Clinical Activity Analysis Set.

8.2.2. Examination of Subgroups

Subgroup analyses may be conducted for selected endpoints. Detailed methodology will be provided in the SAP.

8.2.3. Handling of Missing Data

Unrecorded values will be treated as missing. The appropriateness of the method(s) described for handling missing data may be reassessed and documented in the SAP before database lock. Depending on the extent of missing values, further investigation may be made into the sensitivity of the analysis results to the method(s) specified.

8.2.4. Baseline Evaluations

Demographics, medical history, disease-specific information, and other baseline characteristics collected in this study will be summarized.

8.2.5. Clinical Activity Analyses

Clinical activity of ALN-AS1 will be summarized primarily by descriptive statistics. The clinical activity of ALN-AS1 will be evaluated by the frequency and characteristics of porphyria attacks including duration, treatment, and severity of porphyria attacks and number and duration of visits to a health care setting for acute porphyria care.

Patient diary recordings, including BPI-SF questionnaire scores, will also be summarized.

8.2.6. Pharmacodynamic Analysis

PD analyses will include the evaluation of change in ALA, PBG, and ALAS mRNA levels. Descriptive statistics (eg, mean and standard error of the mean) for observed levels of PD parameters and the relative change from baseline will be presented for each of the postdose follow-up time points.

8.2.7. Pharmacokinetic Analysis

The PK concentration of ALN-AS1 will be determined.

Antidrug antibody results will be tabulated. A by-patient data listing will also be provided.

8.2.8. Safety Analyses

Extent of exposure will be summarized. AEs will be summarized by the Medical Dictionary for Regulatory Activities System Organ Class and Preferred Term. Prior and concomitant medications will be classified according to the World Health Organization drug dictionary.

Incidence of AEs (those events that started after exposure to study drug or worsened in severity after dosing) will be presented. Incidence of AEs will also be presented by maximum severity and relationship to study treatment. The incidence of SAEs and AEs leading to discontinuation of treatment will also be tabulated. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Laboratory shift tables from baseline to worst values will be presented. Abnormal physical examination findings and 12-lead ECG data will be presented in a by-patient data listing.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

Concomitant hemin administration will also be recorded.

8.2.9. Other Analyses

Possible associations between PD parameters (ALA, PBG, and ALAS1 mRNA levels) and clinical activity parameters may also be explored.

Additional exploratory analyses may be conducted on analytes related to targeting the heme biosynthesis pathway.

QOL scores on the EQ-5D-5L questionnaire will also be summarized.

9. STUDY ADMINISTRATION

9.1. Ethical and Regulatory Considerations

This study will be conducted in accordance with the protocol, all applicable regulatory requirements, and the guidelines of Good Clinical Practice (GCP). Compliance with GCP provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

9.1.1. Informed Consent

The Investigator will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to withdraw from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

9.1.2. Ethical Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC, as appropriate. The Investigator must submit written approval before he or she can enroll any patient into the study.

The Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be reapproved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

Initial IRB approval of the protocol, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

The Investigator will submit reports of SAEs as outlined in Section 7.5.6. In addition, the Investigator agrees to submit progress reports to the IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to the Sponsor or designee.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the Sponsor or its designee.

The Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational drug. The Sponsor or designee will provide this information to the Investigator.

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the Sponsor and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB or IEC and the Regulatory Authority for approval before patients are enrolled under the amended protocol.

9.1.3. Study Documentation, Confidentiality, and Records Retention

All documentation relating to the study should be retained for the period of time required by applicable local law. If it becomes necessary for the Sponsor, the Sponsor's designee, applicable IRB/IEC, or applicable regulatory authorities to review or audit any documentation relating to

the study, the Investigator must permit direct access to all source documents/data. Records will not be destroyed without informing the Sponsor in writing and giving the Sponsor the opportunity to store the records for a longer period of time at the Sponsor's expense.

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to the Sponsor or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to the Sponsor or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to the Sponsor (eg, signed ICFs) should be maintained by the Investigator in strict confidence.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. The Sponsor must approve any transfer of information not directly involved in the study.

In compliance with local and/or regional regulations, this clinical study may be registered and study results may be posted on public registries, such as ClinicalTrials.gov.

9.1.4. End of the Study

The end of the study is defined as last patient last visit.

9.1.5. Discontinuation of the Clinical Study

The Sponsor reserves the right to discontinue the study for clinical or administrative reasons at any time. If the clinical study center does not recruit at a reasonable rate, the study may be discontinued at that clinical study center. Should the study be terminated and/or the clinical study center closed for whatever reason, all documentation and study drug pertaining to the study must be returned to the Sponsor or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up. Patients should then be withdrawn from the study.

9.2. Data Quality Control and Quality Assurance

9.2.1. Data Handling

Study data must be recorded on case report forms (paper and/or electronic) provided by the Sponsor or designee on behalf of the Sponsor. Case report forms must be completed only by persons designated by the Investigator. If eCRFs are used, study data must be entered by trained clinical study center personnel with access to a valid and secure eCRF system. All data entered into the eCRF must also be available in the source documents. Corrections on paper CRFs must be made so as to not obliterate the original data and must be initialed and dated by the person who made the correction.

9.2.2. Study Monitoring

The clinical monitor, as a representative of the Sponsor, has an obligation to closely follow the study conduct at the clinical study center. The monitor will visit the Investigator and clinical

study center periodically and will maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the Investigator and personnel.

The monitor will review source documents, systems and CRFs to ensure overall quality and completeness of the data and to confirm study procedures are complied with the requirements in the study protocol. The Sponsor, or its designee, will be allowed to conduct clinical study center visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, patient charts and study source documents, and other records relative to study conduct.

9.2.3. Audits and Inspections

Periodically, the Sponsor or its authorized representatives audit clinical investigative study centers as an independent review of core trial processes and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. A regulatory authority, an IEC or an IRB may visit the clinical study center to perform audits or inspections, including source data verification. The Investigator should contact the Sponsor, or its designee, immediately if contacted by a regulatory agency about an inspection.

9.3. Publication Policy

It is intended that after completion of the study, the data are to be submitted for publication in a scientific journal and/or for reporting at a scientific meeting. A copy of any proposed manuscript must be provided and confirmed received at the Sponsor at least 30 days before its submission, and according to any additional publication details in the Investigator Agreement.

10. LIST OF REFERENCES

1. Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005 Mar 15;142(6):439-50.

2. Siegesmund M, van Tuyll van Serooskerken AM, Poblete-Gutierrez P, Frank J. The acute hepatic porphyrias: current status and future challenges. Best Pract Res Clin Gastroenterol. 2010 Oct;24(5):593-605.

3. Poblete-Gutierrez P, Wiederholt T, Merk HF, Frank J. The porphyrias: clinical presentation, diagnosis and treatment. Eur J Dermatol. 2006 May-Jun;16(3):230-40.

4. Balwani M, Desnick RJ. The porphyrias: advances in diagnosis and treatment. Blood. 2012 Nov 29;120(23):4496-504.

5. Puy H, Gouya L, Deybach JC. Porphyrias. Lancet. 2010 Mar 13;375(9718):924-37.

6. Floderus Y, Sardh E, Moller C, Andersson C, Rejkjaer L, Andersson DE, et al. Variations in porphobilinogen and 5-aminolevulinic acid concentrations in plasma and urine from asymptomatic carriers of the acute intermittent porphyria gene with increased porphyrin precursor excretion. Clin Chem. 2006 Apr;52(4):701-7.

7. Anderson K. Disorders of heme biosynthesis. 8th ed. CR S, editor: McGraw-Hill, New York 2001.

8. Dowman JK, Gunson BK, Mirza DF, Bramhall SR, Badminton MN, Newsome PN. Liver transplantation for acute intermittent porphyria is complicated by a high rate of hepatic artery thrombosis. Liver Transpl. 2012 Feb;18(2):195-200.

9. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. J Inherit Metab Dis. 2005;28(3):277-86.

10. Deybach J-C, Puy H. 86 - Acute Intermittent Porphyria: From Clinical to Molecular Aspects. In: Guilard KMKMS, editor. The Porphyrin Handbook. Amsterdam: Academic Press; 2003. p. 23-41.

11. Bylesjo I, Wikberg A, Andersson C. Clinical aspects of acute intermittent porphyria in northern Sweden: a population-based study. Scand J Clin Lab Invest. 2009;69(5):612-8.

12. James MF, Hift RJ. Porphyrias. Br J Anaesth. 2000 Jul;85(1):143-53.

13. Chemmanur AT, Bonkovsky HL. Hepatic porphyrias: diagnosis and management. Clin Liver Dis. 2004 Nov;8(4):807-38, viii.

14. Stein P, Badminton M, Barth J, Rees D, Stewart MF. Best practice guidelines on clinical management of acute attacks of porphyria and their complications. Ann Clin Biochem. 2013 May;50(Pt 3):217-23.

15. Bonkovsky HL. Neurovisceral porphyrias: what a hematologist needs to know. Hematology Am Soc Hematol Educ Program. 2005:24-30.

16. Panhematin. (hemin for injection). 2013.

17. Normosang. (human hemin). 2007.

18. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. Proc Natl Acad Sci U S A. 1977 May;74(5):2118-20.

19. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med. 1993 Sep 13;153(17):2004-8.

20. Bonkovsky HL, Singh RH, Jafri IH, Fiellin DA, Smith GS, Simon D, et al. A randomized, controlled trial of treatment of alcoholic hepatitis with parenteral nutrition and oxandrolone. II. Short-term effects on nitrogen metabolism, metabolic balance, and nutrition. Am J Gastroenterol. 1991 Sep;86(9):1209-18.

21. Herrick AL, McColl KE, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. Lancet. 1989 Jun 10;1(8650):1295-7.

22. Bissell DM. Treatment of acute hepatic porphyria with hematin. J Hepatol. 1988 Feb;6(1):1-7.

23. Green D, Ts'ao CH. Hematin: effects on hemostasis. J Lab Clin Med. 1990 Feb;115(2):144-7.

24. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. Am J Med. 2001 Feb 15;110(3):240.

25. Elder G, Harper P, Badminton M, Sandberg S, Deybach JC. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis. 2013 Sep;36(5):849-57.

26. Anderson K. Heme arginate for prevention of acute porphyric attacks (abstract). Acta Haematologica. 1997;98((suppl 1):120).

27. Bonkovsky HL, Guo JT, Hou W, Li T, Narang T, Thapar M. Porphyrin and heme metabolism and the porphyrias. Compr Physiol. 2013 Jan;3(1):365-401.

28. Vaishnaw AK, Gollob J, Gamba-Vitalo C, Hutabarat R, Sah D, Meyers R, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001 May 24;411(6836):494-8.

30. Kauppinen R, von und zu Fraunberg M. Molecular and biochemical studies of acute intermittent porphyria in 196 patients and their families. Clin Chem. 2002 Nov;48(11):1891-900.

31. Division of Clinical Pharmacology, Indiana University, P450 Drug Interaction Table. 2013 [Accessed 06 February 2015]; Available from: http://medicine.iupui.edu/clinpharm/ddis/main-table/.

32. Atkinson TM M, TR, Sit L, Passik P, Scher, HI, Cleeland C, and Basch E. The Brief Pain Inventory and its “Pain at its Worst in the last 24 Hours” Item: Clinical Trial Endpoint Considerations. Pain Med. 2010 March;11(3):337–46.

33. Levey AS SL, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

34. Herdman M GC, Lloyd A, Janssen M, Kind P, Parkin D, Bonsel G, Badia X. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727-36.

11. APPENDICES

11.1. Pharmacokinetic Assessment Time Points

Table 3 contains a detailed schedule for the collection of blood samples for PK analysis.

Table 3: Pharmacokinetic Time Points

Study Day	Protocol Time (hh:mm)	PK Blood
Day 1 and Month 12 (D361) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X
	06:00 (±20 minutes)	X
Month 1 (Day 31) ±7 days, Month 3 (Day 91) ±7 days, Month 6 (Day 181) ±7 days, Month 18 (Day 541) ±10, and Month 24 (Day 721) ±10 days	Predose (within 60 minutes before dosing)	X
	02:00 (±5 minutes)	X

Abbreviations: hh=hours; mm=minutes; PK=pharmacokinetic.

11.2. Schedules of Assessments for Every Month Dosing Regimen

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	-60 to -1	D1	D31±7	D61±7	D91±7	D121±7/ D151±7	D181±7	211±7/ 241±7	271±10	301±7/ 331±7	361±10	391±7/ 421±7	451±10	481±7/ 511±7	541±10
Informed Consent	X														
Medical History/Disease History[c]	X	X													
Demographics	X														
Inclusion/ Exclusion Criteria	X	X													
Physical Examination[d]	X	X	X	X	X	X	X		X		X				X
Body Weight, BMI, and Height[e]	X	X	X	X	X	X	X	X	X	X	X		X		X
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Triplicate 12-Lead ECG[g]	X	X			X						X				
Clinical Laboratory Assessment[h]	X		X	X	X		X		X		X		X		X
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration[j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X	X	X	X	X	X		X		X		X		X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]		X	X	X	X		X		X		X				X

Table 4: Schedule of Assessments: Screening/Baseline through Month 18 (Every Month Dosing Regimen)

Study Visit (M)	Screening/ Baseline[a]	Treatment Period (Screening/Baseline through Month 18)													
			M1	M2	M3	M4/ M5	M6	M7/ M8[b]	M9	M10/ M11[b]	M12	M13/ M14[b]	M15[b]	M16/ M17[b]	M18
Study Visit (D±Visit Window)	**-60 to -1**	**D1**	**D31±7**	**D61±7**	**D91±7**	**D121±7/ D151±7**	**D181±7**	**211±7/ 241±7**	**271±10**	**301±7/ 331±7**	**361±10**	**391±7/ 421±7**	**451±10**	**481±7/ 511±7**	**541±10**
Blood Sample for PK Analysis[m]		X	X		X		X				X				X
Antidrug Antibodies[n]		X	X		X		X				X				X
Porphyria Attack Laboratory Sample Collection Kit and Instructions[o]	X														
EQ-5D-5L Questionnaire[p]	X						X				X				X
Diary Review (including BPI-SF)[q]		X													
AEs		Continuous													
Concomitant Medications[r]		Continuous													

Abbreviations: AE=adverse event; ALAS1=5-aminolevulinic acid synthase 1; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Patients are not required to complete Screening/Baseline assessments if the assessments in the previous study (ALN-AS1-001) were completed within 60 days of administration of the first dose of ALN-AS1 in this study. If more than 60 days have elapsed since the last assessment, clinical laboratory tests and ECGs will be repeated before administration of ALN-AS1. Results should be available before the administration of the first dose of ALN-AS1.

b After a patient has completed 6 months of ALN-AS1 administration in this study, and where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the

Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q3M through M12, and thereafter Q6M.

c See Section 7.1 for details on the interval medical history/disease history to be recorded at Screening/Baseline. Ongoing AEs from the previous study (ALN-AS1-001) will be captured as medical history.

d A complete physical examination will be performed at Screening/Baseline, and D1. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.

e Height will be measured at Screening/Baseline only.

f Vital signs include blood pressure, heart rate, oral body temperature, and respiratory rate. On D1, vital signs will be collected within 1 hour predose; and 2 hours (±10 minutes) postdose. On all other dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.

g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On D1, ECGs will be performed within 1 hour predose and 2 hours (±10 minutes) postdose. On all other dosing days, ECGs will be collected within 1 hour predose.

h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.

i A serum pregnancy test will be performed at Screening/Baseline; thereafter, urine pregnancy tests will be performed. Results must be available before dosing.

j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. For weight-based dosing, weight measured on the dosing day will be used to calculate the dose of ALN-AS1. After M12, weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.

k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.

l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.

m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).

n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.

o Urine samples should be collected within 24 hours of the start of clinical intervention for a porphyria attack (eg, hemin or glucose administration) and within 24 hours of the end of clinical intervention for a porphyria attack through M39. If patients are unable to report to the clinical study center during a porphyria attack, laboratory sample collection kits will be provided that contain instructions for collecting and sending urine samples to the clinical study center, if possible. These urine samples may also be analyzed for exploratory biomarkers.

p In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but not more than once in a 6 month period), if possible.

q Patients or caregivers will be provided with a diary to record acute porphyria attacks, pain assessments, and narcotic use on a daily basis through M9; thereafter, acute porphyria attacks will be recorded in the diary. The BPI will be completed on a weekly basis as part of the patient diary through M9, then Q3M.

r Medications that are ongoing from the previous study (ALN-AS1-001), started between the previous study and this study, and started after signing informed consent in this study, will be captured as concomitant medication(s).

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

Study Visit (M)	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
	M19/ M20[a]	M21[a]	M22/ M23[a]	M24	M25/ M26[a]	M27[a]	M28/ M29[a]	M30	M31/ M32[a]	M33[a]	M34/ M35[a]	M36/ EOT	M39[b]	M42
Study Visit (D±Visit Window)	571±7/601±7	631±10	661±7/691±7	721±10	751±7/781±7	811±10	841±7/871±7	901±10	931±7/961±7	991±10	1021±7/1051±7	1081±10	1171±10	1351±10
Physical Examination[d]				X				X				X	X	
Body Weight, BMI, and Height[e]				X				X					X	
Vital Signs[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Triplicate 12-Lead ECG[g]				X									X	
Clinical Laboratory Assessment[h]		X		X		X		X		X		X	X	
Pregnancy Test[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration[j]	X	X	X	X	X	X	X	X	X	X	X	X		
Urine Sample for PBG, ALA, and Creatinine Analysis[k]		X		X		X		X		X		X	X	X
Blood and Urine Sample for Exploratory Biomarker Analysis[l]				X				X				X	X	
Blood Sample for PK Analysis[m]				X										

Table 5: Schedule of Assessments: Month 19 through End of Study (Every Month Dosing Regimen)

	Treatment Period (Month 19 through EOS)												Posttreatment Follow-up	ALA/PBG Monitoring/ EOS[c]
Study Visit (M)	**M19/ M20[a]**	**M21[a]**	**M22/ M23[a]**	**M24**	**M25/ M26[a]**	**M27[a]**	**M28/ M29[a]**	**M30**	**M31/ M32[a]**	**M33[a]**	**M34/ M35[a]**	**M36/ EOT**	**M39[b]**	**M42**
Study Visit (D±Visit Window)	**571±7/601±7**	**631±10**	**661±7/691±7**	**721±10**	**751±7/781±7**	**811±10**	**841±7/871±7**	**901±10**	**931±7/961±7**	**991±10**	**1021±7/1051±7**	**1081±10**	**1171±10**	**1351±10**
Antidrug Antibodies[n]				X								X		
EQ-5D-5L Questionnaire[o]				X				X				X		
Diary Review (including BPI-SF)[p]	X													
AEs	Continuous													
Concomitant Medications	Continuous													

Abbreviations: AE=adverse event; ALA=delta-aminolevulinic acid; BMI=body mass index; BPI-SF=Brief Pain Inventory-Short Form; D=day; ECG=electrocardiogram; EOS = End of Study; EOT = End of Treatment; M=month; PBG= porphobilinogen; PK=pharmacokinetics; Q=every; QOL=quality of life; SC=subcutaneous.

Notes:

- White boxes represent visits to the clinical study center or when patients will be contacted by phone; grey boxes represent study visit that may be conducted while the patient is at home.
- When scheduled at the same time points, assessments of vital signs and 12-lead ECGs should be performed first, before the physical examinations and blood/urine sample collections.

a Where applicable country and local regulations and infrastructure allow, study visits and ALN-AS1 administration may take place at the patient's home by a healthcare professional. Home visits may occur at the discretion of the Investigator, based on safety and tolerability; however, patients are required to complete at least 1 visit at the clinical study center Q6M.

b Patients who discontinue treatment will be asked to complete the M39 visit assessments. If a patient chooses to withdraw from the study, every effort should be made to conduct early the assessments performed at the M39 visit. See Section 5.3 for details on removal from treatment or assessment.

c The ALA/PBG Monitoring/EOS visit will be conducted at the clinical study center or via phone contact, unless treatment continues with ALN-AS1. Patients will be provided with laboratory sample collection kits and instructions for collecting and sending urine samples to the clinical study center. Urine samples should not be collected during a porphyria attack.
d A complete physical examination will be performed at the posttreatment follow-up visit. At all other visits, a targeted physical examination will be performed. On dosing days, the physical examination will be performed predose. On days when ALN-AS1 is not administered, the physical examination can occur at any time during the visit. See Section 7.5.3 for details on the physical examination.
e Height will be measured at Screening/Baseline only.
f Vital signs include blood pressure, heart rate, oral body temperature, and respiratory rate. On a dosing days, vital signs will be collected within 1 hour predose. On days when ALN-AS1 is not administered, vital signs can be collected at any time during the visit. Vital signs will be measured in the seated or supine position (should be consistent for each patient), after the patient has rested comfortably for 10 minutes.
g Triplicate 12-lead ECGs will be performed in the seated or supine position after the patient has rested comfortably for 10 minutes. On dosing days, ECGs will be collected within 1 hour predose.
h Clinical laboratory parameters are described in Section 7.5.5. On dosing days, blood samples for clinical laboratory evaluations will be collected within 1 hour predose; results are not required before dosing. On days when ALN-AS1 is not administered, blood samples can be collected at any time during the visit.
i Urine pregnancy tests will be performed. Results must be available before dosing.
j ALN-AS1 will be administered by SC injection according to the Pharmacy Manual. See Section 6.2.2 for ALN-AS1 dose and dosing regimen. Weight measured on the nearest previous visit will be used to calculate the weight-based dose of ALN-AS1.
k Spot urine samples will be collected for measurement of ALA, PBG, and creatinine levels. On dosing days, samples should be collected predose.
l On dosing days, blood and urine samples for ALAS1 mRNA (and possible biomarker) analysis will be collected within 1 hour predose.
m Blood samples for PK analysis will be collected at the time points listed in Table 3 (Appendix Section 11.1).
n On dosing days, blood samples for antidrug antibody analysis should be collected within 1 hour predose.
o In addition to the time points indicated, the EQ-5D-5L questionnaire should be completed once during the treatment period if a patient has a porphyria attack (but, not more than once in a 6 month period), if possible.
p Patients or caregivers will be provided with a diary to record acute porphyria attacks. The BPI will be completed as part of the patient diary Q3M.