CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

212194Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	212194
Priority or Standard	Priority
Submit Date(s)	November 15, 2018; January 22, 2019; June 4, 2019
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Division/Office	Division of Hematology Products/Office of Drug Evaluation I
Review Completion Date	November 19, 2019
Established/Proper Name	Givosiran
(Proposed) Trade Name	GIVLAARI
Pharmacologic Class	Small interfering ribonucleic acid (RNA)
Applicant	Alnylam Pharmaceuticals, Inc.
Dosage form	Injection
Applicant proposed Dosing Regimen	2.5 mg/kg once monthly by subcutaneous injection
Applicant Proposed Indication(s)/Population(s)	Treatment of acute hepatic porphyria (AHP) in adults (b) (4)
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	64081000 | Porphobilinogen synthase deficiency (disorder) |
Recommendation on Regulatory Action	Regular Approval
Recommended Indication(s)/Population(s) (if applicable)	GIVLAARI is an aminolevulinate synthase 1-directed small interfering RNA indicated for the treatment of adults with acute hepatic porphyria (AHP)
Recommended Dosing Regimen	2.5 mg/kg administered once monthly by subcutaneous (SC) injection

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OPQ=Office of Pharmaceutical Quality
OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
DRISK=Division of Risk Management
COA=Clinical Outcomes Assessment
IRT/QT=Interdisciplinary Review Team for QT Studies

Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
OCS	Office of Computational Science

OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

1 Executive Summary

1.1. Product Introduction

Givosiran (Givlaari) is a small interfering ribonucleic acid (RNA) (siRNA) that inhibits aminolevulinic acid synthase 1 (ALAS1). The proposed indication is as follows.

- Givlaari (givosiran) is an aminolevulinate synthase 1-directed small interfering RNA indicated for the treatment of adults with acute hepatic porphyria (AHP)

The proposed dosing of givosiran is 2.5 mg/kg administered subcutaneously (SC) once monthly.

1.2. Conclusions on the Substantial Evidence of Effectiveness

Data supporting the recommendation for the approval of givosiran for the treatment of adult patients with AHP was obtained from the study ALN-AS1-003 titled, "A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias", also known as the ENVISION study. Eligible patients were randomized 1:1 to receive givosiran 2.5 mg/kg administered SC or placebo during the 6 month double-blind period. In this study, inclusion criteria specified a minimum of 2 porphyria attacks requiring hospitalization, urgent healthcare visit, or intravenous (IV) hemin administration at home in the 6 months prior to study entry. Hemin use during the study was permitted for the treatment of acute porphyria attacks. The median age of patients studied was 37.5 years (range 19 to 65 years), 89% of patients were female, and 78% were white. Givosiran and placebo arms were balanced with respect to historical porphyria attack rate, hemin prophylaxis prior to study entry, and patient reported measures of pain symptoms between attacks. The study enrolled 98 adult (age ≥ 18 years) patients with AHP (48 patients in the givosiran arm and 46 patients in the placebo arm). Efficacy in the 6-month double-blind period was measured by the rate of porphyria attacks that required hospitalizations, urgent healthcare visit, or IV intravenous hemin administration at home. The efficacy of givosiran over placebo is demonstrated by a decrease in the attack rate observed among patients with AHP who were treated with givosiran compared to those who received placebo. Among patients with AHP in the ENVISION study the estimated attack rate over the 6 month double-blind treatment period was 2 attacks (95% CI: 1, 3 attacks) per patient in the givosiran arm compared to 14 attacks (95% CI: 10, 20 attacks) per patient in the placebo arm. Secondary efficacy endpoints considered supportive of the efficacy of givosiran for the treatment of AHP included changes in urinary aminolevulinic acid (ALA) and porphobilinogen (PBG) concentration. Urinary ALA and PBG concentration levels decreased and then were maintained during the givosiran treatment period compared to placebo.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Acute hepatic porphyria is a rare disease that is the result of increased levels of toxic heme intermediate molecules in the body. These heme intermediates result in acute painful attacks and increase the risk of neurovisceral damage systemically. Hemin (approved for marketing in 1983) is the only approved drug available for treatment of AHP and has an indication that is limited to female patients with acute intermittent porphyria (AIP) attacks related to the menstrual cycle after initial carbohydrate therapy is known or suspected to be inadequate. Among patients with AHP in the ENVISION study the estimated attack rate over the 6 month double-blind treatment period was 2 attacks (95% CI: 1, 3 attacks) per patient in the givosiran arm (2.5mg/kg administered SC once monthly) compared to 14 attacks (95% CI: 10, 20 attacks) per patient in the placebo arm. Givosiran offers adult patients with AHP another prophylactive therapeutic option which, potentially, may be more conveniently administered compared to Hemin. Generally, treatment with givosiran appears to be tolerable. A higher proportion of patients in the givosiran arm (10/48 (21%) patients) compared to the placebo arm (4/46 (9%) patients) reported serious adverse events (SAEs). Of the SAEs, only device-related infection (2/48 (4%) patients in the givosiran arm) was reported in two or more patients treated with givosiran in the study. The most frequently occurring (≥20% incidence) adverse events (AEs) reported in patients treated with givosiran were nausea (27%) and injection site reactions (25%). The risks of givosiran therapy for the proposed indication will be handled in product labeling. The benefit-risk analysis favors approval of givosiran for the treatment of adult patients with AHP.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Acute hepatic porphyria (AHP) is a rare disease with a prevalence of 5-10 cases/100,000 people in the US and effects primarily females (age range15-45 years). The induction of ALAS1 results in increased production and accumulation of toxic heme intermediates delta aminolevulinic acid (ALA) and porphobilinogen (PBG) in the plasma and urine. Clinically, the accumulation of toxic heme intermediates results in acute attacks characterized by severe abdominal pain, muscle weakness, seizures, psychiatric dysfunction, irreversible neurologic damage and increased risk of hepatic malignancy. (Bissell, 2015)	Acute hepatic porphyria is a rare disease that is the result of increased levels of toxic heme intermediate molecules in the body. These heme intermediates result in acute painful attacks and increase the risk of neurovisceral damage systemically.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current Treatment Options	• Panhematin® (Hemin for Injection, approved for marketing in 1983) is an intravenously administered iron-containing metalloporphyrin ALAS1 inhibitor that is derived from processed red blood cells. Panhematin is indicated for the amelioration of recurrent attacks of acute intermittent porphyria temporally related to the menstrual cycle in susceptible women, after initial carbohydrate therapy is known or suspected to be inadequate. Panhematin is not typically stocked in hospital pharmacies and must be ordered from the manufacturer which can delay therapy. Liver transplants, when available, can also be considered for this disease. (Lichtman, 2003)	Hemin (approved for marketing in 1983) is the only approved drug available for treatment of AHP and has an indication that is limited to female patients with AIP attacks related the menstrual cycle after initial carbohydrate therapy is known or suspected to be inadequate.
Benefit	• Data supporting the recommendation for the approval of givosiran for the treatment of adult patients with AHP was obtained from the study ALN-AS1-003 titled, "A Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias", also known as the ENVISION study. Eligible patients were randomized 1:1 to receive givosiran 2.5 mg/kg administered SC or placebo during the 6 month double blind period. The study enrolled 98 adult (age ≥ 18 years) patients with AHP (48 patients in the givosiran arm and 46 patients in the placebo arm). Efficacy in the 6-month double-blind period was measured by the rate of porphyria attacks that required hospitalizations, urgent healthcare visit, or IV intravenous hemin administration at home. Among patients with AHP in the ENVISION study the estimated attack rate over the 6 month double-blind treatment period was 2 attacks (95% CI: 1, 3 attacks) per patient in the givosiran arm compared to 14 attacks (95% CI: 10, 20 attacks) per patient in the placebo arm.	Among patients with AHP in the ENVISION study the estimated attack rate over the 6 month double-blind treatment period was 2 attacks (95% CI: 1, 3 attacks) per patient in the givosiran arm (2.5mg/kg administered SC once monthly) compared to 14 attacks (95% CI: 10, 20 attacks) per patient in the placebo arm. Givosiran offers adult patients with AHP another prophylactive therapeutic option which, potentially, may be more conveniently administered compared to Hemin.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	• All 94 patients enrolled in the ENVISION study completed the double blind 6 month assessment (n=48 patients in the givosiran treatment arm and n=46 patients in the placebo treatment arm). Patients received givosiran for a median of 6 months (range 3-6 months) and placebo for a median of 6 months (range 5-6 months). Although a higher proportion of patients in givosiran arm (10/48 (21%) patients) and compared to the placebo arm (4/46 (9%) patients) reported serious adverse events (SAEs), only device related infection (2/48 (4%) patients in the givosiran arm) was reported in at least two or more patients in the study. The most frequently occurring (≥20% incidence) adverse events (AEs) reported in patients treated with givosiran were nausea (27%) and injection site reactions (25%). The risks of givosiran therapy for the proposed indication will be addressed in product labeling. No Risk Management Evaluation Strategy is proposed (REMS).	Generally, treatment with givosiran appears to be tolerable. A higher proportion of patients in givosiran arm (10/48 (21%) patients) compared to the placebo arm (4/46 (9%) patients) reported SAEs. Of the SAEs, only device-related infection (2/48 (4%) patients in the givosiran arm) was reported in at least two or more patients in the study. The most frequently occurring (≥20% incidence) adverse events (AEs) reported in patients treated with givosiran were nausea (27%) and injection site reactions (25%). The risks of givosiran therapy for the proposed indication will be addressed in product labeling. The benefit-risk analysis favors approval of givosiran for the treatment of adult patients with AHP.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

☒	**The patient experience data that were submitted as part of the application include:**			Section of review where discussed, if applicable [e.g., Section 6.1 Study endpoints]
	☒	Clinical outcome assessment (COA) data, such as		
		☒	Patient reported outcome (PRO) ***Clinical Reviewer Comment:*** *Patient reported outcomes (PRO) data obtained in the ENVISION study (ALN-AS1-003) were prespecified as exploratory endpoints.* (b) (4) [redacted]	See ENVISION study Clinical Study Report (CSR) section 11.2, 11.3 and 11.4.
		□	Observer reported outcome (ObsRO)	
		□	Clinician reported outcome (ClinRO)	
		□	Performance outcome (PerfO)	
	□	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		
	□	Natural history studies		
	□	Patient preference studies (e.g., submitted studies or scientific publications)		
	□	Other: (Please specify):		
□	**Patient experience data that were not submitted in the application, but were considered in this review:**			
	□	Input informed from participation in meetings with patient stakeholders		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		
	☐	Other		

☐	**Patient experience data was not submitted as part of this application.**

Kathy Robie Suh, MD, PhD
Cross Discipline Team Leader

2 Therapeutic Context

2.1. Analysis of Condition

Acute hepatic porphyria (AHP) is a rare disease with a prevalence of 5-10 cases/100,000 people in the US and affects primarily females (age range15-45 years). AHP occurs as a result of an autosomal dominant mutation that leads to deficiency of aminolevulinic acid dehydratase and porphobilinogen deaminase which are enzymes in the heme biosynthesis pathway. The rate limiting step in heme synthesis is the enzyme aminolevulinic acid synthase 1 (ALAS1) which is controlled by feedback repression via the end-product heme. ALAS1 is induced in response to a decrease in the endogenous heme pool in the liver which can occur with stressors such as: fasting, hormonal alterations or cytochrome P450 inducing drugs. The induction of ALAS1 results in increased production and accumulation of toxic heme intermediates delta aminolevulinic acid (ALA) and porphobilinogen (PBG) in the plasma and urine. Clinically, the accumulation of toxic heme intermediates results in acute attacks characterized by severe abdominal pain, muscle weakness, seizures, psychiatric dysfunction, irreversible neurologic damage and increased risk of hepatic malignancy. (Bissell, 2015)

2.2. Analysis of Current Treatment Options

Management of AHP attacks often requires hospitalization. Patients are initially treated with supportive care, intravenous fluid administration, carbohydrate loading, analgesics, antiemetics and removal of known precipitating factors. Panhematin® (Hemin for Injection, approved for marketing in 1983) is an intravenously administered iron containing metalloporphyrin ALAS1 inhibitor that is derived from processed red blood cells. Panhematin is indicated for the amelioration of recurrent attacks of acute intermittent porphyria temporally related to the menstrual cycle in susceptible women, after initial carbohydrate therapy is known or suspected to be inadequate. The recommended Panhematin dose is 3-4 mg/kg infused over 15 minutes in a large vein or central venous catheter once daily for a period of 3-5 days. Prior to administration of Panhematin the drug must be filtered in order to remove particulates. Symptoms generally improve in patients after 2-5 days of hematin treatment accompanied by a decrease in ALA and PBG production. Panhematin is not typically stocked in hospital pharmacies and must be ordered from the manufacturer which can delay therapy. Liver transplants, when available, can also be considered for this disease. (Lichtman, 2003) Givosiran (2.5 mg/kg administered SC once monthly) is a small interfering RNA (siRNA) that inhibits aminolevulinic acid synthase 1 (ALAS1). Inhibition of ALAS1 reduces the downstream synthesis of ALA and PBG. Givosiran offers adult patients with AHP another therapeutic option which, potentially, may be more conveniently administered compared to Hemin. The reviewer's table below summarizes the most relevant approved drug product for the treatment of patients with AHP.

Table 1. Approved Products for the Indication

Product Name	Year of Marketing Approval	Indication	Dosing
Hemin for Injection (Panhematin®)	1983	PANHEMATIN is a hemin for injection indicated for amelioration of recurrent attacks of acute intermittent porphyria temporally related to the menstrual cycle in susceptible women, after initial carbohydrate therapy is known or suspected to be inadequate.	3-4 mg/kg infused of 15 minutes in a large vein or central venous catheter once daily for a period of 3-5 days.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Givlaari (givosiran) is a new molecular entity and not currently marketed in the United States.

3.2. Summary of Presubmission/Submission Regulatory Activity

On March 18, 2015, the Applicant, Alnylam Pharmaceuticals, Inc., submitted a pre-IND meeting request to seek feedback on the design of their proposed clinical study to evaluate givosiran (ALN-AS1) for the treatment of acute intermittent porphyria (AIP). The Agency's feedback was provided via meeting preliminary comments dated June 1, 2015.

On August 13, 2015, IND 126094 was submitted to the Agency to provide for a protocol, Study ALN-AS1-001, entitled, "*A Phase 1, Single-ascending Dose, Multiple-ascending Dose, and Multi-dose Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of Subcutaneously Administered ALN-AS1 in Patients with AIP*." The Applicant was notified that they may proceed with their proposed investigation on September 11, 2015.

Since the activation of the IND, givosiran has been granted the following regulatory designations and special agreements:

- August 29, 2016: Orphan designation for the treatment of acute hepatic porphyria (AHP).
- May 23, 2017: Breakthrough therapy designation for the prophylaxis of attacks in patients with AHP.
- June 14, 2018: Special Protocol – Agreement to (b) (4) carcinogenicity study (b) (4)
- August 31, 2018: Special Protocol – Agreement to (b) (4) carcinogenicity study (b) (4)

With regards to significant regulatory interactions, the following PDUFA meetings were held/scheduled:

- June 14, 2017: Type B pre-phase 3/initial breakthrough therapy meeting held to discuss the design of the Sponsor's proposed phase 3 study, ALN-AS1-003, as well as the adequacy of the overall givosiran development program to support a New Drug Application (NDA).
- February 20, 2018: Feedback provided via type B CMC meeting preliminary comments regarding the givosiran CMC development program.

- June 25, 2018: Feedback provided via type B breakthrough therapy meeting preliminary comments in which the Agency indicated general agreement with the Sponsor's proposed NDA content and format, including a plan for rolling submission.
- April 26, 2019: Additional feedback provided via type B pre-NDA meeting preliminary comments regarding the planned final (clinical) rolling component of NDA 212194.

On July 2, 2019, the Applicant was also notified that they may proceed with their proposal for the treatment use of givosiran for the treatment of AHP as provided by Protocol ALN-AS1-005, entitled "*Expanded Access Protocol of Givosiran for Patients with AHP*."

The rolling components of NDA 212194 were received on November 15, 2018, January 22, 2019, and June 4, 2019, respectively, which proposed the registration of givosiran for the treatment of AHP in adults (b) (4). The Applicant requested priority review, which was granted by the Agency at the time of filing on August 2, 2019.

In regards to foreign regulatory activity, the Applicant submitted a Marketing Authorization Application (MAA) to the European Medicines Agency (EMA) proposing givosiran for the treatment of acute hepatic porphyria (AHP) on July 1, 2019.[1]

[1] http://investors.alnylam.com/news-releases/news-release-details/alnylam-submits-marketing-authorization-application-european

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Three study sites were chosen for evaluation for the Office of Scientific Investigation (OSI), i.e., study site 404 (Dr. Montgomery Bissell; San Francisco, USA) in which 5 patients were enrolled (n=4 patients givosiran), study site 241 Dr. Ulrich Stozel; Chemnitz, DEU Western EU) in which 5 patients were enrolled (n=4 patients givosiran) and study site #405 (Dr. Herbert Bonkovsky, Winston-Salem, USA) in which 5 patients were enrolled (n=2 patients givosiran). These study sites consisted of the highest total number of patients by site enrolled onto the givosiran treatment arm (total 10/48 (21%) patients). Enrollment at other study sites was very disperse. Dr. Anthony Orencia (Clinical Reviewer in the OSI) states in his review (final signature date October 2, 2019) that the study data derived from these clinical sites, based on the inspections, are considered reliable and the study in support of this application appears to have been conducted adequately. The inspection of the sponsor's site found no deficiencies with oversight and monitoring of the trial. In general, the sponsor maintained adequate oversight of the clinical trial and appeared to be in compliance with Good Clinical Practice.

4.2. Product Quality

Dr. Anamitro Banerjee (Application Technical Lead in the Office of Product Quality (OPQ)) recommends approval of the marketing application for givosiran under NDA 212194 is his review (final signature date October 21, 2019) from a product quality perspective. Dr. Banerjee states that all the facilities to be used for drug substance and drug product manufacturing, packaging, labeling and testing (release and stability) were evaluated and were found to have acceptable compliance history and experience.

4.3. Clinical Microbiology

Dr. Banerjee notes in his review of the marketing application for givosiran under NDA 212194 is his review (final signature date October 21, 2019) that the Dr. Renee Marcsisin-Rogers (Clinical Microbiology Reviewer in the OPQ) recommended approval of the application from a clinical microbiology perspective (final signature dated October 15, 2019). (See Clinical Microbiology review N212194MR01.docx, dated 10/09/2019, in Panorama for complete information).

4.4. Devices and Companion Diagnostic Issues

Not applicable. No devices or companion diagnostics are proposed for this application of givosiran.

Clinical Reviewer Comment Section 4: *I agree with Dr. Orencia's (Clinical Reviewer in the OSI) review and recommendation (final signature date October 2, 2019). Dr. Banerjee (Application*

Technical Lead in OPQ) did not identify any significant chemistry, manufacturing, controls or clinical microbiology concerns in his review of the marketing application for givosiran under NDA 212194 for the treatment of AHP.

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

The nonclinical development program for givosiran (also known as ALN-AS1) was conducted in in vitro assays and in various animal species including the mouse, rat, rabbit, and cynomolgus monkey to evaluate the pharmacology, pharmacokinetics, general toxicology, genotoxic potential, and reproductive and developmental effects. Givosiran is a small interfering ribonucleic acid (siRNA) that targets 5′aminolevulinate synthase 1 (ALAS1) messenger RNA (mRNA), the first and rate-limiting enzyme in the production of heme. The drug has been designed for delivery to the liver through the conjugation of the 3′-end of the sense strand to a trivalent N-acetylgalactosamine (GalNAc) moiety. The asialoglycoprotein receptor (ASGPR) is a member of the C-type lectin family of receptors that binds glycoproteins with terminal galactose or GalNAc residues and is expressed on the cell surface of hepatocytes; therefore, the conjugation to the GalNAc moiety results in delivery of the siRNA to the hepatocytes in the liver. Givosiran has been assigned an established pharmacological class of "aminolevulinate synthase 1-directed small interfering RNA". In addition to being pharmacologically active in humans, based on the nonclinical data described below, givosiran is also pharmacologically active in both rats and monkeys but is not pharmacologically active in rabbits.

Givosiran demonstrated *in vitro* activity in the human hepatoma cell line Hep3B by inhibiting ALAS-1 mRNA with an IC_{50} value of 0.026 nM. In a study assessing the activity of potential givosiran antisense strand terminal truncation metabolites, the givosiran drug substance decreased ALAS1 mRNA in Hep3B cells with approximately 69% and 16% mRNA remaining (31% and 84% reduction) for 0.1 nM and 10 nM concentrations of siRNA, respectively. In comparison to givosiran, siRNAS with antisense strands truncated from the 5′ terminus of the siRNA duplex did not demonstrate activity. In contrast, truncations of the 3′ terminus of the antisense strand retained full or partial activity; specifically, removal of the first 5 bases did not affect activity at a concentration of 10 nM siRNA. These results suggest that metabolites with truncations of the 3′ terminus of the antisense strand may contribute to the in vivo activity of givosiran.

The *in vivo* activity of givosiran was assessed by evaluating the effects on ALAS1 levels following single- and repeat-dose subcutaneous administration of givosiran in rats and monkeys and investigating the effects in disease models of acute intermittent porphyria (AIP) in mice and rats. AIP is a subtype of acute hepatic porphyria. Subcutaneous administration of single and repeated doses of givosiran dose-dependently suppressed ALAS1 mRNA levels in the liver of rats with approximately 58% suppression following a single subcutaneous dose of 10 mg/kg and 63% suppression following 4 weekly subcutaneous doses of 5 mg/kg. In a study evaluating the timecourse of the reduction of ALAS1 mRNA levels in the liver of rats administered givosiran weekly for 8 weeks, the maximum reduction in ALAS1 mRNA ranged from 70% to 80% and occurred after 6 to 8 doses of givosiran. The suppression of ALAS1 mRNA levels in the liver was reversible with levels returning to baseline by 28 days after the final dose. To have a less

invasive approach than serial liver biopsies to monitor ALAS1 mRNA levels in monkeys and humans, a circulating extracellular RNA detection (cERD) assay was developed to measure exosomal RNA isolated from serum and urine. Results of a pharmacodynamics and pharmacokinetics study in cynomolgus monkeys administered givosiran as a single dose or as a multiple dose regimen for up to 8 weeks demonstrated consistency between ALAS1 mRNA levels measured in serum and urine using cERD and intra-hepatic levels at the same time points after givosiran dosing. In monkeys, a single subcutaneous administration of 10 mg/kg resulted in a 70% suppression in ALAS1 mRNA and 8-week dosing regimens produced a maximum reduction in serum levels of ALAS1 mRNA of approximately 80%. Levels of serum ALAS1 mRNA returned to baseline approximately 4 weeks after the single subcutaneous 10 mg/kg dose and approximately 30-40 days after the last dose in the multiple dose regimens. Based on these results with the cERD assay in monkeys, the cERD method was used to measure ALAS1 mRNA levels in the repeat-dose studies in monkeys and in clinical serum and urine samples from patients and healthy volunteers.

Pharmacology studies in disease models of AIP (a subtype of acute hepatic porphyria) in mice and rats were conducted to evaluate the effects of givosiran on the toxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG) associated with acute hepatic porphyria and the correlation of the reduction of ALAS1 mRNA with changes in these heme intermediates. In both models, the animals have a decrease in porphobilinogen deaminase (PBGD) activity and administration of phenobarbital results in the induction of ALAS1 mRNA and ALA and PBG; this phenobarbital treatment mimics the increase in ALA and PBG plasma levels that occur with acute attacks in patients. Studies with multiple weekly doses of givosiran were used to investigate the ability of givosiran to prevent phenobarbital-induced increases in ALAS mRNA or ALA and PBG and studies with single-dose administration of givosiran tested the ability of the drug to reduce or suppress phenobarbital-induced production of ALAS mRNA or ALA and PBG. Four weekly administrations of the subcutaneous 3 mg/kg dose of givosiran prevented the production of ALA and PBG and a single subcutaneous dose (20 mg/kg in mice and 10 mg/kg in rats) of givosiran reduced ALA and PBG in both the mouse and rat models of AIP. The effects were dose-dependent in the studies that tested multiple dose levels of givosiran, and in both studies in the rat model of AIP, the level of ALAS1 mRNA in the liver correlated with ALA and PBG levels in the urine.

In an in vitro secondary pharmacology study, givosiran did not significantly suppress the mRNA of any of the potential off-targets predicted from an *in silico* analysis of the antisense strand of givosiran. A safety pharmacology study assessed the effects of a single subcutaneous dose of 150 mg/kg givosiran on cardiovascular and respiratory function in male telemetered cynomolgus monkeys. Givosiran produced no clear drug-related effects on cardiovascular and respiratory function under the conditions tested.

Pharmacokinetics data indicate that following subcutaneous administration, givosiran is rapidly eliminated from the plasma with a half-life of 3 hours after a single dose of 10 mg/kg in rats and 5.5 hours after a single dose of 30 mg/kg in monkeys. Givosiran is distributed to both the liver

and kidney, with high exposures and long half-lives. Following a single subcutaneous dose of 10 mg/kg in rats, the half-life was approximately 120 hours in the liver and 167 to 178 hours in the kidney. Quantitative whole-body autoradiography confirmed that givosiran is distributed to a limited number of tissues, with high exposures observed in the liver, kidneys, kidney cortex, and lymph nodes. Radioactivity was cleared from all tissues by 672 hours (28 days) after dosing with the exception of the liver, kidneys, and injection site. [^{3}H]givosiran was metabolized after subcutaneous administration to rats via hydrolysis. In the plasma, the majority of the radioactivity was associated with unchanged givosiran that accounted for 54% of the total radioactivity exposure through 48 hours. AS(N-1)3' givosiran (also known as M15 and AD-62763) was a major circulating metabolite contributing 13% of the total radioactivity exposure. In addition to the major metabolite, five other metabolites of the antisense strand of givosiran were tentatively identified. These metabolites were generated after cleavage of one or more nucleotides either from 3' and 5' ends. Based on the excretion data in the bile duct-cannulated/jugular vein-cannulated rats, the renal and biliary excretion were the major routes of elimination of [^{3}H]givosiran-derived radioactivity with approximately 30% excreted in the urine and 27% excreted in the bile.

Repeat-dose toxicology studies were conducted to assess the toxicity of givosiran. The studies were conducted using the subcutaneous route of administration, which is consistent with the intended clinical route of administration; however, animals were dosed once weekly instead of the once monthly dose schedule used to treat patients. In a 26-week rat study, Sprague-Dawley rats were subcutaneously administered vehicle or givosiran (3, 10, or 30 mg/kg) once weekly for 26 weeks followed by a 13-week recovery period. The study also included a fertility and early embryonic development assessment using treated males and untreated females. In the main study, eight rats were found dead; no causes of death were determined except in one recovery male that had granulocytic leukemia. The main target of toxicity of givosiran was the liver based on multiple clinical pathology parameters and histological findings. These included increases in total bilirubin and liver enzymes. Microscopic findings in the liver included single cell necrosis, increased mitoses, and hepatocellular karyomegaly. Tubular basophilic granules were observed in the kidney and may be related to accumulation of polyanionic oligonucleotide molecules in lysosomes. Muscle degeneration occurred at the injection site, as well as local erythema. Following repeated administration of givosiran, kidney and liver exposures were markedly higher than plasma concentrations, which is consistent with the distribution data in the pharmacokinetic studies.

In a 39-week monkey study, cynomolgus monkeys were subcutaneously administered vehicle or givosiran (10, 30, or 100 mg/kg) once weekly for 39 weeks followed by a 13-week recovery period. No treatment-related mortality was observed. Consistent with the findings in the rats, the liver was also the main target organ of toxicity in monkeys. Clinical chemistry findings included increased liver enzymes (e.g., ALT, GGT) and microscopic findings included hepatocellular single cell necrosis, hepatocellular basophilic granules, and Kupffer cell basophilic granules. Lymph nodes had macrophage vacuolation. Mild degeneration of the heart was observed in one male at the high dose of 100 mg/kg at the end of recovery. This finding,

taken together with increased creatine kinase at the end of dosing and recovery, indicates the potential for muscle/cardiac toxicity. High dose female monkeys had elevated creatinine compared to controls (+43% at end of dosing; +26% at end of recovery). Givosiran exposure was much greater in the liver compared to kidney or plasma in monkeys.

Givosiran was not mutagenic in the in vitro bacterial reverse mutation test or clastogenic in the in vitro chromosomal aberrations assay in human peripheral blood lymphocytes or in the in vivo bone marrow micronucleus assay in rats. Carcinogenicity studies with givosiran (b) (4) are currently ongoing and will be submitted to the NDA when completed.

Givosiran had no effect on male fertility or early embryonic development in untreated females in a repeat-dose chronic toxicity study in rats where male rats were dosed for 26 weeks and then a subset of dosed males were mated with untreated females. A combination fertility and embryo-fetal development (EFD) study was conducted in female rats with givosiran administered subcutaneously once weekly at 0, 3, 10, or 30 mg/kg for 4 doses before mating, and then once daily at doses of 0, 0.5 , 1.5, or 5 mg/kg/day on gestational days (GD) 6 to 17. Because of low systemic half-life of givosiran (and its active metabolite), a dosing holiday between the fertility and the EFD phase of the study, and lack of cumulative toxicities with this drug, any effects on the fertility may be attributed to the 3, 10, 30 mg/kg dose levels and any EFD effects may be related to the 0.5, 1.5, or 5 mg/kg/day dose levels. Givosiran caused maternal toxicity at the mid (10 mg/kg then 1.5 mg/kg/day) and high (30 mg/kg then 5 mg/kg/day) dose levels characterized by changes in clinical pathology. No effects on fertility were observed at any dose level of givosiran; therefore, the NOAEL for female fertility was 30 mg/kg in rats. Givosiran did not produce any clear dose-dependent effects on embryo-fetal survival and there were no givosiran-related fetal malformations. The only givosiran-related fetal skeletal variation was incompletely ossified pubis of the pelvis at 5 mg/kg/day.

In an embryo-fetal development study in female rabbits, administration of givosiran once daily at doses of 0, 0.5, 1.5, or 5 mg/kg/day on GD 7-19 or as one single dose of 20 mg/kg on GD 7, resulted in maternal toxicity at all dose levels tested characterized by decreased food consumption, maternal body weight gain, red blood cell parameters at all dose levels, gross pathology signs in the liver at 1.5 and 5 mg/kg/day, and increased clinical pathology findings (ALT, AST, fibrinogen, platelets, and reticulocytes) at 5 mg/kg/day. Complete litter loss occurred in 2 females at 1.5 mg/kg/day, 4 females at 5.0 mg/kg/day, and 5 females at 20 mg/kg including abortions in one female at 5 mg/kg/day and two females at 20 mg/kg. Treatment with givosiran at 1.5 and 5 mg/kg/day and 20 mg/kg resulted in increased postimplantation loss due to an increase in resorptions (early, late, and total) leading to a decrease in the number of live fetuses and the total number of fetuses. While givosiran did not produce any fetal malformations in rabbits, treatment with givosiran at 20 mg/kg/day on GD 7 resulted in an increase in skeletal variations of the sterbebrae. The NOAEL for embryofetal toxicity was 0.5 mg/kg/day in rabbits. Pharmacodynamics measurement on GD 22 indicated that givosiran did not reduce hepatic ALAS1 transcript levels in pregnant rabbits at any dose level, confirming that givosiran is not pharmacologically active in rabbits.

In a pre- and postnatal development study in rats, givosiran (0, 3, 10, or 30 mg/kg) was administered to pregnant rats approximately every 6 days on GD 7, 13, and 19 and lactation days (LD) 6, 12, and 18 . Treatment with givosiran did not produce maternal toxicity and there were no givosiran-related effects on pup mortality, growth, sexual maturation, behavior, mating and fertility, or ovarian and uterine parameters in the F1 generation rats.

Due to the limited AUC information in the animals (particularly in rabbits), the animal to human comparisons in Section 8.1 of the label were made using doses; based on body surface area scaling. In addition, because of the difference between the dosing schedule of the embryo-fetal development studies in rats and rabbits (daily dosing) and the clinical dosing schedule (once monthly administration), the maximum recommended human dose (MRHD) of 2.5 mg/kg/month was normalized by dividing by 28 days, resulting in a value of 0.089 mg/kg/day. Using this method, the 1.5 mg/kg/day dose in rabbits that produced an increase in postimplantation loss was 5 times the normalized MRHD based on body surface area, and the 5 mg/kg/day dose in rats that was associated with skeletal variation in the EFD study was 9 times the normalized MRHD based on body surface area.

5.2. Referenced NDAs, BLAs, DMFs

None

5.3. Pharmacology

Primary Pharmacology

A. In vitro studies

Study Title/Study No.	Findings
In Vitro Identification of AS1-GalNAc siRNA Candidates by Transfection in Support of Lead Selection for ALN-AS1/ Study (b) (4) 14037	The objective of this study was to identify siRNA molecules for clinical development based on the transcript for human ALAS1 by transfection in the human hepatoma cell line Hep3B or free uptake in primary cynomolgus monkey hepatocytes. Givosiran (AD-60519) was selected as the lead siRNA with an IC_{50} of 0.026 nM (26 pM) for transfection in Hep3B cells.
Single Nucleotide Polymorphisms in the ALN-60519 Target Region/ Study (b) (4) 4024	A search of the NCBI dbSNP database determined that there were no single nucleotide polymorphisms or other polymorphisms (insertions or deletions) within the ALAS1 gene region targeted by givosiran that could impact activity. The nearest single nucleotide polymorphisms were 9 nucleotides upstream and 13 nucleotides downstream of the target region.
In Vitro Activity of Potential Givosiran Antisense Strand (A-122227) Terminal Truncation Metabolites/ Study (b) (4) 18001	Compared to the givosiran drug substance (% ALAS1 mRNA remaining compared to negative control: 69.1% for 0.1 nM siRNA and 16.4 % for 10 nM siRNA), siRNAs with antisense strands truncated from the 5′ terminus of the siRNA duplex did not demonstrate activity. In contrast, truncations of the 3′ terminus of the antisense strand retained full or partial activity; at 10 nM, removal of the first 5 bases did not affect activity.

B. In vivo studies

Effects on ALAS1 Levels Following Single- and Repeat-Dose Administration of Givosiran in Rats and Monkeys

The suppression of ALAS1 mRNA levels in the liver was evaluated following subcutaneous administration of single and repeated doses of givosiran in female wild-type Sprague Dawley rats. ALAS1 mRNA levels were measured by determining the relative abundance of ALAS1 mRNA relative to the housekeeping gene GAPDH in liver tissue lysates. In the single dose study (b) (4) 14008), rats were injected with PBS or givosiran (1, 2.5, 5, or 10 mg/kg) and sacrificed 72 hours after dosing. A single dose of givosiran dose-dependently suppressed ALAS1 mRNA levels in the liver of rats with approximately 35%, 42%, and 58% suppression at 2.5, 5, and 10 mg/kg givosiran, respectively. In the repeat-dose study (b) (4) 14009), rats were injected with PBS or givosiran (1.25, 2.5, or 5 mg/kg) once weekly for a total of 4 doses and sacrificed 72 hours after the 4th dose. Four weekly doses of givosiran also dose-dependently suppressed ALAS1 mRNA levels in the liver with approximately 34%, 55%, and 63% suppression at 1.25, 2.5, and 5 mg/kg givosiran, respectively.

In an additional study in male and female Sprague Dawley rats (b) (4) 18002), rats were injected subcutaneously with 2.5 mg/kg for 8 weekly doses or a loading dose of 5 mg/kg followed by 7 weekly doses of 1 mg/kg. ALAS1 mRNA levels were quantified from liver biopsies at specified timepoints throughout the designated dosing phase of givosiran. ALAS1 mRNA levels in the liver were suppressed in rats for both multiple dosing regimens of givosiran. The maximum reduction in ALAS1 mRNA ranged from 70% to 80% and occurred after 6 to 8 doses of givosiran, which is between 42 days (1008 hours) and 49 days (1176 hours) after the first dose. The suppression of ALAS1 mRNA levels in the liver was reversible with levels returning to baseline by 28 days after the final dose.

Since ALAS1 is not a secreted serum protein, the ability to monitor the kinetics of drug activity and liver ALAS1 mRNA recovery is limited without conducting serial liver biopsies. In order to have a less invasive approach to monitor ALAS1 mRNA levels in monkeys and humans, a circulating extracellular RNA detection (cERD) assay was developed to measure exosomal RNA isolated from serum and urine. In a pharmacodynamics and pharmacokinetics study in cynomolgus monkeys (Study 20053262; (b) (4) 14027), ALAS1 mRNA levels were measured from serum and urine samples using the cERD assay as well as liver biopsies following subcutaneous administration of givosiran as a single dose or as a multiple dose regimen for up to 8 weeks. Results demonstrated good consistency between ALAS1 mRNA levels measured in serum and urine using cERD and intra-hepatic levels at the same time points after givosiran dosing. A single subcutaneous dose of givosiran resulted in a dose-dependent suppression of ALAS1 mRNA with approximately 20% suppression at 1 mg/kg and 70% suppression at 10 mg/kg on Day 4 after dosing. Levels of serum ALAS1 mRNA returned to baseline approximately 2 weeks after the 1 mg/kg dose and 4 weeks after the 10 mg/kg dose. Several different repeat-dose regimens were tested in monkeys including 2.5 mg/kg or 5 mg/kg once weekly for 8 weeks, 5

mg/kg twice weekly for 8 weeks, and 5 mg/kg daily for 3 doses in Week 1 followed by 2.5 or 5 mg/kg once weekly for 7 weeks. All 8-week dosing regimens produced a maximum reduction in serum levels of ALAS1 mRNA of approximately 80% following 5 to 8 weeks of givosiran dosing and ALAS1 levels returned to baseline approximately 30-40 days after the last dose. Based on these results, the cERD method was used to assess the effects of givosiran on ALAS1 mRNA reduction in the repeat-dose studies in monkeys and was used for the measurement of ALAS1 mRNA levels in biorepository serum and urine samples obtained from patients with AIP and healthy volunteers.

Studies in Mouse and Rat Acute Intermittent Porphyria (AIP) Models

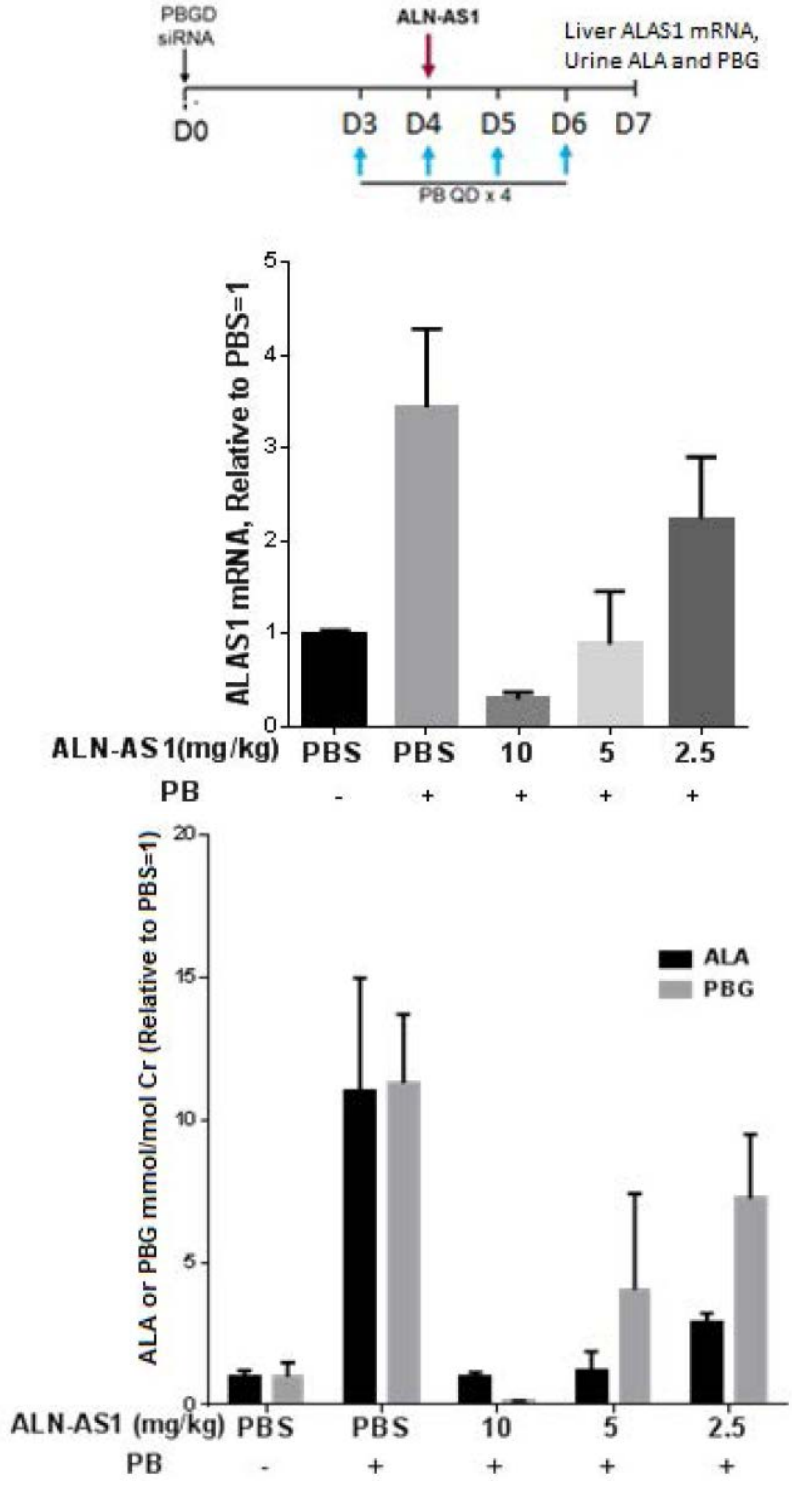
Pharmacology studies were conducted in disease models of AIP in mice and rats to evaluate the effects of givosiran on the heme intermediates ALA and PBG that accumulate in acute hepatic porphyria causing toxicity. Additionally, the correlation of the reduction of ALAS1 mRNA with changes in these heme intermediates was also evaluated in the rat model. The mouse model of AIP consists of a compound heterozygote for PBGD mutations and manifests similar biochemical features of human AIP such as decreased PBGD activity in the liver (approximately 70% reduction). Treatment of these mice with the cytochrome P450-inducing drug phenobarbital results in 3- to 5-fold induction of ALAS1 mRNA and 50- to 200-fold induction of ALA and PBG; this phenobarbital treatment mimics the increase in ALA and PBG plasma levels that occur with acute attacks in patients.

In a prophylaxis study (b) (4)14012), AIP mice were injected subcutaneously with givosiran (0.3, 1, or 3 mg/kg) or PBS weekly on Days 0, 6, 13, and 20 and were then administered phenobarbital intraperitoneally once daily on three consecutive days (110 mg/kg on Day 20, 120 mg/kg on Day 21, and 130 mg/kg on Day 22) to induce ALAS1 levels and stimulate the hem synthesis pathway. Serum was collected on Day 23 to measure ALA and PBG. Compared to the PBS-treated control group that received phenobarbital, treatment with givosiran almost completely prevented the phenobarbital-induced production of ALA or PBG at the 3 mg/kg dose and prevented approximately 80% of the maximal serum ALA and 68% of the serum PBG production at the 1 mg/kg dose. In another study (b) (4)14050), AIP mice were injected intraperitoneally once daily on 4 consecutive days with phenobarbital (90 mg/kg on Day 0, 100 mg/kg on Day 1, 110 mg/kg on Day 2, and 90 mg/kg on Day 3) and diethyldithiocarbamate (20 mg/kg) to achieve more sustained increases in ALA and PBG levels. The mice were then injected with either a single subcutaneous dose of givosiran (20 mg/kg) or PBS on Day 2 or two intravenous doses of Panhematin (4 mg/kg; the current standard of care for patients with AIP) on Days 2 and 3. Levels of ALA and PBG were measured in serum collected 4, 16, 24, 28, and 40 hours after the initial injection of givosiran or Panhematin. Under the conditions of the assay, a single dose of 20 mg/kg givosiran produced a greater reduction in ALA and PBG levels compared with the two days of Panhematin treatment. ALA levels were approximately 31 µmol/L after PBS treatment, 16 µmol/L after Panhematin treatment, and 5 µmol/L after givosiran treatment, 40 hours after the first injection. PBG levels were 32 µmol/L after PBS treatment, 15 µmol/L after Panhematin treatment, and 5 µmol/L after givosiran treatment, 40 hours after the first injection.

The Applicant developed a rat model of AIP in which dosing rats intravenously with a PBGD-specific siRNA results in a reduction in PBGD mRNA of approximately 80% in the rat liver. Similar to the AIP mouse model, phenobarbitol injected daily for 3 to 4 days in PBGD-deficient rats resulted in an induction of both ALAS1 mRNA and in the intermediates ALA and PBG, mimicking an attack state. In an acute treatment study (b) (4)14010), female Sprague Dawley rats were injected intravenously with the PBGD-specific siRNA (AD-55542) on Day 0 and were administered phenobarbital (75 mg/kg) intraperitoneally once daily on 4 consecutive days (Days 3, 4, 5, and 6) to induce ALAS 1 levels and stimulate the heme synthesis pathway. On Day 4, rats were also injected subcutaneously with PBS or givosiran (2.5, 5, or 10 mg/kg). Overnight urine was collected on the morning of Day 7 to measure ALA and PBG levels and liver ALAS1 mRNA levels were measured in rats sacrificed on Day 7 (see the study design in figure below). Treatment with a single dose of givosiran resulted in a dose-dependent suppression of ALAS1 in the rat model of AIP and at the 10 mg/kg dose the levels of ALA and PBG heme intermediates measured in the urine were comparable to the PBS control (see figure below). In a multiple dose study in the rat model of AIP (b) (4)14011), female rats were injected subcutaneously with PBS or givosiran (0.3, 1, or 3 mg/kg) weekly on Days 0, 7, 14, and 21. The rats were injected intravenously with the PBGD-specific siRNA (AD-55542) on Day 18 and were administered phenobarbital (75 mg/kg) intraperitoneally once daily on 4 consecutive days (Days 21, 22, 23, and 24). Overnight urine was collected on the morning of Day 25 to measure ALA and PBG levels and liver ALAS1 mRNA levels were measured in rats sacrificed on Day 25. Findings in the multiple dose study were similar to the single dose study, with 4 weekly administrations of the 3 mg/kg dose preventing the phenobarbital induced increases in ALAS1 mRNA levels in the liver and the overproduction of ALA and PBG in the urine. The effects of givosiran were also dose-dependent in the multiple dose study, and in both studies in the rat model of AIP, the level of ALAS1 mRNA in the liver correlated with ALA and PBG levels in the urine.

Figure 1. ALAS1 mRNA and Urinary ALA and PBG Levels After a Single Subcutaneous Dose Acute Treatment with Givosiran in a Rat AIP Model

(excerpted from Applicant's submission)



ALN-AS1=givosiran; PB= phenobarbitol

Secondary Pharmacology

Study title/ number: In Vitro Off-Target Analysis of ALN-60519, the ALAS1-Targeting siRNA Component of ALN-AS1/ (b) (4)14038

An in vitro analysis of mRNA suppression by givosiran was conducted on 6 potential off-target transcripts (target genes: OR2A5, GTF2E1, ARMCX4, XIRP2, SCAF8, TXLNG2P) predicted from an *in silico* analysis of the antisense strand of givosiran. Inhibition of the of the potential off-target mRNA was measured following givosiran exposure (concentration range of 37.5 fM to 10 nM) using quantitative PCR (qPCR) with gene specific TaqMan assays in the hepatocyte cell line HepG2. One potential off-target, ARMCX4, was not expressed in HepG2. A second potential off-target, XIRP2, was expressed at a level near the lower limit of quantitation for qPCR with data unavailable at one of the concentrations of givosiran tested. Givosiran did not significantly suppress any of the potential off-targets in a concentration-dependent manner. XIRP2 was moderately silenced (24% silencing; not clear at what concentration; IC_{50} was not established). By contrast, the intended target of givosiran, ALAS1, was suppressed with an IC_{50} value of 88 pM in this assay.

Safety Pharmacology

Study title/ number: ALN-AS1: A cardiovascular and Respiratory Safety Pharmacology Study in the Cynomolgus Monkey Using Telemetry/ AS1-NCD14-019

In a GLP safety pharmacology study of both cardiovascular and respiratory function, male telemetered cynomolgus monkeys (5 total) were subcutaneously administered a single dose of vehicle (0.9% sodium chloride for injection) on Day 1 and a single dose of givosiran (150 mg/kg) on Day 15. Evaluations included clinical signs, arterial blood pressures (systolic, diastolic, pulse and mean arterial pressures), heart rate, quantitative electrocardiographic intervals (PR, QRS, QT, and heart rate adjusted QT interval [QTc]), and body temperature. Qualitative ECG was conducted two times predose (at least 30 minutes apart) and 1 (T_{max}), 2, 3, 8, 12, 18, 24, and 72 hours postdose. Potential effects on respiratory function were evaluated based on ventilator parameters (tidal volume, respiratory rate and derived minute volume) at predose, 4, and 72 hours postdose. Results indicated that in one of the 5 males tested, the QTc interval was decreased by 12.4 msec (5%) following a single dose of 150 mg/kg compared to the animal's own vehicle (control) value.

5.4. ADME/PK

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td colspan="2">Absorption</td></tr>
<tr><td>Pharmacokinetic Study of ALN-AS1 and Metabolite in Sprague Dawley Rats After a Single Subcutaneous Administration / Study AS1-DSM18-010</td><td>A single dose of givosiran (10 mg/kg) was administered by subcutaneous injection to male Sprague Dawley rats (n=3). Concentrations of givosiran and the metabolite (M15 or AD-62763; 3'-truncated at the antisense) were reported as the antisense-based duplex concentrations. The lower limit of quantitation was 10 ng/mL in plasma.

Plasma Pharmacokinetics of Givosiran and AD-62763 After a Single Dose of 10 mg/kg in Rats
<table>
<tr><th rowspan="2">Parameter</th><th colspan="2">Mean</th></tr>
<tr><th>Givosiran</th><th>AD-62763 (metabolite)</th></tr>
<tr><td>C_{max} (µg/mL)</td><td>1.06</td><td>0.190</td></tr>
<tr><td>AUC_{last} (hour* µg/mL)</td><td>3.00</td><td>0.626</td></tr>
<tr><td>AUC_{0-24} (hour* µg/mL)</td><td>3.24</td><td>0.702</td></tr>
<tr><td>$t_{1/2}$ (hours)</td><td>3.0</td><td>8.2</td></tr>
</table></td></tr>
<tr><td>Pharmacokinetics of Givosiran Following Subcutaneous Administration in the Cynomolgus Monkey/ Study AS1-DSM18-009</td><td>A single dose of givosiran (30 mg/kg) was administered by subcutaneous injection to male cynomolgus monkeys (n=3). Concentrations of givosiran and the metabolite (AD-62763) were reported as the antisense-based duplex concentrations. The lower limit of quantitation was 10 ng/mL in plasma.

Plasma Pharmacokinetics of Givosiran and AD-62763 After a Single Dose of 30 mg/kg in Monkeys
<table>
<tr><th rowspan="2">Parameter</th><th colspan="2">Mean</th></tr>
<tr><th>Givosiran</th><th>AD-62763 (metabolite)</th></tr>
<tr><td>C_{max} (µg/mL)</td><td>2.42</td><td>1.67</td></tr>
<tr><td>AUC_{last} (hour* µg/mL)</td><td>26.4</td><td>19.4</td></tr>
<tr><td>AUC_{0-24} (hour* µg/mL)</td><td>28.3</td><td>21.2</td></tr>
<tr><td>$t_{1/2}$ (hours)</td><td>5.5</td><td>5.1</td></tr>
</table></td></tr>
<tr><td colspan="2">Distribution</td></tr>
<tr><td>Binding of ALN-AS1 to Mouse, Rat, Monkey, or Human Plasma Proteins/ Study AS1-DSM18-008</td><td>In general, the plasma protein binding of givosiran (1-50 µg/mL) was similar across species with the mean percentage ranging from approximately 10-91% for mouse plasma, 28-93% for rat plasma, 26-90% for monkey plasma, and 21-92% for human plasma. For all species tested, the percentage of protein binding decreased as the concentration of givosiran increased, with the highest protein binding observed at 1 µg/mL (90-93%).</td></tr>
<tr><td>[^{3}H]ALN-AS1: Metabolism, Excretion and Mass Balance, and Tissue Distribution via Quantitative Whole-Body Autoradiography in Male Sprague Dawley Rats Following a Single Subcutaneous Administration/ Study AS1-DSM17-018</td><td>Tissue distribution of the radioactivity was measured using quantitative whole-body autoradiography following a single subcutaneous dose of 10 mg/kg of [^{3}H]givosiran with a specific radioactivity of 2.65 mCi/mL per mg of givosiran (265 mCi/mg per mg of siRNA) in male intact Sprague Dawley rats (1 animal/time point). At 0.5, 1, 2, 4, 6, 24, 48, 96, 168, 336, 672, and 1344 hours after dosing, rats were sacrificed via cardiac puncture under anesthesia, blood was collected, and the carcasses were prepared for quantitative whole-body autoradiography.

[^{3}H]givosiran-derived radioactivity was distributed to a limited number of tissues. The highest tissue-to-plasma concentration ratios were</td></tr>
</table>

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td></td><td>observed in the liver (255 at 6 hours), kidney cortex (36.6 at 24 hours), lymph nodes (36.4 at 48 hours), and kidneys (32.7 at 24 hours). The tissues with the highest AUC_{0-t} values (ng equivalents·hours/g) were the liver (14,200,000), kidney cortex (3,350,000), and kidneys (3,340,000). Radioactivity was cleared from all tissues by 672 hours after dosing with the exception of the liver, kidneys, and dose site. The elimination half-life of the radioactivity was 116 hours in the kidney cortex, 171 hours in the kidneys, and 137 hours in the liver. At 1344 hours after dosing, the radioactivity was still quantifiable at the injection site.</td></tr>
<tr><td>The Pharmacokinetics, Bioavailability, Tissue Distribution and Pharmacodynamics of ALN-AS1 After a Single Intravenous, or Single or Multiple Subcutaneous, Doses to Sprague-Dawley Rats/ Study AS1-NCD14-003</td><td>The review of this study is focused on the exposures in the plasma and the biodistribution to tissues following a single subcutaneous dose of 10 mg/kg to rats. Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, 168, 336, 504, 672, 1008, and 1344 hours after dosing. Tissue samples were collected and biodistribution was assessed at 0.25, 2, 4, 8, 24, 96, 168, 336, 504, 672, 1008, and 1344 hours after dosing.

Combined Male and Female Mean Givosiran Pharmacokinetic Parameters in Rat Plasma After Single Subcutaneous Administration of 10 mg/kg
<table>
<tr><th>Parameter</th><th>Mean</th></tr>
<tr><td>T_{max} (hours)</td><td>1.1</td></tr>
<tr><td>C_{max}(µg/mL)</td><td>1.07</td></tr>
<tr><td>AUC_{0-t} (hour* µg/mL)</td><td>2.79</td></tr>
<tr><td>AUC_{0-2} (hour* µg/mL)</td><td>1.48</td></tr>
<tr><td>$t_{1/2\beta}$ (hours)</td><td>2.7</td></tr>
<tr><td>Absolute bioavailability (%)</td><td>24</td></tr>
</table>
$t_{1/2\beta}$=Apparent terminal elimination half-life

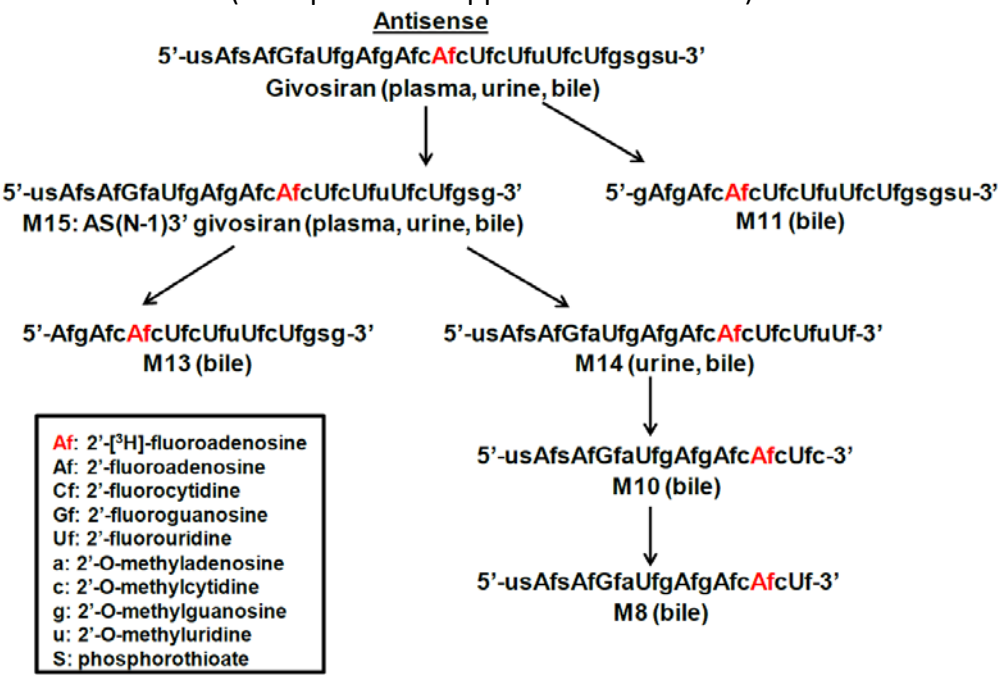
Mean Givosiran Pharmacokinetic Parameters in Rat Tissues After Single Subcutaneous Administration of 10 mg/kg
<table>
<tr><th rowspan="2">Tissue</th><th colspan="2">C_{max} (µg/g)</th><th colspan="2">AUC_{0-t} (hour* µg/g)</th><th colspan="2">$t_{1/2\beta}$ (hours)</th></tr>
<tr><th>Males</th><th>Females</th><th>Males</th><th>Females</th><th>Males</th><th>Females</th></tr>
<tr><td>Liver</td><td>197</td><td>220</td><td>10494</td><td>14690</td><td>120</td><td>121</td></tr>
<tr><td>Kidney</td><td>18.0</td><td>19.9</td><td>2614</td><td>3763</td><td>178</td><td>167</td></tr>
<tr><td>Adrenal</td><td>0.44</td><td>0.47</td><td>6.56</td><td>2.14</td><td>NR</td><td>NR</td></tr>
<tr><td>Heart</td><td>0.47</td><td>0.37</td><td>8.93</td><td>6.99</td><td>NR</td><td>30.5</td></tr>
<tr><td>Jejunum</td><td>0.50</td><td>0.37</td><td>9.04</td><td>6.76</td><td>NR</td><td>NR</td></tr>
<tr><td>Lung</td><td>0.58</td><td>0.48</td><td>9.76</td><td>8.62</td><td>25.4</td><td>24.1</td></tr>
<tr><td>Pancreas</td><td>1.73</td><td>1.17</td><td>28.1</td><td>22.2</td><td>NR</td><td>38.5</td></tr>
<tr><td>Spleen</td><td>0.53</td><td>0.67</td><td>9.87</td><td>9.33</td><td>32.3</td><td>37.3</td></tr>
<tr><td>Thymus</td><td>0.28</td><td>0.28</td><td>1.64</td><td>1.80</td><td>NR</td><td>NR</td></tr>
<tr><td>Thyroid</td><td>0.51</td><td>0.52</td><td>9.56</td><td>9.34</td><td>NR</td><td>NR</td></tr>
<tr><td>Testes</td><td>0.29</td><td>NA</td><td>6.00</td><td>NA</td><td>NR</td><td>NA</td></tr>
</table>
NR=Not reportable NA=Not Applicable, tissue not present in females
$t_{1/2\beta}$=Apparent terminal elimination half-life</td></tr>
<tr><td colspan="2">Metabolism</td></tr>
<tr><td>[^{3}H]ALN-AS1: Metabolism, Excretion and Mass Balance, and Tissue Distribution via Quantitative Whole-Body Autoradiography in Male Sprague Dawley Rats Following a</td><td>Metabolism of the radioactivity was determined following a single subcutaneous dose of 10 mg/kg of [^{3}H]givosiran with a specific radioactivity of 2.65 mCi/mL per mg of givosiran (265 mCi/mg per mg of siRNA) in male intact or bile duct-cannulated/jugular vein-cannulated Sprague Dawley rats. Quantification of the metabolites present in plasma, urine, bile, and feces was based on the</td></tr>
</table>

Type of Study	Major Findings
Single Subcutaneous Administration/ Study AS1-DSM17-018	chromatographic profiles of radioactivity in each sample. [^{3}H]givosiran was metabolized after subcutaneous administration to rats via hydrolysis. • In the plasma, the majority of the radioactivity was associated with unchanged givosiran that accounted for 54% of the total radioactivity exposure through 48 hours (AUC_{0-48}). AS(N-1)3' givosiran (M15; AD-62763) was a major circulating metabolite contributing 13% of the total radioactivity exposure. • In the urine, the majority of the unknown radioactivity (M1, a mixture of degraded drug-related components) was eluted at the void volume and accounted for 38.7% of the dose in intact rats and 18.8% in bile duct cannulated rats. Unchanged givosiran in the urine accounted for 3.4% and 3.9% of the dose and the M15 metabolite accounted for 0.97% and 1.96% of the dose in intact and bile duct cannulated rats, respectively. • In the bile, approximately 6% of the dose was recovered as unchanged givosiran and the AS(N-1)3' givosiran (M15) metabolite accounted for approximately 5% of the dose. • No unchanged givosiran was recovered in the feces from intact rats. All radioactivity recovered in the feces was associated with unknown components. In addition to the major metabolite AS(N-1)3' givosiran (M15; AD-62763), five other metabolites of the antisense strand of givosiran were tentatively identified. These metabolites were generated after cleavage of one or more nucleotides either from 3' and 5' ends. See the figure below this table for the proposed biotransformation pathways of [^{3}H]givosiran in rats.
Excretion	
[^{3}H]ALN-AS1: Metabolism, Excretion and Mass Balance, and Tissue Distribution via Quantitative Whole-Body Autoradiography in Male Sprague Dawley Rats Following a Single Subcutaneous Administration/ Study AS1-DSM17-018	The excretion of the radioactivity was determined following a single subcutaneous dose of 10 mg/kg of [^{3}H]givosiran with a specific radioactivity of 2.65 mCi/mL per mg of givosiran (265 mCi/mg per mg of siRNA) in male intact or bile duct-cannulated/jugular vein-cannulated Sprague Dawley rats. The elimination of radioactivity in the urine and feces was determined through 1344 hours after dosing in the intact rats (4 total) and the excretion of radioactivity in bile, urine, and feces was determined through 168 hours after dosing in the bile duct-cannulated/jugular vein-cannulated rats (6 total). In the intact rats, most of the [^{3}H]givosiran-derived radioactivity was excreted within 336 hours after dosing, primarily in the urine. By 1344 hours after dosing, the overall mean recovery of the radioactivity in the intact rats was 84.1%, with 57.8% excreted in the urine (including urine wipe) and 14.3% excreted in the feces. In the bile duct-cannulated/jugular vein-cannulated rats, by 168 hours after dosing the mean overall recovery of radioactivity was 86.6%, with 30.4% excreted in the urine, 27.4% excreted in the bile, and 1.42% excreted in the feces. These results indicate that the renal and biliary excretion were the major routes of elimination of [^{3}H]givosiran-derived radioactivity.

Type of Study	Major Findings
A Subcutaneous Injection Seminal Fluid Transfer Study of ALN-AS1 in Male New Zealand White Rabbits/ Study INV-DSM16-057	A single subcutaneous dose of givosiran (2.5 or 20 mg/kg) was administered to male New Zealand White rabbits (n=5/group). Semen samples were collected at predose (baseline) and at 8, 24, 72, and 216 hours after dosing. Givosiran was detectable at all time points at both dose levels, with C_{max} occurring at 8 hours postdose. Seminal fluid exposure increased in a greater than dose-proportional manner after a single subcutaneous injection at 2.5 and 20 mg/kg.

Figure 2. Proposed Biotransformation Pathways of [^{3}H]Givosiran in Rats

(excerpted from Applicant's submission)



5.5. Toxicology

5.5.1. General Toxicology

Study title / number: ALN-AS1: A 26-Week Repeat Dose Subcutaneous Injection Toxicity, Fertility and Early Embryonic Development (to Implantation) Study in the Albino Rat Followed by a 13-Week Recovery Period/ AS1-GLP15-022

- The main target of toxicity was the liver based on multiple clinical pathology parameters and histological findings.
- Tubular basophilic granules were observed in the kidney.
- Muscle degeneration occurred at the injection site.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 3, 10, or 30 mg/kg once weekly for 26 weeks followed by a 13 week recovery period
Route of administration:	Subcutaneous injection; 5 mL/kg dose volume
Formulation/Vehicle:	0.9% sodium chloride for injection
Species/Strain:	Rat/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	30/sex/group
Age:	At initiation of treatment, rats were 53 to 56 days old
Satellite groups/ unique design:	Toxicokinetics: 3/sex for control group and 24/sex for givosiran-treated groups. The study was also designed to assess the effect of givosiran on male fertility and early embryonic development in untreated females (see Section 5.5.4).
Deviation from study protocol affecting interpretation of results:	Yes

Observations and Results:

Parameters	Major findings
Mortality	Eight rats were found dead during the study. No causes of death were determined except in one recovery male. Main dosing phase: control (2); 3 mg/kg (3); 10 mg/kg (1) Recovery phase: 3 mg/kg (1) - granulocytic leukemia Toxicokinetics satellite: control (1)
Clinical Signs	Local erythema was observed at all dose levels of givosiran
Body Weights	Unremarkable
Ophthalmoscopy	Unremarkable
Hematology	Platelets were reduced -20% on Day 184 (end of dosing) at 30 mg/kg in females compared to control.

Clinical Chemistry

Parameters at End of Dosing, Day 184	% Change from Control			
	Males		Females	
	10 mg/kg	30 mg/kg	10 mg/kg	30 mg/kg
APTT	-	↓18***	-	↓12***
Total bilirubin	↑46*	↑128***	-	↑81**
AST	-	↑23**	-	↑40
ALP	-	↑46***	-	↑132***
Cholesterol	↓32***	↓22*	-	↑19*
Triglycerides	-	↑27	-	↑88***
Total protein	-	↓4*	↓4*	↓7***
Albumin	-	↑5**	↓9***	↓12***
Globulin	↓11**	↓17**	↑6	↑6
A/G	↑17***	↑28***	↓14***	↑16***
Phosphate	↑17***	↑12**	↑4	↑13**

Parameter at End of Recovery, Day 275	% Change from Control					
	Males			Females		
	3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Creatine kinase	↓58**	↓62***	↓63***	↓31	↓17	↓13
Total bilirubin	-	-	↑75	-	-	-
AST	-	-	↑184	-	-	-
ALT	-	-	↑72	-	-	-
Cholesterol	-	↓26*	↓34**	-	-	-
Total protein	-	-	-	-	-	↓10**
Albumin	-	-	-	↓9	↓10	↓14**
A/G	-	-	↑12	-	↓12	↓10
Phosphate	↑14	↑23**	↑22*	-	-	-

↓=Decreased; ↑=Increased; - = not toxicologically or statistically significant; *=Significantly different from control, p≤0.05; **=Significantly different from control, p≤0.01; ***=Significantly different from control, p≤0.001

Urinalysis

Unremarkable

Gross Pathology

Increased incidence of pale discoloration of liver (≥3 mg/kg in males and females) and liver enlargement (≥10 mg/kg in females)

Organ Weights

Relative Organ Weights (BW basis)	% Change from Control					
	Males			Females		
	3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Adrenal gland	-	-	↓13	-	↓10	↓19**
Thyroid gland	-	-	↑11	-	-	↑21**
Kidney	-	-	13***	-	-	↑23***
Liver	-	↓18***	↓24***	-	-	-
Spleen	↓15*	-	-	↓10	-	-

↓=Decreased; ↑=Increased; - = not toxicologically or statistically significant; *=Significantly different from control, p≤0.05;**=Significantly different from control, p≤0.01; ***=Significantly different from control, p≤0.001

Histopathology

Adequate battery: Yes

			Males				Females			
		Dose Level	0	3	10	30	0	3	10	30
		No. Examined	20	18	19	20	20	20	20	20
Kidney	Basophilic granules; tubular	Minimal	0	0	19	0	0	0	19	8
		Mild	0	0	0	20	0	0	0	12
Liver	Vacuolation; hepatocellular	Minimal	8	12	9	0	4	13	1	0
		Mild	1	1	6	20	2	2	10	0
		Moderate	0	0	4	0	0	0	9	0
		Marked	0	0	0	0	0	0	0	20
	Single cell necrosis	Minimal	0	1	5	9	0	0	2	6
		Mild	0	0	1	11	0	0	0	14
	Increased mitoses	Minimal	0	0	5	3	0	2	5	4
		Mild	0	0	0	17	0	0	0	16
	Basophilic granules; Kupffer cell	Minimal	0	0	2	4	0	0	0	15
		Mild	0	0	0	16	0	0	0	5
	Pigmentation; Kupffer cell	Minimal	1	0	2	6	0	1	1	16
		Mild	0	0	0	12	0	0	0	4
	Karyomegaly; hepatocellular	Minimal	0	0	0	0	0	0	0	16
	Increased hematopoiesis	Minimal	0	0	0	0	0	0	4	4
	Focus of cellular alteration; eosinophilic	Minimal	0	0	0	2	0	0	1	4
		Mild	0	0	0	2	0	0	0	0
Pancreas	Angiectasis; islet of Langerhans	Minimal	0	0	2	12	0	0	6	8
		Mild	0	0	0	1	0	0	0	11
Injection site	Vacuolation; macrophage	Minimal	0	9	8	6	0	3	10	11
		Mild	0	0	1	12	0	0	1	8
	Degeneration; cutaneous muscle	Minimal	3	4	3	7	3	0	5	7
		Mild	0	0	1	10	0	0	0	0
		Moderate	0	0	0	1	0	0	0	0

Toxicokinetics	

	Day	3 mg/kg	10 mg/kg	30 mg/kg
Plasma Exposure				
C_{max} (µg/ml)	1	0.3	1.6	6.3
	183	0.4	0.8	5.4
AUC_{0-2} (h*µg/mL)	1	0.4	1.9	8.0
	183	0.5	1.5	8.8
$t_{1/2}$(h)	183	2.1	3.1	3.1
Liver Exposure (µg/g)				
C_{max} (µg/g)	1	31.6	108	326
	183	41.4	208	489
AUC_{0-24} (h*µg/g)	1	573	1997	6157
	183	777	4141	9503
$t_{1/2}$(h)	183	25.9	26.8	58.0
Kidney Exposure (µg/g)				
C_{max} (µg/g)	1	5.0	26.4	144
	183	28.7	91.6	931
AUC_{0-24} (h*µg/g)	1	96.9	462	3157
	183	516	1908	15887
$t_{1/2}$(h)	183	NR	121	NR

Study title / number: ALN-AS1: A 39-Week Subcutaneous Injection Toxicity and Toxicokinetic Study in the Juvenile Cynomolgus Monkey Followed by a 13-Week Recovery Period / AS1-GLP15-018

- The main target organ of toxicity was the liver based on clinical pathology and microscopic findings including hepatocellular single cell necrosis.
- Mild degeneration of the heart was noted in one male at the high dose at the end of recovery; creatine kinase was elevated at the end of dosing and recovery.
- High dose (100 mg/kg) female monkeys had elevated creatinine compared to control (+43% at end of dosing; +26% at end of recovery).

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 10, 30, or 100 mg/kg once weekly for 39 weeks followed by a 13 week recovery period

Route of administration: Subcutaneous injection; 1 mL/kg dose volume

Formulation/Vehicle: 0.9% sodium chloride for injection

Species/Strain: Cynomolgus monkeys

Number/Sex/Group: 6/sex/group

Age: At initiation of treatment, monkeys were 13 to 17 months old

Satellite groups / unique design: Main study animals (3/sex/group) were necropsied on Day 275 (Week 39) and recovery animals (3/sex/group) were necropsied on Day 365.

Deviation from study protocol affecting interpretation of results: No

Observations and Results:

<table>
<tr><th>Parameters</th><th>Major findings</th></tr>
<tr><td>Mortality</td><td>No mortality related to ALN-AS1 treatment</td></tr>
<tr><td>Clinical Signs</td><td>Unremarkable</td></tr>
<tr><td>Body Weights</td><td>For the mid dose (30 mg/kg) relative to controls, body weights were 24% higher at the end of dosing and 33% higher at the end of recovery</td></tr>
<tr><td>Ophthalmoscopy</td><td>Unremarkable</td></tr>
<tr><td>Hematology</td><td>Unremarkable at the end of dosing. For end of recovery, see table.
<table>
<tr><th rowspan="3">Parameter
Recovery (Day 359/360)</th><th colspan="6">% Change from Control</th></tr>
<tr><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><th>10 mg/kg</th><th>30 mg/kg</th><th>100 mg/kg</th><th>10 mg/kg</th><th>30 mg/kg</th><th>100 mg/kg</th></tr>
<tr><td>Neutrophils</td><td>↑23</td><td>↑197</td><td>↑165</td><td>-</td><td>-</td><td>↑76</td></tr>
<tr><td>Monocytes</td><td>↑60</td><td>↑66</td><td>↑63</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>RBC</td><td>-</td><td>-</td><td>-</td><td>↓5</td><td>↓14</td><td>↓6</td></tr>
<tr><td>Hemoglobin</td><td>-</td><td>-</td><td>-</td><td>↓7</td><td>↓17*</td><td>↓6</td></tr>
<tr><td>Hematocrit</td><td>-</td><td>-</td><td>-</td><td>↓11</td><td>↓17*</td><td>↓7</td></tr>
<tr><td>Platelets</td><td>↑24</td><td>↑26</td><td>↑41</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Fibrinogen</td><td>↓14</td><td>-</td><td>-</td><td>↓22</td><td>↓27</td><td>↓32</td></tr>
</table>
↓=Decreased; ↑=Increased; - = not toxicologically or statistically significant; *=Significantly different from control, p≤0.05; **=Significantly different from control, p≤0.01; ***=Significantly different from control, p≤0.001</td></tr>
<tr><td>Clinical Chemistry</td><td>
<table>
<tr><th rowspan="3">Parameter</th><th rowspan="3">Day</th><th colspan="4">% Change from Control</th></tr>
<tr><th colspan="2">Male</th><th colspan="2">Female</th></tr>
<tr><th>30 mg/kg</th><th>100 mg/kg</th><th>30 mg/kg</th><th>100 mg/kg</th></tr>
<tr><td>Creatine kinase</td><td>275</td><td>-</td><td>↑319</td><td>↑133</td><td>-</td></tr>
<tr><td></td><td>359/360</td><td>-</td><td>↑97</td><td>↑43</td><td>-</td></tr>
<tr><td>AST</td><td>275</td><td>-</td><td>↑55</td><td>-</td><td>-</td></tr>
<tr><td>ALT</td><td>275</td><td>-</td><td>↑65</td><td>-</td><td>↑30</td></tr>
<tr><td></td><td>359/360</td><td>↑70</td><td>↑77*</td><td>-</td><td>↑62</td></tr>
<tr><td>ALP</td><td>275</td><td>↑36</td><td>↑62</td><td>↑30</td><td>↑52</td></tr>
<tr><td></td><td>359/360</td><td>-</td><td>-</td><td>-</td><td>↑66</td></tr>
<tr><td>GGT</td><td>275</td><td>↑23</td><td>↑61*</td><td>-</td><td>↑64*</td></tr>
<tr><td></td><td>359/360</td><td>-</td><td>↑36</td><td>-</td><td>↑70</td></tr>
<tr><td>Cholesterol</td><td>275</td><td>-</td><td>↑45*</td><td>-</td><td>-</td></tr>
<tr><td></td><td>359/360</td><td>-</td><td>↑34</td><td>↑23</td><td>↑35</td></tr>
<tr><td>Total bilirubin</td><td>275</td><td>↑15</td><td>↑14</td><td>-</td><td>-</td></tr>
<tr><td></td><td>359/360</td><td>-</td><td>-</td><td>-</td><td>↑66</td></tr>
<tr><td>Creatinine</td><td>275</td><td>↑19</td><td>-</td><td>-</td><td>↑43**</td></tr>
<tr><td></td><td>359/360</td><td>-</td><td>-</td><td>-</td><td>↑26</td></tr>
</table>
↓=Decreased; ↑=Increased; - = not toxicologically or statistically significant; *=Significantly different from control, p≤0.05; **=Significantly different from control, p≤0.01; ***=Significantly different from control, p≤0.001</td></tr>
<tr><td>Urinalysis</td><td>Unremarkable</td></tr>
<tr><td>Gross Pathology</td><td>Unremarkable</td></tr>
<tr><td>Organ Weights</td><td>Unremarkable</td></tr>
</table>

Histopathology Adequate battery: Yes	Mild degeneration of the heart was observed in one male at 100 mg/kg) at the end of recovery. Compared to control, an increased incidence and severity of hepatocellular single cell necrosis, hepatocellular basophilic granules, Kupffer cell basophilic granules, and lymph node macrophage vacuolation were still present at the end of recovery (see table below for histopathology at end of dosing).

			Males				Females			
		Dose Level	0	10	30	100	0	10	30	100
		No. Examined	3	3	3	3	3	3	3	3
Lymph node, Axillary	Macrophage Vacuolation	Minimal	0	2	0	1	0	1	0	0
		Mild	0	0	2	1	0	2	1	1
		Moderate	0	0	1	1	0	0	2	2
Lymph node, Cervical	Macrophage Vacuolation	Minimal	0	0	1	0	0	0	2	0
		Mild	0	1	1	1	0	1	1	1
		Moderate	0			1	0	0		2
Lymph node, Mesenteric	Macrophage Vacuolation	Minimal	0	0	2	0	0	0	2	0
		Mild	0	0	1	1	0	0	0	1
		Moderate	0	0	0	2	0	0	1	2
Lymph node, Mandibular	Macrophage Vacuolation	Minimal	0	0	1	3	0	0	3	1
		Mild	0	0	0	0	0	0	0	1
		Moderate	0	0	0	0	0	0	0	1
Injection site	Macrophage Vacuolation	Minimal	0	0	3	1	0	3	1	1
		Mild	0	0	0	1	0	0	1	1
		Moderate	0	0	0	1	0	0	0	0
	Infiltration, mononuclear cell	Minimal	0	0	1	1	0	1	0	0
		Mild	0	0	1	0	0	0	1	1
		Moderate	0	0	0	1	0	0		1
Liver	Basophilic granules, Kupffer cell	Minimal	0	0	1	0	0	0	2	0
		Mild	0	0	2		0	0	1	0
		Moderate	0	0		3	0	0		3
	Basophilic granules, hepatocellular	Minimal	0	0	2	0	0	0	2	0
		Mild	0	0	0	3	0	0	1	2
		Moderate	0	0	0		0	0	0	1
	Single cell necrosis; hepatocellular	Minimal	0	0	0	3	0	0	0	1
Lung	Inflammation; interstitial	Mild	0	0	0	1	0	0	0	0
	Infiltration, mixed cell	Minimal	0	0	0	1	0	0	0	2

Toxicokinetics	

	Day	10 mg/kg	30 mg/kg	100 mg/kg
Plasma Exposure				
C_{max} (µg/ml)	1	1.1	4.7	14.6
	274	1.9	6.0	19.2
AUC_{0-24} (h*µg/mL)	1	5.4	33.7	153
	274	7.3	34.3	187
$t_{1/2}$(h)	1	2.4	2.6	3.7
	274	1.8	3.3	3.4
Liver Exposure (µg/g)	275	497	2658	4769
	365	54	548	808
Kidney Exposure (µg/g)	275	10	49	141
	365	0.3	0.5	1.5

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title/ number: ALN-AS1: Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli/ AS1-NCD14-015

Key Study Findings:

- Givosiran did not increase the number of revertant colonies in any of the tester strains in the presence or absence of metabolic activation; therefore, givosiran was negative for mutagenicity in the reverse mutation assay.

GLP compliance: Yes
Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537; *Escherichia coli* tester strain WP2*uvrA*; +/- S9 activation; tested at concentrations of 50, 158, 500, 1581, and 5000 µg/plate in the definitive study
Study is valid: Yes

In Vitro Assays in Mammalian Cells

Study title/ number: ALN-AS1: In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes/ AS1-NCD14-016

Key Study Findings:

- Givosiran was negative for clastogenicity in the in vitro chromosome aberration assay in whole blood human lymphocyte cultures in the presence or absence of metabolic activation at concentrations of 128, 256, and 500 µg/mL.

GLP compliance: Yes
Test system: Whole blood human peripheral blood lymphocytes; +/- S9 activation; exposure to givosiran of 4 or 21 hours in the absence of S9 mix and 4 hours in the presence of S9 mix; tested concentrations of 1, 2, 4, 8, 16, 32, 64, 128, 256, and 500 µg/mL in both the presence and absence of metabolic activation, concentrations of 128, 256, and 500 µg/mL selected for detailed chromosomal aberration analysis

Study is valid: Yes

Givosiran did not cause any increases in the proportion of aberrant metaphases at the experimental points evaluated compared to negative controls. There were chromosome gaps at concentrations ≥ 128 µg/mL givosiran after 4 hours of treatment in the presence of metabolic activation (+S9) and after 21 hours of treatment in the absence of metabolic activation (-S9), which were not concentration-dependent.

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)
Study title/ number: ALN-AS1: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow/ AS1-NCD14-017

Key Study Findings:

- Givosiran did not induce an increase in the incidence of micronucleated polychromatic erythrocytes at doses up to 2000 mg/kg; therefore, givosiran was negative for micronucleus induction and in vivo clastogenicity.

GLP compliance: Yes
Test system: Sprague-Dawley rats; males and females in dose range finding assay, males only in definitive assay; single subcutaneous dose of 0, 500, 1000, or 2000 mg/kg givosiran; 24 hour (all doses) or 48 hour (0 and 2000 mg/kg only) bone marrow collection
Study is valid: Yes

5.5.3. Carcinogenicity

(b) (4) carcinogenicity study (b) (4) are currently ongoing. The label will be updated with the results of the carcinogenicity studies once they are submitted to the NDA.

5.5.4. Reproductive and Developmental Toxicology

Fertility, Early Embryonic Development, and Embryo-Fetal Development
Study title/ number: ALN-AS1: A 26-Week Repeat Dose Subcutaneous Injection Toxicity, Fertility and Early Embryonic Development (to Implantation) Study in the Albino Rat Followed by a 13-Week Recovery Period/ AS1-GLP15-022

Key Study Findings

- Givosiran had no effect on fertility or early embryonic development in a repeat-dose chronic toxicity study in rats where male rats were dosed for 26 weeks and then a subset of dosed males were mated with untreated females. The NOAEL was 30 mg/kg givosiran.

Conducting laboratory and location: (b) (4)

GLP compliance:	Yes
Methods	
Dose and frequency of dosing:	0, 3, 10, or 30 mg/kg once weekly for 26 weeks followed by a 13 week recovery period
Route of administration:	Subcutaneous injection; 5 mL/kg dose volume
Formulation/Vehicle:	0.9% sodium chloride for injection
Species/Strain:	Rat/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	20/sex/group for assessing fertility and early embryonic development
Satellite groups:	Toxicokinetics: 3/sex for control group and 24/sex for givosiran-treated groups
Study design:	The study was designed to assess the effect of givosiran on male fertility and early embryonic development in untreated females. Givosiran was dosed once weekly for 26 weeks, at doses of 0 (0.9% NaCl), 3, 10, or 30 mg/kg, to 30 rats/sex/group. Main study animals (20/sex/group) were necropsied on Day 184 and recovery animals (10/sex/group) were necropsied on Day 275. Ten males/group from the main (necropsy Day 184) and recovery (necropsy Day 275) populations (total of 20 males/group) were selected for a cohabitation phase of the study. Twenty naïve, untreated females/group with normal estrous cycles were used for functional fertility and early embryonic development phase assessments.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results
Findings were unremarkable, including observations for mating index, fertility index, conception rate, mean days to mating, spermatozoac counts, sperm motility, numbers of corpora lutea, implantation sites, live and dead embryos, resorptions, and the pre- and post-implantation losses.

Study title/ number: ALN-AS1: A Subcutaneous Injection Fertility and Embryo-Fetal Development Study in Sprague Dawley Rats/ AS1-GLP16-011

Key Study Findings

- Givosiran caused maternal toxicity at the mid dose (10 mg/kg then 1.5 mg/kg/day) and high dose (30 mg/kg then 5 mg/kg/day) characterized by clinical pathology findings

including increased AST, triglyceride, and potassium levels at both dose levels and increased ALT, ALP, and phosphorus levels and reduced albumin levels at 30 mg/kg then 5 mg/kg/day.

- No effects on fertility were observed at any dose level of givosiran; therefore, the NOAEL for female fertility was the high dose of 30 mg/kg in rats.
- Givosiran did not produce any clear dose-dependent effects on embryo-fetal survival and there were no givosiran-related fetal malformations. The only givosiran-related fetal skeletal variation was incompletely ossified pubis of the pelvis at the high dose (5 mg/kg/day given during the period of organogenesis); this dose was associated with maternal toxicity.

Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes

Methods

Dose and frequency of dosing

Prior to cohabitation:	0, 3, 10, or 30 mg/kg once weekly for 4 doses at 22 days, 15 days, 8 days, and 1 day before cohabitation
During the period of organogenesis:	0, 0.5, 1.5, or 5 mg/kg/day once daily on GD 6-17
Route of administration:	Subcutaneous injection; 5 mL/kg dose volume
Formulation/Vehicle:	0.9% sodium chloride for injection
Species/Strain:	Rat/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	24 females/group
Satellite groups:	Toxicokinetics: 3 females for control group and 9 females/group for givosiran-treated groups; caesarean section on GD 18
Study design:	Female rats were administered vehicle or givosiran (3, 10, or 30 mg/kg) once weekly starting 22 days before cohabitation and were cohabitated with untreated males for a maximum of 14 days until evidence of mating. When a female did not mate within 7 days, another dose of givosiran was administered. The day on which positive evidence of copulation was observed was considered GD 0. Females were then dosed with vehicle or givosiran (0.5, 1.5, or 5 mg/kg/day) once daily on GD 6-17 and necropsy/cesarean section was conducted on GD 21.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

<table>
<tr><th>Parameters</th><th>Major findings</th></tr>
<tr><td>Mortality</td><td>No givosiran-related mortality; one control female was euthanized on GD 18 due to a leg injury</td></tr>
<tr><td>Clinical Signs</td><td>Unremarkable</td></tr>
<tr><td>Body Weights</td><td>Unremarkable</td></tr>
<tr><td>Clinical Pathology</td><td>Changes in Clinical Chemistry
<table>
<tr><th rowspan="2">Parameter</th><th colspan="3">% Change from Control</th></tr>
<tr><th>3 mg/kg then 0.5 mg/kg/day</th><th>10 mg/kg then 1.5 mg/kg/day</th><th>30 mg/kg then 5 mg/kg/day</th></tr>
<tr><td>AST</td><td>-</td><td>↑32*</td><td>↑66***</td></tr>
<tr><td>ALT</td><td>-</td><td>-</td><td>↑27***</td></tr>
<tr><td>ALP</td><td>-</td><td>-</td><td>↑32</td></tr>
<tr><td>Triglyceride</td><td>↑43</td><td>↑94*</td><td>↑159*</td></tr>
<tr><td>Potassium</td><td>-</td><td>↑8*</td><td>↑11**</td></tr>
<tr><td>Phosphorus</td><td>-</td><td>-</td><td>↑12*</td></tr>
<tr><td>Albumin</td><td>-</td><td>-</td><td>↓18*</td></tr>
<tr><td>Albumin/Globulin ratio</td><td>-</td><td>-</td><td>↓14***</td></tr>
</table>
*=Significantly different from control, p≤0.05
**=Significantly different from control, p≤0.01
***= Significantly different from control, p≤0.001
↓=Decreased ↑=Increased -=No effect in this group</td></tr>
<tr><td>Fertility</td><td>Unremarkable</td></tr>
<tr><td>Necropsy findings
Cesarean Section Data</td><td>One female at 0.5 mg/kg/day and one female at 1.5 mg/kg/day had complete litter loss with all resorbed fetuses, which contributed to a slightly increased number of early resorptions at 0.5 mg/kg/day (n=20; mean=1.1) compared to controls (n=8; mean=0.5) and lead to increased postimplantation loss at 0.5 mg/kg/day (8.1%) and at 1.5 mg/kg/day (7.4%) compared to controls (3.3%). These findings were not statistically significant and were not observed at the high dose of 5 mg/kg/day and thus not considered drug related.</td></tr>
<tr><td>Necropsy findings
Offspring</td><td>5 mg/kg/day: Skeletal variation of incompletely ossified pubis of the pelvis occurred in 3 fetuses (2.4%) from 3 litters (11.1%) and was not observed in the concurrent control group; this fetal and litter incidence also exceeded the historical control range.</td></tr>
<tr><td>Toxicokinetics</td><td>On GD 17, plasma concentrations of givosiran were below the lower limit of quantitation (<50.0 ng/mL) at all time points at 0.5 mg/kg/day and were generally below the limit of quantitation at 1.5 mg/kg/day; toxicokinetics were not calculated for these groups. Liver concentrations of givosiran were measured in all dose groups; however, givosiran concentrations in the placenta were measurable in only 51% of the placenta samples collected for the 5 mg/kg/day group and were not measurable for the low and mid dose groups. The concentrations of givosiran were not measurable in any of the fetuses for any of the givosiran treated groups on GD 18.</td></tr>
</table>

Toxicokinetics in the Plasma of Pregnant Rats at 5 mg/kg/day on GD 17

Toxicokinetic Parameter	Mean
C_{max} (µg/mL)	0.340
T_{max} (hours)	0.5
AUC_{0-8} (µg·hr/mL)	1.26
AUC_{0-24} (µg·hr/mL)	1.64
$T_{1/2}$ (hours)	4.0

Mean Maternal Rat Liver and Placenta Tissue Concentrations (µg/g) on GD 18

Organ	Givosiran Treatment Group		
	3 mg/kg then 0.5 mg/kg/day	10 mg/kg then 1.5 mg/kg/day	30 mg/kg then 5 mg/kg/day
Liver	23.5	133	347
Placenta	NC	NC	0.388

NC= Not calculated

Pharmacodynamics

Hepatic ALAS1 mRNA expression was dose-dependently reduced at all doses of givosiran compared to controls on GD 18 and GD 21.

Pharmacodynamics: ALAS1 mRNA Expression

Day	% Change from Control		
	0.5 mg/kg/day	1.5 mg/kg/day	5 mg/kg/day
GD 18	↓70	↓89	↓92
GD 21	↓57	↓80	↓88

↓=Decrease

Study title/ number: ALN-AS1: An Embryo-Fetal Development Study by Subcutaneous Injection in Rabbits/ AS1-GLP16-018

Key Study Findings

- Givosiran caused maternal toxicity at all dose levels tested (0.5, 1.5, and 5 mg/kg/day and 20 mg/kg) characterized by decreased food consumption, maternal body weight gain, and red blood cell parameters at all dose levels, gross pathology signs in the liver at 1.5 and 5 mg/kg/day, and increased clinical pathology findings (ALT, AST, fibrinogen, platelets, and reticulocytes) at 5 mg/kg/day.
- Complete litter loss occurred in 2 females at 1.5 mg/kg/day, 4 females at 5.0 mg/kg/day, and 5 females at 20 mg/kg including abortions in one female at 5 mg/kg/day and two females at 20 mg/kg. Treatment with givosiran at 1.5 and 5 mg/kg/day and 20 mg/kg resulted in increased postimplantation loss due to an increase in resorptions (early, late, and total) leading to a decrease in the number of live fetuses and the total number of fetuses.
- Treatment with givosiran at 20 mg/kg/day on GD 7 resulted in an increase in skeletal variations (asymmetric and misshapen sternebrae and isolated ossification sites of sternebrae).
- The NOAEL for developmental toxicity was 0.5 mg/kg/day in rabbits.

Conducting laboratory and location: (b) (4)

GLP compliance:	Yes

Methods

Dose and frequency of dosing:	0, 0.5, 1.5, or 5 mg/kg/day, once daily on GD 7-19 or 20 mg/kg once on GD 7
Route of administration:	Subcutaneous injection; 5 mL/kg dose volume
Formulation/Vehicle:	0.9% sodium chloride for injection
Species/Strain:	Rabbit/New Zealand White [Crl:KBL(NZW)]
Number/Sex/Group:	20 females/group
Satellite groups:	Toxicokinetics: 2 females for control group and 3 females/group for givosiran-treated groups; termination on GD 22
Study design:	Pregnant rabbits were administered vehicle or givosiran (0.5, 1.5, or 5 mg/kg/day) once daily on GD 7-19 or a single administration of 20 mg/kg givosiran on GD 7. The single administration of 20 mg/kg was intended to model the clinical dosing schedule of one injection per month. Main study animals were euthanized and the necropsy and ovarian/uterine examinations were conducted on GD 29.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	**5 mg/kg/day:** One female aborted on GD 24 and was euthanized. **20 mg/kg:** Two females aborted (one on GD 20 and one on GD 24) and were euthanized.
Clinical Signs	**Aborting dams:** Red aborted tissue **Food consumption:** Reduced for all givosiran-treated groups compared to controls; ↓23-31% for entire dosing period (GD 7-20) and ↓17-23% for the entire study period (GD 7-28)
Body Weights	**Aborting dams:** **5 mg/kg/day:** Aborting female lost 14% of body weight from GD 10-24. **20 mg/kg:** One female lost 17% of body weight from GD 7-24 and the other female lost 5% body weight from GD 16-19. In all givosiran-treated groups, females lost maternal body weight and/or had a reduction in mean maternal body weight gain compared to controls during the dosing period (GD 7-20) or during the entire study period (GD 7-28); these changes were dose-dependent for the groups administered

givosiran daily GD 7-19.

Maternal Body Weight Gains

Interval	Mean Change in Grams (% Change from Control)				
	0 mg/kg/day	0.5 mg/kg/day	1.5 mg/kg/day	5 mg/kg/day	20 mg/kg
GD 7-10	36.1	-1.0 (↓103)	10.4 (↓71)	-53.5* (↓248)	-51.2* (↓242)
GD 7-20	216.3	57.9* (↓73)	21.5* (↓90)	-73.2** (↓134)	-46.2** (↓121)
GD 20-28	111.3	103.3 (↓7)	117.5	71.5 (↓36)	140.2
GD 7-28	327.6	161.2 (↓51)	139.0 (↓58)	15.7** (↓95)	133.5 (↓59)

*=Significantly different from control, p≤0.05
**=Significantly different from control, p≤0.01
↓=Decreased

Clinical pathology

Changes in Hematology, Coagulation, and Clinical Chemistry

Parameter	% Change from Control			
	0.5 mg/kg/day	1.5 mg/kg/day	5 mg/kg/day	20 mg/kg
RBC	↓10*	↓10*	↓15**	↓6
Hemoglobin	↓11**	↓12**	↓17**	↓6
Hematocrit	↓9*	↓10*	↓14**	-
Monocytes	-	↑130**	↑144**	-
Platelets	↑45	↑43	↑68**	↑21
Reticulocytes	↑14	↑79	↑123*	↑102
Fibrinogen	↑27	↑34	↑86**	↑19
AST	-	↑18	↑133**	-
ALT	-	↑40	↑203*	-

*=Significantly different from control, p≤0.05
**=Significantly different from control, p≤0.01
↓=Decreased ↑=Increased -=No effect in this group

Necropsy findings

Abortions: One female in the 5 mg/kg/day group and two females in the 20 mg/kg group aborted on GD 20 or GD 24.

Liver: Multifocal tan discoloration of the liver was observed in 1 female at 0.5 mg/kg/day, 5 females at 1.5 mg/kg/day, and 11 females at 5 mg/kg/day. At 5 mg/kg/day, one of the incidences of multifocal discoloration was the female that aborted; this liver was also abnormally firm in consistency.

Cesarean Section Data

Summary of Pregnancy and Animals with Fetuses

Number of females:	0 mg/kg/day	0.5 mg/kg/day	1.5 mg/kg/day	5 mg/kg/day	20 mg/kg
In study	**20**	**20**	**20**	**20**	**20**
Pregnant	18	18	19	19	19
With live fetuses	18	18	17	15	14
With all dead or resorbed fetuses	0	0	2	4	5
Aborted	0	0	0	1	2
Complete litter loss	0	0	2	3	3

In aborting dams:

5 mg/kg/day: The litter of the aborting female consisted of 8 dead fetuses and 3 late resorptions.

20 mg/kg: The litter of one female consisted of 8 dead fetuses and 1 late resorption and the litter of the other female consisted of 11 dead fetuses.

In remaining animals:
Findings included a decrease in the total number of fetuses and the number of live fetuses and an increase in total resorptions, early resorptions, late resorptions, and postimplantation loss.

Summary of Uterine Examination Findings

Parameter	Mean (% Change from Control)				
	0 mg/kg/day	0.5 mg/kg/day	1.5 mg/kg/day	5 mg/kg/day	20 mg/kg
Total fetuses	10.72	10.11	9.82 (↓8)	8.13 (↓24)	7.64* (↓29)
# of live fetuses	10.67	10.06	8.79 (↓18)	6.78* (↓36)	6.29** (↓41)
# of dead fetuses	0.06	0.06	0	0	0
Total resorptions	0.33	0.78	1.79 (↑437)	2.94** (↑783)	4.00** (↑1100)
Early resorptions	0.22	0.56	1.53 (↑587)	2.50** (↑1025)	3.71** (↑1568)
Late resorptions	0.11	0.22	0.26 (↑137)	0.44 (↑300)	0.29 (↑165)
Postimplantation loss (%)	3.9	8.0	16.2 (↑316)	35.4** (↑808)	39.3** (↑910)

*=Significantly different from control, p≤0.05
**=Significantly different from control, p≤0.01
↓=Decreased ↑=Increased

Necropsy findings
Offspring
[malformations, variations, etc.]

20 mg/kg: Increased litter and fetal instances of skeletal variations compared to concurrent control that also exceeded the historical control range.

Skeletal Variations

Skeletal Variation Findings		Number Affected (%)	
		0 mg/kg/day (Control)	20 mg/kg
Number of fetuses examined		192	107
Number of litters examined		18	14
Variation	Measure		
Asymmetric sternebrae	Fetuses	1 (0.5)	5 (4.7)
	Litters	1 (5.6)	4 (28.6)
Isolated ossification sites of sternebrae	Fetuses	0 (0)	5 (4.7)
	Litters	0 (0)	3 (21.4)
Misshapen (irregularly shaped) sternebrae	Fetuses	2 (1.0)	6 (5.6)
	Litters	2 (11.1)	3 (21.4)

Toxicokinetics

Toxicokinetics in the Plasma of Pregnant Rabbits

Dose	GD Day	C_{max} (µg/mL)	T_{max} (hours)	T_{last} (hours)	$AUC_{0\text{-}8}$ (µg·hr/mL)
1.5 mg/kg/day	7	0.0996	1.7	2.0	NA
	19	0.0940	1.3	2.0	NA
5 mg/kg/day	7	0.453	2.0	8.0	2.24
	19	0.443	1.5	8.0	2.20
20 mg/kg	7	2.99	1.5	8.0	13.5

NC= Not calculated, below limit of quantitation
NA= Not Applicable
GD=Gestational Day

All plasma concentration values of givosiran in the 0.5 mg/kg/day group were below the lower limit of quantitation (<50.0 ng/mL) on GD 7 and GD 19. AUC values were not calculated at 1.5 mg/kg/day.

	Mean Maternal Rabbit Liver Tissue Concentrations (µg/g) on GD 22 **Givosiran Treatment Group** **0.5 mg/kg/day** | **1.5 mg/kg/day** | **5 mg/kg/day** | **20 mg/kg** 7.47 | 64.6 | 562 | 4.51 The concentrations of givosiran were below the limit of quantitation in all placentas and fetuses for all givosiran treated groups on GD 22.
Pharmacodynamics	Hepatic ALAS1 transcript levels were not reduced on GD 22 in pregnant rabbits at any of the givosiran dose levels. These data confirm that givosiran is not pharmacologically active in rabbits.

Prenatal and Postnatal Development

Study title/ number: ALN-AS1: A Subcutaneous Injection Developmental and Perinatal/Postnatal Reproduction Study in Sprague Dawley Rats, Including a Postnatal Behavioral/Functional Evaluation / AS1-GLP17-009

Key Study Findings

- Treatment with givosiran on GD 7, 13, and 19 and on LD 6, 12, and 18 in pregnant rats did not result in maternal toxicity or produce any effects on gestation, parturition, lactation, or maternal behavior. There were no givosiran-related effects on pup mortality, growth, sexual maturation, behavior, mating and fertility, or ovarian and uterine parameters in the F1 generation rats.

Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes

Methods

Dose and frequency of dosing:	0, 3, 10, or 30 mg/kg approximately every 6 days on GD 7, 13, and 19 and Lactation Days (LD) 6, 12, and 18 for a total of 6 doses
Route of administration:	Subcutaneous injection; 5 mL/kg dose volume
Formulation/Vehicle:	0.9% sodium chloride for injection
Species/Strain:	Rat/Sprague Dawley [Crl:CD(SD)]
Number/Sex/Group:	22 females/group
Satellite groups:	Toxicokinetics: 5 females/group; blood and milk samples collected on LD 12; termination on LD 12 and blood samples from F1 pups
Study design:	Pregnant females (F0 dams) were administered vehicle or givosiran every 6 days on GD 7, 13, and 19 and LD 6, 12, and 18 for a total of 6 doses. Females were allowed to deliver the F1 litters and rear the F1 pups to weaning on LD/post natal day (PND) 21 to evaluate the

	effects on the F0 dams and F1 generation litters through weaning. F1 rats selected for continuation (1/sex/litter; 22/sex/group) were evaluated for sexual maturation and behavior assessments included passive avoidance on PND 24 and PND 31 and M-Shaped water maze testing beginning on PND 70. F1 animals were mated for assessment of reproductive function and uterine parameters were evaluated in F1 females on GD 13.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Generation	Major Findings
F0 Dams	Unremarkable
F1 Generation	Unremarkable
F2 Generation	Unremarkable

Toxicokinetics

The maternal plasma concentrations of givosiran at 2 hours after dosing on LD12 increased at a greater than dose proportional rate; concentrations are presented in the table below. The concentrations of givosiran in the plasma of all pups was below the lower level of quantification on LD/PND 12. Consistent with this finding, the concentrations of givosiran in the milk of the dams was also below the lower level of quantification 2 hours after dosing on LD 12, with the exception of one dam (concentration of 229 ng/mL) in the 30 mg/kg dose group.

Table 2. Concentration of Givosiran in Maternal Plasma on LD 12

Dose Level (mg/kg)	Mean Concentration (ng/mL)
3	95.5
10	435
30	2712

5.5.5. Other Toxicology Studies

Impurity Qualification

The proposed specifications for the givosiran drug substance impurities require qualification. In the table below, the dose of each impurity based on mg/kg at the proposed specification for the recommended clinical dose of 2.5 mg/kg givosiran once monthly is compared to the dose of the impurity (in mg/kg) administered to the animals at the 10 mg/kg/week dose in the 26-week repeat-dose toxicology study in rats (Study #AS1-GLP15-022).

Table 3. Impurity Qualification with Doses Based on mg/kg for Impurities for Givosiran Drug Substance

Impurity (RRT)	Proposed Specification		Levels/Doses in Animal Toxicology Study (AS1-GLP15-022; 26-week rat) DS BATCH 15D002		Qualification Determination
	%	Dose (mg/kg)	%	Dose (mg/kg)	
DS_{AX}^{1} = (b) (4)				(b) (4)	Qualified
DS_{AX}^{2} >					Qualified
DS_{AX}^{3} >					Qualified
DS_{AX}^{4} >					Qualified
DS_{RP}^{1} =					Qualified
DS_{RP}^{2} >					Qualified
DS_{RP}^{3} >					Qualified
DS_{RP}^{4} >					Qualified

DS= drug substance
AX= Impurities from AX-HPLC (anion exchange)
RP= Impurities from Ion pair reverse phase-HPLC

Brenda J Gehrke, PhD
Primary Reviewer

Matthew D Thompson, PhD
Primary Reviewer

6 Clinical Pharmacology

6.1. Executive Summary

The applicant seeks approval for givosiran for the treatment of acute hepatic porphyria (AHP) in adults (b) (4). The proposed dosage regimen is 2.5 mg/kg once monthly (QM) by subcutaneous injection.

The primary evidence of efficacy supporting the 2.5 mg/kg dose is based on the demonstrated reduction in composite porphyria attacks in ENVISION (Study ALN-AS1-003), a randomized placebo-controlled clinical trial. The study consisted of a 6 month double blind period (DB, completed) and an open-label extension (OLE, ongoing) period. The study evaluated givosiran at dose level of 2.5 mg/kg subcutaneously once monthly (QM) during the DB period, and at dose levels of 1.25 mg/kg QM or 2.5 mg/kg QM during OLE period. The lower dose of 1.25 mg/kg QM was introduced as a down-titration dose in patients who transaminase elevations. The dose of 1.25 mg/kg achieve significant ALA and PBG reduction with minimal effect on transaminase elevation.

(b) (4)

The clinical pharmacology review focused on evaluating the acceptability of the proposed dosage regimen, the clinical pharmacology data (b) (4), and dosing recommendations for drug interactions.

6.1.1. Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in this NDA and recommends approval of givosiran for the treatment of AHP in adults (b) (4). The key review issues are summarized below (Table 4).

Table 4. Summary of Clinical Pharmacology Recommendations

Review Summary	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Primary evidence of effectiveness is based on a placebo-controlled ENVISION trial. Refer to section 7 for further details.
General dosing instructions	• General dosing 2.5 mg/kg QM through subcutaneous injection. *Reviewer's comment: The proposed dosing regimen is supported by dose-response analysis, safety data at 2.5 mg/kg QM, and PK/PD modeling and simulation.*

	• Dose Modification for Adverse Reactions In patients with severe or clinically significant transaminase elevations, who have a dose interruption and subsequently improve, reduce the dose to 1.25 mg/kg once monthly. In patients who resume dosing at 1.25 mg/kg once monthly without recurrence of severe or clinically significant transaminase elevations, the dose may be increased to the recommended dose of 2.5 mg/kg once monthly. *Reviewer's comment:* (b) (4) *the 1.25 mg/kg QM dosage also achieves clinically relevant ALA and PBG reductions with minimal effects on transaminase elevation and a potentially better overall safety profile. Therefore, FDA recommends 1.25 mg/kg QM as the* (b) (4) *resuming dose following transaminase elevation recovery. In patients receiving the resuming dose of 1.25 mg/kg QM without recurrence of severe or clinically significant transaminase elevations, the dose may be increased to the recommended dose of 2.5 mg/kg QM.*
Dosing in patient subgroups (intrinsic and extrinsic factors)	No therapeutic individualization is required for givosiran based on intrinsic or extrinsic factors. Givosiran is metabolized by nucleases and is not a substrate, inhibitor, or inducer of major CYP450 isozymes (CYPs) or transporters. No clinically meaningful differences in givosiran PK were observed in patients with mild, moderate or severe renal impairment, or in patients with mild hepatic impairment. Givosiran has not been studied in patients with moderate or severe hepatic impairment, patients with end-stage renal disease or in patients on dialysis. The body weight-based dosing regimen is appropriate as body weight is a significant covariate affecting the PK of givosiran. After correcting for body weight (with body weight-based dosing), body weight was not identified as a significant covariate for the PK/PD model. (b) (4)
Drug Interaction	Givosiran increased $AUC_{0\text{-}inf}$ by 3 fold for caffeine (CYP1A2 substrate) and by 2.4 fold for dextromethorphan (CYP2D6 substrate). The following recommendation regarding the concomitant use of CYP1A2 and 2D6 substrates was concluded: • Avoid concomitant use of givosiran with CYP1A2 or CYP2D6 substrates, for which minimal concentration changes may lead to serious or life-threatening toxicities.

Labeling	Generally acceptable upon the applicant's agreement to the FDA revisions to the label with specific content and formatting change recommendations. Clinical pharmacology labeling recommendations are detailed in section 11.

6.1.2. Post-Marketing Requirements and Commitments

None.

6.2. Summary of Clinical Pharmacology Assessment

The PK of givosiran were evaluated by monitoring the antisense strand of givosiran, with concentrations reported as the full-length double-stranded siRNA (ALN-60519), in plasma and urine. Metabolite profiling identified AS(N-1)3' givosiran as a major metabolite (>10% of givosiran) in plasma and urine. This pharmacologically active metabolite, with equal potency to givosiran, is formed by the loss of 1 nucleotide (uridine) from the 3'-end of the antisense strand.

6.2.1. Pharmacology and Clinical Pharmacokinetics

The PK of givosiran and its active metabolite [AS(N-1)3' givosiran] were evaluated following single and multiple dosing in patients that are chronic high excreters and in patients with AHP as summarized in Table 5.

Table 5. Pharmacokinetic Parameters of Givosiran and Its Active Metabolite

		Givosiran	AS(N-1)3' Givosiran
General Information			
Steady-State Exposure	**C_{max} [Mean (CV%)]**	321 ng/mL (51%)	123 ng/mL (64%)
	AUC_{24} [Mean (CV%)]	4130 ng·h/mL (43%)	1930 ng·h/mL (63%)
Dose Proportionality		• Steady-state maximum plasma concentration (C_{max}) and area under the curve (AUC) for givosiran and AS(N-1)3' givosiran increase proportionally over the 0.35 mg/kg to 2.5 mg/kg once monthly dose range (0.14 to 1-fold the approved recommended dosage). • C_{max} and AUC for givosiran and AS(N-1)3' givosiran increase slightly greater than proportionally at doses greater than 2.5 mg/kg once monthly.	
Accumulation		• No accumulation of givosiran or AS(N-1)3' givosiran was observed following multiple dosing.	
Absorption			
T_{max} [Median (range)]		3 (0.5-8) hours	7 (1.5-12) hours
Distribution			

Apparent Central Volume of Distribution (Vz/F) [Mean (RSE%)][a]	10.4 L (2.3%)	
Protein Binding	90%[b]	Not evaluated
Organ Distribution	Givosiran and AS(N-1)3' givosiran distribute primarily to the liver after subcutaneous dosing.	
Elimination		
Half-Life [Mean (CV%)]	6 hours (46%)	6 hours (41%)
Apparent Clearance [Mean (CV%)][a]	35.1 L/hr (18%)	64.7 L/hr (33%)
Metabolism		
Primary Pathway	Givosiran is metabolized by nucleases to oligonucleotides of shorter lengths. Givosiran is not a substrate of CYP enzymes[c].	
Active Metabolite	The active metabolite, AS(N-1)3' givosiran, is equipotent to givosiran in plasma and the AUC_{0-24} represents 45% of givosiran AUC, at the approved recommended givosiran dosage.	
Excretion		
Primary Pathway	The dose recovered in urine was 5 to 14% as givosiran and 4 to 13% as AS(N-1)3' givosiran[d].	

[a] Based on population PK model estimation.
[b] Givosiran plasma protein binding was concentration-dependent and decreased with increasing givosiran concentrations (from 92% at 1 µg/mL to 21% at 50 µg/mL).
[c] Based on in vitro study result.
[d] After single and multiple subcutaneous doses of givosiran 2.5 mg/kg and 5 mg/kg.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

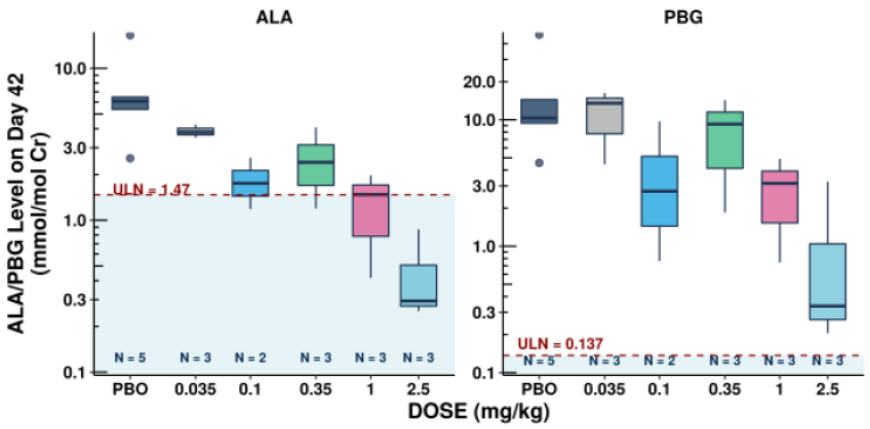
The applicant proposed givosiran dosage is 2.5 mg/kg QM through subcutaneous injection for the treatment of patients with AHP. This is the same dosage as studied in the ENVISION trial.

Clinical symptoms of AHP result from the accumulation of the toxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG) due to induced expression of ALAS1. Givosiran is a double-stranded small RNA that causes degradation of ALAS1 mRNA in hepatocytes through RNA interference, thereby leading to reduction of neurotoxic ALA and PBG concentrations and subsequent reductions in composite porphyria attacks. Achievement of ALA/PBG reduction provides supportive evidence for the proposed 2.5 mg/kg QM givosiran dosage.

Selection of the givosiran 2.5 mg/kg QM dosage is supported by the following evidence:

- Dose dependent reductions in urinary ALAS1 mRNA, ALA, and PBG levels over the 0.035 to 2.5 mg/kg single dose range in subjects that are chronic high excreters (CHE) in Study 001 A (Figure 3).

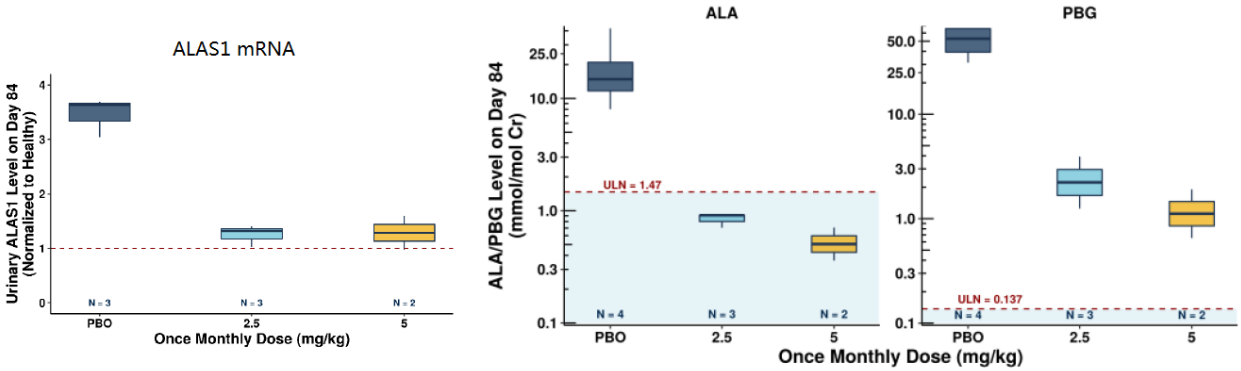
Figure 3. Relationship between Givosiran Dose and Absolute Urinary ALA and PBG Levels in CHE Subjects



Source: Population PK/PD Report, Figures 11.2.23

- Comparable levels of the ALAS1 mRNA reduction at the 2.5 mg/kg and 5.0 mg/kg doses in patients with AIP. The 2.5 mg/kg QM dose resulted in near-maximal reductions in urinary ALA and PBG concentrations in study 001C (Figure 4).

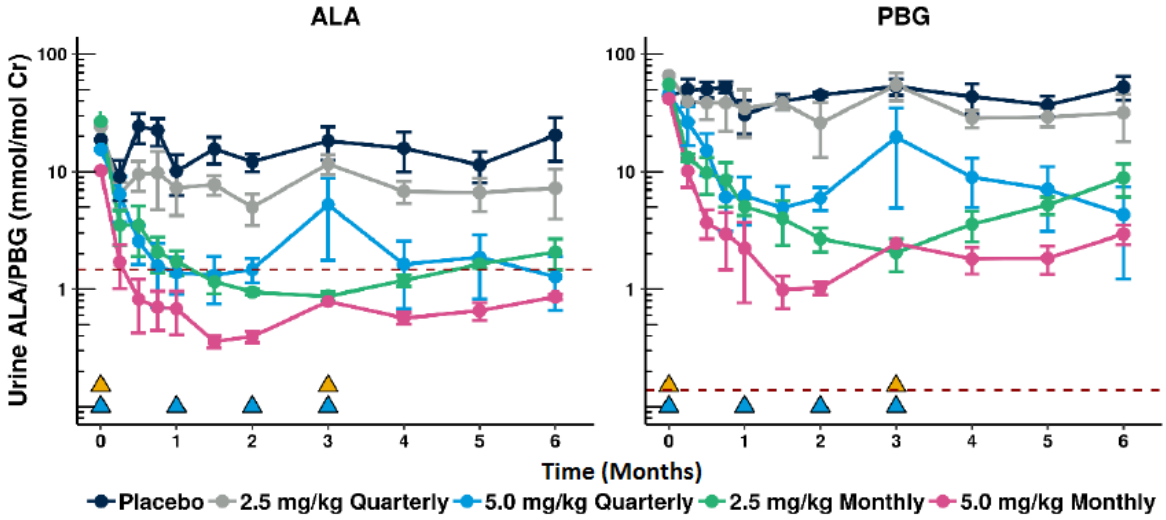
Figure 4. Comparison of Urinary ALAS1 mRNA, ALA and PBG Reduction on Day 84 After 2.5 and 5 mg/kg QM Doses in AIP Patients



Source: Population PK/PD Report, Figures 11.2.24. and 11.2.26.

- ALA and PBG levels were maintained for the entire QM dosing interval with no evidence of recovery of the biomarkers between dose administrations. In contrast, the once-quarterly dosing interval was associated with greater variability in ALA and PBG levels with a trend toward biomarker recovery at the end of the 3-month dosing interval (Figure 5).

Figure 5. Urinary ALA and PBG Levels After Once Monthly and Once Quarterly Doses of Givosiran in AIP Patients



Source: Population PK/PD Report, Figures 11.2.27

- The 2.5 mg/kg QM dosage was generally well tolerated in AIP patients, as shown in the ENVISION trial, where serious adverse events (AEs) were reported in 20.8% of patients in the givosiran group and 8.7% of patients in the placebo group. Out of total 48 patients, one patient in the givosiran group had a related SAE of abnormal LFT and permanently discontinued treatment after an ALT elevation of >8 × ULN. A total of 2 patients had AEs that led to treatment interruption in the 6-month DB period.
- Model-based analysis suggests that the 2.5 mg/kg QM dose is predicted to lower urinary ALA to within the normal range in a majority of patients with AHP (Figure 4). A comparison of the 1.25, 2.5 and 5 mg/kg QM regimens suggests that doses at or above 2.5 mg/kg QM are in the plateau portion of the dose-response curve for ALA (Table 6).

Table 6. Model-Predicted Steady State AAR Based on ALA Absolute Model

Treatment	Dose	Mean [Median] (5^{th} – 95^{th} Interval)		Proportion of Patients (%)				
		ALA, mmol/mol Cr	AAR, attacks/year	AAR <12	AAR <8	AAR <4	AAR <2	AAR<1
Placebo	Placebo	18.1 [14.6] (4.82 – 44.3)	**9.88** [6.75] (0.858 – 29.6)	70.9	**56.2**	**32.7**	**15.9**	**6.34**
Givosiran QM	1.25 mg/kg	3.49 [1.98] (0.433 – 12.3)	3.44 [1.87] (0.254 – 11.9)	95.1	89.7	74.1	52.2	30.4
	2.5 mg/kg	2.24 [1.38] (0.261 – 7.32)	**2.90** [1.65] (0.234 – 9.81)	96.9	**92.4**	**78.0**	**56.5**	**33.8**
	5 mg/kg	1.63 [0.967] (0.209 – 4.95)	2.68 [1.53] (0.223 – 8.92)	97.5	93.7	80.4	59.2	35.7
Givosiran Q3M	1.25 mg/kg	7.65 [4.87] (0.87 – 23.0)	5.48 [2.94] (0.354 – 19.4)	87.8	79.0	59.5	38.3	20.2
	2.5 mg/kg	5.17 [3.54] (0.614 – 16.5)	**4.30** [2.34] (0.293 – 15.1)	92.2	**85.0**	**66.9**	**45.0**	**25.1**
	5 mg/kg	4.20 [2.46] (0.438 – 11.9)	3.76 [2.03] (0.27 – 13.2)	93.9	87.9	71.3	49.6	28.4

Source: Givosiran ALA Attack Analysis, Table 13.Therapeutic Individualization

No therapeutic individualization is required for givosiran based on intrinsic or extrinsic factors. Givosiran is metabolized by nucleases and is not a substrate, inhibitor, or inducer of cytochrome p450 isozymes (CYPs) or transporters. No clinically relevant PK differences were observed in patients with mild, moderate or severe renal impairment, compared to patients with normal renal function, or in patients with mild hepatic impairment, compared to patients with normal hepatic function. PK/PD analysis indicated that baseline age, body weight, renal impairment, sex, and race (East Asian versus non-East Asian) were not statistically significant

covariates on givosiran PK. Givosiran has not been studied in patients with moderate or severe hepatic impairment, patients with end-stage renal disease or in patients on dialysis.

Body weight was a significant covariate on the absorption rate constant, where higher body weight was associated with slower absorption and delayed T_{max} supporting the body-weight based dosing. The difference in mean plasma givosiran exposure in 40 kg and 130 kg patients were predicted to be clinically insignificant (within 23%) as compared to of that observed in a typical 66.2 kg patient. The body weight based 2.5 mg/kg QM dose yields similar urinary ALA reduction across the observed body weight range of 40 kg to 130 kg, supporting that the body weight-based regimen is appropriate.

Dose Adjustment for Adverse Events

It is recommended that givosiran dosing should be interrupted upon severe or clinically significant transaminase elevations. Once transaminase levels resolve, (b) (4) (b) (4) resume therapy; 1.25 mg/kg QM (b) (4) In patients who resume at 1.25 mg/kg QM and without further recurrence of severe or clinically significant transaminase elevations, the dose may be increased to 2.5 mg/kg QM. The dose of 1.25 mg/kg achieved clinically relevant ALA and PBG reductions with minimal effects on transaminase elevation, leading to a potentially better overall safety profile compared to 2.5 mg/kg QM dose. Therefore, FDA recommends resuming therapy with 1.25 mg/kg QM (b) (4) following recovery from severe transaminase elevation. In patients receiving the resuming dose of 1.25 mg/kg QM without recurrence of severe or clinically significant transaminase elevations, the dose may be increased to the recommended dose of 2.5 mg/kg QM.

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

The givosiran PK profile was consistent across all 4 clinical studies (Study 001, 002, 003, 004). AS(N-1)3' givosiran the major circulating metabolite in plasma with approximately 45% exposure relative to givosiran and with equal potency as givosiran. Givosiran exhibited linear, time-independent kinetics in plasma. There was no accumulation of givosiran or AS(N-1)3' givosiran in plasma after repeated monthly or quarterly dosing of 2.5 mg/kg.

Key pharmacology and PK characteristics are summarized in Table 7. Refer to Table 5 for observed and predicted PK parameters.

Table 7. Key Pharmacology and PK characteristics of Givosiran

Pharmacology	
Mechanism of Action	Givosiran degrades *ALAS1* mRNA in hepatocytes through RNA interference, which leads to reduced circulating levels of the neurotoxic intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG), factors that have been associated with acute porphyria attacks or other disease manifestations of AHP.
QT Prolongation	No large mean increase in QTc (i.e. > 20 ms) was detected at the 2.5 mg/kg QM dose level in 94 patients based on the evaluation of QTc prolongation potential in the ENVISION trial.
General Information	
Bioanalysis	The PK of givosiran was evaluated by monitoring the antisense strand of givosiran, with concentrations reported as the full-length double-stranded siRNA (ALN-60519), in plasma and urine. Validated liquid chromatography mass spectrometry-high resolution accurate mass (LC-MS/HRAM) and liquid chromatography tandem mass spectrometry (LC-MS/MS) assays with the lower

	limit of quantitation (LLOQ) of 10-20 ng/mL in plasma and 50 ng/mL in urine were used to measure givosiran concentrations. Plasma and urine samples were analyzed for the PD biomarkers ALA and PBG to understand the primary effect of ALAS1 reduction. Total ALA and PBG concentrations in urine and plasma samples were measured using validated and sensitive LC-MS/MS assays with an LLOQ of 10 ng/mL. Urine ALA and PBG levels were normalized to time-matched urine creatinine (Cr) concentrations and expressed as mmol/mol Cr. Details are described in section 19.4.
Renal Impairment	No clinically relevant difference in givosiran and AS(N-1)3' givosiran exposure was observed in patients with mild, moderate or severe renal impairment compared to patient with normal renal function.
Hepatic Impairment	Compared to patients with normal hepatic function, mild hepatic impairment had no effect on PK or PD of givosiran. Givosiran has not been studied in patients with moderate or severe hepatic impairment.
Healthy Subjects vs Patients	Givosiran has not been evaluated in healthy subjects. The clinical development program for givosiran consists of clinical pharmacology studies in subjects that are chronic high excreters (CHE) who carry a genetic mutation associated with AHP but do not have active neurovisceral attacks; these subjects have elevated ALA and PBG levels, but the levels are generally lower than those observed in patients with AHP. Analysis indicated that givosiran PK in subjects that are CHE are not statistically significantly different from that in patients with AHP.
PK/PD/Efficacy Relationship	After SC dosing, givosiran plasma concentrations decline to below the limit of quantification after 24 hours post dose with a short plasma half-life of 10 hours. Despite short the half-lives of givosiran and its metabolite in the plasma, the PD effects can last for weeks to months after a single SC dose. The long duration of the PD effect does not directly correlate with the transient plasma concentrations, indicating that the driver of PD is the exposure of givosiran in the liver. A mixed-effects Markov model was used to characterize the relationship between urinary ALA reduction and the probability of an attack occurring in AHP patients. The modeling predicted that lowering of urinary ALA towards the normal range predicted a clinically meaningful reduction in the rate of AHP attacks
Immunogenicity	There was 1 case of treatment-induced ADA in 131 subjects who received givosiran across the 4 clinical studies.
DDI: Effect of Givosiran on other drugs	Hepatic heme serves as the prosthetic moiety of CYP enzymes in liver. Since givosiran acts by inhibition of ALAS1, it can potentially lower hepatic heme content and thereby reduce the activity of CYP enzymes. A dedicated drug-drug interaction study indicated that givosiran increased caffeine (CYP1A2 substrate) $AUC_{0\text{-}inf}$ by 3 fold; increased dextromethorphan (CYP2D6 substrate) $AUC_{0\text{-}inf}$ by 2.4 fold for; increased omeprazole (CYP2C19 substrate) $AUC_{0\text{-}inf}$ by 1.6 fold; increased midazolam (CYP3A4 substrate) $AUC_{0\text{-}inf}$ by 1.4 fold; and increased losartan (CYP2C9 substrate) $AUC_{0\text{-}inf}$ by 1.1 fold.

6.3.2. Clinical Pharmacology Questions

Is 1.25 mg/kg optimal for resuming givosiran dosing following recovery from transaminase elevation?

Yes. The dose regimen of 1.25 mg/kg QM is optimal for resuming givosiran dosing following recovery from transaminase elevation. This conclusion is supported by biomarker and clinical observations in patients who resumed dosing at 1.25 mg/kg QM (Figure 6 and Table 8), and PK/PD modeling and simulation results (Table 6).

Following an alanine aminotransferase (ALT) elevation to >5.4x ULN after 4 doses in in one patient ((b) (6)), the dose of givosiran was interrupted and subsequently resumed on Month 7 (Month 1 of the OLE period) at a reduced dose of 1.25 mg/kg QM following ALT elevation recovery. Subsequent liver function test (LFT) results have been normal. In order to generate additional data at this dose level, evaluation of the 1.25 mg/kg QM dose is proposed for patients crossing over to the OLE period under amendment 3 of the ENVISION trial. Patients who crossed over to the OLE period prior to implementation of amendment 3, received a 2.5 mg/kg QM dose, and will remain on that dose until completion. Starting at the Month 13 Visit, patients in the 1.25 mg/kg monthly treatment group who experience inadequate disease control may have their monthly dose increased to 2.5 mg/kg givosiran.

The resumption of dosing with 1.25 mg/kg QM is acceptable from efficacy perspective as clinically relevant reductions in ALA levels were observed with both dose levels [median (Q1, Q3) for 2.5 mg/kg QM and 1.25 mg/kg QM: -13.6 (-16.9, -9.3) and -8.2(-15.7, -4.1), respectively, source: Study 003 CSR 1 Table 42]. None of the 37 patients who received givosiran 1.25 mg/kg QM (all 37 patients has more than 3 month follow-up) needed dose escalation to 2.5 mg/kg QM because of inadequate disease control. In addition, the model predicted annualized attack rate (AAR) generally overlaps for the for 2.5 mg/kg QM and 1.25 mg/kg QM dosages (median (95% CI) : 2.9 (0.2, 9.8) and 3.4(0.3, 11.9), respectively (Givosiran ALA Attack Analysis Table 13]. The overlapping model predicted AAR occurs despite the lower magnitude of reduction in urinary ALA levels with 1.25 mg/kg compared to 2.5 mg/kg (Table 6).

During the DB period, givosiran 2.5 mg/kg QM resulted in mild ALT elevations (>ULN to ≤3×ULN) in 39.6% patients and ALT elevations >3×ULN in 14.6% (7 patients). Data from the OLE period indicate that the dose regimen of 1.25 mg/kg QM had minimal effect on transaminase elevation. The 1.25 mg/kg QM dosage had fewer safety events, as indicated by the lower rate of serious/sever AEs compared to givosiran 2.5 mg QM (Table 8).

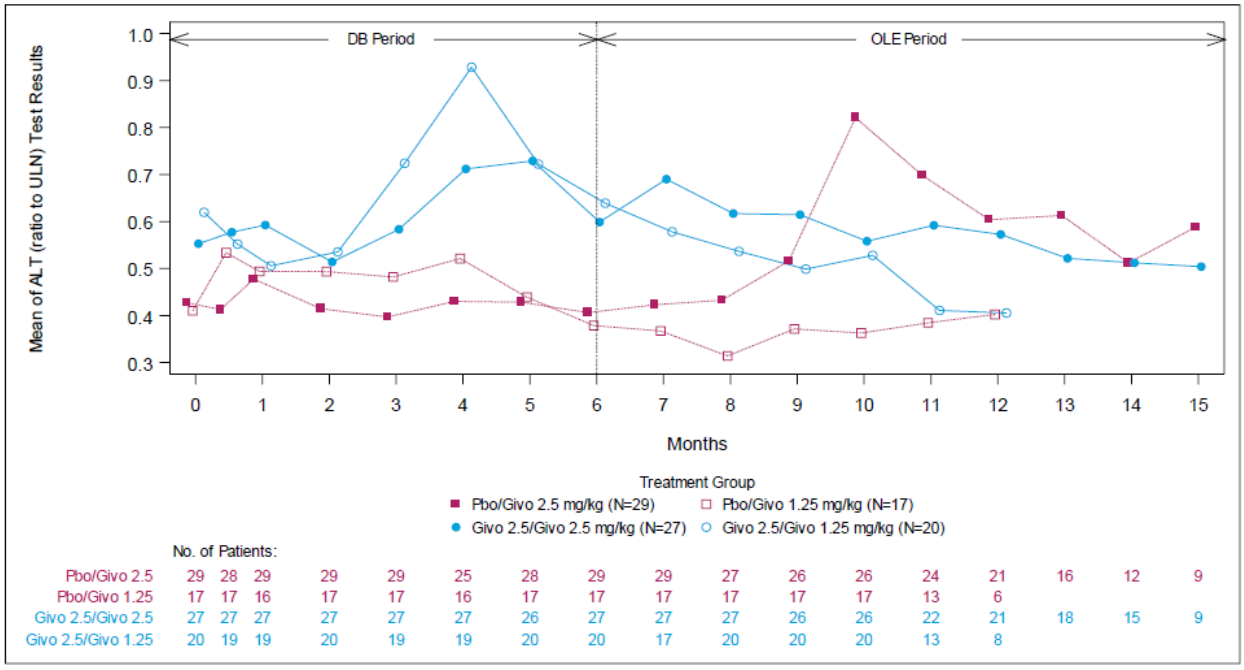
Table 8. Rate of Serious or Severe AE Rate Across Different Dose Regimens

n/N (%)	PBO-2.5 mg/kg	PBO-1.25 mg/kg	2.5-2.5 mg/kg	2.5-1.25 mg/kg
At least 1 Serious AE	3 /29 (10.3)	2 /17 (11.8)	9 /27 (33.3)	3 /20 (15)
At least 1 Severe AE	4 /29 (13.8)	3 /17 (17.6)	9 /27 (33.3)	2/20 (10)

Source: Reviewer's analysis

Transient ALT elevations between 3 and 5×ULN were observed in 5 patients in the 2.5 mg/kg dose group, but none were observed at 1.25 mg/kg. The mean ALT profile over time indicated that the transaminase elevation peaks following approximately 4 doses and is transient in nature, which may due to the development of tolerability (Figure 6).

Figure 6. Mean of ALT (Ratio to ULN) Over Time



Source: Study 003 SUR#1 Figure 14.3.5.3.2.1

Taken together, FDA considered 1.25 mg/kg as the optimal dose for resuming givosiran treatment following transaminase elevation recovery, with the benefit of reducing the probability of transaminase elevation in patients who previously experienced clinically significant transaminase elevation. Selection of 1.25 mg/kg, (b) (4) in the dose reduction instructions for the proposed package insert. The lower dose of 1.25 mg/kg has a lower magnitude of ALA/PBG reduction and a numerically higher predicted AAR rate, as compared to the standard dose of 2.5 mg/kg. Re-escalating back to 2.5 mg/kg QM without recurrence of severe or clinically significant transaminase elevations is permitted.

(b) (4)

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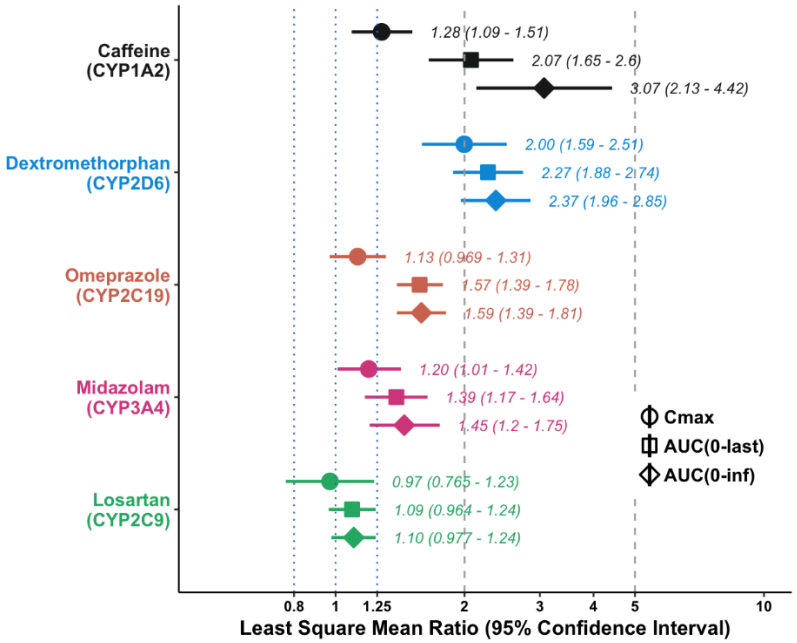
Is the Single Dose Drug-Drug Interaction (DDI) Study Adequate to Address the Drug Interaction Potential with CYP Substrate?

Yes. The potential for a PD based DDI by givosiran was investigated in a dedicated DDI study in subjects that are CHE (Study 004), with a cocktail approach to assess the interaction of givosiran with five major CYP enzymes (CYP1A2, 2C9, 2C19, 2D6, and 3A4). The study design was adequate to evaluate the drug-drug interaction potential of givosiran as an inhibitor or inducer of substrates of major CYP enzymes.

Small interfering RNA molecules do not directly inhibit or induce CYP450 enzymes, as supported by results from in vitro human liver microsomes and hepatocytes studies. However, givosiran may potentially reduce the CYP enzyme activity of by reducing hepatic heme content, the prosthetic moiety of CYP enzymes in liver, since givosiran acts by inhibiting ALAS1, the first and rate limiting enzyme in the heme biosynthesis pathway in the liver. Nonclinical DDI studies may not reflect the expected change in CYP450 activity in patients with AHP receiving givosiran in clinical studies, given the mechanism of this possible drug interaction and the inconsistent and contradictory results observed from DDI studies (AS1-DSM17-003 and AS1-NCD14-014) in monkeys. As such, the DDI potential by givosiran on major CYP isozymes was investigated in a dedicated DDI study 004 in subjects that are CHE. In this study, AUC and Cmax of midazolam (CYP3A4), caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), and dextromethorphan (CYP2D6) on Days 1 (before givosiran dosing) and 36 (28 days after a single 2.5 mg/kg dose of givosiran) were compared. A comparison of the cocktail drug exposure before and 28 days after

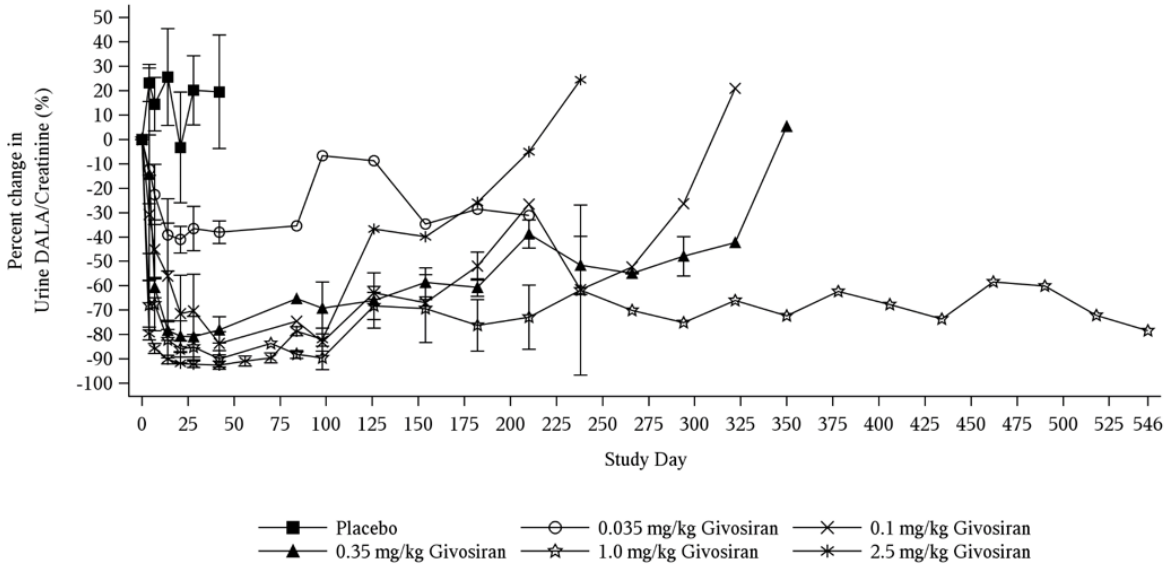
givosiran show that givosiran treatment increased AUC_{inf} by approximately 3-fold for caffeine, approximately 2-fold for dextromethorphan, and approximately 1.5 fold for omeprazole and midazolam. The givosiran treatment did not change the AUC_{inf} for losartan (Figure 9).

Figure 9. GLSM Mean Ratio (Day 36 /Day 1) and 90% CI for C_{max}, AUC_{last}, and AUC_{inf} for Cocktail Drugs



In contrast to conventional small molecule drug interactions in which the greatest magnitude of the drug interaction is achieved when the investigational drug and index substrate drugs are coadministered, the evaluation of givosiran drug interaction potential was conducted by comparing to the cocktail drug exposure 28 days post givosiran dose. The lag time of 28 days was based on the observation that the maximum decrease of ALA and PBG is achieved between Day 21 and Day 28 postdose.

Figure 10. Mean (±SEM) Percent Change from Baseline Urine ALA after Single Dosing in CHE Subjects



Source: Study 001 CSR Figure 5

The changes in ALA/PBG levels are expected to rapidly translate into changes in CYP450 activity as supported by the following evidence:

- Short turnover rate of heme and ALA/PBG:
  In the liver, heme biosynthetic enzymes turn over rapidly (within hours), permitting the liver to quickly alter heme levels in response to changing metabolic requirements (PMID: 16839620). For example, the half-life of ALA synthase enzyme in rat liver mitochondria was estimated to be about 35 min (PMID: 6789140), while ALA itself has been reported to have a short half-life of < 1 hour (PMID: 11961050).
- Rapid conversion from ALA into heme in the liver:
  It was determined that radiolabeled ALA was incorporated in the hepatic heme pool within 30 minutes in rat (PMID: 4007401). In a similar study, radiolabeled carbon monoxide, a specific degradation product of heme, was detected within minutes of administration of a radiolabeled dose of ALA(PMID: 970967). In addition, a rapid and ALA concentration-dependent increase in heme synthesis was demonstrated after treatment for just 30 minutes in human liver homogenates (PMID: 4004804).
- Rapid effect of changing heme level on CYP450 enzymes activity:
  The effect of heme on the activity of CYP450 enzymes is also expected to be rapid based on the rapid turnover rates of most CYP450 enzymes in liver. Half-lives of major CYP450 enzymes in human liver have been estimated to range from 23 to 104 hours (Table 10).

Table 10. Half-lives of CYP Enzymes

CYP450 Enzyme	Half-Life (hr)
CYP1A2	36
CYP2A6	26
CYP2B6	32
CYP2C8	23
CYPC29	104
CYP2C19	26
CYP2D6	70
CYP2E1	27
CYP3A4	79
CYP3A5	36
CYP4A11	75

Adapted from PMID: 10997941

In normal subjects and patients with variegate porphyria, a single dose of heme arginate resulted in a 45-50% increase in activity of CYP2D6 and CYP3A4 within a few hours (PMID: 8033500). Similarly, a rapid increase in CYP450-mediated antipyrine metabolism was observed after intravenous infusions of heme arginate in normal women and in women suffering from acute intermittent porphyria (AIP) (PMID: 1713408).

Furthermore, study ALN-AS1-004 was conducted in subjects that are CHE. Givosiran treatment resulted in a reduction in ALAS1 mRNA levels to below normal levels (ratio relative to healthy = 0.37). This level of suppression is greater than that observed in patients with AIP with repeated monthly dosing of givosiran (ratio relative to healthy = 1.32). Given the greater reduction in ALAS1 mRNA observed in the DDI study, these results likely represent the worst-case scenario since ALAS1 mRNA levels in patients with AIP patients are expected to be >1 after givosiran dosing.

Taken together, given that 1) maximal reduction of ALA and PBG was achieved and maintained around 28 days; 2) there is no clinically significant lag time is expected between changes in ALA/PBG and changes in CYP enzyme activities; and 3) a greater level of ALAS1 mRNA reduction was achieved in the DDI study compared to that expected in the target population, the results from the dedicated DDI study ALN-AS1-004 reflect the worst-case scenario for the drug interaction potential of givosiran.

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7 Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

The efficacy and safety of givosiran was assessed in a single Phase 3 pivotal study. Additional supportive efficacy data was obtained from a single exploratory Phase 1 study as follows. Table 11 provides additional information on these studies in this review below.

- ALN-AS1-003 (ENVISION; Study 003 (NCT03338816)): Phase 3 Randomized, Double-blind, Placebo-Controlled Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias.
- ALN-AS1-001: A Phase 1, Single-Ascending Dose, Multiple-Ascending Dose, and Multi-Dose Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of Subcutaneously Administered ALN-AS1 (Givosiran) in Patients With Acute Intermittent Porphyria (AIP).

Table 11. Summary of Efficacy Studies. SOURCE FDA-generated.

Study ID	Design	Objective	Arms	Number of Patients	Patient Population	Duration of Treatment
ALN-AS1-003 (ENVISION)	Phase 3 randomized (1:1), multicenter, double-blind, placebo-controlled study to assess the efficacy and safety of givosiran relative to placebo.	The primary objective was to assess the efficacy of givosiran in AIP patients as measured by the rate of porphyria attacks that require hospitalization, urgent care visit, or in-home IV hemin administration.	1) experimental arm is 2.5 mg/kg givosiran; 2) placebo	A total of n=94 patients were randomized: 48 givosiran, 46 placebo.	Patients with documented diagnosis of AHP (AIP, HCP, VP, or ADP)	Q1M for 6 months.
ALN-AS1-001 (Part C)	Phase 1 multicenter, randomized (3:1), double-blind, placebo-controlled study to assess the safety and tolerability of givosiran. The study consists of a run-in period, a treatment period, and a follow-up period.	The primary objective was to evaluate the safety and tolerability of givosiran in chronic high excreters (CHE) AIP patients and in AIP patients who experienced recurrent attacks (Part C).	Five arms: 1) 2.5 mg/kg Q1M; 2) 2.5 mg/kg Q3M; 3) 5.0 mg/kg Q1M; 4) 5.0 mg/kg Q3M; 5) Placebo.	In part C, a total of n=17 patients were randomized: 4 to placebo; 3 to 2.5mg/kg Q1M; 3 to 2.5 mg/kg Q3M; 3 to 5.0mg/kg Q1M; 4 to 5.0mg/kg Q3M.	Patients with documented AIP who experienced recurrent acute porphyria attacks.	Patients are treated with their respective doses for a total of 12 weeks.

7.2. Review Strategy

Study ALN-AS1-003 (ENVISION (Study 003)) was the pivotal study upon which the efficacy and safety of givosiran was assessed. The efficacy and safety reviews will focus on this study. In addition, the efficacy review will also provide a brief summary of the supportive Study ALN-AS1-001.

8 Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study ALN-AS1-003 (ENVISION)

Trial Design

Study ALN-AS1-003 (ENVISION (Study 003)) was the pivotal study to establish the efficacy and safety of givosiran and upon which the application was based. It was a Phase 3, randomized (1:1), multicenter, double-blind (DB), placebo-controlled study to assess the efficacy and safety of subcutaneously administered givosiran in patients ≥ 12 years with documented diagnosis of acute intermittent porphyria (AIP) and any non-AIP acute hepatic porphyria (AHP), which includes hereditary coproporphyria (HCP), variegated porphyria (VP), and aminolevulenic acid (ALA) dehydratase deficient porphyria (ADP).

Hemin Usage. Although hemin prophylaxis was not permitted in this study, patients on hemin prophylaxis prior to enrollment were eligible if they satisfied the attack entry criterion (per inclusion criterion 4 in Table 12). Hemin usage post-randomization to treat acute attacks was permitted as clinically indicated.

Stratification. Randomization was stratified by

- AHP type: AIP versus non-AIP, the latter included HCP, VP, ADP. AIP patients were further stratified by
- Hemin prophylaxis usage at screening: yes versus no
- Historical annualized attack rate (AAR). There were 2 cut-offs, depending on hemin prophylaxis usage at the time of screening:
  - hemin prophylaxis: < 7 versus ≥ 7 in the past 12 months.
  - no hemin prophylaxis: < 12 versus ≥ 12 in the past 12 months.

Although the second stratification factor (AAR) had two thresholds, there were only two levels: Low and High.

Inclusion/Exclusion Criteria. Table 12 summarized the proposed inclusion and exclusion criteria.

Table 12. Inclusion and Exclusion Criteria for Study 003. SOURCE FDA-generated.

	Inclusion Criteria	**Exclusion Criteria**
1	Be willing and able to comply with the study requirements and to provide written informed consent per local and national requirements. In the case of patients under the age of legal consent, legal guardian must provide written informed consent and the patient should provide assent per local and national requirements and institutional standards.	Any condition (e.g., medical concern or alcohol or substance abuse), which in the opinion of the Investigator, would make the patient unsuitable for dosing or which could interfere with the study compliance, the patient's safety and/or the patient's participation in the 6-month treatment period of the study. This includes significant active and poorly controlled (unstable) cardiovascular, neurologic, gastrointestinal, endocrine, renal or psychiatric disorders unrelated to porphyria identified by key laboratory abnormalities or medical history.
2	Age $\geq$ 12 years	Any of the following laboratory parameter assessments at Screening
		- Alanine aminotransferase (ALT) > 2 × ULN
		- Total bilirubin > 1.5 × ULN. Patients with elevated total bilirubin that is secondary to documented Gilbert's syndrome are eligible if the total bilirubin is < 2 × ULN
		- International normalized ratio (INR) > 1.5 (patients on an anticoagulant [e.g., warfarin] with an INR < 3.5 will be allowed)

3	Documented diagnosis of AIP, HCP, VP, or ADP based on clinical features, at least one documented urinary or plasma PBG or ALA value $\geq 4 \times$ ULN within the past year prior to or during Screening, AND one of the following: Either documented genetic evidence of mutation in a porphyria-related gene, defined as ANY of the following: - AIP: mutation in the hydroxymethylbilane synthase gene (HMBS; also referred to as the porphobilinogen deaminase [PBGD] gene) - HCP: mutation in the coproporphyrinogen oxidase (CPOX) gene - VP: mutation in the protoporphyrinogen oxidase (PPOX) gene - ADP: mutation in the aminolevulinic acid dehydratase (ALAD) homozygous or compound heterozygous genes Or, if the results of a patient's genetic testing do not identify a mutation in a porphyria-related gene (< 5% of cases), a patient may be eligible for the study if they have both clinical features and diagnostic biochemical criteria consistent with AHP	Estimated Glomerular Filtration Rate (eGFR) < 30 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease (MDRD) formula
4	Have active disease, with at least 2 porphyria attacks requiring hospitalization, urgent healthcare visit or treatment with IV hemin at home within the 6 months prior to Screening	On an active liver transplantation waiting list, or anticipated to undergo liver transplantation during the blinded study treatment period
5	Willing to discontinue and/or not initiate use of prophylactic hemin at the time of Screening and for the duration of the study	History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to N-acetylgalactosamine (GalNAc)
6	Have adequate venous access for study sample collection as judged by the investigator	History of intolerance to subcutaneous injection

7	Be willing to comply with the contraceptive requirements during the study period	Known active HIV infection; or evidence of current or chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection
8		Currently enrolled in another investigational device or drug study, or less than 30 days or 5 half-lives (whichever is longer) since ending another investigational device or drug study, or receiving other investigational agent
9		Females who are pregnant, breast-feeding, or planning to become pregnant during the study
10		History of recurrent pancreatitis, or acute pancreatitis with disease activity within the past 12 months prior to Screening
11		Has a major surgery planned during the first 6 months of the study
12		History of serious infection within one month prior to Screening
13		Had a malignancy within 5 years prior to Screening, except for basal or squamous cell carcinoma of the skin, cervical in-situ carcinoma, or breast ductal carcinoma, that has been successfully treated

Sample Size. Study 003 was designed to test the null hypothesis that the annualized attack rates (AAR) were the same between the givosiran and placebo arms. Per the SAP, a sample size of 74 patients was sufficient to induce a 90% power under the alternative hypothesis that the AAR for the givosiran arm is 45% lower than the placebo arm. Sample size calculation was based on the negative binomial model. Additional design parameters used in the calculation included

- Placebo: AAR = 8 porphyria attacks per year (SD=5)
- Givosiran: AAR = 4.4 porphyria attacks per year (SD=3)
- two-sided $\alpha = 0.05$

NOTE Due to rapid enrollment, the actual number of AHP patients randomized was 94, 89 of whom were AIP.

Interim Analysis. Study 003 planned for an unblinded interim efficacy analysis based on the ALA endpoint, to occur when 40 AIP patients have completed at least 3 months of the DB period. The purpose was to support a submission for accelerated approval. An alpha of 0.001 was allocated for this purpose.

Objectives

Primary. The primary objective was to assess the efficacy of givosiran in AIP patients as measured by the rate of porphyria attacks that require hospitalization, urgent care visit, or in-home IV hemin administration.

Secondary. Secondary objectives included the evaluation of the effect of givosiran on

- urinary aminolevulinic acid (ALA) in patients with AIP
- urinary porphobilinogen (PBG) in patients with AIP
- hemin usage in patients with AIP
- the rate of porphyria attacks that require hospitalization, urgent care visit, or in-home IV hemin administration in patients with ***any*** AHP.
- symptoms of pain, nausea, and fatigue in patients with AIP
- the Physical Component Summary (PCS) from the Short-Form Health Survey (SF12) in patients with AIP
- the safety and tolerability in patients with AIP

Study Endpoints

Primary. The primary endpoint was the annualized rate of porphyria attacks (AAR) that require hospitalization, urgent care visit, or in-home IV hemin administration.

Secondary. Secondary endpoints included

- urinary ALA in patients with AIP at 3 months. **NOTE:** Postbaseline ALA values measured within 3 days after hemin use during the 6 month DB period are treated as missing and excluded from analysis.
- urinary ALA in patients with AIP at 6 months.
- urinary PBG in patients with AIP at 6 months. **NOTE:** Postbaseline PBG values measured within 3 days after hemin use during the 6 month DB period are treated as missing and excluded from analysis.

- annualized rate of administered hemin doses in patients with AIP over the 6-month DB treatment period
- annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP over the 6-month DB treatment period
- daily worst pain score as measured by Brief Pain Inventory-Short Form (BPI-SF) numeric rating scale (NRS) in patients with AIP over the 6-month DB treatment period
- daily worst fatigue score as measured by Brief Fatigue Inventory-Short Form (BFI-SF) NRS in patients with AIP over the 6-month DB treatment period
- daily worst nausea score as measured by NRS in patients with AIP over the 6-month DB period
- change from baseline in the Physical Component Summary (PCS) of the SF-12 in patients with AIP at 6 months

Statistical Analysis Plan

Analysis Sets. Efficacy analyses made use of the following analysis sets:

- Full Analysis Set (**FAS**) consisted of all randomized patients who received at least one dose of study drug. Cohort membership was based on randomization.
- AIP patients in FAS (**FAS_{AIP}**) consisted of FAS patients who were AIP. The primary endpoint was based on this analysis set.
- Per Protocol Set (**PPS**) consisted of all randomized AIP patients who received at least 4 doses (> 60%) of study drug during the 6 months DB period, were followed for collection of attacks through 6 months and did not experience major protocol violations. PPS was applied to only the primary efficacy endpoint.

Safety analyses were based on the Safety Analysis Set which consisted of all patients who received at least one dose of study drug; cohort membership was based on actual treatment received.

Multiplicity. Due to the planned interim analysis, which used up $\alpha_1 = 0.001$, the final analysis of the primary endpoint had access to only $\alpha_2 = 0.049$. Overall Type I error was controlled using a hierarchical fixed sequence testing procedure based on the order listed in the Study Endpoints Section.

Efficacy Analyses. AAR (primary endpoint) and days of hemin use (a secondary endpoint) were analyzed using a negative binomial count regression model where, per FDA's interpretation of the SAP, the linear predictor was described by

$$\log\lambda_i = \log(f_i) + \alpha_0 + \alpha_1 H_i + \alpha_2 R_i + \alpha_3 Z_i \qquad (1)$$

such that

- i indexes patients
- λ_i is the attack intensity for patient i
- f_i is the length of follow-up for patient i during the 6 month DB period.
- H_i is baseline hemin prophylaxis use indicator (1 for "Yes"; 0 for "No")
- R_i is the baseline historical AAR indicator (1 for "High"; 0 for "Low")

Linear mixed models (SAP, p. 25) were used to characterize the longitudinal ALA and PBG behavior. Per the SAP (p 25), the mean structure was given by

$$E(Y_{ij}; b_i, Y_{0i}, S_i, t_{ij}, Z_i) = b_i + \beta_0 + \beta_1 Y_{0i} + \beta_2 S_i + \beta_3 t_{ij} + \beta_4 Z_i + \beta_5 Z_i \times t_{ij}$$

Where

- b_i is a subject specific random effect
- Y_{0i} is baseline ALA/PBG
- S_i is a vector containing the two stratification factors (baseline hemin use and historical AAR)
- t_{ij} is the time (month) of the j-th visit
- Z_i is a treatment indicator (1 for givosiran; 0 for placebo)

Safety Assessments

Adverse events were characterized and graded according to National Institutes of Health - Common Terminology Criteria for Adverse Events (NCI-CTCAE) v 4.03 criteria. Patients were monitored according to the study schedule shown in the sponsor's table shown below through 6 months of the study. Patients were monitored at baseline, week 2 and then monthly thereafter. After the 6 month double blind treatment period patients were monitored monthly.

Table 13. ENVISION Study Schedule (6 Month Double Blind Period)

		6-Month Treatment Period							
Study Visit	Screening Period	Randomization[a]	Week 2	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6[b,c]
Study Day (±Visit Window)	Day -60 to Day -1	(Baseline) Day 1	Day 15 (±2)	Day 29 (±7)	Day 57 (±7)	Day 85 (±7)	Day 113 (±7)	Day 141 (±7)	Day 169 (±7)
Informed Consent (and Assent, if applicable)	X								
Medical History[d]	X	X							
Demographics	X								
Inclusion/Exclusion Criteria	X								
Randomization		X							
Physical Examination[e]	X	X		X	X	X			X
Body Weight, Height, and BMI[f]	X	X		X	X	X	X	X	X
FSH and Serology[g]	X								
Vital Signs[h]	X	X	X	X	X	X	X	X	X
Single 12-Lead ECG[i]	X								
Triplicate 12-Lead ECGj		X						X	X
Clinical Laboratory Assessment[k]	X	X	X	X	X	X	X	X	X
Pregnancy Test[l]	X	X		X	X	X	X	X	X
Study Drug Administration[m]		X		X	X	X	X	X	X[l]
Urine Sample for ALA and PBG[n]	X	X	X	X	X	X	X	X	X
Urine ALAS1 mRNA[n]	X		X	X	X	X			X
Samples for Exploratory Biomarkers (urine, plasma, serum)[o]		X				X			X
DNA Sample for Porphyria Genotyping[p]	X								
Exploratory DNA Sample (optional)[q]		X							
Serum ADA[r]		X		X		X			X
Blood Sample for PK[s]		X		X		X		X	X

Urine Sample for PK (patients at East Asian Study Centers only)[t]		X							X
QOL Assessments[u]	X					X			X
PGIC[v]									X
PPEQ[w]									X
Daily Electronic Diary Entries[x]	X	Daily							
Adverse Events[y]		Continuous							
Concomitant Medications		Continuous							

Abbreviations: ADA=anti-drug antibody; AE=adverse event; ALAS1=aminolevulinic acid synthase 1; ALA= aminolevulinic acid; ALT=alanine aminotransferase; BMI=body mass index; DNA=deoxyribonucleic acid; EC=ethics committee; ECG=electrocardiogram; EOS=end of study; FSH=follicle-stimulating hormone; HCV=hepatitis C virus; INR=international normalized ratio; IRB=institutional review board; LFT=liver function test(s); M=month; mRNA=messenger ribonucleic acid; PBG= porphobilinogen; PGIC=Patient Global Impression of Change; PK=pharmacokinetics; PPEQ = Porphyria Patient Experience Questionnaire; QOL=quality of life; SAE=serious adverse event; SC=subcutaneous; TBL=total bilirubin.

Notes:

- White boxes represent visits to the clinical study center; grey boxes represent study visits that may be conducted by a home healthcare professional, where applicable country and local regulations and infrastructure allow. In the event that a patient is unable to come to the study center for a scheduled clinical study visit or the procedure (s) cannot be completed during the visit, vital sign assessments, laboratory sample collection, and/or study drug administration may be conducted by a home healthcare professional, where applicable country and local regulations and infrastructure allow, after consultation with the medical monitor.
- If, in the Investigator's judgment, lab abnormalities are likely to be transient, laboratory tests can be retested. INR and other laboratory values can be retested during Screening providing the patient can be evaluated for eligibility and randomized within the allowed Screening period.
- A patient who does not meet all study eligibility criteria due to a transient condition observed at Screening (eg, prohibited medications that were subsequently discontinued, variability in ALT measurements) will be allowed to return for rescreening. A patient will be re-consented if rescreening occurs outside of the 60-day screening window. In this case, all screening procedures must be repeated.

[a] Randomization may occur on Day 1 or on the business day prior.

[b] Patients who discontinue treatment during the 6-month treatment period will be asked to complete the remainder of scheduled study visits and assessments (except for study drug administration) through Month 6, as well as a safety follow-up assessment at 3 months after the final dose of study drug (Table 3).

[c] Patients who crossed over to the OLE period prior to implementation of amendment 3 (ie, under amendment version 1 or 2) and are receiving 2.5 mg/kg once monthly will remain on that dose. Upon entry to the OLE period under amendment version 3, patients will cross over to receive a 1.25 mg/kg once monthly dose of givosiran.

[d] Medical history collected will incorporate the patient's porphyria history, including their typical attack characteristics, triggers, and treatment, as well as central venous access history, iron overload history, and prior liver disease history.

[e] A complete physical examination will be performed at Screening, Day 1, and Month 6. At all other visits, a targeted physical examination will be performed as described in Section 7.5.3. The physical examination will be performed prior to dosing.

[f] Dosing weight (in kilograms) will be collected during the clinical study center visits. Weight collected at either the previous study center visit or current study center visit may be used for dose calculations. The Screening Visit weight will be used for dose-calculation on Study Day 1. Height (in centimeters) will be measured and BMI calculated at clinic visits every 6 months (on Day 1 and Month 6). BMI will be automatically calculated within the database.

[g] Serology includes hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti-HCV antibody. FSH testing may be performed to confirm suspected post-menopausal status.

[h] Vital signs include blood pressure, heart rate, body temperature, and respiratory rate and will be measured when patients are in a seated or supine position (should be consistent across study visits with each patient) after the patient has rested for 10 min. On the Day 1 and Month 6 visit, vital signs will be collected predose and 2 hours postdose (± 10 minutes). On all other dosing days, vital signs will be collected predose.

[i] Single 12-lead ECG will be performed in the supine position after the patient has rested comfortably for 5 minutes. ECGs will be collected prior to any blood draws.

[j] Triplicate 12-lead ECGs will be performed at predose and 2-hours (± 10 minutes) post-dose on the Day 1, Month 5, and Month 6 visits, using validated ECG services equipment from a central facility; triplicate measurements should be separated by approximately 1 minute with the patient in the supine position after he or she has rested comfortably for 5 minutes. When ECG and blood sample collection (eg, for PK assessment) occur at the same time, ECG assessments should be performed before blood samples are drawn. In a prespecified subset of approximately 24 patients at prespecified study centers (including all patients at East Asian study centers [in Japan, South Korea, and Taiwan]), an additional ECG (with paired PK assessment) at 24 hours (±2 hours) post-dose will be collected at Day 1 and Month 6.

[k] Clinical laboratory parameters will include: serum chemistry including LFTs, lipase, amylase, hematology, coagulation, and urinalysis. Blood and urine samples for clinical laboratory evaluations will be collected predose. Serum ferritin assessments will be collected at Study Day 1 and Month 6. D-dimer assessments will be performed at Screening, Day 1, Month 3, and Month 6; as a clinical research parameter only, d-dimer results will not be communicated to the study centers. Results from ALT, TBL, and INR tests collected within the 14 days prior to dosing must be reviewed by the Investigator before study drug administration. Locally analyzed results may be reviewed to allow study drug administration, but additional samples for central analysis must also be collected on the day of dosing prior to dose administration.

[l] For females of childbearing potential, a serum pregnancy test will be performed at Screening; thereafter, serum or urine pregnancy tests will be performed monthly. Results must be available before dosing.

[m] At the Month 6 visit, the first open-label dose of givosiran in the extension study will be administered after all scheduled 6-month assessments (for the completion of the double-blind period) have been performed. The urine ALA and PBG sample must be collected prior to the first open-label dose of givosiran. If the ALA and PBG sample collection is delayed due to hemin usage within 4 days of the Month 6 visit, the open-label dose should be delayed as well.

[n] In patients who were on hemin prophylaxis prior to study, collection of the Screening urine samples for ALA, PBG, and ALAS1 mRNA must occur when the patient is not having an attack, and ≥4 days after prophylactic hemin discontinuation and after their last hemin dose Two screening urine samples (collected on 2 different days) should be collected for ALA/PBG; one screening urine sample should be collected for ALAS1 mRNA. During the study, spot urine samples for ALA, PBG, and ALAS1 mRNA measurement should be collected prior to study drug dosing; however, if hemin is used for an attack, scheduled urinary ALA, PBG and ALAS1 mRNA will be collected 4 days (+4 days) after the patient's last hemin dose. At the Month 6 visit, the first open-label dose of givosiran should not be administered until the Month 6 urine sample for ALA, PBG have been collected. Where applicable country and local regulations and infrastructure allow, Screening and on-study urine samples for ALA, PBG, and ALAS1 mRNA assessments may be collected by a home healthcare professional, sent to the study center by mail, or brought to the study center at the next visit.

[o] Where allowed per local regulations and IRB/EC approval, serum, plasma, and urine samples for exploratory biomarker testing will be collected prior to dosing.

[p] Genetic testing of porphyria-related genes is only required in patients without prior documented genetic testing for porphyria. See Section 7.6.2 for details.

[q] Where allowed per local regulations, IRB/IEC approval, and patient consent (and assent, where applicable), a voluntary blood sample may be collected for later DNA analysis. See Section 7.6.2 for details.

[r] Sample to be collected predose.

[s] Blood samples for PK analysis will be collected in all patients at the time points listed in Table 8. An additional 24-hour (±2 hour) PK assessment (with paired ECG measurement) will be performed on Day 1 and Month 6 in approximately 24 patients from preselected study centers. In patients at East Asian study

centers (in Japan, South Korea, or Taiwan), blood samples for PK analysis will be collected at the time points listed in Table 9 (which includes all those time points listed in Table 8).

[t] In patients at East Asian study centers, spot (predose) and pooled (postdose) urine samples will be collected for PK analysis on Day 1 and Month 6 at the time points listed in Table 10.

[u] QOL questionnaires to be completed include SF-12, EQ-5D-5L, and days of missed work/school.

[v] The PGIC assesses a patient's perceived overall health status change since the beginning of the study using a single-item scale.

[w] The PPEQ is a set of questions to assess treatment experience and impacts to the patient's life that are not collected by the other QOL assessments.

[x] During the screening period, patients and caregivers will record a minimum of 4 days of eDiary entries for pain, nausea, fatigue, and analgesic use to provide a baseline. These baseline entries must be collected on days when the patient is not experiencing a porphyria attack. During the treatment period through the Month 12 visit, eDiary entries for pain, nausea, fatigue and analgesic use will occur daily. Potential porphyria attacks will be reported in the eDiary when they occur throughout the duration of the study (from screening through the EOS visit). eDiary training will be completed at the study center at Screening. Further details on eDiary training can be found in the Study Manual.

[y] Serious adverse events (SAEs) will be collected starting at the time that informed consent is signed and through the duration of the study; AEs will be collected starting at the time the first dose of study drug is administered (Study Day 1) through the duration of the study.

Sponsor's table ENVISION study protocol

Protocol Amendments

Three amendments (summarized in Table 14) were made to the protocol.

Table 14. Protocol Amendments for Study 003. SOURCE FDA-generated, as summarized from the protocol.

Amendment	Motivation	Description
3	To generate additional efficacy data at dose 1.25 mg/kg. Effectively, patients who enter OLE after completing the 6 months double-blind period AND after the implementation of Amendment 3 are assigned to dose 1.25 mg/kg, regardless of elevated liver transaminase. **NOTE:** The 1.25 mg/kg down-titration is in response to observing liver transaminase elevation.	1) Added givosiran dose 1.25 mg/kg QM during OLE; 2) Guidance for increasing dose from 1.25 mg/kg QM to 2.5 mg/kg QM in patients who tolerate 1.25 mg/kg QM but with inadequate disease control; 3) Add statistical analysis to evaluate durability of the treatment effect. Patients who crossed over to OLE prior to A3 will be given 2.5 mg/kg QM. After 6 months of OLE, those with inadequate disease control are put on 2.5 mg/kg.
2	Reports of elevated liver transaminase	1) Require review of pre-dose LFT prior to administration of givosiran; 2) Implement standard hepatic assessment panel if patients develop significant ALT elevation; 3) Provide specific guidance for re-challenge using a lower dose in patients whose ALT resolves after study drug dosing has been withheld due to ALT elevation; 4) Expand the medical history collection to include a specific

	inquiry into iron overload and other liver disease.
1	1) Address potential for anaphylactic reaction; 2) Add two QoL measures (PGIC and PPEQ); 3) Update guidance and procedures on patient withdrawal from study; 4) Clarified that ALA/PBG levels measured during screening can be used as entry criteria.

Compliance with Good Clinical Practices

The ENVISION study was conducted under International Conference on Harmonization Good Clinical Practice Guidelines and Declaration of Helsinki Guidelines. A signed written informed consent form (ICF) was required in order to enroll into the study. An Institutional Review Board (IRB) and Independent Ethics Committee (IEC) reviewed and monitored the study.

Data Quality and Integrity

The data supporting the application were of sufficient quality.

Patient Disposition

Patient dispositions are summarized in Table 15.

Table 15. Patient Disposition for Study 003. SOURCE FDA-generated.

	Givosiran	Placebo	Overall
Full Analysis Set (AHP)	48 (100%)	46 (100%)	94 (100%)
Non-AIP	2 (4%)	3 (7%)	5 (5%)
AIP	46 (96%)	43 (93%)	89 (95%)
Number Treated (Safety Set)	48 (100%)	46 (100%)	94 (100%)
Per Protocol Set	46 (96%)	42 (91%)	88 (94%)
Completed 6 Mos Assessment	48 (100%)	46 (100%)	94 (100%)
Non-AIP	2 (4%)	3 (7%)	5 (5%)
AIP	46 (96%)	43 (93%)	89 (95%)
Met all IE Criteria	45 (94%)	43 (93%)	88 (94%)
Non-AIP	2 (4%)	3 (7%)	5 (5%)
AIP	43 (90%)	40 (87%)	83 (88%)
Treated in OL Extension	47 (98%)	46 (100%)	93 (99%)
Non-AIP	1 (2%)	3 (7%)	4 (4%)
AIP	46 (96%)	43 (93%)	89 (95%)

DB Treatment Discontinuation	1 (2%)	1 (1%)
Non-AIP	1 (2%)	1 (1%)

Protocol Violations/Deviations

Major protocol violations associated with 6 AIP patients are enumerated in Table 16.

Table 16. Major Protocol Deviations in Study 003. SOURCE FDA-generated.

Site	Country	Subject	Arm	Violation	Epoch
201	GBR	(b) (6)	Placebo	The syringe was not masked with the blinding strip prior to study drug administration during the 6-month double-blind period.	DB Period: On days 1, 43, 60, 85, 120, 148
201	GBR		Givosiran	The syringe was not masked with the blinding strip prior to study drug administration during the 6-month double-blind period.	DB Period: On days 1, 29, 57, 85, 110
201	GBR		Placebo	The syringe was not masked with the blinding strip prior to study drug administration during the 6-month double-blind period.	DB Period: On days 1, 30, 58, 88
201	GBR		Givosiran	The syringe was not masked with the blinding strip prior to study drug administration during the 6-month double-blind period.	DB Period: On days 1, 29
407	USA		Givosiran	The patient did not meet an inclusion criterion and was enrolled in the study (inclusion criterion 6: Be willing to comply with the contraceptive requirements during the study period.	Screening Period: Day 1
431	CAN		Placebo	The patient did not meet an inclusion criterion and was enrolled in the study (criterion number 3, patient didn't experience 2 porphyria attacks requiring hospitalization, urgent healthcare visit or treatment with IV	Screening Period: Day -46

	hemin at home within 6 months prior to screening.

Statistical Reviewer Comment*. The unmasking of the syringes in 4 patients is unlikely to impact the estimated number of porphyria attacks and ALA/PBG lab results and similarly for the 2 patients who did not meet the specified inclusion criterion.*

Table of Demographic Characteristics

Categorical baseline demographic characteristics, as presented in Table 17 (for AHP) and Table 18 (for AIP), were well-balanced. Continuous baseline demographic characteristics, as presented in Table 19 (for AHP) and Table 20 (for AIP), were also balanced.

Table 17. Categorical Baseline Demographic Characteristics in all Randomized AHP Patients in Study 003. SOURCE FDA-generated.

Attribute	Givosiran	Placebo	Total
Overall	48 (51%)	46 (49%)	94 (100%)
Age Group			
<38	22 (46%)	25 (54%)	47 (50%)
>=38	26 (54%)	21 (46%)	47 (50%)
Gender			
F	43 (90%)	41 (89%)	84 (89%)
M	5 (10%)	5 (11%)	10 (11%)
Race			
White	39 (81%)	34 (74%)	73 (78%)
Asian	8 (17%)	7 (15%)	15 (16%)
Black		1 (2%)	1 (1%)
Other	1 (2%)	4 (9%)	5 (5%)
Region			
North America	16 (33%)	18 (39%)	34 (36%)
Europe	23 (48%)	19 (41%)	42 (45%)
Other	9 (19%)	9 (20%)	18 (19%)
BMI			
< 25	28 (58%)	26 (57%)	54 (57%)
>= 25	20 (42%)	20 (43%)	40 (43%)

Table 18. Categorical Baseline Demographic Characteristics in all Randomized AIP Patients in Study 003. SOURCE FDA-generated.

Attribute	Givosiran	Placebo	Total
Overall	46 (52%)	43 (48%)	89 (100%)
Age Group			
<38	20 (43%)	23 (53%)	43 (48%)
>=38	26 (57%)	20 (47%)	46 (52%)
Gender			
F	41 (89%)	39 (91%)	80 (90%)
M	5 (11%)	4 (9%)	9 (10%)
Race			
White	37 (80%)	33 (77%)	70 (79%)
Asian	8 (17%)	6 (14%)	14 (16%)
Other	1 (2%)	4 (9%)	5 (6%)
Region			
North America	16 (35%)	17 (40%)	33 (37%)
Europe	22 (48%)	18 (42%)	40 (45%)
Other	8 (17%)	8 (19%)	16 (18%)
BMI			
< 25	27 (59%)	24 (56%)	51 (57%)
>= 25	19 (41%)	19 (44%)	38 (43%)

Table 19. Continuous Baseline Demographic Characteristics in all Randomized AHP Patients in Study 003. SOURCE FDA-generated.

Attribute	Givosiran	Placebo	Total
Age			
N	48	46	94
Mean (Stddev)	40.13 (12.11)	37.43 (10.5)	38.81 (11.37)
Min / Max	19 / 65	20 / 60	19 / 65
Q1 / Median / Q3 / P90	29.5 / 42 / 47.5 / 57	30 / 36 / 45 / 54	30 / 37.5 / 47 / 57
Weight (kg)			
N	48	46	94
Mean (Stddev)	65.85 (15.63)	67.88 (16.82)	66.84 (16.17)
Min / Max	39.5 / 131.3	41.5 / 115.7	39.5 / 131.3
Q1 / Median / Q3 / P90	56.75 / 64.1 / 72.3 / 84	58.8 / 65.1 / 78.2 / 91	57 / 64.95 / 73 / 84
Height (cm)			
N	48	46	94
Mean (Stddev)	164.38 (8.09)	163.26 (8.67)	163.83 (8.35)
Min / Max	149 / 190	142 / 185	142 / 190
Q1 / Median / Q3 / P90	159.5 / 164.5 / 168.5 / 172	158 / 162.5 / 170 / 172	158 / 163.5 / 169 / 172
BMI (kg/m^2)			
N	48	46	94
Mean (Stddev)	24.31 (5.15)	25.49 (6.38)	24.89 (5.78)
Min / Max	16.4 / 44.9	16.6 / 49.7	16.4 / 49.7
Q1 / Median / Q3 / P90	20.95 / 24.2 / 27.2 / 29.9	21.3 / 24.7 / 26.7 / 32.3	21.1 / 24.45 / 26.9 / 30.4
Years Since Porphyria Dx			
N	48	46	94
Mean (Stddev)	11.09 (11.18)	8.25 (8.47)	9.7 (10)
Min / Max	0.21 / 43.29	0.06 / 38.52	0.06 / 43.29
Q1 / Median / Q3 / P90	2.66 / 6.98 / 16.33 / 29.38	2.25 / 6.11 / 10.81 / 19.22	2.39 / 6.46 / 12.97 / 27.3
Age at Porphyria Dx			
N	48	46	94
Mean (Stddev)	30.08 (11.77)	30.17 (8.73)	30.13 (10.34)

Min / Max	5 / 58.07	16.88 / 51.43	5 / 58.07
Q1 / Median / Q3 / P90	23.06 / 29.54 / 39.94 / 46.09	23.92 / 28.67 / 35.01 / 45.05	23.79 / 29.25 / 35.46 / 45.05
Historical AAR			
N	48	46	94
Mean (Stddev)	12.08 (8.95)	10.65 (9.24)	11.38 (9.07)
Min / Max	4 / 34	0 / 46	0 / 46
Q1 / Median / Q3 / P90	4 / 8 / 18 / 26	4 / 7 / 14 / 22	4 / 8 / 16 / 24

Table 20. Continuous Baseline Demographic Characteristics in all Randomized AIP Patients in Study 003. SOURCE FDA-generated.

Attribute	Givosiran	Placebo	Total
Age			
N	46	43	89
Mean (Stddev)	40.67 (12.05)	37.3 (10.54)	39.04 (11.41)
Min / Max	19 / 65	20 / 60	19 / 65
Q1 / Median / Q3 / P90	30 / 43 / 48 / 57	30 / 36 / 45 / 54	30 / 38 / 47 / 57
Weight (kg)			
N	46	43	89
Mean (Stddev)	65.71 (15.91)	68.5 (16.69)	67.06 (16.26)
Min / Max	39.5 / 131.3	41.5 / 115.7	39.5 / 131.3
Q1 / Median / Q3 / P90	56.6 / 64.1 / 71.6 / 84	59.3 / 65.2 / 78.2 / 91	57.1 / 65 / 73 / 85.3
Height (cm)			
N	46	43	89
Mean (Stddev)	164.35 (8.27)	163.47 (8.69)	163.92 (8.44)
Min / Max	149 / 190	142 / 185	142 / 190
Q1 / Median / Q3 / P90	159 / 164 / 169 / 172	158 / 163 / 170 / 172	158 / 163 / 169 / 172
BMI (kg/m^2)			
N	46	43	89
Mean (Stddev)	24.27 (5.24)	25.66 (6.34)	24.94 (5.8)
Min / Max	16.4 / 44.9	17.8 / 49.7	16.4 / 49.7
Q1 / Median / Q3 / P90	20.9 / 24.2 / 27 / 29.9	21.5 / 24.7 / 26.7 / 32.3	21.2 / 24.5 / 26.7 / 30.4
Years Since Porphyria Dx			
N	46	43	89
Mean (Stddev)	11.47 (11.27)	8.44 (8.69)	10 (10.16)
Min / Max	0.21 / 43.29	0.06 / 38.52	0.06 / 43.29
Q1 / Median / Q3 / P90	2.8 / 7.18 / 17.34 / 29.38	2.25 / 6.45 / 12.75 / 19.22	2.43 / 6.64 / 13.93 / 28.62
Age at Porphyria Dx			
N	46	43	89

Mean (Stddev)	30.26 (11.99)	29.9 (8.35)	30.09 (10.33)
Min / Max	5 / 58.07	16.88 / 47.21	5 / 58.07
Q1 / Median / Q3 / P90	23 / 29.67 / 40.04 / 46.09	23.92 / 29.25 / 35.01 / 44.1	23.11 / 29.5 / 35.46 / 45.05
Historical AAR			
N	46	43	89
Mean (Stddev)	12.13 (9.09)	10.93 (9.48)	11.55 (9.24)
Min / Max	4 / 34	0 / 46	0 / 46
Q1 / Median / Q3 / P90	4 / 8 / 18 / 26	4 / 8 / 14 / 22	4 / 8 / 16 / 26

Baseline Disease Characteristics

Baseline disease characteristics, as presented in Table 21 (AHP) and Table 22 (AIP), were balanced.

Table 21. Baseline Disease Characteristics in all Randomized AHP Patients in Study 003. SOURCE FDA-generated.

Attribute	Givosiran	Placebo	Total
Overall	48 (51%)	46 (49%)	94 (100%)
Prior hemin prophylaxis			
Yes	20 (42%)	18 (39%)	38 (40%)
No	28 (58%)	28 (61%)	56 (60%)
Prior use of opioids			
Yes	14 (29%)	13 (28%)	27 (29%)
No	34 (71%)	33 (72%)	67 (71%)
Historical AAR			
Low	24 (50%)	25 (54%)	49 (52%)
High	24 (50%)	21 (46%)	45 (48%)

Table 22. Baseline Disease Characteristics in all Randomized AIP Patients in Study 003. SOURCE FDA-generated.

Attribute	Givosiran	Placebo	Total
Overall	46 (52%)	43 (48%)	89 (100%)
Prior hemin prophylaxis			
Yes	20 (43%)	17 (40%)	37 (42%)
No	26 (57%)	26 (60%)	52 (58%)
Prior use of opioids			
Yes	14 (30%)	12 (28%)	26 (29%)
No	32 (70%)	31 (72%)	63 (71%)
Historical AAR			
Low	23 (50%)	23 (53%)	46 (52%)
High	23 (50%)	20 (47%)	43 (48%)

Efficacy Results – Primary Endpoint

Over the 6 months double-blind treatment period, the estimated attack intensity in the

- Placebo arm was approximately 6.6 (95% CI: 4.5, 9.5) attacks per person, or roughly 14.3 (95% CI: 9.9, 20.7) attacks on an annualized basis.

- Givosiran arm was approximately 1.8 (95% CI: 1.2, 2.7) attacks per person, or roughly 3.9 (95% CI: 2.6, 5.8) attacks on an annualized basis.

As shown by these results, Study 003 met its primary endpoint. The efficacy data suggested that patients in the givosiran arm experienced 70% (95% CI: 53%, 84%) fewer porphyria attacks than patients in the placebo arm. In absolute terms, patients in the givosiran arm experienced approximately 4.8 (95% CI: 2.2, 7.3) fewer attacks on average within the 6 months double-blind period. On an annualized basis, patients in the givosiran arm experienced 10.4 (95% CI: 4.9, 15.9) fewer attacks per year.

Statistical Reviewer Comment*. The efficacy results shown here and in Table 23 below were based on FDA's analyses. Where the Sponsor used a count regression negative binomial model with offsets and adjusting for baseline stratification factors [hemin use and historical attack rates; see expression (1)], FDA's analyses simply compared the porphyria attack intensities between givosiran and placebo arms, without these additional modeling features.*

Subgroups. Porphyria attacks stratified by various subpopulations are presented in Table 23. The treatment effect as quantified by the rate ratio was consistent across subgroups. As noted in the previous ***Statistical Reviewer Comment***, the values displayed here were obtained based on FDA's analytical approach and therefore are slightly different from the Sponsor's. Nevertheless, both the FDA's and the Sponsor's approaches agreed with respect to the general conclusion.

Table 23. Attack Intensity in the 6 Month Double-Blind Period, Stratified by Subpopulations. SOURCE FDA analysis.

Subgroup	N	Percent	Givosiran	Placebo	Rate Ratio	L95	U95
Overall	89	100%	1.8	6.6	0.27[†]	0.16	0.47
Sex							
Female	80	90%	1.9	6.2	0.3	0.17	0.53
Male	9	10%	1.2	10.5	0.11	0.02	0.66
Age							
<38	43	48%	1.5	5.8	0.25	0.11	0.56
>=38	46	52%	2.1	7.5	0.28	0.13	0.58
Race							
White	70	79%	1.8	6.6	0.27	0.15	0.5
Asian	14	16%	1.9	8.3	0.23	0.06	0.88
Other	5	6%	2	4.3	0.47	0.02	8.93
Region							
North America	33	37%	1	5	0.2	0.08	0.5

Europe	40	45%	2.5	9.2	0.28	0.13	0.58
Other	16	18%	1.4	4.1	0.33	0.09	1.18
BMI							
< 25	51	57%	1.6	6.4	0.26	0.12	0.53
>= 25	38	43%	2.1	6.9	0.3	0.13	0.68
Prior hemin use and historical AAR							
No Hemin, Low AAR	31	35%	0.6	1.7	0.36	0.14	0.91
No Hemin, High AAR	21	24%	2.4	8.1	0.29	0.13	0.66
Hemin, Low AAR	15	17%	1	7	0.14	0.05	0.43
Hemin, High AAR	22	25%	3.3	12.7	0.26	0.12	0.56
Prior chronic opioid use							
Yes	26	29%	2.5	5.8	0.43	0.16	1.14
No	63	71%	1.5	6.9	0.22	0.11	0.42

† $p < 0.001$

Statistical Reviewer Comment*: In general, efficacy statements made about specific subgroups are viewed as exploratory as the sample size is usually small. Nevertheless, in patients with a history of no prior hemin use and a history of low attack rates, it is not clear whether such patients would benefit in a meaningful way given that on average, givosiran patients experienced approximately 1 fewer attack than placebo patients in the 6 months double blind period. The purpose of this statement is to put into perspective what a 60% reduction in attack rates actually means in a subpopulation with low attack intensity.*

Efficacy Results – Secondary and other relevant endpoints

Secondary endpoints were listed in the Study Endpoints Section.

ALA/PBG. As noted in the Statistical Analysis Plan Section, the planned analyses for ALA and PBG in AIP patients specified the use of longitudinal mixed models to characterize the average ALA and PBG over the 6 months double-blind period. Contrasts at 3 and 6 months from these models were used to quantify the difference in ALA and PBG levels. Table 24 summarized the Sponsor's results in AIP patients, as reported in the CSR (Section 11.2.1, 11.2.2).

Table 24. Differences in Average ALA and PBG in AIP Patients in the Double-Blind Period. SOURCE Excerpted from Sponsor's CSR, Table 21.

	Placebo (N=43)	Givosiran (N=46)	Difference
Month 3 Average ALA	19.9 (17.03, 22.89)	1.7 (-1.05, 4.56)	-18.2 (-22.26, -14.16)
Month 6 Average ALA	23.1 (18.09, 28.21)	4.0 (-0.69, 8.71)	-19.1 (-26.04, -12.23)
Month 6 Average PBG	49.1 (39.24, 58.97)	12.9 (3.66, 22.15)	-36.2 (-49.7, -22.69)

***Statistical Reviewer Comment**. Note that the longitudinal modeling exercise excluded the baseline value. Additionally, it is unclear that ALA and PBG were normally distributed and therefore, they should not be modeled as such; tests for normality were not likely to be informative. FDA results presented below in Table 25 and Table 26 were semiparametric where only the first and second moments were specified and estimation was performed by solving a generalized estimating equation; uncertainty was approximated by a sandwich estimator. In particular, FDA's mean structure was described by the model* $1 + Z + Visit + Z \times Visit$ *and the second moment was approximated by an independence working correlation. The baseline value was incorporated into the outcome vector. Here,* Z *was a treatment indicator (1 for givosiran and 0 for placebo) and Visit was a time factor with values 0, 0.5, 1, 2, 3, 4, 5, 6, where 0 denoted baseline. Table 25 summarized the results of FDA's analyses in AIP patients and Table 26 summarized results in AHP patients. Given the different methodological approaches between FDA and the Sponsor, it was not unexpected that the difference between ALA and PBG at 3 and 6 months, as estimated by FDA, were slightly different from the Sponsor's. Nevertheless, the general conclusion that givosiran induced a reduction in ALA and PBG over the 6 months double-blind period continued to hold.*

Table 25. Differences in Average ALA and PBG in AIP Patients in the Double-Blind Period. SOURCE FDA's analyses based on GEE.

	Placebo (N=43)	Givosiran (N=46)	Difference
Month 3 Average ALA	19.2 (15.1, 23.4)	2.0 (1.1, 2.9)	-17.2 (-21.4, -12.9)
Month 6 Average ALA	20.4 (13.9, 26.9)	4.3 (1.8, 6.8)	-16.1 (-23.0, -9.2)
Month 6 Average PBG	46.1 (33.2, 58.9)	13.2 (6.6, 19.8)	-32.8 (-47.2, -18.4)

Table 26. Differences in Average ALA and PBG in AHP Patients in the Double-Blind Period. SOURCE FDA's analyses based on GEE.

	Placebo (N=43)	Givosiran (N=46)	Difference
Month 3 Average ALA	18.6 (14.7, 22.5)	2.0 (1.1, 2.8)	-16.6 (-20.6, -12.6)
Month 6 Average ALA	19.7 (13.6, 25.7)	4.2 (1.8, 6.6)	-15.4 (-21.9, -8.9)
Month 6 Average PBG	44.9 (32.8, 56.9)	12.9 (6.5, 19.4)	-31.9 (-45.6, -18.2)

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summarized the longitudinal ALA averages over the double-blind period. Consistent with the lower number of attacks, ALA levels in the givosiran arm showed a decrease after treatment initiation with some degree of maintenance[2].

[2] Note that this statement is approximate in the sense that there is no pre-defined criteria for defining what constitutes maintenance.

Figure 11. Average ALA Over the Double-Blind Period Relative to Individual ALA Contours in Patients in the Placebo Arm. SOURCE FDA's analysis.

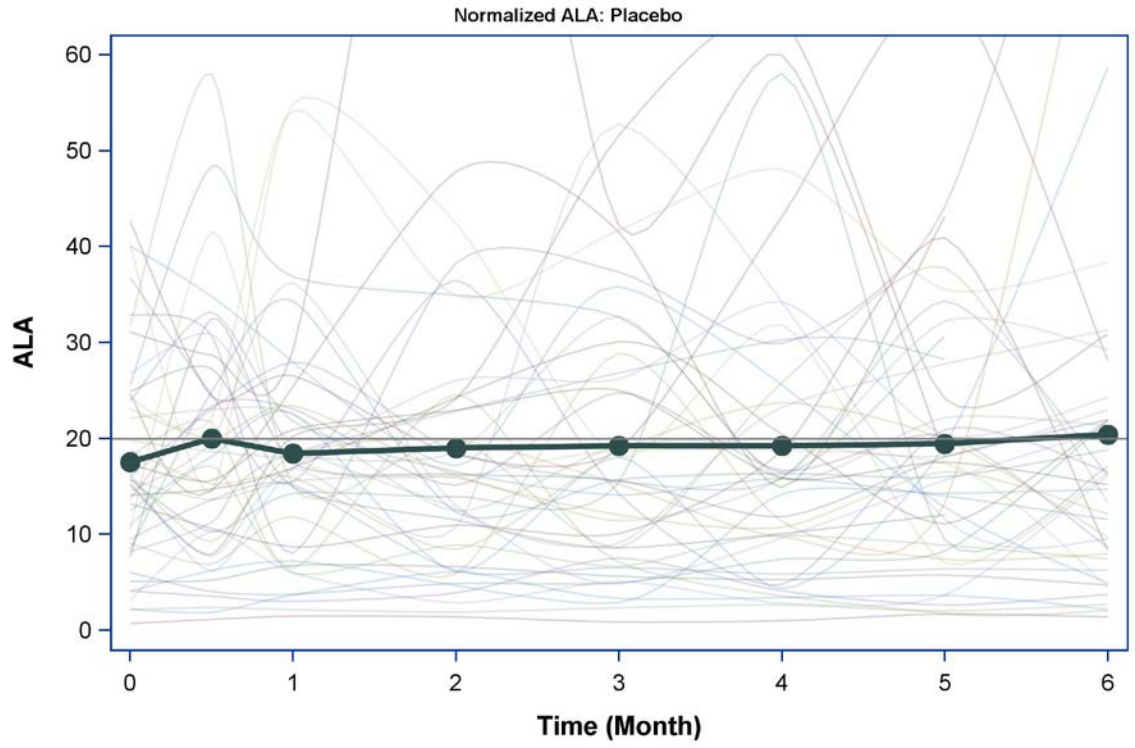


Figure 12. Average ALA Over the Double-Blind Period Relative to Individual ALA Contours in Patients in the Givosiran Arm. SOURCE FDA's analysis

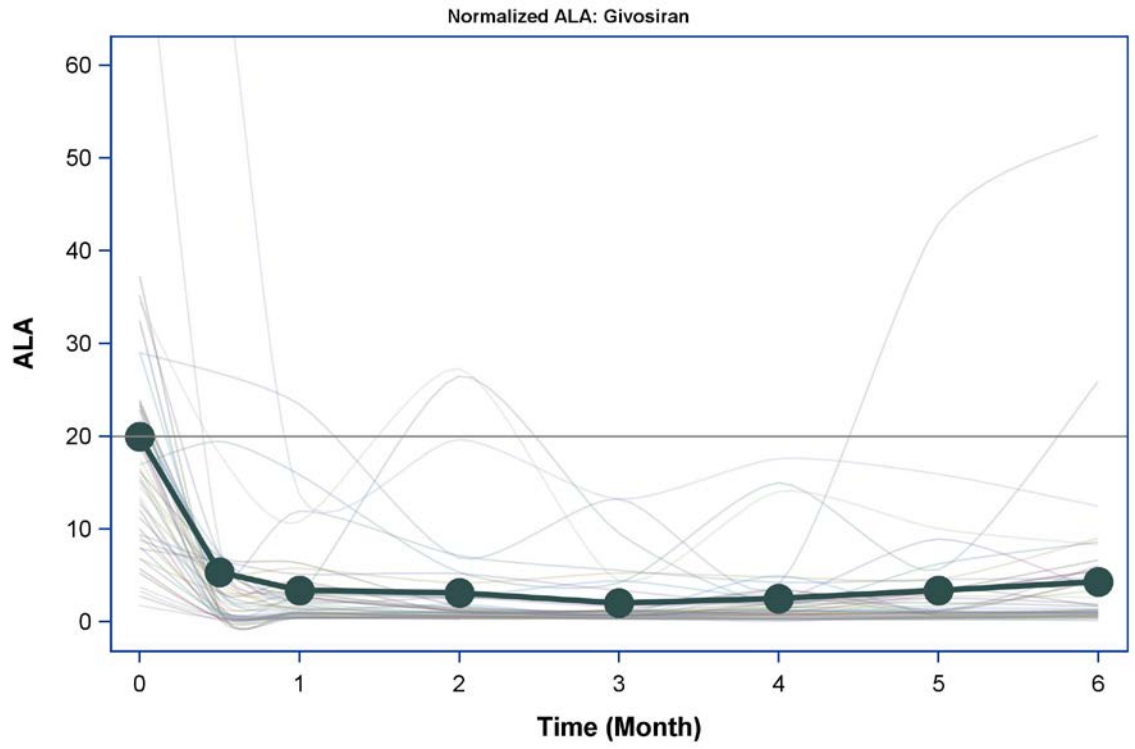


Figure 13 and Figure 14 summarized the longitudinal PBG averages over the double-blind period. Consistent with the lower number of attacks, PBG levels in the givosiran arm showed a decrease after treatment initiation with some degree of maintenance.

Figure 13. Average PBG Over the Double-Blind Period Relative to Individual PBG Contours in Patients in the Placebo Arm. SOURCE FDA's analysis.

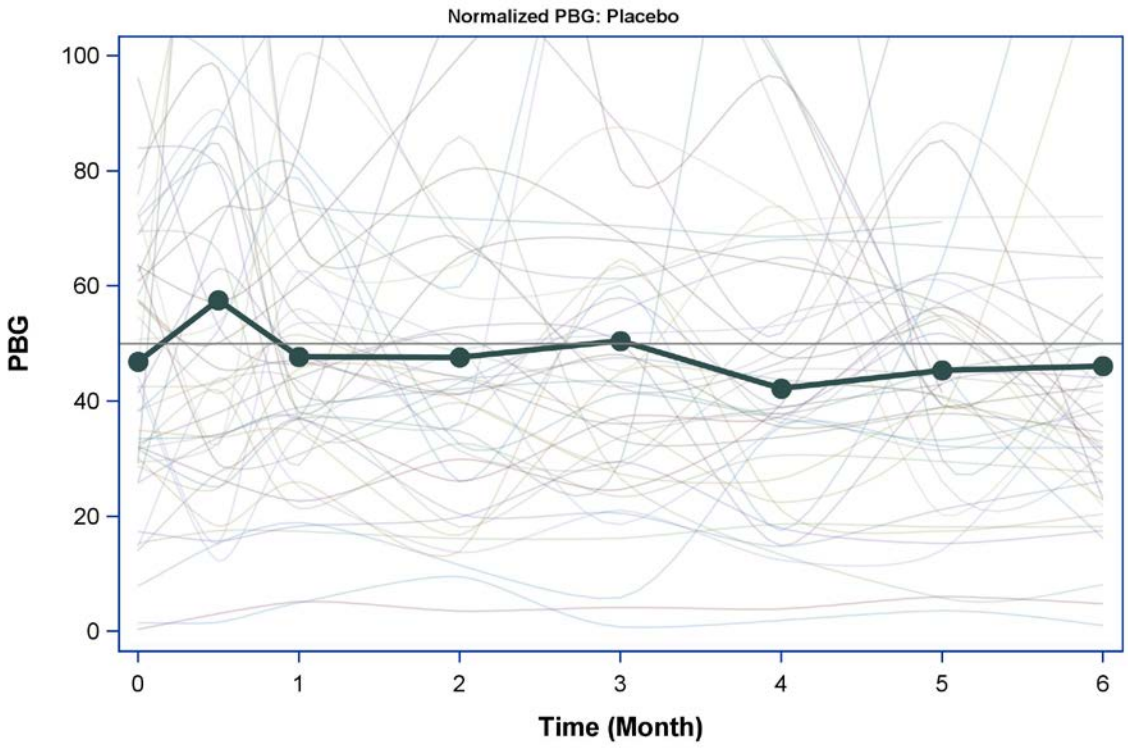
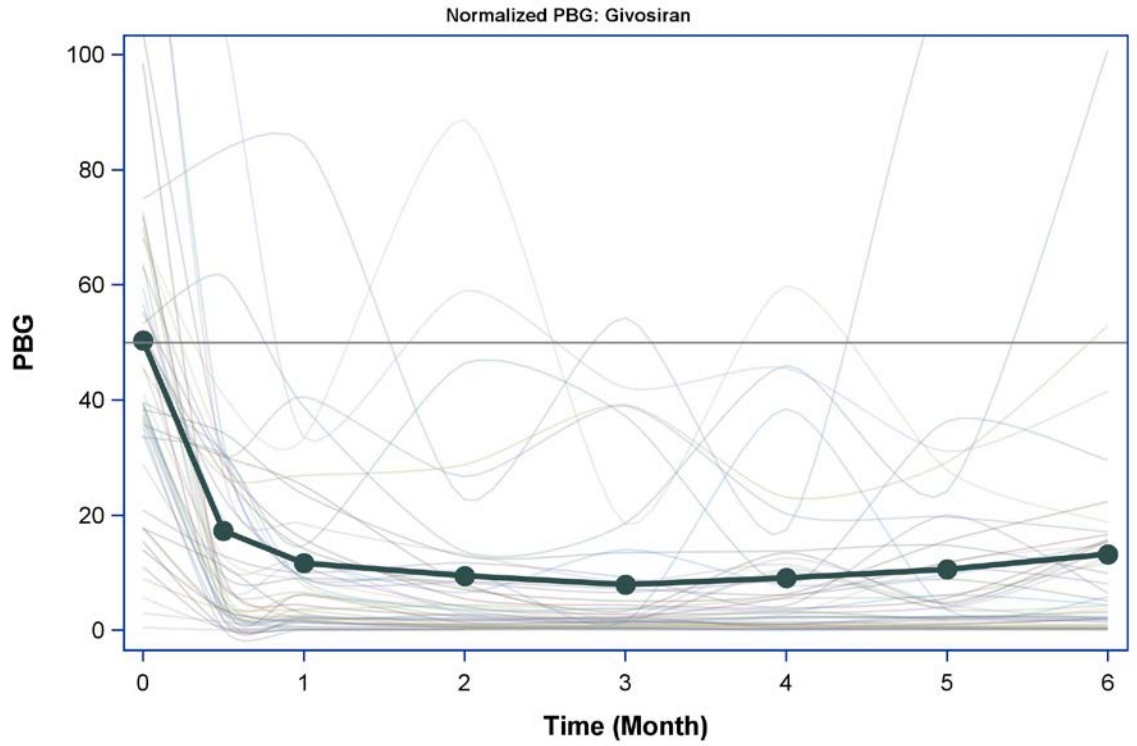


Figure 14. Average PBG Over the Double-Blind Period Relative to Individual PBG Contours in Patients in the Givosiran Arm. SOURCE FDA's analysis.



Days of Hemin Use. With respect to the number of days of hemin use in AIP patients over the 6 months double-blind treatment period, the estimated average days of hemin administrations in the

- Placebo arm was approximately 13.6 (95% CI: 8.1, 22.6) days, or 29.4 (95% CI: 17.6, 49.1) days of hemin use on an annualized basis.
- Givosiran arm was approximately 4.2 (95% CI: 2.5, 7.0) days, or 9.1 (95% CI: 5.5, 15.2) days of hemin use on an annualized basis.

These data suggested that, on average, patients on givosiran require 70% (95% CI: 36%, 85%) fewer days of hemin administrations within the 6 months double-blind period. In absolute terms, this was approximately 9.3 (95% CI: 2.1, 16.5) fewer days of hemin administrations or 20.2 (95% CI: 4.4, 39.9) days on an annualized basis.

The results for AHP patients were similar; the estimated average days of hemin administration in the

- Placebo arm was approximately 12.7 (95% CI: 7.6, 21.3) days, or 27.7 (95% CI: 16.5, 46.4) days of hemin use on an annualized basis.

- Givosiran arm was approximately 4.7 (95% CI: 2.8, 7.9) days, or 10.2 (95% CI: 6.1, 17.1) days of hemin use on an annualized basis.

These data suggested that, on average, patients on givosiran require 60% (95% CI: 23%, 82%) fewer days of hemin administrations within the 6 months double-blind period. In absolute terms, this was approximately 8.0 (95% CI: 1.0, 15.0) fewer days of hemin administrations; or approximately 17.4 (95% CI: 2.2, 32.6) days on an annualized basis.

Porphyria Attacks in AHP Patients. In AHP patients, the estimated attack intensity over the 6 months double-blind treatment period in the

- Placebo arm was approximately 6.5 (95% CI: 4.5, 9.3) attacks per person, or roughly 14.0 (95% CI: 9.7, 20.1) attacks on an annualized basis.
- Givosiran arm was approximately 1.9 (95% CI: 1.3, 2.8) attacks per person, or roughly 4.0 (95% CI: 2.7, 6.0) attacks on an annualized basis.

The data suggested that patients in the givosiran arm experienced an attack intensity that was approximately 70% (95% CI: 50%, 83%) lower than patients in the placebo arm. In absolute terms, patients in the givosiran arm experienced approximately 4.6 (95% CI: 2.1, 7.0) fewer attacks on average within the 6 months double-blind period. On an annualized basis, patients in the givosiran arm experienced approximately 10 (95% CI: 4.6, 15.3) fewer attacks per year.

Daily Worst Pain Score. With respect to daily worst pain score as captured by the Brief Pain Inventory-Short Form (BPI-SF) numeric rating scale (NRS), the data did not suggest a givosiran advantage over placebo, at least in the statistical sense as $p = 0.053$ (based on the pre-specified analysis method outlined in the SAP).

Statistical Reviewer Comment*: As noted above, givosiran did not demonstrate a worst pain score advantage over placebo, at least in the statistical sense. This suggested an incompatibility between observed reduction in porphyria attacks (with statistical significance) and observed reduction in worst pain scores (without statistical significance). While this is a convenient interpretation, it was not clear whether this was actually the case. There were some notable limitations to this analysis.*

First, while AUC has been used extensively in the context of studying drug activity and PK/PD properties, it lacked an intuitive clinical interpretation. For weekly change from baseline, the AUC over the 6 months can be viewed as being proportional to a difference between an average of the post-baseline pain scores and the baseline pain score; for the i-th patient, $AUC_i = \left(\sum_{j=1}^{24} w_j Y_{ij}\right) - w_0 Y_{i0}$*. The interpretation of the weights were not obvious; they simply arose as a by product of computing the area under the curve. Thus, it was not clear that the AUC characterization of worst pain scores can sufficiently summarize the pain contours over time; the lack of statistical significance may simply be a reflection of this. Note this problem of AUC interpretation would still persist even if there were statistical significance and regardless of*

analytical methodology.

Second, it is possible that BPI-SF cannot adequately characterize pain in the context of this disease population and in the context of the longitudinal follow-up specific to this study. This can be viewed as a problem of using an instrument that does not have the appropriate assay sensitivity.

Third, although the study was enriched with patients with at least 2 attacks that require hospitalization, urgent care visit, or IV hemin administration at home, within the 6 months prior to Screening, it was possible that for patients with no prior history of hemin use and having a history of "Low" porphyria attacks, there really was no difference in pain scores, not just in the statistical sense but also in the clinical sense. Although the study was not large enough to provide this level of granular information, this view was not incompatible with the subgroup analysis provided at the bottom of the Efficacy Results: Primary Endpoint Section; there we observed an estimated average of 1 fewer attack between givosiran and placebo in patients with no prior history of hemin use and low historical AAR.

For all of these reasons, the statistical conclusion reached for this endpoint was simply that the data were not capable of supporting a givosiran advantage over placebo with respect to worst pain scores over the 6 months double-blind period.

Other Secondary Endpoints. Given that worst pain scores failed statistical significance, all subsequent secondary endpoints were viewed as exploratory endpoints.

Durability of Response

Not applicable as the primary endpoint is not response. However, to the extent that givosiran acts to reduce ALA and PBG, the data suggested that average levels of ALA and PBG declined as early as 15 days after treatment and remain below baseline for the duration of the 6 months double-blind period.

Persistence of Effect

See Durability of Response.

Additional Analyses Conducted on the Individual Trial

There were no additional efficacy analyses other than those discussed above.

Clinical Reviewer comment for section 8.1: *The objectives, design, efficacy, endpoint, ethical, safety assessment and statistical considerations for the ENVISION study are acceptable from a clinical perspective. The study is well designed to evaluate the benefit and risk of givosiran for the treatment of adult patients with AHP. The treatment arms are balanced in terms of key enrollment criteria, i.e., mean age (40 years (SD 12 years) in the givosiran arm compared to 37*

years (SD 11 years) in the placebo arm), sex (43/48 (90%) female patients in the givosiran arm compared to 41/46 (89%) female patients in the placebo arm), mean historical annualized attack rate (12 attacks/year (SD 9 attacks/year) in the givosiran arm compared to 11 attacks/year (SD 9 attacks/year) in the placebo arm) and prior hemin prophylaxis (20/48 (42%) in the givosiran arm compared to 18/46 (39%) in the placebo arm). The efficacy of givosiran over placebo is demonstrated by a decrease in the attack rate overserved among patients with AHP who were treated with givosiran compared to those who received placebo. Among patients with AHP in the ENVISION study the estimated attack rate over the 6 month double-blind treatment period was 2 attacks (95% CI: 1, 3 attacks) per patient in the givosiran arm compared to 14 attacks (95% CI: 10, 20 attacks) per patient in the placebo arm. Urinary ALA and PBG levels also decreased and then were maintained during the givosiran treatment period compared to placebo. I agree with the Statistical Reviewer's comments that Patient Report Outcomes (PRO) data from the ENVISION study was not capable of supporting a givosiran advantage over placebo with respect to worst pain scores over the 6 months double-blind period.

8.1.3 ALN-AS1-001 (Part C)

Trial Design

Data from Part C of Study ALA-AS1-001 provided additional supporting information about efficacy. The Agency considered this study as exploratory. For completeness, an overview of the study design and a general summary of the results are provided below.

Part C was a multi-dose randomized study to examine safety, tolerability, PD, clinical activity, and PK of givosiran in AIP patients with recurrent attacks. The following arms were considered:

- 2.5 mg/kg Q3M
- 5.0 mg/kg Q3M
- 2.5 mg/kg Q1M
- 5.0 mg/kg Q1M
- Placebo

Part C contained the following periods.

- **Run-In Period**. The run-in period was approximately 4 to 24 weeks (168 days). Patients with at least 1 attack (intense abdominal or back pain that require hospitalization, hemin use, or treatment consisting of increased carbohydrate intake and/or administration of pain medication [opioid and non-opioid]) in this run-in period were randomized in the treatment period (see below).
- **Treatment Period (TP)**. Patients were randomized 3:1 to receive givosiran SC doses or placebo. Patients randomized to givosiran were dosed over 12 weeks (84 days), starting from day 0.

- **Follow-up Period (FP)**. After the 12 weeks Treatment Period, patients were followed for approximately 12 weeks after last dose. The follow-up period provided for continued assessment of safety, tolerability, PK/PD, and clinical activity.

The combined TP+FP period spanned approximately 168 days (6 months).

Objectives

Primary The primary objective of Study 001 was to evaluate the safety and tolerability of givosiran in chronic high excreter (CHE) AIP patients and in AIP patients who experienced recurrent attacks (Part C).

Secondary Additional objectives were

- To characterize the pharmacokinetics (PK) of givosiran in AIP patients who were CHE and in AIP patients who experienced recurrent attacks.
- To assess the PD effects of givosiran on plasma and urine levels of delta aminolevulinic acid (ALA) and porphobilinogen (PBG) in AIP patients who were CHE and in AIP patients who experienced recurrent attacks.

Study Endpoints

Exploratory clinical activity endpoints for Part C included
- number of porphyria attacks
- number of hemin doses administered

Pharmacodynamic endpoints such as urinary ALA and PBG were also assessed.

8.1.4 Study Results

Patient Disposition

The following is a summary of patient disposition in Part C:

- **Patients Randomized**
  - Placebo: 4
  - Givosiran: 13 (N=3 for 2.5 mg/kg Q3M; N=4 for 5.0 mg/kg Q3M; N=3 for 2.5 mg/kg QM; N=3 for 5.0 mg/kg QM)
- **Patients Treated**
  - Placebo: 4
  - Givosiran: 13

- **Patients Who Completed**
  - Placebo: 4
  - Givosiran: 12 (1 patient in 5.0 mg/kg QM withdrew early)

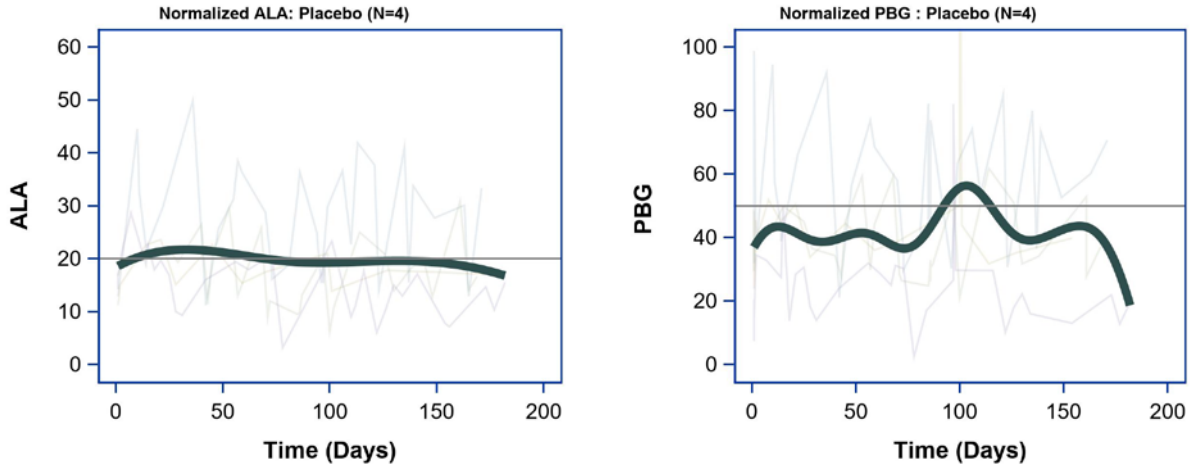
In general, most randomized patients completed the study.

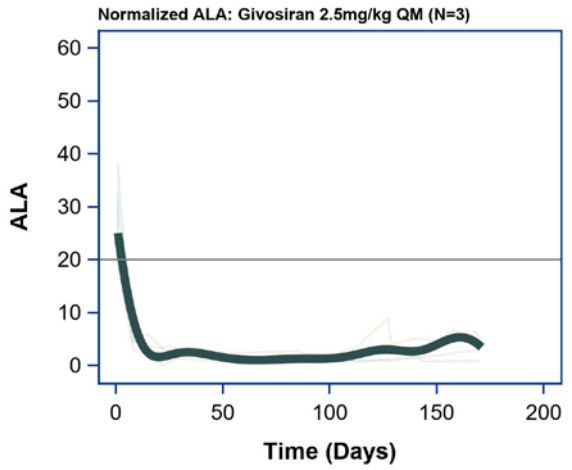
Results Pertaining to ALA, PBG

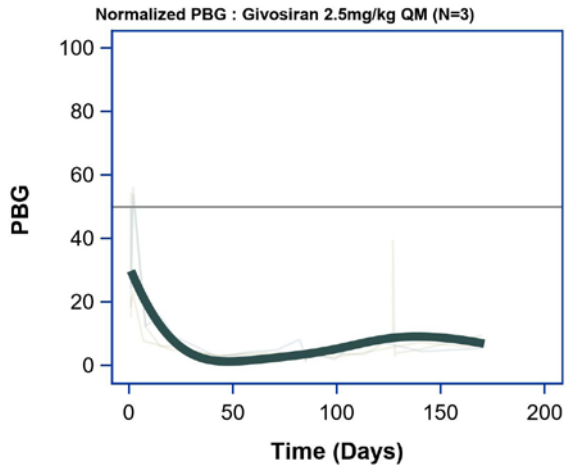
As observed in **Error! Reference source not found.** below, the lowering of ALA and PBG levels in patients exposed to givosiran resembled those in the pivotal 003 study, although the quarterly administration of both the 2.5 mg/kg and 5.0 mg/kg appeared less impactful at lowering ALA and PBG as compared to the monthly administration.

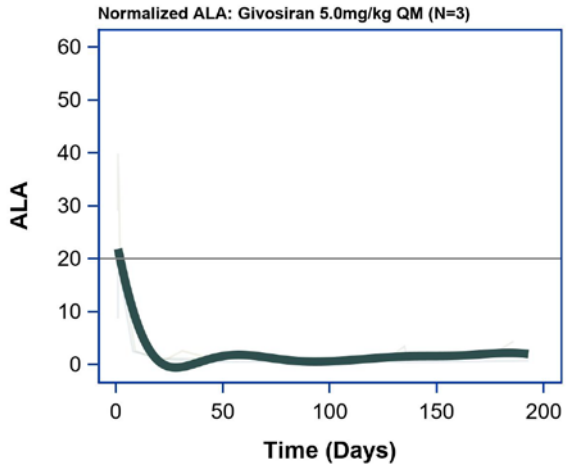
Figure 15. Creatine Normalized Urine ALA and PBG Levels Over Time in Study 001. SOURCE FDA's analyses.

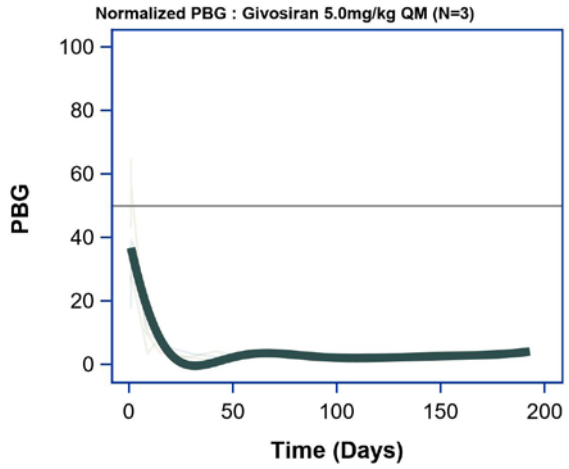
ALA **PBG**

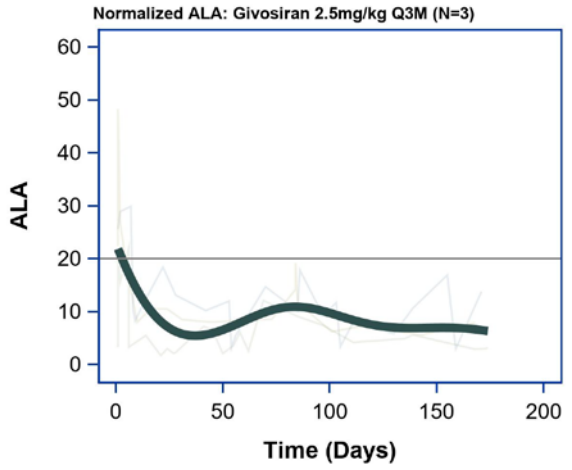


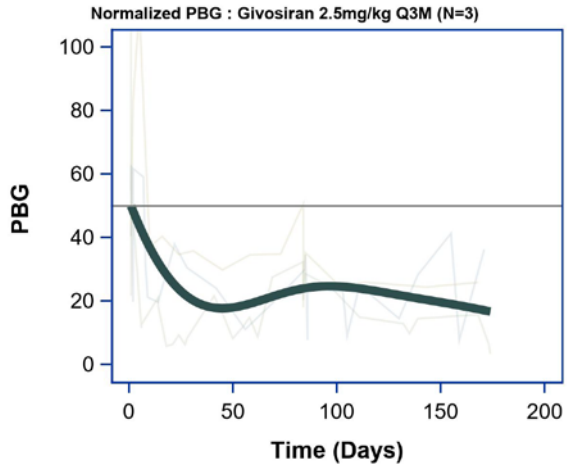

Normalized ALA: Givosiran 2.5mg/kg QM (N=3)
ALA
Time (Days)

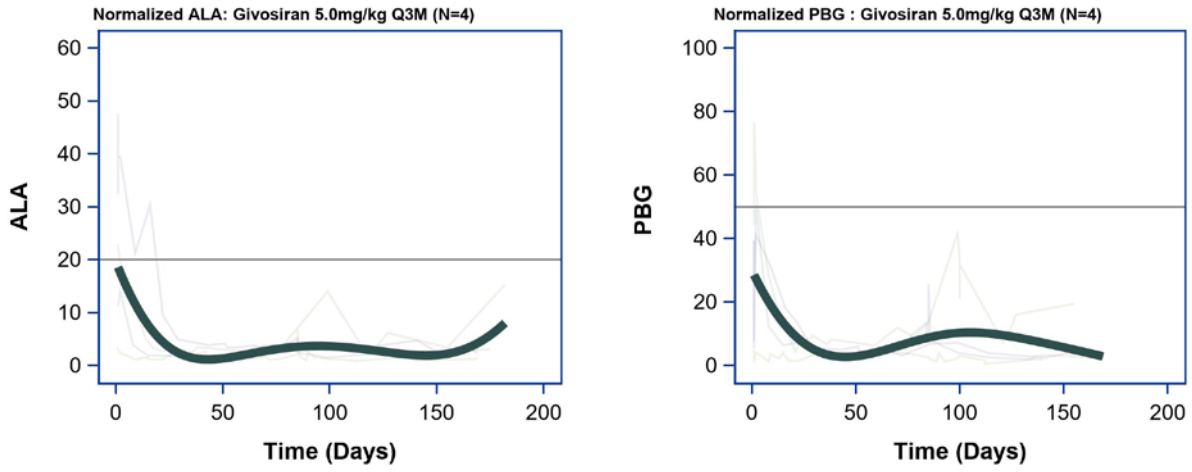

Normalized PBG : Givosiran 2.5mg/kg QM (N=3)
PBG
Time (Days)


Normalized ALA: Givosiran 5.0mg/kg QM (N=3)
ALA
Time (Days)


Normalized PBG : Givosiran 5.0mg/kg QM (N=3)
PBG
Time (Days)


Normalized ALA: Givosiran 2.5mg/kg Q3M (N=3)
ALA
Time (Days)


Normalized PBG : Givosiran 2.5mg/kg Q3M (N=3)
PBG
Time (Days)



Results Pertaining to Porphyria Attacks

The CSR (pg. 114) for Study 001 also reported a lower annualized attack rate in patients exposed to givosiran (N=13) compared to placebo (N=4):

- 16.7 per year for placebo
- 7.2 per year for givosiran

a difference of about 10 attacks per year.

NOTE: To the extent that Study 001 was small and was not capable of providing robust statements about efficacy, the results pertaining to ALA, PBG, and porphyria attack rates were in the same direction as the efficacy conclusions observed in pivotal Study 003.

8.1.5 Integrated Review of Effectiveness

Not applicable. The single pivotal ENVISION study supports the proposed indication for givosiran for the treatment of adult patients with AHP.

8.2 Review of Safety

8.2.1 Safety Review Approach

The safety database for givosiran for the treatment of adult patients with AHP consists of data obtained from the single pivotal ENVISION study (n=94 patients of which n=48 patients in the givosiran treatment arm and n=46 patients in the placebo treatment arm).

8.2.2 Review of the Safety Database

Overall Exposure

All 94 patients enrolled in the ENVISION study completed the double-blind 6 month assessment (n=48 patients in the givosiran treatment arm and n=46 patients in the placebo treatment arm). Patients received givosiran for a median of 6 months (range 3-6 months) and placebo for a median of 6 months (range 5-6 months). Of these patients, 47 patients received ≥5 months of treatment.

One patient was discontinued from the study prematurely due to elevated liver transaminases. The patient had received three doses of givosiran during the double blind treatment period and was not enrolled in the open label extension portion of the study. The case is described below.

- Patient (b) (6) withdrew from the study after completion of the 6 month double blind treatment period. This patient was a female age (b) (6) years with a diagnosis of VP and an associated past medical history of elevated serum hepatic enzymes. Three years after diagnosis of the disease the patient was enrolled in the ENVISION study. Prior to enrollment the patient's serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were mildly elevated at 57 U/L (1.3× upper limit of normal (ULN)) and 46 U/L (1.2×ULN), respectively. There is no available report for the patient's serum total bilirubin (TBili) prior to enrollment. At the time of enrollment in the ENVISION study the patient's serum ALT, AST and TBili were within normal limits. The patient was treated with givosiran 2.5mg/kg administered subcutaneously (SC) once monthly for a period of three months. The patient's serum ALT and ALT increased after the third dose of study drug (ALT 172 U/L (4.2xULN) and AST 95 U/L (2.8xULN)) and the serum TBili was within normal limits. Givosiran therapy was discontinued. Clinical laboratory evaluation did not reveal other potential causes of the liver enzyme elevation, e.g., viral infection, fibrosis or other disease process. Prior to the third dose of study drug the patient stated that she had consumed a large quantity of health food supplement containing Peruvian ginseng, omega 3 fatty acids, magnesium, vitamin B12 and vitamin D. There is no report if the patient also discontinued consumption of the health food supplements. After three months of follow-up the patient's serum transaminases returned to normal limits. The patient was not enrolled in the open label portion of the ENVISION study.

8.2.3 Safety Results

Deaths

No deaths were reported in the ENVISION study

Serious Adverse Events

Few serious adverse events (SAEs) in either treatment arm were reported in the study. There

were 10/48 (21%) patients in givosiran arm and 4/46 (9%) patients in the placebo arm that reported serious adverse events. Of the SAEs Only device related infection (2/48 (4%) patients in the givosiran arm compared to 1/46 (2%) patient in the placebo arm) and chronic renal insufficiency (0/48 (0%) patients in the givosiran arm compared to 2/26 (4%) patients in the placebo arm) were reported in at least two or more patients in either treatment arm during the study.

Discontinuations Due to Adverse Effects

One patient was prematurely discontinued from the study. The case is discussed in detail in section 8.2.2 Review of the Safety Database in this review above.

Significant Adverse Events

The most frequently occurring (≥20% incidence) adverse events (AEs) reported in patients treated with givosiran were nausea (13/48 (27%)) and injection site reactions (12/48 (25%)). Among the 12 patients with injection site reactions the highest severity of the reaction was reported to be mild among 11/12 (92%) patients and moderate in 1/12 (8%) patient. There was 1/48 (2%) patient who reported recall injection site reaction consisting of erythema at a prior injection site after subsequent givosiran dose administration. The reviewer's table below shows the AEs that were reported ≥ 5% more frequently among patients in the givosiran arm.

Table 27. AEs Reported in ≥ 5% More Patients in the Givosiran Arm Compared to the Placebo Arm

Adverse Event	Givosiran N=48 (n, %)	Placebo N=46 (n, %)
Nausea	13 (27%)	5 (11%)
Injection Site Reaction	12 (25%)	0 (0%)
Rash*	8 (17%)	2(4%)
Increased Serum Creatinine#	7 (15%)	2 (4%)
Increased Serum Hepatic Transaminases	6 (13%)	1 (2%)
Fatigue	5 (10%)	2 (4%)

*Term includes pruritus, eczema, erythema, rash, rash pruritic, urticaria; #Term includes blood creatinine increased, glomerular filtration rate decreased, chronic kidney disease (decreased eGFR, MDRD equation)
Reviewer's table derived from ENVISION CSR and Integrated Summary of Safety (ISS)

Laboratory Findings

Overall, no significant differences were observed among treatment groups regarding clinical laboratory test abnormalities in the ENVISION study. There were 7/48 (15%) patients in the givosiran treatment arm that had a renal AE (increased serum creatinine, decreased eGFR, etc.) Overall, among patients treated with givosiran the median change eGFR (evaluated using the

Modification of Diet in Renal Disease Study (MDRD) equation) or serum creatinine over six months of therapy was low. For example, the median change eGFR over six months of therapy with givosiran was 8%. The reviewer's table below shows the median serum creatinine and eGFR at baseline and the median serum creatinine and eGFR after six months of givosiran therapy.

Table 28. Change in Serum Creatinine and EGFR*

	Givosiran N=48 N (%)	**Placebo N=46 N (%)**
Median (Range) Serum Creatinine at Baseline (µmol/L)	82 (53-194)	83 (45-184)
Median (Range) Serum Creatinine after 6 months Therapy (µmol/L)	82 (47-241)	80 (41-178)
Change (Range) from Baseline (%)	7% (-19, 49%)	0% (-41, 38%)
Median eGFR (Range) at Baseline	70 (31, 124)	67 (26, 151)
Median eGFR (Range) after 6 months Therapy	68 (24, 141)	71 (27, 166)
Change (Range) from Baseline (%)#	-8% (-36, 29%)	0% (-32, 86%)

*EGFR (ml/min/1.73m^2) evaluated using the Modification of Diet in Renal Disease Study (MDRD) equation; #Negative % change represents decrease in eGFR; SD=Standard deviation.
Reviewer's table derived from ENVISION CSR

Vital Signs/Electrocardiograms (ECGs)

Overall, no significant changes in vital signs or electrocardiograms (ECGs) were reported in the safety database among patients treated with givosiran.

QT

Dr. Nan Zheng (Clinical Reviewer in the Interdisciplinary Review Team for QT studies (QT-IRT)) states in his review (final signature date August 8, 2019) that the effect of givosiran on potential QT prolongation was evaluated in the ENVISION study and that no large QTc prolongation effect (i.e., >20 ms) of givosiran was observed in this QT assessment.

Immunogenicity

Overall, in the clinical development program for givosiran the sponsor stated that 1/111 (1%)

patient had an anaphylactic reaction. The case is discussed below. No patients in the ENVISION study were reported to have an anaphylactic reaction.

- Patient (b) (6), was a female age (b) (6) years with a history of AIP and a past medical history of multiple hypersensitivities including: allergic asthma, food allergies, atopic dermatitis and facial edema following latex exposure. The patient was enrolled in study ALN-AS1-002 titled, "A Multicenter, Open-label Extension Study to Evaluate the Long-term Safety and Clinical Activity of Subcutaneously Administered Givosiran in Patients with Acute Intermittent Porphyria who have Completed a Previous Clinical Study with ALN-AS1". This was a phase 1/2 multicenter, open-label extension study to evaluate the long-term safety and clinical activity of givosiran in patients with AIP who completed Part C of Study ALN-AS1-001. Study ALN-AS1-001 is discussed in detail in section 8.1.3 ALN-AS1-001 (Part C) in this review above. The patient had a SAE of anaphylactic reaction after the first dose of givosiran 2.5 mg/kg administered SC on study Day 1 of in study ALN-AS1-002. Within 3 minutes of givosiran administration the patient developed urticaria at the injection site that extended to the limbs, reported facial swelling and hypotension. There were no symptoms of airway compromise reported. The patient was treated with epinephrine, intravenous (IV) fluid, corticosteroids and antihistamines. The event was reported to have resolved the same day. The patient was discharged from the hospital on the same day. Anti-drug antibody clinical laboratory evaluation (IgM, IgG and IgE) was reported to be negative. The event was considered by the investigator to be definitely related to givosiran. The patient previously received 2 doses of givosiran 2.5 mg/kg (total 5.0 mg/kg) administered SC approximately 3 months apart in study ALN-AS1-001. There was approximately a 4 month interval between the last dose of givosiran administered in study ALN-AS1-001 and her dose of givosiran administered on study ALN-AS1-002. Study drug was discontinued by the investigator and the patient withdrew from the study.

In the ENVISION study 1/48 (2%) patient with AHP developed anti-drug antibodies (ADA) during treatment with givosiran. The case is discussed below.

- Patient (b) (6) was a female age (b) (6) years with a history of AHP. She had no other significant past medical history and was enrolled in the ENVISION study. The patient was treated with placebo. The patient received 6 doses of placebo uneventfully. Baseline and periodic clinical laboratory testing for ADA per protocol was negative. The patient was enrolled in the open label extension portion of the study and was treated with givosiran 1.25mg/kg administered SC once monthly. After the first dose of givosiran the patient tested positive for givosiran ADA. The patient had a low ADA titer (reported to be 50 U). No serious adverse events (SAEs), anaphylactic reactions, hypersensitivity or injection site reactions were reported for this patient. During placebo treatment the patient's urinary ALA level ranged from 9nmol/mL to 58nmoL/mL. After givosiran therapy the patient's urinary ALA level was 4nmol/mL. The patient was discontinued from the study after having received one dose of givosiran.

8.2.5 Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

Patient reported outcomes (PRO) data obtained in the ENVISION study (ALN-AS1-003) were prespecified as exploratory endpoints. See the statistical review of PRO under section 8.1 Review of Relevant Individual Trials Used to Support Efficacy. The Clinical Reviewer agrees with the Statistical Reviewer's comment that the Clinical Outcomes Assessment (COA) data from the ENVISION study was not capable of supporting a givosiran advantage over placebo with respect to worst pain scores over the 6 months double-blind period.

8.2.6 Additional Safety Explorations

Pediatrics Safety

No pediatric patients were enrolled in the ENVISION study or in the broader givosiran clinical development program.

Overdose and Drug Abuse Potential

Givosiran 2.5mg/kg is administered SC once monthly. The proposed givosiran product label recommends that medical support is available to appropriately manage anaphylactic reactions when administering givosiran. There is no abuse potential for givosiran.

Safety Concerns Identified Through Postmarket Experience

Currently givosiran is not marketed anywhere in the world.

Clinical Reviewers comment for section 8.2: *All 94 patients enrolled in the ENVISION study completed the double blind 6 month assessment (n=48 patients in the givosiran treatment arm and n=46 patients in the placebo treatment arm). Patients received givosiran for a median of 6 months (range 3-6 months) and placebo for a median of 6 months (range 5-6 months). Of these patients, 47 patients received ≥5 months of treatment. One patient was discontinued from the study prematurely due to elevated liver transaminases after the third dose of givosiran (ALT 172 U/L (4.2xULN) and AST 95 U/L (2.8xULN)) and the serum TBili was within normal limits). Generally, treatment with givosiran appears to be tolerable. Although a higher proportion of patients in the givosiran arm (10/48 (21%) patients) compared to the placebo arm (4/46 (9%) patients) reported SAEs, only device-related infection (2/48 (4%) patients in the givosiran arm compared to 1/46 (2%) patient in the placebo arm) was reported in at least two or more patients in the study. The most frequently occurring (≥20% incidence) adverse events (AEs) reported in patients treated with givosiran were nausea (27%) and injection site reactions (25%). Overall in the clinical development program for givosiran (n=111 patients) anaphylactic reaction was reported in 1 patient (1%). In the ENVISION study 1/48 (2%) patient with AHP developed anti-drug antibodies (ADA) during treatment with givosiran. Therefore, key AEs identified in the safety review of data from the ENVISION study were an increased risk for injection site reaction, renal toxicity (decreased eGFR), hepatotoxicity (elevated transaminases)*

and anaphylaxis.

8.3 Statistical Issues

There were no major statistical issues that have the potential to invalidate the efficacy of givosiran.

8.4 Conclusions and Recommendations

The benefit-risk analysis favors the approval of givosiran for the treatment of adult patients with AHP. Acute hepatic porphyria (AHP) is a rare disease with a prevalence of 5-10 cases/100,000 people in the US and affects primarily females (age range 15-45 years). AHP occurs as a result of an autosomal dominant mutation that leads to deficiency of aminolevulinic acid dehydratase and porphobilinogen deaminase which are enzymes in the heme biosynthesis pathway. The rate limiting step in heme synthesis is the enzyme aminolevulinic acid synthase 1 (ALAS1) which is controlled by feedback repression via the end-product heme. ALAS1 is induced in response to a decrease in the endogenous heme pool in the liver which can occur with stressors such as: fasting, hormonal alterations or cytochrome P450 inducing drugs. The induction of ALAS1 results in increased production and accumulation of toxic heme intermediates delta aminolevulinic acid (ALA) and porphobilinogen (PBG) in the plasma and urine. Clinically, the accumulation of toxic heme intermediates results in acute attacks characterized by severe abdominal pain, muscle weakness, seizures, psychiatric dysfunction, irreversible neurologic damage and increased risk of hepatic malignancy. (Bissell, 2015) Management of AHP attacks often requires hospitalization. Patients are initially treated with supportive care, intravenous fluid administration, carbohydrate loading, analgesics, antiemetics and removal of known precipitating factors. Panhematin® (Hemin for Injection, approved for marketing in 1983) is an intravenously administered iron containing metalloporphyrin ALAS1 inhibitor that is derived from processed red blood cells. Panhematin is indicated for the amelioration of recurrent attacks of acute intermittent porphyria (AIP) temporally related to the menstrual cycle in susceptible women. The recommended Panhematin dose is 3-4 mg/kg infused over 15 minutes in a large vein or central venous catheter once daily for a period of 3-5 days. Prior to administration of Panhematin the drug must be filtered in order to remove particulates. Symptoms generally improve in patients after 2-5 days of hematin treatment accompanied by a decrease in ALA and PBG production. Panhematin is not typically stocked in hospital pharmacies and must be ordered from the manufacturer which can delay therapy. Liver transplants, when available, can also be considered for this disease. (Lichtman, 2003) Givosiran (2.5 mg/kg administered SC once monthly) is a small interfering RNA (siRNA) that inhibits aminolevulinic acid synthase 1 (ALAS1). Inhibition of ALAS1 reduces the downstream synthesis of ALA and PBG. Givosiran offers adult patients with AHP another therapeutic option which, potentially, may be more conveniently administered compared to Hemin.

Study ALN-AS1-003 (ENVISION) was the pivotal study upon which the efficacy of givosiran was established. The primary endpoint was the rate of porphyria attacks observed during the 6 months double-blind period in AIP patients. From a Statistical Reviewer's perspective, the primary endpoint was met, as demonstrated by a 70% (95% CI: 53%, 84%) reduction in porphyria attacks that require hospitalization, urgent care visits, or in-home IV hemin administration. Additional secondary endpoints also provided corroborating evidence. Based on FDA's analyses, the 6-month average:

- ALA level for givosiran AIP patients was 16.1 (95% CI: 9.2, 23.0) lower than that of placebo.
- PBG level for givosiran AIP patients was 32.8 (95% CI: 18.4, 47.2) lower than that of placebo.
- days of hemin use for givosiran AIP patients is 70% (95% CI: 36%, 85%) fewer than that of placebo.

Although the sponsor's results were slightly dissimilar to FDA's results, the efficacy of givosiran continued to hold irrespective of analytical approaches taken.

Study ALN-AS1-001 was a first-in-human study of givosiran. Part C of Study 001, a small randomized study (N = 17) with a run-in period, provided additional data that supported the efficacy of givosiran. Specifically, the following endpoints were in the same direction as those reported in Study 003:

- ALA and PBG levels of patients exposed to givosiran were lower than placebo during the 6 months period that spanned treatment and follow-up.
- Attack rate of patients exposed to givosiran was lower than placebo during the 6 months period that spanned treatment and follow-up.

Overall, the totality of the data from the ENVISION pivotal study (Study 003) and the exploratory Study 001 demonstrated that givosiran was efficacious.

From the Clinical Reviewer's perspective, the objectives, design, efficacy, endpoint, ethical, safety assessment and statistical considerations for the ENVISION study are acceptable. The study is well designed to evaluate the benefits and risks of givosiran for the treatment of adult patients with AHP. The study enrolled 98 adult (age ≥ 18 years) patients with AHP (48 patients in the givosiran arm and 46 patients in the placebo arm). The treatment arms were balanced in terms of key enrollment criteria, i.e., mean age (40 years (SD 12 years) in the givosiran arm compared to 37 years (SD 11 years) in the placebo arm), sex (43/48 (90%) female patients in the givosiran arm compared to 41/46 (89%) female patients in the placebo arm), mean historical annualized attack rate (12 attacks/year (SD 9 attacks/year) in the givosiran arm compared to 11 attacks/year (SD 9 attacks/year) in the placebo arm) and prior hemin prophylaxis (20/48 (42%) in the givosiran arm compared to 18/46 (39%) in the placebo arm). The efficacy of givosiran over placebo is demonstrated by a decrease in the attack rate observed among patients with AHP who were treated with givosiran compared to those who received placebo. Among

patients with AHP in the ENVISION study the estimated attack rate over the 6 month double-blind treatment period was 2 attacks (95% CI: 1, 3 attacks) per patient in the givosiran arm compared to 14 attacks (95% CI: 10, 20 attacks) per patient in the placebo arm. Urinary ALA and PBG levels also decreased and then were maintained during the givosiran treatment period compared to placebo. I agree with the Statistical Reviewer's review and comments regarding the efficacy results for givosiran (for the treatment of adult patients with AHP) that were obtained from the ENVISION study and study ALN-AS1-001.

From the Clinical Reviewer's perspective, generally, treatment with givosiran appears to be tolerable. The safety database consists of data obtained from 94 patients enrolled in the ENVISION study. All 94 patients enrolled in the ENVISION study completed the double blind 6 month assessment (n=48 patients in the givosiran treatment arm and n=46 patients in the placebo treatment arm). Patients received givosiran for a median of 6 months (range 3-6 months) and placebo for a median of 6 months (range 5-6 months). Of these patients, 47 patients received ≥5 months of treatment. One patient was discontinued from the study prematurely due to elevated liver transaminases after the third dose of givosiran (ALT 172 U/L (4.2xULN) and AST 95 U/L (2.8xULN)) and the serum TBili was within normal limits). Although a higher proportion of patients in givosiran arm (10/48 (21%) patients) compared to the placebo arm (4/46 (9%) patients) reported SAEs, only device-related infection (2/48 (4%) patients in the givosiran arm) was reported in at least two or more givosiran treated patients in the study. The most frequently occurring (≥20% incidence) adverse events (AEs) reported in patients treated with givosiran were nausea (27%) and injection site reactions (25%). In the ENVISION study 1/48 (2%) patient with AHP developed anti-drug antibodies (ADA) during treatment with givosiran. The benefit-risk analysis favors approval of givosiran for the treatment of adult patients with AHP.

Kunthel By, PhD
Primary Statistical Reviewer

Yu-Te Wu, PhD
Statistical Team Leader

Andrew Dmytrijuk, MD
Primary Clinical Reviewer

Kathy Robie Suh, MD, PhD
Clinical Team Leader/
Cross Discipline Team Leader

9 Advisory Committee Meeting and Other External Consultations

No Oncology Drug Advisory Committee (ODAC) Meeting or other external consultations are required for the current application for givosiran for the treatment of AHP.

10 Pediatrics

The sponsor requests exemption from studies required under the Pediatric Research Equity Act (PREA) for givosiran for the treatment of AHP in pediatric patients age birth to 17 years because givosiran was granted an Orphan Drug Designation on August 29, 2016 for the treatment of AHP. (b) (4)

.

(b) (4)

(b) (4)

Postmarketing Commitment (PMC) (final wording of the PMC and PMC Schedule Milestone dates will be based on agreement with the sponsor).

- Conduct a controlled trial in pediatric patients to evaluate the dose, clinical outcomes, and safety of givosiran for the treatment of pediatric patients age greater than or equal to 12 years to less than 17 years with acute hepatic porphyria. Appropriate sampling must be incorporated to explore exposure-response relationships for measures of pharmacodynamic biomarkers, safety, and efficacy. The final protocol should be agreed upon with the Agency.

PMC Schedule Milestones:	Preliminary Protocol Submission	08/2020
	Final Protocol Submission:	02/2021
	Study/Trial Completion:	02/2026
	Final Report Submission:	02/2027

Clinical Reviewer comment for Section 10: The sponsor's request for exemption from studies required under the Pediatric Research Equity Act (PREA) for givosiran for the treatment of AHP in pediatric patients age birth to 17 years should be granted because givosiran was granted an Orphan Drug Designation on August 29, 2016 for the treatment of AHP. A PMC with the following wording for givosiran for the treatment of pediatric patients age 12 years to 17 years should be issued (final wording of the PMC and PMC Schedule Milestone dates will be based on agreement with the sponsor).

- *Conduct a controlled trial in pediatric patients to evaluate the dose, clinical outcomes, and safety of givosiran for the treatment of pediatric patients age greater than or equal to 12 years to less than 17 years with acute hepatic porphyria. Appropriate sampling must be incorporated to explore exposure-response relationships for measures of pharmacodynamic biomarkers, safety, and efficacy. The final protocol should be agreed upon with the Agency.*

PMC Schedule Milestones:	*Preliminary Protocol Submission*	*08/2020*
	Final Protocol Submission:	*02/2021*
	Study/Trial Completion:	*02/2026*
	Final Report Submission:	*02/2027*

11 Labeling Recommendations

11.1 Prescription Drug Labeling

The reviewers' table below summarizes the key labeling changes proposed by each review discipline. Final wording of the label will depend on agreement with the sponsor.

Table 29. Key Labeling Changes Proposed by Each Review Discipline

Summary of Significant Labeling Changes (High level changes and not direct quotations)		
Section	**Sponsor Proposed Labeling**	**FDA Proposed Labeling**
Highlights		
Indication	(b) (4)	FDA proposed wording is as follows (b) (4) GIVLAARI is an aminolevulinate synthase 1-directed small interfering RNA indicated for the treatment of adults with acute hepatic porphyria (AHP).
Drug Interactions	(blank in proposed labeling)	Sensitive CYP1A2 and CYP2D6 Substrates: Avoid concomitant use with CYP1A2 and CYP2D6 substrates where minimal concentration changes may lead to serious or life-threatening toxicities. (7.1)
Full Prescribing Information		
1. Indication	(b) (4)	FDA proposed wording is (b) (4) GIVLAARI is an aminolevulinate synthase 1-directed small interfering RNA indicated for the treatment of adults with acute hepatic porphyria (AHP).

2.1 Recommended Dosage	(b) (4)	FDA proposed 1.25 mg/kg once monthly as the (b) (4) resuming dose regimen following transaminase elevation recovery.
5.4 Injection Site Reactions	(blank in proposed labeling)	FDA proposed wording is: injection site reactions have been reported in 25% of patients receiving GIVLAARI in the placebo-controlled trial. Symptoms included erythema, pain, pruritus, rash, discoloration, or swelling around the injection site. Among 12 patients with reactions the highest severity of the reaction was mild among 11 (92%) patients and moderate in one (8%) patient. One (2%) patient experienced a single, transient, recall reaction of erythema at a prior injection site with a subsequent dose administration. (6.1).
12. Clinical Pharmacology	12.2 Pharmacodynamics ...	FDA added the following: 'The pharmacodynamic effects of GIVLAARI were evaluated in chronic high excreters treated with 0.035 to 2.5 mg/kg single dose and patients with AHP treated with 2.5 to 5 mg/kg once monthly and 2.5 to 5 mg/kg once quarterly doses via subcutaneous injection.'
12. Clinical Pharmacology	12.3 Pharmacokinetics ...	FDA made format changes by summarizing the PK data into a summary table. FDA also made editorial changes to improve readability and clarity for health care

		providers.
14. Clinical Studies	14.1 ENVISION Study (b) (4) [redacted])	FDA proposed that the sponsor present primary efficacy endpoint data. (b) (4) [redacted]

12 Risk Evaluation and Mitigation Strategies (REMS)

No Risk Evaluation and Mitigation Strategy (REMS) is proposed for givosiran for the treatment of adult patients with AHP.

13 Postmarketing Requirements and Commitment

(b) (4)

the sponsor should fulfill the following Postmarketing Commitment (PMC) (final wording of the PMC and PMC Schedule Milestone dates will be based on agreement with the sponsor). *See section 10 Pediatrics in this review above.*

14 Division Director (DHOT)

Haleh Saber, PhD
Deputy Division Director
OOD/DHOT

15 Division Director (OCP)

Brian Booth, PhD
Deputy Division Director
OCP/DCPV

(proxy signature by Nam A. Rahman, PhD, Division Director)

16 Division Director (OB) Comments

Thomas Gwise, PhD
Division Director
OB/DBIX

17 Division Director (Clinical) Comments

(This section is based in part on the reviews of Drs. Andrew Dmytrijuk and Kathy Robie Suh).

Background: Alnylam Pharmaceuticals, Inc. submitted NDA 212194 on November 15, 2018, January 22, 2019 and on June 4, 2019 in which it requested approval of givosiran (Givlaari) for the following indication: for the treatment of adults with acute hepatic porphyria (AHP). Givosiran is a 5'- aminolevulinate synthase 1 (ALS) directed small interfering RNA (ALSiRNA) which is connected to N-acetyl-galactosamine (NAGAL) for delivery into hepatocytes. Hepatocytes have on their membrane a receptor which internalizes when bound by NAGAL.

In patients with AHP, levels of 5'aminolevulinate synthase, which is a product at the beginning of the heme synthesis pathway, along with porphobilinogen (PBG), the next step in the heme synthetic pathway increase as result of a positive feedback regulatory loop in the liver cell and mutations in the heme synthetic pathway which reduce the levels of heme produced in the liver cells of patients with AHP.

The increased levels of ALS and PBG are responsible for the induction of 6-12 neurovisceral pain crises (NVPC) per year in patients with AHP. Subcutaneous monthly administration of givosiran to patients with AHP is predicted to decrease the levels of ALS and PBG and therefore the number of neurovisceral pain crises per year.

The request for approval of the proposed indication relies on Study ALN-AS1-003 (Envision trial) which is a phase 3 double blind placebo-controlled study which randomized 94 patients with PBG or ALS levels ≥4XULN due to AHP, 1:1 between 2.5 mg/kg of monthly x 6 givosiran vs placebo. The primary endpoint was the annualized rate of porphyria attacks that require hospitalization, urgent care visit or home IV hemin administration.

Efficacy Results: The number of attacks (NVPC) observed on the placebo arm in the 6 months blinded period was 6.5 (95% CI: 4.5, 9.3) and 1.9 (95% CI: 1.3, 2.8) on the givosiran arms respectively. On an annualized rate, there were approximately 10 fewer attacks per patient on the givosiran vs the placebo arm. The data show that the levels of ALS and PBG fall as early as 15 days after treatment and remain below baseline for the duration of the 6 months double blind period. Study LN-AS1-003 met its primary endpoint.

Safety Results: In study ALN-AS1-003, 48 patients received 6 monthly subcutaneous injections of givosiran and 46 patients received 6 monthly subcutaneous injections of placebo. There were no deaths attributable to the drug. The most frequently (≥20%) observed adverse reactions reported in patients given givosiran were nausea (27%) and injection site reactions (25%).

Benefit Risk Discussion: There was demonstration of remarkable efficacy. The toxicity was manageable. The benefit risk ratio was favorable.

Regulatory Recommendation: The Supervisory Associate Division Director (Albert Deisseroth) agrees with the recommendations of the review divisions for Approval.

Albert Deisseroth, MD, PhD
Supervisory Associate Division Director
ODE1/DHP

18 Office Director (or designated signatory authority) Comments

This application was reviewed by the Oncology Center of Excellence (OCE) per the OCE Intercenter Agreement. My signature below represents an approval recommendation for the clinical portion of this application under the OCE.

Richard Pazdur, MD
Acting Director
OOD

19 Appendices

19.1 References

Bissell, D.M. and Wang, B.: Acute haptic porphyria. J. Clin. Trans. Hepatology. 2015; 3:17-26.

Lichtman, M. A. et al.: Williams Manual of Hematology 6th ed. 2003.

19.2 Financial Disclosure

In the ENVISION study there were four study sites that reported a financial disclosure ranging from $33,293.80 to $331,667.27. Each of the four study sites enrolled few (range 0-3 patients) patients in the givosiran treatment arm. It is not expected that the data of any one of these study sites would significantly impact the overall results of the ENVISION study. The study sites are listed in the reviewer's table below.

Table 30. Financial Disclosures ENVISION Study

Study Site	Investigator	Financial Interest	Enrollment Number (n)	Proportion of 48 Patients Enrolled in Givosiran Arm (%)
(b) (6)	(b) (6)	$161709.06	(b) (6)	(b) (6)
		$331667.27		
		$38308.76		
		$41950.00		

Reviewer's table derived from NDA 212194 Module 1.3.4 supporting document 9 and ENVISION CSR

19.3 Nonclinical Pharmacology/Toxicology

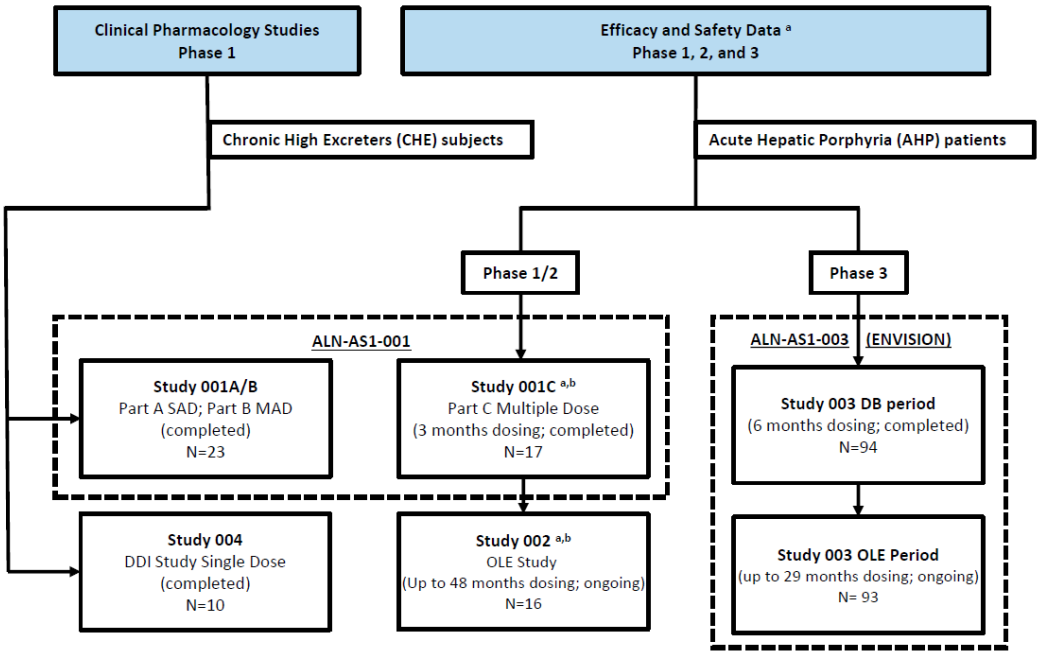
No additional nonclinical data.

19.4 OCP Appendices (Technical documents supporting OCP recommendations)

19.4.1 Overview of the Clinical Pharmacology Program

The clinical development program for givosiran consists of four clinical pharmacology studies conducted in subjects that are CHE and studies supporting efficacy and safety in patients with symptomatic AHP (Figure 16).

Figure 16. Summary of Clinical Pharmacology Program



19.4.2 Summary of Bioanalytical Method Validation and Performance

An overview of the bioanalytical methods used to evaluate key endpoints in clinical studies and their corresponding validations/qualification results are presented in Table 31. All bioanalytical assays were validated, except for the exploratory assays for IgE and the *ALAS1* mRNA.

Table 31. Overview of Bioanalytical Methods

Analyte	Sample Matrix	Method Description	Assay Range	LLOQ	Accuracy Results (% Bias)	Precision Results (%CV)	Validation Report Reference	Clinical Study
Givosiran	Plasma	LC-MS/ HRAM	20.0 – 1000 ng/mL	20.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	(b) (4)14-138	Studies 001, 002, 003
Givosiran	Plasma	LC-MS/MS	10.0 – 5000 ng/mL	10.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	8361960	Study 004
Givosiran	Urine	LC-MS /HRAM	50.0 – 5000 ng/mL	50.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	(b) (4)14-140	Studies 001, 003
AS(N-1)3'givosiran	Plasma	LC-MS/MS	10.0 – 5000 ng/mL	10.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	8361960	Studies 001, 003, 004
AS(N-1)3'givosiran	Urine	LC-MS/MS	50.0 – 5000 ng/mL	50.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	8373238	Studies 001, 003
ALA	Plasma	LC-MS/MS	10.0 – 5000 ng/mL	10.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	(b) (4)14-147	Study 001
ALA	Urine	LC-MS/MS	10.0 – 3000 ng/mL	10.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	(b) (4)14-148	Studies 001, 002, 003, 004
PBG	Plasma	LC-MS/MS	10.0 – 5000 ng/mL	10.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	(b) (4)14-147	Study 001
PBG	Urine	LC-MS/MS	10.0 – 3000 ng/mL	10.0 ng/mL	≤15% (≤20% at LLOQ)	≤15% (≤20% at LLOQ)	(b) (4)14-148	Studies 001, 002, 003, 004
ADA	Serum	ELISA	NA	37.4 ng/mL	NA	≤25%	3000749	Studies 001, 002, 003, 004
ALAS1 mRNA	Serum, urine	cERD	NA	0.1 pg (Ct value ≥38)	NA	<20% (serum); <25% (urine)	Not validated[a]	Studies 001, 004

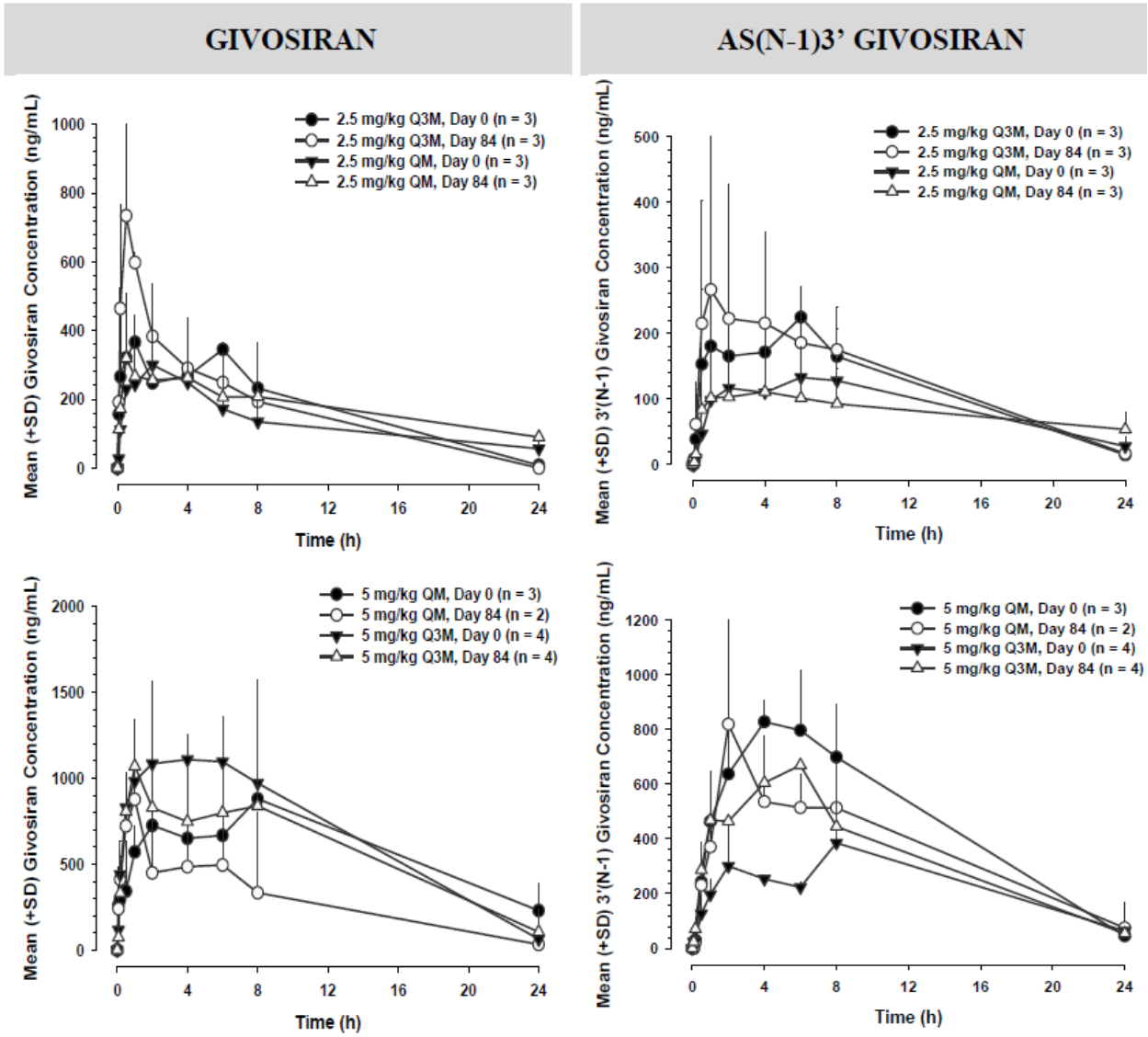
Source: 2.7.1 Summary of Biopharmaceutic Studies and Associated Analytical Methods, Table 4.

19.4.3 Clinical PK Assessments

Plasma concentrations of givosiran and AS(N-1)3' givosiran were determined following single and multiple dosing in Studies 001, 002, and 003, from a total of 125 subjects with 14 subjects that are CHE and 111 AHP patients who received at least one dose of givosiran and had at least one postdose evaluable concentration.

Mean plasma concentration-time profiles for givosiran and AS(N-1)3' givosiran after the administration of 2.5 mg/kg or 5.0 mg/kg QM and Q3M in AIP patients from Part C of Study 001 are presented in Figure 17.

Figure 17. Mean (+ SD) Plasma Concentration-Time Profiles for Givosiran and AS(N-1)3' Givosiran after Multiple Quarterly (x 2 Doses) or Monthly (x 4 Doses) Dosing in AIP Patients



The PK of givosiran after monthly and quarterly dosing in AIP patients was comparable to that in subjects that are CHE. The AUC of givosiran and AS(N-1)3' givosiran were comparable between Day 0 and Day 84, indicating no accumulation in plasma after once monthly or quarterly dosing (Table 32).

Table 32. PK Parameters of Givosiran(A) and AS(N-1)3' Givosiran (B) after Multiple Once Quarterly (x 2 Doses) or Once Monthly (x 4 Doses) Doses of Givosiran in AIP Patients

(A)

PK Parameter	2.5 mg/kg Q3M		5.0 mg/kg Q3M		2.5 mg/kg QM		5.0 mg/kg QM	
	DAY 0 (N=3)	DAY 84 (N=3)	DAY 0 (N=4)	DAY 84 (N=4)	DAY 0 (N=3)	DAY 84 (N=3)	DAY 0 (N=3)	DAY 84 (N=2)[a]
t_{max} (h)[b]	0.98 (0.17 – 3.97)	0.50 (0.50 – 0.53)	5.00 (2.00 – 7.98)	0.73 (0.08 – 8.00)	2.00 (1.00 - 2.00)	0.50 (0.17 – 2.00)	4.00 (0.50 – 8.00)	3.50 (1.00, 6.00)[c]
C_{max} (ng/mL)	427 (30.8)	734 (40.9)	1280 (24.4)	7880 (174.5)	279 (14.0)	321 (50.8)	975 (61.8)	984 (979, 988)[c]
$AUC_{0\text{-last}}$ (ng·h/mL)	2530 (46.0)	2560 (42.4)	12700 (38.3)	12400 (47.4)	3030 (66.3)	4130 (43.2)	7830 (6.7)	5870 (780, 11000)[c]
$AUC_{0\text{-}24}$ (ng·h/mL)	3890 (38.1)	4900 (25.5)	14500 (27.8)[e]	14400 (36.6)[e]	4120 (3380, 4850)[c]	4130 (43.2)	8280 (8020, 8540)[c]	10900
$t_{1/2}$ (h)	4.62[d]	NR	5.77[d]	6.72 (54.7)[e]	9.32[d]	9.46[d]	9.29 (8.06, 10.5)[c]	NR
f_e (%)	4.58 (32.4)	6.45 (26.3)	10.9 (20.7)	11.4 (34.4)	13.5 (27.3)	9.63 (24.6)	8.30 (21.9)	13.7[d]

(B)

PK Parameter	2.5 mg/kg Q3M		5.0 mg/kg Q3M		2.5 mg/kg QM		5.0 mg/kg QM	
	DAY 0 (N=4)	DAY 84 (N=3)	DAY 0 (N=4)	DAY 84 (N=4)	DAY 0 (N=3)	DAY 84 (N=3)	DAY 0 (N=3)	DAY 84 (N=4)[a]
t_{max} (h)[b]	0.98 (0.50 – 5.95)	1.02 (0.97 – 4.00)	6.00 (5.98 – 6.00)	7.21 (0.50 – 8.00)	8.00 (2.00 – 8.00)	2.00 (1.00 – 6.00	4.00 (4.00 – 8.00)	3.50 (1.00, 6.00)[c]
C_{max} (ng/mL)	215 (22.4)	298 (61.0)	797 (27.7)	2090 (151.9)	132 (24.3)	123 (64.1)	411 (66.3)	632 (595, 669)[c]
$AUC_{0\text{-last}}$ (ng·h/mL)	1970 (24.1)	2150 (39.8)	7980 (39.7)	9180 (63.4)	1880 (13.4)	1930 (62.7)	3680 (9.5)	3690 (318, 7060)[c]
$AUC_{0\text{-}24}$ (ng·h/mL)	2930 (58.8)	2530 (49.8)	5040[d]	10500 (60.2)[e]	1880 (13.4)	1930 (62.7)	3590 (3460, 3720)[c]	7040[d]
$t_{1/2}$ (h)	10.00[d]	7.46[d]	NR	4.73[d]	NR	NR	8.88 (8.13, 9.63)[c]	NR
MR_{Cmax}	0.513 (8.1)	0.380 (41.8)	0.628 (27.2)	0.437 (34.1)	0.490 (39.0)	0.404 (52.5)	0.414 (4.4)	0.642 (0.608, 0.677)[c]
MR_{AUC}	0.713 (21.4)	0.496 (34.8)	0.492[d]	0.754 (0.599, 0.908)[c]	0.437 (0.397, 0.477)[c]	0.449 (36.7)	0.434 (0.405, 0.464)[c]	0.643[d]
f_e (%)	4.19 (32.3)	4.66 (7.2)	10.8 (31.6)	9.46 (50.9)	7.20 (51.1)	5.80 (45.7)	4.57 (66.1)	13.2[d]

19.4.4 Population PK Analyses

Descriptive statistics of baseline demographics for continuous demographic data are presented in Table 33 and descriptive statistics of categorical demographic data are presented in Table 34.

The PK population included a total of 125 subjects. There were 111 (88.8%) AHP patients and 14 (11.2%) subjects that are CHE. Subjects were primarily female (88.0%) and white (80.0%). There were 12 subjects of East Asian origin (9.6%). Median (range) age and body weight were 38.0 years (19.0 - 65.0) and 66.2 kg (39.5 - 131), respectively. Median values for age and body weight were comparable across studies. Median eGFR was 69.3 mL/min/1.73 m2, with individual values ranging from 26.0 to 151 mL/min/1.73 m2. Since eGFR greater than 120 mL/min/1.73m2 were considered as normal renal function, eGFR were capped to 120 mL/min/1.73m2 for the covariate analysis.

Table 33. Baseline Characteristics of PK Population by Study (continuous covariates)

Baseline Characteristics	Mean (SD) Median [Minimum, Maximum]			
	ALN-AS1-001 (N=27)	ALN-AS1-002 (N=16)	ALN-AS1-003 (N=94)	Overall* (N=125)
Age (years)	39.3 (11.2) 38.0 [21.0, 59.0]	37.4 (12.0) 39.5 [21.0, 60.0]	38.8 (11.4) 37.5 [19.0, 65.0]	39.0 (11.3) 38.0 [19.0, 65.0]
Weight (kg)	73.3 (15.8) 70.9 [44.5, 118]	75.8 (18.5) 70.4 [44.5, 118]	66.8 (16.2) 65.0 [39.5, 131]	69.0 (16.8) 66.2 [39.5, 131]
eGFR[a] (mL/min/1.73 m^2)	77.7 (25.5) 71.2 [38.5, 141]	75.1 (27.5) 74.4 [38.5, 126]	72.9 (26.4) 67.0 [26.0, 151]	74.0 (26.4) 69.3 [26.0, 151]

Abbreviations: eGFR=estimated glomerular filtration rate; PK=pharmacokinetic; SD= standard deviation.
[a] eGFR were capped to 120 mL/min/1.73 m^2 for the covariate analysis. A total of 9 subjects had eGFR values > 120 mL/min/1.73 m^2
* Subjects appearing in more than one study were only counted once in the overall column, keeping their first available value

(Source: Applicant's Population PK Report, Table 4)

Table 34. Baseline Characteristics of PK Population by Study (categorical covariates)

Baseline Characteristics	Count (%)			
	ALN-AS1-001 (N=27)	ALN-AS1-002 (N=16)	ALN-AS1-003 (N=94)	Overall[a] (N=125)
Sex				
Male	3 (11.1%)	2 (12.5%)	10 (10.6%)	15 (12.0%)
Female	24 (88.9%)	14 (87.5%)	84 (89.4%)	110 (88.0%)
Race				
White	23 (85.2%)	13 (81.2%)	73 (77.7%)	100 (80.0%)
Black or African American	2 (7.4%)	2 (12.5%)	1 (1.1%)	3 (2.4%)
Asian	2 (7.4%)	1 (6.2%)	15 (16.0%)	17 (13.6%)
Native Hawaiian/Pacific Islander	0 (0%)	0 (0%)	1 (1.1%)	1 (0.8%)
Other	0 (0%)	0 (0%)	4 (4.3%)	4 (3.2%)
Race Group				
Non-East Asian	27 (100%)	16 (100%)	82 (87.2%)	113 (90.4%)
East Asian	0 (0%)	0 (0%)	12 (12.8%)	12 (9.6%)
Renal Function (eGFR)				
Normal (≥90 mL/min/1.73 m^2)	8 (29.6%)	4 (25.0%)	21 (22.3%)	30 (24.0%)
Mild RI (60-89 mL/min/1.73 m^2)	12 (44.4%)	7 (43.8%)	46 (48.9%)	59 (47.2%)
Moderate RI (30-59 mL/min/1.73 m^2)	7 (25.9%)	5 (31.2%)	26 (27.7%)	35 (28.0%)
Severe RI (15-29 mL/min/1.73 m^2)	0 (0%)	0 (0%)	1 (1.1%)	1 (0.8%)
Hepatic Function[b]				
Normal	24 (88.9%)	15 (93.8%)	84 (89.4%)	112 (89.6%)
Mild HI	3 (11.1%)	1 (6.2%)	8 (8.5%)	11 (8.8%)
Moderate HI	0 (0%)	0 (0%)	1 (1.1%)	1 (0.8%)
Severe HI	0 (0%)	0 (0%)	1 (1.1%)	1 (0.8%)
Patient Type				
AHP	13 (48.1%)	16 (100%)	94 (100%)	111 (88.8%)
CHE	14 (51.9%)	0 (0%)	0 (0%)	14 (11.2%)

Abbreviations: AHP=acute hepatic porphyria; CHE=chronic high excreters; HI=hepatic impairment; NCI-ODWG=National Cancer Institute Organ Dysfunction Working Group; PK=pharmacokinetic; RI=renal impairment.
[a] Subjects appearing in more than one study were only counted once in the overall column, keeping their first available value
[b] Patients were categorized based on the NCI-ODWG classification. A total of 3 patients had Gilbert's syndrome (one each with mild, moderate, and severe hepatic impairment) may have confounded total bilirubin measurement and categorization of hepatic impairment.

(Source: Applicant's Population PK Report, Table 5)

Development of the Covariate Model

Although the effect of body weight was already included in the structural model on clearance (CL23, CLH, and Q) and volume (V4) parameters using fixed allometric exponents (0.75 for clearance and 1 for volume parameters, respectively), residual trends were observed between the random effects (ETA values) of CLH and body weight (Appendix 1, Section 12.16) as well as between ETA values of Ka and body weight. As a result of the above exploratory analysis, an additional effect of body weight on these parameters was evaluated using an additional exponent to account for potential deviation relative to allometric scaling exponents[i.e., (Weight/66.2)0.75 + (Weight/66.2)Estimated].

The covariate eGFR was already included in the structural model to explain the renal clearance of givosiran and AS(N-1)3' givosiran.

Given the low incidence of ADA (n=5) in the clinical studies (Table 6), the presence of ADA was not formally tested in the covariate analysis.

PK-covariate relationships formally evaluated are listed in Table 8. The covariate analysis was performed using a stepwise forward inclusion (ΔOFV of 6.63, p <0.01 for 1 degree of freedom) and backward exclusion (ΔOFV of 10.82, p <0.001 for 1 degree of freedom) procedure.

Table 35. Population PK Model of Givosiran and AS(N-1)3' Givosiran: Covariates Tested

Covariates	PK Parameters
Age	CL_{23}, CL_H
Body Weight (additional effect)	CL_{23}, CL_H, K_a, Q, V_2, V_3 and V_4
Sex	CL_{23}, CL_H
Patient Type (AHP vs CHE)	CL_{23}, CL_H
Race (East Asian vs non-East Asian)	CL_{23}, CL_H
Hepatic impairment category (mild versus normal) (based on NCI-ODWG classification)	CL_{23}, CL_H

Abbreviations: AHP=acute hepatic porphyria; CHE=chronic high excreters; CL_{23}= parent to metabolite conversion clearance; CL_H =hepatic clearance; K_a=first order absorption rate constant; NCI-ODWG=National Cancer Institute Organ Dysfunction Working Group; PK=pharmacokinetic; Q=inter-compartmental clearance between central and peripheral compartment; V_2= volume of distribution for parent in central compartment, V_3= volume of distribution for metabolite in central compartment, V_4= volume of distribution of peripheral compartment

(Source: Applicants Population PK Report, Table 8)

The applicant's final PK parameter estimates are presented in Table 36.

Table 36. Final PK Model Parameter Estimates

PK Parameters	Population Estimates	IIV (%)	RSE[a] (%)	95% CI Lower	95% CI Upper	Shrinkage (%)
K_a (h^{-1})	**0.135** × (Weight/66.2)$^{-0.972}$	40.4	3.6	0.117	0.156	16.3
CL_{23} (L/h)	**13.2** × (Weight/66.2)$^{0.190*}$	NA	1.3	12.4	14.1	NA
East Asian	× 0.674					
CL_H (L/h)	**18.9** × (Weight/66.2)$^{0.75}$	28.2	1.7	17.2	20.9	24.8
Dose	× (Dose/2.5)$^{-0.271}$					
East Asian	× 0.703					
Q (L/h)	**43.8** × (Weight/66.2)$^{0.75}$	NA	2.7	35.9	53.4	NA
V_2 (L)	**10.4** × (Weight/66.2)	NA	2.3	9.33	11.6	NA
V_3 (L)	**10.4** × (Weight/66.2)	NA	2.3	9.33	11.6	NA
V_4 (L)	**69.4** × (Weight/66.2)	NA	4.1	49.4	97.6	NA
Residual Error (Parent)	**40.6%**	NA	2.2	39.1	42.1	NA
Residual Model (Metabolite)	**39.9%**	NA	3.4	37.5	42.5	NA
OFV	-719.169	NA	NA	NA	NA	NA

Abbreviations: CI=confidence interval; CL_{23}= apparent conversion of parent to metabolite; CL_H=hepatic clearance; IIV= inter-individual variability; K_a=first-order absorption rate constant; NA=not applicable; OFV=objective function value; PK=pharmacokinetic; Q= apparent inter-compartmental clearance between central and peripheral compartment; RSE=relative standard error; V_2=apparent volume of distribution for parent in central compartment, V_3=apparent volume of distribution for metabolite in central compartment, V_4=apparent volume of distribution of peripheral compartment

Note: IIV, RSE, 95% CI and shrinkage are presented for PK parameter estimates only. RSE and 95% CI for covariate effects are presented in Appendix 1, Section 12.25.

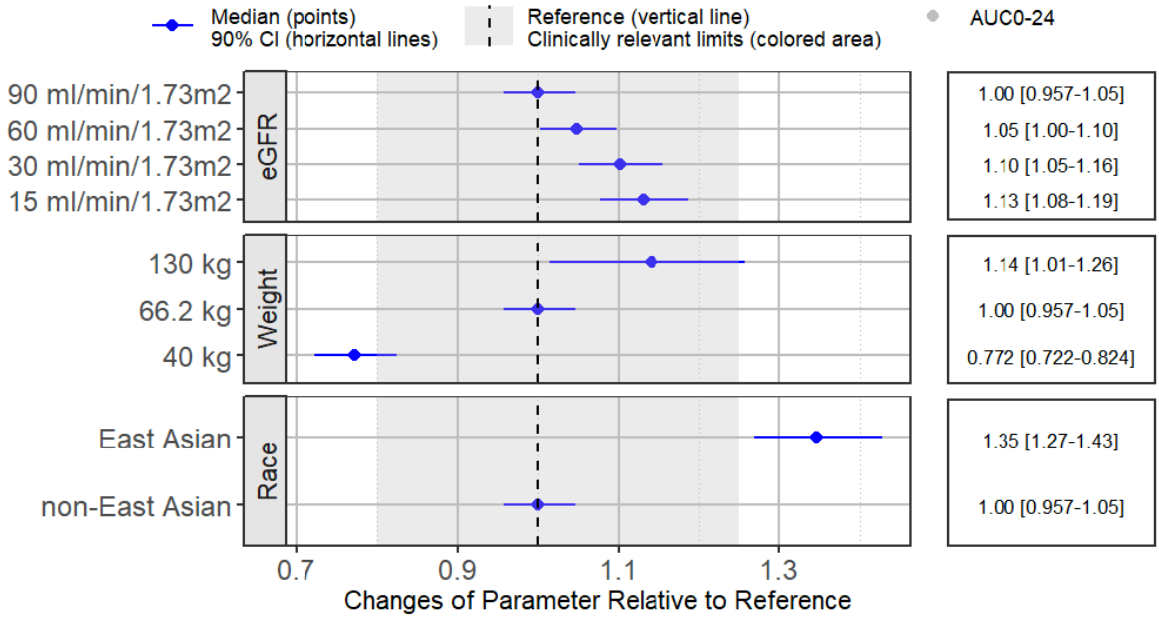
[a] PK parameters (Ka, CL_{23}, CL_H, Q, V_2, V_3, V_4 and residual errors) were estimated on log scale and raw RSE (%) are reported

* The exponent (0.190) for the effect of body weight on CL_{23} was assessed using a theoretical allometric model (0.75) and an additional exponent (-0.560) to account for potential deviation relative to theoretical model as follows: (Weight/66.2)$^{0.75}$ + (Weight/66.2)$^{-0.560}$

(Source: Applicant's Population PK Report, Table 10)

A graphical representation of magnitude of the covariate effects on AUC_{0-24} and Cmax of givosiran are given in Figure 18 and Figure 19, respectively; and on AUC_{0-24} and Cmax of AS(N-1)3' givosiran are given in Figure 20 and Figure 21, respectively. The covariate effects of baseline age, patient population (CHE versus AHP), sex, hepatic function (normal versus mild hepatic impairment) were not statistically significant in the final PK model.

Figure 18. Givosiran Forest Plot: Covariate Effects on AUC_{0-24}



Abbreviations: AHP=acute hepatic porphyria; AUC_{0-24}=area under the plasma concentration-time curve from time of dosing to 24 hours poste dose; CI=confidence interval; eGFR=estimated glomerular filtration rate; QM=once monthly
Note: The circles and the horizontal segments represent median and 90% CI of covariate effect relative to the median of the typical patients population (n=500). The shaded area represents effect size of 80% -125%. The typical patients was defined as a 66.2-kg non-East Asian patient with AHP, with and eGFR of 90 mL/min/1.73 m^2 treated with 2.5 mg/kg QM givosiran.

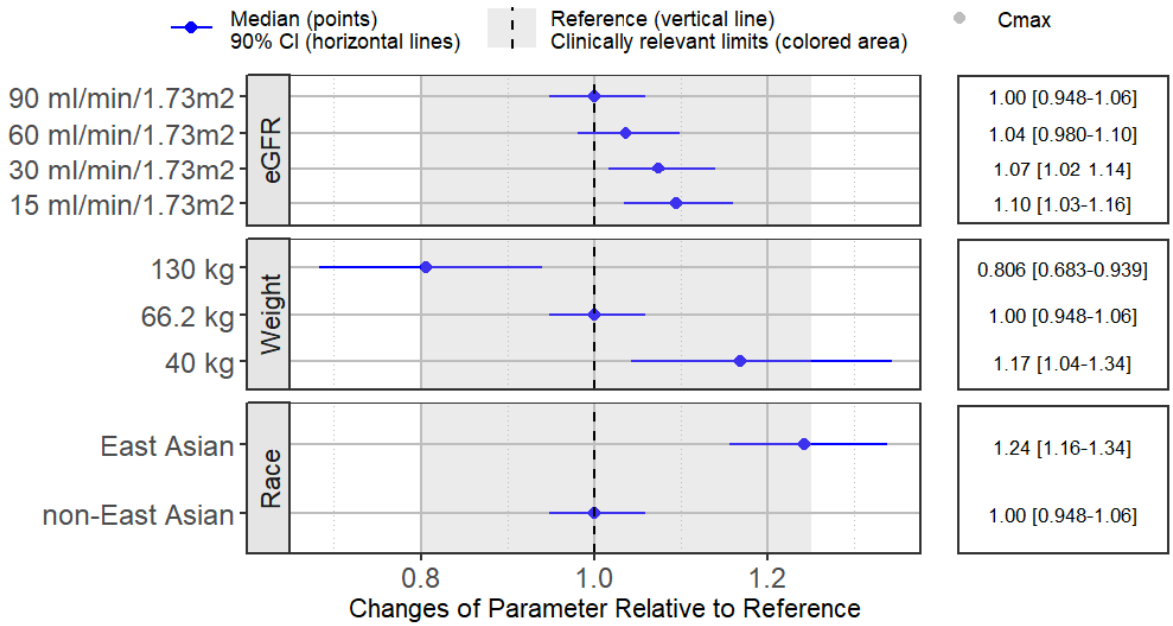
(Source: Applicant's Population PK Report, Figure 9)

Except for race (East Asian origin) and body weight, none of the other evaluated covariates impacted AUC_{0-24} of givosiran (point estimate of the covariate effect contained within 80 – 125% of reference).

A typical patient of East Asian origin is predicted to have a 35% higher mean AUC_{0-24} of givosiran than the median value observed in the overall population.

A typical patient of 40 kg is predicted to have a 23% lower mean AUC0-24 of givosiran than the median value observed in the overall population (with higher bound of the 90% CI contained within 80-125% of reference).

Figure 19. Givosiran Forest Plot: Covariate Effects on Cmax



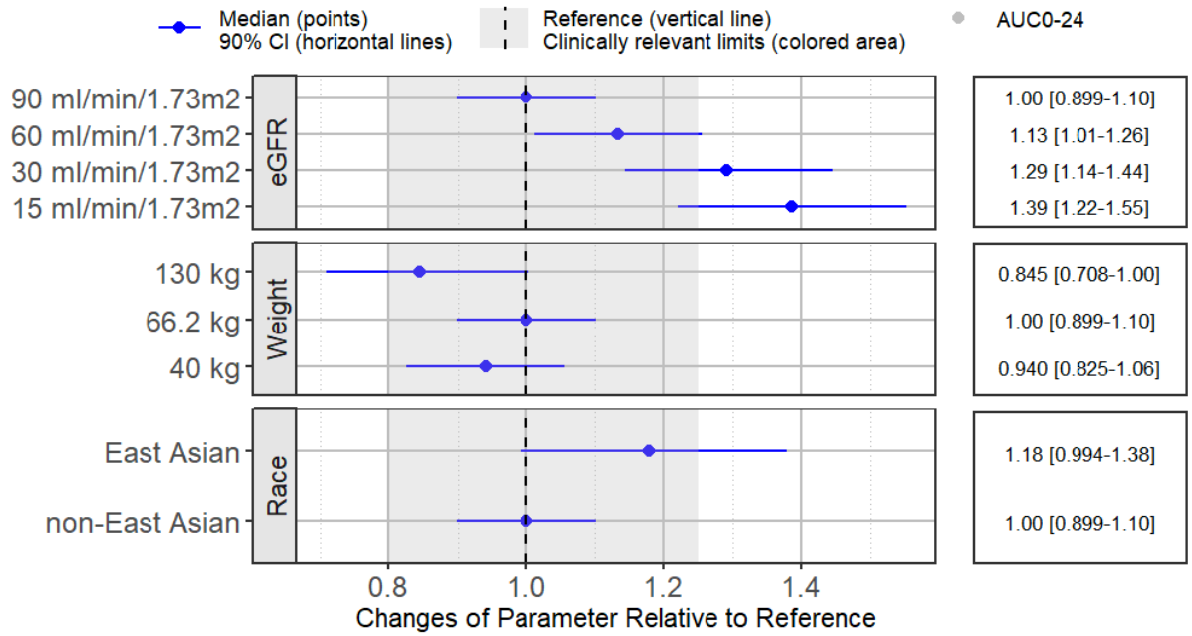
Abbreviations: AHP=acute hepatic porphyria; CI=confidence interval; C_{max}= maximum observed plasma concentration; eGFR=estimated glomerular filtration rate; QM=once monthly

Note: The circles and the horizontal segments represent median and 90% CI of covariate effect relative to the median of the typical patients population (n=500). The shaded area represents effect size of 80% -125%. The typical patients was defined as a 66.2-kg non-East Asian patient with AHP, with and eGFR of 90 mL/min/1.73 m^2 treated with 2.5 mg/kg QM givosiran.

(Source: Applicant's Population PK Report, Figure 10)

The point estimate of effect sizes for all of the covariates tested were all within the 80 – 125% equivalence window.

Figure 20. AS(N-1)3' Givosiran Forest Plot: Covariate Effects on AUC_{0-24}

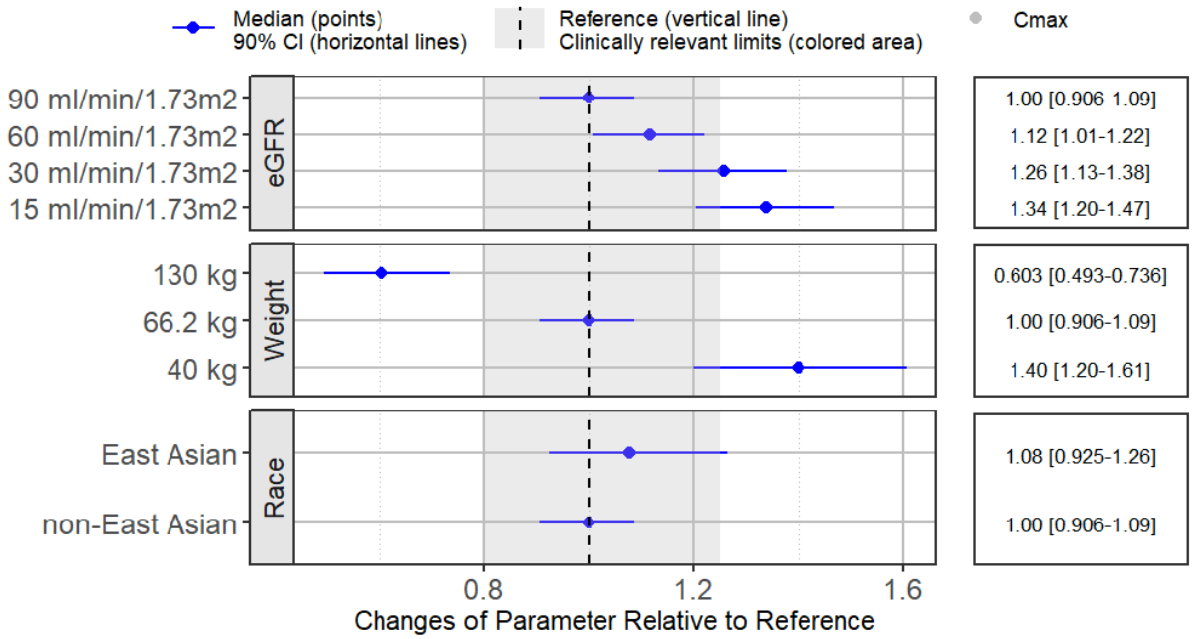


Abbreviations: AHP=acute hepatic porphyria; AUC_{0-24}=area under the plasma concentration-time curve from time of dosing to 24 hours poste dose; CI=confidence interval; eGFR=estimated glomerular filtration rate; QM=once monthly
Note: The circles and the horizontal segments represent median and 90% CI of effect relative to the median of the typical patients population (n=500). The shaded area represents effect size of 80% -125%. The typical patients was defined as a 66.2-kg non-East Asian patient with AHP, with and eGFR of 90 mL/min/1.73 m^2 treated with 2.5 mg/kg QM givosiran.

(Source: Applicant's Population PK Report, Figure 11)

A trend of increasing AUC_{0-24} with increasing severity of renal function was observed. The predicted AUC_{0-24} in patients with severe renal impairment was 39% higher relative to the median value observed in the typical population (eGFR of 90 mL/min/1.73m2).

Figure 21. AS(N-1)3' Givosiran Forest Plot: Covariate Effects on C_{max}



Abbreviations: AHP=acute hepatic porphyria; CI=confidence interval; C_{max}= maximum observed plasma concentration; eGFR=estimated glomerular filtration rate; QM=once monthly
Note: The circles and the horizontal segments represent median and 90% CI of covariate effect relative to the median of the typical patients population (n=500). The shaded area represents effect size of 80% -125%. The typical patients was defined as a 66.2-kg non-East Asian patient with AHP, with and eGFR of 90 mL/min/1.73 m^2 treated with 2.5 mg/kg QM givosiran.

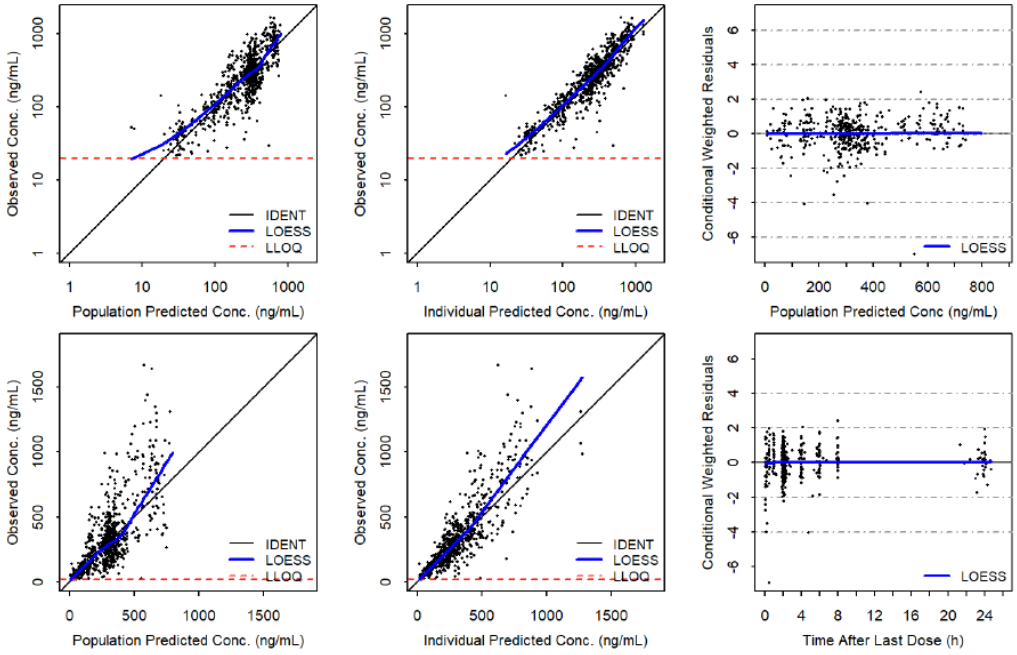
(Source: Applicant's Population PK Report, Figure 12)

Typical patients with body weight of 40 or 130 kg are expected to have a mean Cmax of AS(N-1)3' givosiran 40% higher or 40% lower than the mean value observed in the typical patient with median body weight of 66.2 kg.

A typical patient with a severe renal impairment (eGFR = 15 ml/min/1.73m2) is expected to have a median Cmax of AS(N-1)3' givosiran that is 34% higher than the mean value observed in the typical population with normal renal function (eGFR of 90 ml/min/1.73m2).

Goodness-of-fit of the final model for givosiran and AS(N-1)3' givosiran are presented in Figure 22 and Figure 23.

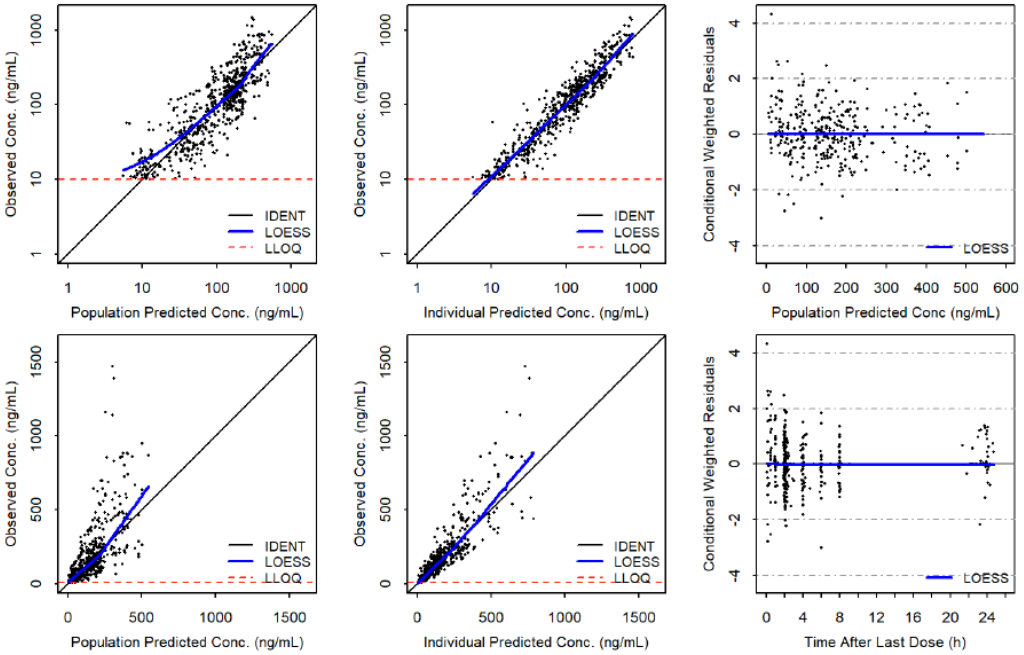
Figure 22. Final Population PK Model Goodness-of-fit Plots for Givosiran



Abbreviations: Conc=concentration; IDENT=identity line; LOESS=locally weighted scatterplot smoothing; LLOQ=lower limit of quantitation; PK=pharmacokinetic

(Source: Applicant's Population PK Report, Figure 13)

Figure 23. Final Population PK Model Goodness-of-fit Plots for AS(N-1)3' Givosiran



Abbreviations: Conc=concentration; IDENT=identity line; LOESS=locally weighted scatterplot smoothing; LLOQ=lower limit of quantitation; PK=pharmacokinetic

(Source: Applicant's Population PK Report, Figure 14)

PK Predictions in (b) (4) Adults

The final population PK model was used to perform simulations to explore the effect of body weight on exposure parameters of givosiran and AS(N-1)3' givosiran. (b) (4)

(b) (4)

Table 37. Descriptive Statistics of Predicted Givosiran PK and Exposure in Typical [redacted] (b) (4) Adult Patients (2.5 mg/kg Givosiran Monthly)

Parameters	[redacted] (b) (4) Givosiran	
	Adult (66.2 kg)	Adult (130 kg)
CL/F (L/h)		
Mean (SD)	38.1 (5.51)	52.1 (9.23)
Geometric Mean (CV%)	37.7 (14.0%)	51.3 (17.2%)
Median	37.4	51.0
[5th -95th Interval]	[30.5-47.6]	[39.5-68.0]
$T_{1/2}$ terminal (h)		
Mean (SD)	5.84 (2.32)	11.5 (4.85)
Geometric Mean (CV%)	5.45 (37.2%)	10.7 (40.4%)
Median	5.39	10.6
[5th -95th Interval]	[3.15-10.2]	[5.55-20.5]
AUC_{0-24} (ng*h/mL)		
Mean (SD)	4060 (636)	4730 (1110)
Geometric Mean (CV%)	4010 (16.1%)	4600 (24.3%)
Median	4050	4630
[5th -95th Interval]	[3020-5160]	[3030-6800]
C_{max} (ng/mL)		
Mean (SD)	344 (111)	277 (90.8)
Geometric Mean (CV%)	327 (32.5%)	263 (33.5%)
Median	323	259
[5th -95th Interval]	[198-548]	[156-442]

Abbreviations: AUC_{0-24}=area under the concentration-time curve from time of dosing up to 24 hours post dose; CL/F=apparent clearance; C_{max}=maximum concentration after the dose; CV=geometric coefficient of variation; PK=pharmacokinetic; SD=standard deviation; $T_{1/2}$= half-life
Note: PK parameters were predicted from simulations (500 replicates by scenario)

(Source: Applicant's Population PK Report, Table 13)

The respective mean AUC_{0-24} values of givosiran in a typical [redacted] (b) (4) 130-kg adult were [redacted] (b) (4) 17% higher than that observed in a typical 66.2-kg adult. The effect of body weight on givosiran AUC_{0-24} is the result of the combination of body weight effects on hepatic clearance (exponent 0.75) and on metabolic clearance (exponent 0.190).

The respective mean Cmax values of givosiran in a typical [redacted] (b) (4) 130-kg adult were [redacted] (b) (4) 19% lower than that observed in a typical 66.2-kg adult. Givosiran Cmax decreases with increasing body weight as absorption rate constant, Ka, decreases with increasing body weight.

Descriptive statistics of PK and exposure parameters of AS(N-1)3' givosiran following monthly administration of 2.5 mg/kg givosiran are presented in Table 38.

Table 38. Descriptive Statistics of PK and Exposure of AS(N-1)3' Givosiran in Typical [redacted] (b) (4) Adult Patients (2.5 mg/kg Givosiran Monthly)

Parameters	AS(N-1)3' Givosiran	
	[redacted] (b) (4) Typical Adult (66.2 kg)	Typical Adult (130 kg)
CL/F (L/h)		
Mean (SD)	74.4 (28.8)	137 (61.3)
Geometric Mean (CV%)	69.8 (36.3%)	126 (42.2%)
Median	68.8 [	125
[5th -95th Interval]	40.3-124]	[66.4-242]
$T_{1/2}$ terminal (h)		
Mean (SD)	5.85 (2.32)	11.6 (4.90)
Geometric Mean (CV%)	5.46 (37.2%)	10.7 (40.4%)
Median	5.41	10.7
[5th -95th Interval]	[3.13-10.2]	[5.60-20.8]
$AUC_{0\text{-}24}$ (ng*h/mL)		
Mean (SD)	2300 (829)	2020 (902)
Geometric Mean (CV%)	2160 (37.4%)	1840 (45.3%)
Median	2200	1880
[5th -95th Interval]	[1200-3850]	[872-3740]
C_{max} (ng/mL)		
Mean (SD)	193 (87.0)	119 (59.8)
Geometric Mean (CV%)	175 (45.7%)	107 (50.5%)
Median	173	106
[5th -95th Interval]	[84.3-374]	[47.5-244]

Abbreviations: $AUC_{0\text{-}24}$=area under the concentration-time curve from time of dosing up to 24 hours post dose; CL/F=apparent clearance; C_{max}=maximum concentration after the dose; CV= geometric coefficient of variation; PK=pharmacokinetic; SD=standard deviation; $T_{1/2}$= half-life
Note: PK parameters were predicted from simulations (500 replicates by scenario)
(Source: Applicant's Population PK Report, Table 14)

The respective mean AUC0-24 values of AS(N-1)3' givosiran in a typical [redacted] (b) (4) 130-kg adult were [redacted] (b) (4) 12% lower than that predicted in a typical 66.2-kg adult. The effect of weight on AUC0-24 of AS(N-1)3' givosiran is the result of combination of body weight effects on AUC0-24 of givosiran, on metabolic formation clearance (exponent 0.190 on CL23), and hepatic clearance (exponent of 0.75 on CLH).

The respective mean Cmax values of AS(N-1)3' givosiran in a typical [redacted] (b) (4) 130-kg adult were [redacted] (b) (4) 38% lower than that predicted in a typical 66.2-kg adult. AS(N-1)3' givosiran Cmax decreases with increasing body weight due to the effect of weight on Ka.

Reviewer's Comments:
The applicant's population PK model appears to capture the central tendency of the data and is therefore reasonable for descriptive labeling purposes and generating individual post hoc estimates for exposure-response analyses.

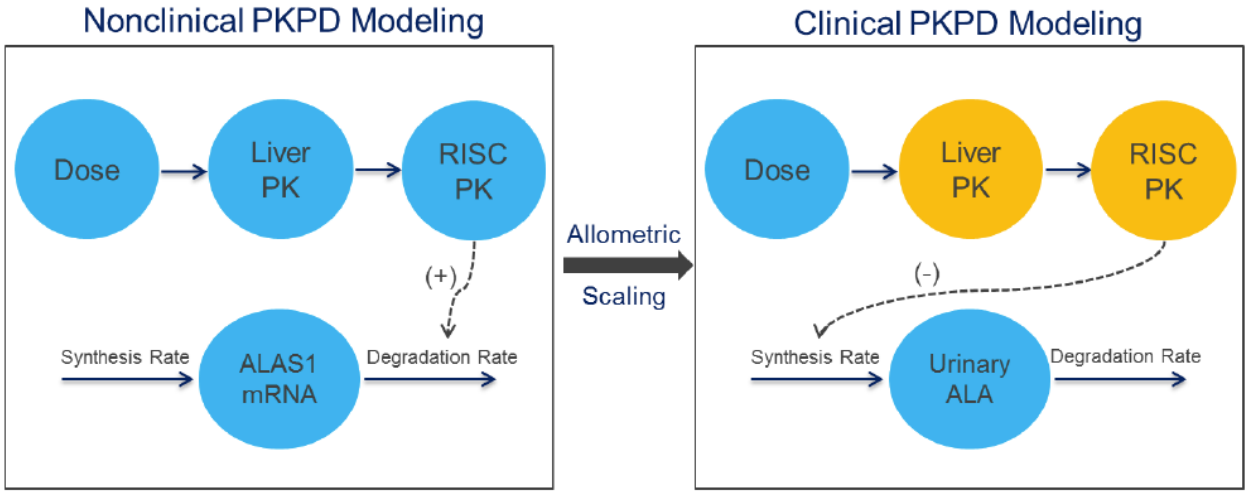
[redacted] (b) (4)

(b) (4)

19.4.5 Exposure-Response Analysis

For exposure-response analyses the applicant utilized exposure metrics derived from concentrations projected in the liver based on allometry and originating from a nonclinical PK/PD model developed with rat data. Their approach to scaling this model is shown in Figure 25 with the blue shaded variables indicating observed data.

Figure 25. Schematic Representation of the Givosiran PK/PD Model



Abbreviations: ALA=aminolevulinic acid; ALAS1=aminolevulinate synthase 1; mRNA=messenger ribonucleic acid; PD=pharmacodynamic; PK=pharmacokinetic, RISC=RNA-induced silencing complex.
Note: Blue shaded area=observed data; orange shaded area=predicted data based on allometric scaling.
(Source: Applicant's PK/PD Report, Figure 3)

In brief the applicant took the following approach:

1. A nonclinical PK/PD model was developed to describe the relationship between observed liver concentrations of active siRNA (givosiran and AS(N-1)3' givosiran), RISC-loaded active siRNA levels, and changes in ALAS1 mRNA following givosiran dosing in rats. An Emax model best described the relationship between RISC-loaded active siRNA levels and the higher degradation rate of ALAS1 mRNA, enabling estimation of an IC50 value.
2. The PK model parameters from the nonclinical model were allometrically scaled to predict liver active siRNA levels in human. Human RISC concentrations of active siRNA were predicted from scaled liver PK and observed PD (urinary ALA levels).
3. Predicted RISC concentrations of active siRNA were modeled to have an inhibitory effect on the synthesis of urinary ALA in human. The relationship between predicted RISC-loaded active siRNA levels and decrease in synthesis of ALA was described as an Imax model with an IC50 value obtained from the nonclinical PK/PD model.

E-R Analysis for Urine ALA Levels:

Summary of Data used in the PK/PD Analysis

The following 3 clinical studies for givosiran were included in the modeling and simulation analysis and summarized in this report: ALN-AS1-001 (Study 001), ALN-AS1-002 (Study 002), and ALN-AS1-003 (Study 003).

Table 39. Summary of Givosiran Clinical Studies and Clinical Pharmacology Data

Study, Status, Data Cutoff	Study Design/Objectives, Location	Dose(s)/Patient Type	Dosage Regimen: Number of Patients	Clinical Pharmacology Data
ALN-AS1-001 (Study 001) *Completed* *Data lock: 23 Oct 2017*	Phase 1, randomized, single-blind SAD (Part A, 001A) and MAD (Part B, 001B), double-blind multiple-dose study (Part C, 001C) to evaluate safety, tolerability, PK, PD, and ADA of givosiran 6 clinical study centers (4 in US, 1 in UK, 1 in Sweden)	Part A: 0.035 mg/kg to 2.5 mg/kg single SC dose, CHE subjects	Placebo: N=5 Givosiran: N=15 0.035 mg/kg (N=3) 0.10 mg/kg (N=3) 0.35 mg/kg (N=3) 1 mg/kg (N=3) 2.5 mg/kg (N=3)	• Plasma and urine concentrations of givosiran and metabolite AS(N-1)3' givosiran • Plasma and urine concentrations of PD biomarkers – ALA, PBG, and *ALAS1* mRNA • Data on clinical activity of givosiran based on annualized rate of porphyria attacks and administered hemin doses[a] • ADA and other safety data
		Part B: 0.35 mg/kg and 1 mg/kg, monthly (x 2), CHE subjects	Placebo: N=2 Givosiran: N=6 0.35 mg/kg (N=3) 1.0 mg/kg (N=3)	
		Part C: 2.5 mg/kg and 5 mg/kg once monthly (x 4) and quarterly (x 2), AIP patients	Placebo: N=4 Givosiran: N=13 2.5 mg/kg Q3M (N=3) 2.5 mg/kg QM (N=3) 5 mg/kg Q3M (N=4) 5 mg/kg QM (N=3)	
ALN-AS1-002 (Study 002) *Ongoing* *Data cut-off: 13 Dec 2018*[b]	Phase 2, open-label, single-arm, long-term extension study to evaluate the long-term safety, and clinical activity of givosiran 5 clinical study centers (3 in US, 1 in UK, 1 in Sweden)	2.5 mg/kg SC once monthly[c], AIP patients who completed Study 001C Dosing up to 3 years	Givosiran: 2.5 mg/kg QM (N=16)	• Plasma concentrations of givosiran • Urine concentration of ALA, PBG, and *ALAS1* mRNA • Data on clinical activity of givosiran based on annualized rate of porphyria attacks and administered hemin doses • ADA and other safety data
ALN-AS1-003 (Study 003) *6-Month Double Blind Period: completed* *Data lock: 27 Feb 2019*	Phase 3, randomized (1:1), double-blind, placebo-controlled study with an open-label extension to evaluate the efficacy and safety of givosiran 36 centers across North America, Europe, Asia, Australia, and Mexico	2.5 mg/kg SC once monthly, AHP patients Dosing for 6 months in the double-blind treatment period	Placebo: N=46 Givosiran 2.5 mg/kg QM: N=48	• Plasma and urine PK data for givosiran and AS(N-1)3' givosiran • Urine PD data – ALA and PBG • Efficacy data for givosiran based on annualized rate of porphyria attacks and administered hemin doses • ADA and safety data
Ongoing; Open-label extension *Data cut-off: 31 Jan 2019*[d]		1.25 mg/kg or 2.5 mg/kg SC once monthly, AHP patients	Givosiran 2.5 mg/kg QM: N=56 Givosiran 1.25 mg/kg QM: N=37	• Plasma and urine concentrations of givosiran and AS(N-1)3' givosiran • Urine concentrations of ALA and PBG • Efficacy data for givosiran based on annualized rate of porphyria attacks and administered hemin doses • ADA and safety data

Abbreviations: ADA=anti-drug antibodies; AHP=acute hepatic porphyria; AIP=acute intermittent porphyria; ALA=aminolevulinic acid; ALAS1=aminolevulinate synthase 1; CHE=chronic high excreters; CYP450=cytochrome P450; MAD=multiple ascending dose; mRNA=messenger ribonucleic acid; PBG= porphobilinogen; PD=pharmacodynamic; PK=pharmacokinetic; Q3M=once every 3 months; QM=once monthly; SAD=single ascending dose; SC=subcutaneous.

[a] For Study 001C only

[b] Analysis for this submission was based on all available data from Study 002 as of the cut-off date of 13 Dec 2018

[c] Subjects received different starting doses in Study 002 before transitioning to the Phase 3 dose of 2.5 mg/kg once monthly

[d] All available data from the open label extension period as of the database lock date of 31 Jan 2019 was used in analysis

(Source: Applicant's PK/PD Report, Table 1)

Table 40. PD Sampling Strategy in Clinical Studies of Givosiran

Study	Population	Urinary ALA Sampling Schedule
001	Part A: CHE subjects	Screening (Days -60 to -2) and admission visits (Days -1 or 0); predose, and at 6 and 8 h postdose on Day 0; on Days 1, 4, 7, 14, 21, 28, and 42; after Day 42 Q2W for 8 weeks, then Q4W until end of study[a]; at the ET visit (if applicable)
	Part B: CHE subjects	Screening (Days -60 to -2) and admission visits (Days -1 or 0); predose, and at 6 and 8 h postdose on Days 0 and 28; on Days 1, 7, 14, 21, 28, 29, 35, 42, 56, and 70; after Day 42 Q2W for 8 weeks, then Q4W until end of study[a]; at the ET visit (if applicable)
	Part C: AIP patients	Run-in (Days -168 to -2) and admission visits (Days -1 or 0); predose, and at 6 and 8 h postdose on Days 0 and 84; on Days 1, 7, 14, 21, 28, 29, 35, 42, 49, 56, 63, 70, 77, 85, 98, 112, 140, 168 and 252; at the ET visit (if applicable)
002	AIP patients	• Q3M regimen: Screening (Days -60 to -1); on Days 1, 14, 31, 61, 91, 181, 271, 361, 451, 541, 631, 721, 811, 901, 991, 1081, 1171, and 1351
		• QM regimen: Screening (Days -60 to -1); on Days 1, 14, 31, 61, 91, 121, 151, 181, 271, 361, 451, 541, 631, 721, 811, 901, 991, 1081, 1171, and 1351
003	AHP patients	• 6-month Treatment Period: Screening (Days -60 to -1); predose on Days 1, 15, 29, 57, 85, 113, 141, and 169 • Open-label Extension Period: Predose on Days 183, 197, 225, 253, 281, 309, 337, 365, 393, 421, 449, 477, 505, 589, 673, 757, 841, 925, and 1009; at the ET visit (if applicable)

Abbreviations: AHP=acute hepatic porphyria; AIP=acute intermittent porphyria; CHE=chronic high excreters; ET=early termination; PD=pharmacodynamic; PK=pharmacokinetic; Q2W=once every 2 weeks; Q3M=once every 3 months; Q4W=once every 4 weeks; QM=once monthly.
[a] In patients where urinary ALA or PBG levels at the Day 42/ET visit had not returned to ≥80% of Day 0 predose levels, urine levels were monitored until ALA or PBG returned to ≥80% of Day 0 predose levels, or until the end of study.

(Source: Applicant's PK/PD Report, Table 2)

Descriptive statistics of the continuous and categorical demographic data included in the PK/PD modeling are summarized in Table 41 and Table 42, respectively.

Table 41. Baseline Characteristics of PK/PD Population by Study (Continuous Covariates)

Baseline Characteristics	Study 001A/B[a] (CHE subjects)	Study 001C /Study 002[b] (AHP patients)	Study 003 (AHP patients)	Total
	N=23	N=17	N=94	N=134
Age (years)				
Mean ±SD	44.4±9.7	37±11.8	38.8±11.4	39.5±11.3
Median (min, max)	47 (30, 64)	39 (21, 60)	37.5 (19, 65)	38 (19, 65)
Weight (kg)				
Mean ±SD	75.9±15.9	75.7±17.9	66.8±16.2	69.5±16.7
Median (min, max)	75 (57.3, 118)	73.1 (44.5, 118.4)	65 (39.5, 131.3)	66.2 (39.5, 131.3)
eGFR (mL/min/1.73 m^2)				
Mean ±SD	79.1±21.4	74.4±26.7	72.9±26.4	74.1±25.6
Median (min, max)	76.2 (51.1, 141.2)	71.2 (38.5, 126.2)	67 (26, 151)	70.4 (26, 151)
Baseline ALA (mmol/mol Cr)				
Mean ±SD	10.2±7.2	17.8±13	18.5±14.1	17±13.3
Median (min, max)	6.8 (2.5, 23.6)	15.6 (1.5, 50.5)	15.8 (0.7, 88.9)	15.3 (0.7, 88.9)

Abbreviations: AHP=acute hepatic porphyria; ALA=aminolevulinic acid; CHE=chronic high excreters, Cr=creatinine; eGFR=estimated glomerular filtration rate; max=maximum; min=minimum; PD=pharmacodynamic; PK=pharmacokinetic; SD=standard deviation.

[a] 3 CHE subjects were treated in both Parts A and B; 2 CHE subjects participated in Part A twice.

[b] Study 002 patients were from Study 001C; 4 out of 16 patients were treated with placebo in Study 001.

(Source: Applicant's PK/PD Report, Table 4)

Table 42. Baseline Characteristics of the PK/PD Population by Study (Categorical Covariates)

Baseline Characteristics	Study 001A/B[a] (CHE subjects)	Study 001C /Study 002[b] (AHP patients)	Study 003 (AHP patients)	Total
	N=23	N=17	N=94	N=134
Sex, n (%)				
Female	18 (78.3%)	15 (88.2%)	84 (89.4%)	117 (87.3%)
Male	5 (21.7%)	2 (11.8%)	10 (10.6%)	17 (12.7%)
Race, n (%)				
White	22 (95.7%)	14 (82.4%)	73 (77.7%)	109 (81.3%)
Black or African American	0 (0%)	2 (11.8%)	1 (1.1%)	3 (2.2%)
Asian	1 (4.3%)	1 (5.9%)	15 (16%)	17 (12.7%)
Native Hawaiian or other Pacific Islander	0 (0%)	0 (0%)	1 (1.1%)	1 (0.7%)
Other	0 (0%)	0 (0%)	4 (4.3%)	4 (3%)
Patient Type, n (%)				
CHE	23 (100%)	0 (0%)	0 (0%)	23 (17.2%)
AHP	0 (0%)	17 (100%)	94 (100%)	111 (82.8%)
East Asian Flag, n (%)				
East Asian	0 (0%)	0 (0%)	12 (12.8%)	12 (9%)
Non-East Asian	23 (100%)	17 (100%)	82 (87.2%)	122 (91%)
Hepatic function, n (%)				
Normal	21 (91.3%)	16 (94.1%)	84 (89.4%)	121 (90.3%)
Mild	2 (8.7%)	1 (5.9%)	8 (8.5%)	11 (8.2%)
Moderate[c]	0 (0%)	0 (0%)	1 (1.1%)	1 (0.7%)
Severe[c]	0 (0%)	0 (0%)	1 (1.1%)	1 (0.7%)
Renal function, n (%)				
Normal	6 (26.1%)	4 (23.5%)	21 (22.3%)	31 (23.1%)
Mild	12 (52.2%)	8 (47.1%)	46 (48.9%)	66 (49.3%)
Moderate	5 (21.7%)	5 (29.4%)	26 (27.7%)	36 (26.9%)
Severe[d]	0 (0%)	0 (0%)	1 (1.1%)	1 (0.7%)

Abbreviations: AHP=acute hepatic porphyria; CHE=chronic high excreters; max=maximum; min=minimum; NCI-ODWG=National Cancer Institute Organ Dysfunction Working Group; PD=pharmacodynamic; PK=pharmacokinetic.
[a] 3 CHE subjects were treated in both Parts A and B; 2 CHE subjects participated in Part A twice.
[b] Study 002 patients were from Study 001C; 4 out of 16 patients were treated with placebo in Study 001.
[c] 2 patients (1 with moderate and 1 with severe hepatic impairment) were pooled into the mild hepatic impairment category in covariate analysis.
[d] 1 patient with severe renal impairment was pooled into the moderate renal impairment category in covariate analysis.

(Source: Applicant's PK/PD Report, Table 5)

A total of 134 patients were in the pooled PK/PD dataset. The pooled population comprised 111 (82.8%) patients with AHP and 23 (17.2%) subjects that are CHE. Patients were predominantly females (87.3%) with median age (range) of 38.0 (19.0, 65.0) years and median body weight

(range) of 66.2 (39.5, 131.3) kg. The median baseline ALA level was approximately 2-fold higher in AHP patients (15.7 mmol/mol Cr) compared to subjects that are CHE (6.80 mmol/mol Cr).

The covariate effects of age, hepatic and renal functions, baseline ALA, patient type (subjects that are CHE versus patients with AHP), and race (East Asian versus non-East Asian) were investigated in population PK/PD models. Since absolute dose (mg) of givosiran received by a patient was based on body weight, the effect of body weight on ALA lowering was investigated by simulations from the final PK/PD model. (b) (4)

There were only 5 non-AIP patients (2 with VP, 2 with HCP, and 1 with other) in Study 003. Two patients were randomized to givosiran treatment group and 3 patients were randomized to placebo group during the DB period. Thus, there were not enough non-AIP patients to evaluate as a covariate in the PK/PD analysis.

The numbers of subjects and urinary ALA samples included in the PK/PD analysis are summarized in Table 43. A total of 2600 measurable urinary ALA samples from 134 subjects in placebo and givosiran treatment groups were available for PK/PD modeling. The Phase 3 Study 003 represented 47.4% of the overall urinary ALA samples included in the population PK/PD. analysis.

Table 43. Summary of Subjects and Urinary ALA Levels Included in the PK/PD Analysis Dataset by Study.

Study	Number of Subjects N (%)	Number of ALA Samples N (%)
Study 001A/B	23 (17.2%)	499 (19.2%)
Study 001C/Study 002	17 (12.7%)	869 (33.4%)
Study 003	94 (70.1%)	1232 (47.4%)
Total	**134 (100%)**	**2600 (100%)**

Abbreviations: ALA=aminolevulinic acid; PD=pharmacodynamic; PK=pharmacokinetic.
(Source: Applicant's PK/PD Report, Table 6)

Base PK/PD Model Development

Development of the population PK/PD model of givosiran on ALA was driven by the mechanistic hypotheses, statistical considerations, and heuristics guided by observed trends in the data. A schematic of the population PK/PD model of ALA is shown in Figure 26.

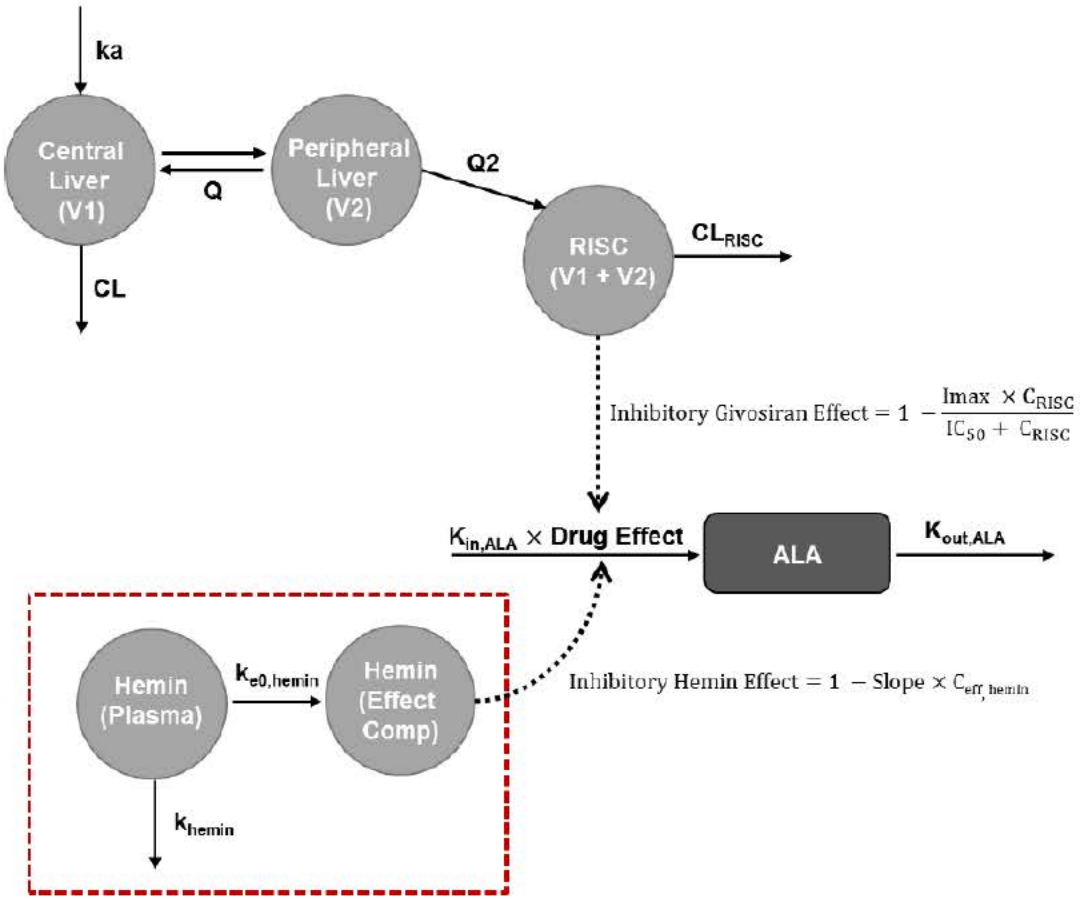
The effect of allometrically scaled concentrations of active siRNA in liver on urinary ALA levels was modeled as an inhibitory effect on the synthesis rate of ALA through an intermediary RISC effect compartment.

In AHP patients, an additive effect of hemin and givosiran was assumed and included in the model as an additional inhibitory effect on the synthesis rate of ALA. The elimination rate constant of ALA was fixed to 0.84 hr-1 (half-life=0.825 hours) from literature.[Floderus 2006]

Active siRNA turnover rate from the peripheral liver compartment to the RISC compartment (Q2) was estimated to account for the equilibrium delay between the liver and RISC compartments, which translated into a delay in PD effect relative to the projected liver concentrations.

Exploratory analysis indicated that baseline ALA levels in subjects that are CHE were approximately 2-fold higher than in AHP patients, and the duration of PD effect was longer in subjects that are CHE. Thus, different formation rate constant of ALA (Kin,ALA) and different IC50,givo were estimated for patients with AHP and subjects that are CHE.

Figure 26. Schematic Representation of Population PK/PD Model for ALA in Humans



Abbreviations: ALA=urinary aminolevulinic acid compartment; $C_{eff,hemin}$=hemin concentration of effect compartment; CL=clearance; CL_{RISC}=clearance from RNA-induced silencing complex (RISC) loaded drug; IC_{50}=concentration required to produce half-maximal effect of givosiran; I_{max}=maximum inhibitory effect of givosiran on $k_{in,ALA}$; ka=uptake rate constant to liver; $k_{e0,hemin}$=first-order rate constant of hemin from plasma to effect compartment; k_{hemin}=elimination rate constant for hemin; $k_{in,ALA}$=zero-order synthesis rate of ALA; $k_{out,ALA}$=first-order degradation rate constant for ALA; Q=intercompartmental clearance; Q2=turnover rate of givosiran from peripheral liver compartment into RISC; V1=volume of distribution of central liver compartment; V2=volume of distribution of peripheral liver compartment.

(Source: Applicant's PK/PD Report, Figure 12)

Differential equations used for the population PK/PD model (indirect response model) of urinary ALA are presented below.

$$\frac{dALA}{dt} = K_{in,ALA} \times GivosiranEffect \times HeminEffect - K_{out,ALA} \times ALA$$

where Kin,ALA is the zero-order formation rate of urinary ALA, and Kout,ALA is the first-order degradation rate of urinary ALA. As part of the above model, "GivosiranEffect" is the effect of

active siRNA concentrations in RISC on Kin,ALA. Drug effect of givosiran was modeled using inhibitory effect model (Imax) as presented below.

$$GivosiranEffect = \left(1 - I_{max,givo} \bullet \frac{C_{RISC}(t)}{IC_{50,\text{givo}} + C_{RISC}(t)}\right)$$

where CRISC(t) is the RISC concentration of active siRNA at time 't' predicted with a PK model. Imax,givo represents maximum inhibition effect of active siRNA on Kin,ALA and IC50,givo represents the RISC concentration of active siRNA reaching 50% of maximum inhibition of givosiran.

For the modeling of hemin effect, hemin plasma PK was not collected in the studies and only dosing information was present in the dataset. Therefore, hemin plasma concentrations were predicted using a 1-compartment model using the literature-reported value for the elimination rate constant 'ke' of 0.0642 hr-1 (half-life is approximately 10.8 hours).[Tokola 1986] However, this could not describe the duration of hemin effect on ALA and resulted in systematic underestimation of duration of hemin effect on urinary ALA. Therefore, effect compartment model was used to explain the time delay between plasma concentration of hemin and the PD response. The inhibitory effect of hemin on $K_{in,ALA}$ was tested using Imax and linear function models. A linear inhibitory model was selected since the model-estimated maximum inhibition of hemin (64.2%) was underestimated by the Imax model. "HeminEffect" is the hemin inhibitory effect on $K_{in,ALA}$. Drug effect of hemin was modeled using linear inhibitory model as presented below.

$$HeminEffect = \left(1 - Slope \bullet C_{eff,hemin}(t)\right)$$

where Ceff,hemin(t) is the effect compartment concentration of hemin at time 't' predicted with a reference value.

Final Population PK/PD Model

The covariate analysis indicated that age, body weight, renal impairment, sex, and race were not significant and therefore were not included the final PK/PD model. Four covariates were retained in the final PK/PD model: patient type (subjects that are CHE versus patients with AHP) on $IC_{50,givo}$ and $K_{in,ALA}$, mild hepatic impairment on $K_{in,ALA}$, and baseline ALA on $I_{max,givo}$. The parameter estimates derived with the final PK/PD model are presented in Table 44.

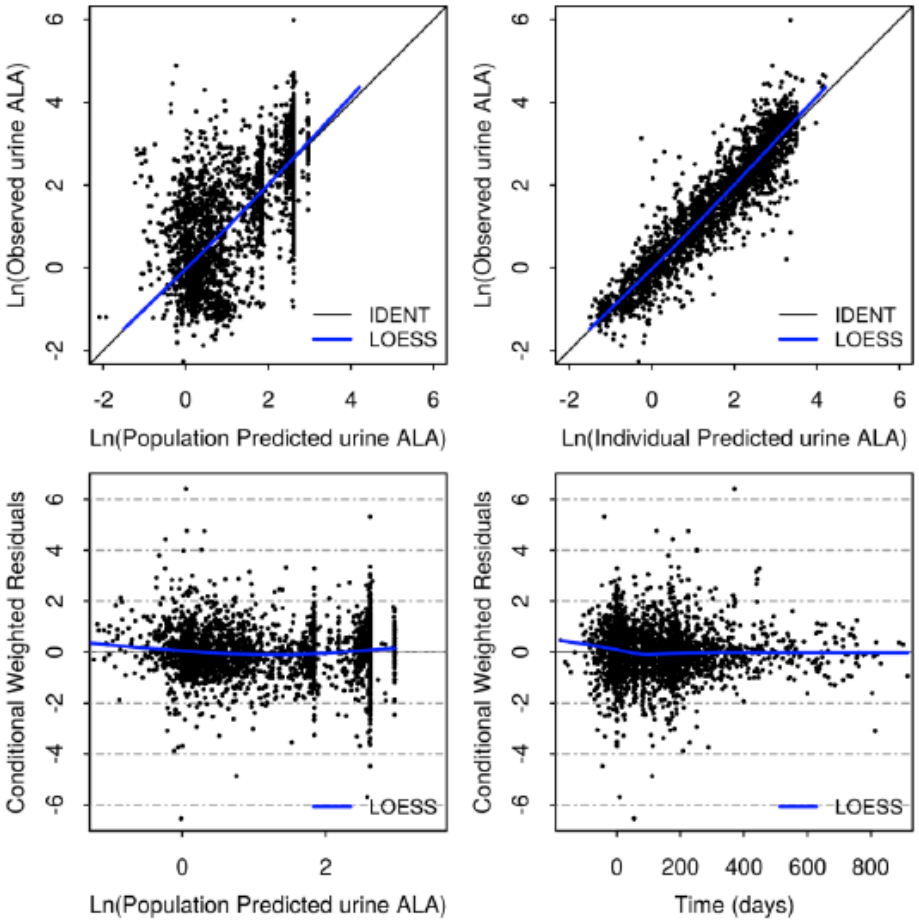
Table 44. Parameter Estimates for the Final Population PK/PD Model

PKPD Parameters	Population Estimates		RSE	IIV (RSE)	Lower 95% CI	Upper 95% CI
	Log transformed	Untransformed				
Ka (h^{-1})	NA	2.85 fixed[a]	NA	NA	NA	NA
CL (g/h)	NA	13.347 fixed[a]	NA	NA	NA	NA
Q (g/h)	NA	1.348 fixed[a]	NA	NA	NA	NA
V1 (g)	NA	6356 fixed[a]	NA	NA	NA	NA
V2 (g)	NA	5208 fixed[a]	NA	NA	NA	NA
Q2 (g/h)	NA	0.0122 fixed[b]	NA	NA	NA	NA
CL_{RISC} (g/h)	NA	20.2	17.4%	NA	13.3	27.1
$K_{eo,hemin}$ (h^{-1})	NA	0.00761	14.8%	NA	0.0054	0.00982
Slope (mL/µg)	NA	0.034	19.9%	58.0% (24.3%)	0.0207	0.0473
$I_{max,givo}$	-0.0448	0.956	12.3%	NA	0.946	0.967
$IC_{50,givo}$ [AHP] (ng/g)	-7.65	0.476[c]	2.43%	144% (9.58%)	0.329[c]	0.683[c]
$IC_{50,givo}$ [CHE] (ng/g)	-9.21	0.100[c]	3.40%		0.054[c]	0.185[c]
$k_{in,ALA}$ [AHP] (mmol/mol*hr)	2.44	11.47	3.14%	68.7% (8.22%)	9.91	13.40
$k_{in,ALA}$ [CHE] (mmol/mol*hr)	1.67	5.312	8.41%		4.027	6.98
$k_{out,ALA}$ (hr^{-1})	-0.174 fixed	0.84 fixed[d]	NA	NA	NA	NA
Change in log($I_{max,givo}$) per log(Baseline ALA)	NA	0.0368	20.5%	NA	0.022	0.0516
Fraction Change in $k_{in,ALA}$ for mild hepatic impairment	0.342	1.41	45.8%	NA	1.036	1.915
Residual Error	NA	52.4%	3.60%	NA	48.7%	56.1%
OFV	-28.827					

Abbreviations: AHP=acute hepatic porphyria; CHE=subjects who are chronic high excreters; CI=confidence interval; CL=clearance; CL_{RISC}=clearance of RISC loaded drug; $IC_{50,givo}$=givosiran concentration in RISC required to reach 50% of maximum inhibition; IIV=inter-individual variability; $I_{max,givo}$=maximum inhibitory effect of givosiran on $k_{in,ALA}$; ka=uptake rate constant to liver; k_{hemin}=elimination rate constant of hemin; $k_{in,ALA}$=zero-order synthesis rate constant for ALA; $k_{out,ALA}$=first-order degradation rate constant for ALA; NA=not applicable; OFV=objective function value; Q=intercompartmental clearance; Q2=turnover rate of givosiran from peripheral liver compartment into RISC; RSE=relative standard error; V1=volume of distribution of central liver compartment; V2=volume of distribution of peripheral liver compartment.
[a] Liver PK parameters for 70 kg patient were fixed to allometrically scaled values from the rat liver PK model.
[b] The slope for hemin effect on $k_{in,ALA}$ was fixed to the estimated slope from the previous PK/PD model (run5).
[c] Values were converted from µg/g to ng/g.
[d] The $k_{out,ALA}$ value for ALA was fixed to 0.84 hr^{-1} from literature.[Floderus 2006]

(Source: Applicant's PK/PD Report, Table 10)

Figure 27. Goodness-of-fit Plots for the Final Population PK/PD Model



Abbreviations: ALA=aminolevulinic acid; LOESS=locally weighted scatterplot smoothing; PD=pharmacodynamic; PK=pharmacokinetic; IDENT=line of identity.
Note: Blue lines represent LOESS lines.

(Source: Applicant's PK/PD Report, Figure 14)

Effect of Body Weight on Givosiran PD

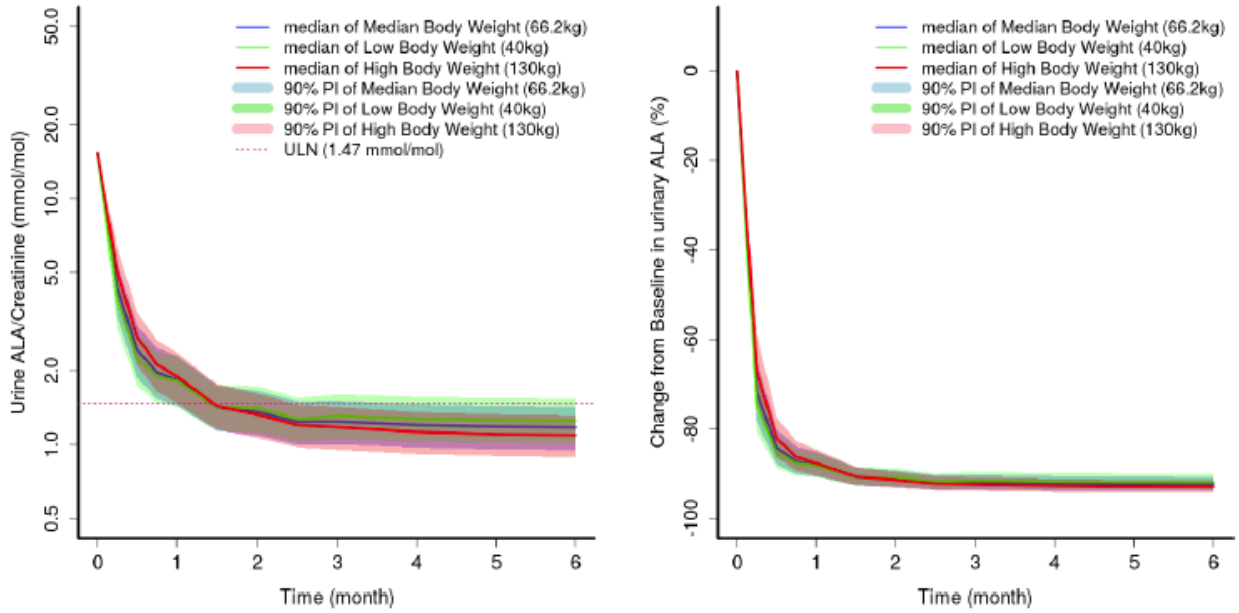
The impact of body weight was incorporated in the PK/PD model using allometric exponents. All clearance parameters were scaled by an exponent of 0.75 and all volume parameters were scaled by an exponent of 1.

Covariate evaluation showed that the impact of body weight on PD parameters (I_{max} and IC_{50}) was not statistically significant. Since the dose of givosiran is based on body weight, patients with lower body weight received a lower absolute dose compared to patients with higher body weight. The impact of body weight on steady state urinary ALA levels was investigated using simulations from the final PK/PD model.

Simulations showed that ALA levels and % reduction in ALA at steady state were similar across the range of body weights observed in clinical studies. PD response in patients weighing 40 kg

(lowest body weight across studies) and 130 kg (highest body weight across studies) were comparable to that in patients weighing 66.2 kg (median body weight across studies; see Figure 35).

Figure 28. Model-Predicted Absolute and Percent Change from Baseline ALA in AHP Patients Weighting 40, 66.2 and 130 kg After 2.5 mg/kg Once Monthly Dose of Givosiran



Abbreviations: AHP=acute hepatic porphyria; ALA=aminolevulinic acid; PI=prediction interval.
(Source: Applicant's PK/PD Report, Figure 19)

(b) (4)

(b) (4)

Table 45. Simulated Steady-State Urinary ALA Levels in (b) (4) Adult Patients After 2.5 mg/kg Monthly Doses of Givosiran

Patients	Mean	SD	Geometric Mean	%CV	5th Percentile	Median	95th Percentile
(b) (4)							
Adults (>18 years)	1.18	0.15	1.17	12.6	0.95	1.18	1.43

Abbreviations: ALA=aminolevulinic acid; CV=coefficient of variation; SD=standard deviation.

(Source: Applicant's PK/PD Report, Table 19)

Reviewer's Comments on the E-R Analysis for Urine ALA:

The applicant's analysis establishes distribution to liver based in part on rat distribution to liver data and allometric scaling principles. While this may be a reasonable approach to estimate the

liver concentrations, some degree of uncertainty remains as to the accuracy of this model for human PK in the liver, since this cannot be measured directly.

The analysis does appear to capture the central tendency of the data, which makes this reasonable for descriptive purposes.

(b) (4)

E-R Analysis for Safety: Serum Creatinine Concentrations and Liver ALT Levels

Methods

Longitudinal serum ALT and SCr measurements obtained following givosiran administration from long-term multiple dose studies in AHP patients (Study 002 and Study 003) were pooled for the analysis.

The Safety Population data included 64 AHP patients who received givosiran 1.25 mg/kg, 2.5 mg/kg, and 5.0 mg/kg once monthly, and 5.0 mg/kg once quarterly.

A summary of relevant safety variables analyzed in this section is presented in Table 46.

Table 46. Baseline Characteristics of Safety Population Treated with Givosiran by Study

Baseline Characteristics	Study 002 (N=16)	Study 003 (N=48)	Safety Population Total (N=64)
SCr (µmol/L)			
Mean ±SD	87.8±26.4	88.1±28.7	88.0±28.0
Median (min, max)	76 (53, 140)	82 (53, 194)	81 (53, 194)
SCr/ULN			
Mean ±SD	0.853±0.242	0.864±0.266	0.861±0.258
Median (min, max)	0.752 (0.525, 1.32)	0.813 (0.535, 1.53)	0.808 (0.525, 1.53)
ALT (U/L)			
Mean ±SD	22.5±13.7	24.3±15.1	23.8±14.7
Median (min, max)	17 (10, 58)	20 (8, 78)	19 (8, 78)
ALT/ULN			
Mean ±SD	0.649±0.41	0.592±0.369	0.606±0.377
Median (min, max)	0.500 (0.294, 1.71)	0.488 (0.195, 1.90)	0.494 (0.195, 1.90)

Abbreviations: ALT=alanine aminotranferase; SCr=serum creatinine; ULN=upper limit of normal; SD=standard deviation.
Note: Baseline values are from central labs.
(Source: Applicant's PK/PD Report, Table 20)

Analysis of ALT Elevations in Studies 002/003

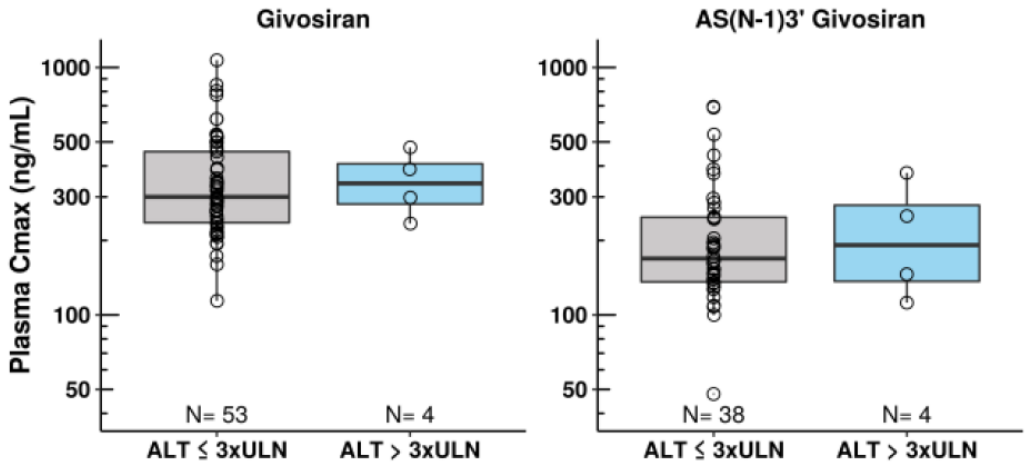
In Study 003, 6/48 patients treated with 2.5 mg/kg once monthly givosiran and 1/46 patients treated with placebo had AEs mapping to the Drug-Related Hepatic Disorders Standardized Medical Dictionary for Regulatory Activities (MedDRA) Query (SMQ). All 7 patients had ALT elevations > 3x ULN, which was the protocol-defined threshold for enhanced monitoring with hepatic assessments. Additionally, another patient (USUBJID=ALN-AS1-003-(b) (6)) had ALT>3x ULN that was not considered as an AE, but was included in the population safety analysis. Therefore, the analysis of correlations between givosiran PK and/or PD and ALT elevations was conducted by categorizing the patients by whether they had any postdose measurement of ALT >3x ULN in Studies 002 and 003.

In Study 003, a total of 7 patients treated with givosiran had ALT elevations >3x ULN. No patient in Study 002 had ALT elevations >3x ULN.

Relationship between ALT Elevations and Givosiran Plasma PK

The applicant concluded that there is no correlation between the maximum postdose ALT levels and plasma Cmax for givosiran or its metabolite (Figure 30).

Figure 30. Study 002/003 – Lack of Relationship Between ALT Elevations and Plasma Cmax for Givosiran and AS(N-1)3' Givosiran



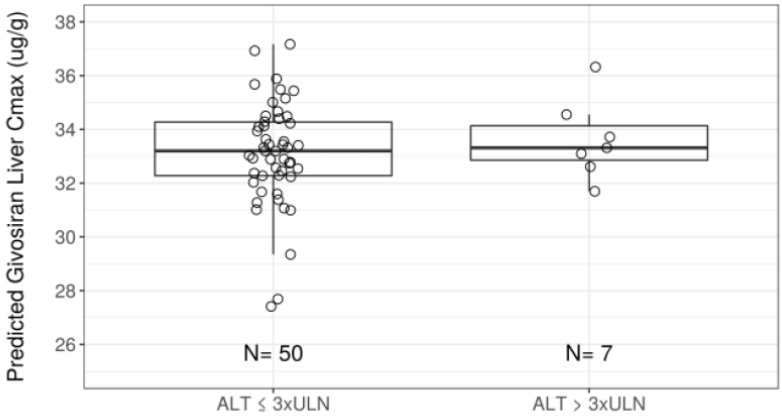
Abbreviations: ALT=alanine aminotranferase; C_{max}= maximum observed plasma concentration; ULN=upper limit of normal.
Note: C_{max} is from Month 6 in Study 002 and from Month 5 in Study 003. All measurable concentrations at Month 6 for Study 002 and Month 5 for Study 003 are shown. AS(N-1)3' givosiran was not measured in Study 002. A total of 4/7 patients with ALT>3xULN had measurable givosiran concentrations, 2 were below the limit of quantitation, and 1 patient had a missing PK sample. A total of 53/57 patients with ALT≤3xULN had measurable givosiran concentrations, 3 had missing PK samples, and 1 patient discontinued.
(Source: Applicant's Population PK/PD Report, Figure 26)

Relationship between ALT Elevations and Givosiran Predicted Liver PK

Based on Figure 31 and Figure 32 the applicant concluded:

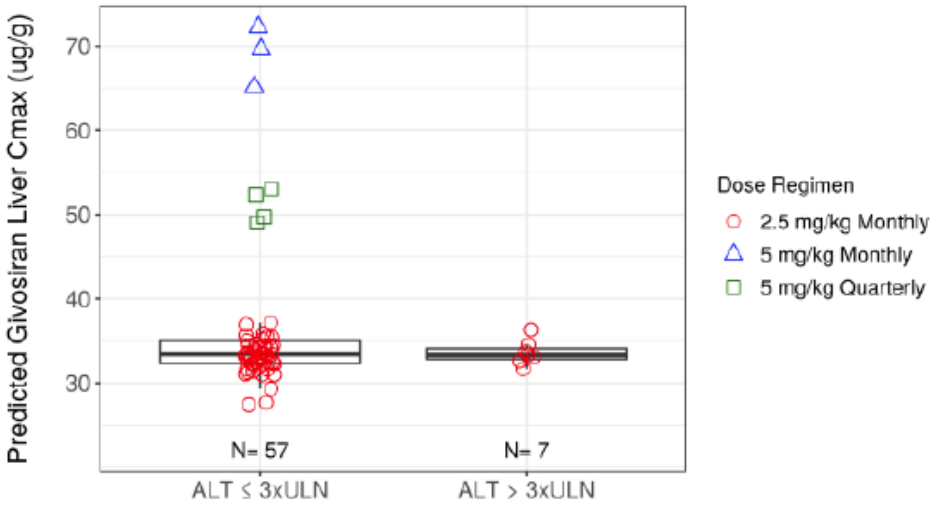
Model-predicted maximum liver Cmax of givosiran in patients who had ALT >3x ULN were comparable to patients without ALT elevations for patients receiving givosiran 2.5 mg/kg monthly indicating that ALT elevations were not correlated with predicted target site exposure of givosiran (Figure 31). A comparison of model-predicted liver concentrations across all dose regimens in Studies 002 and 003 showed a higher liver Cmax for 5 mg/kg monthly and 5 mg/kg quarterly relative to other doses, however, there were no ALT elevations from Study 002 (Figure 32). This indicates that there were no ALT elevations in patients at liver Cmax of approximately 2- to 3-fold higher than the average liver Cmax in patients with ALT >3x ULN following the therapeutic dose of 2.5 mg/kg monthly.

Figure 31. Study 002/003 – Lack of Relationship Between ALT Elevations and Predicted Givosiran Liver Cmax During First 3 Months of Study for Patients Receiving Givosiran 2.5 mg/kg Once Monthly



Abbreviations: ALT=alanine aminotransferase; C_{max}=maximum observed plasma concentration, ULN=upper limit of normal.
Note: In Study 002, 7 patients started with dose regimens different than 2.5 mg/kg once monthly and their data are not presented above. *(Source: Applicant's Population PK/PD Report, Figure 27)*

Figure 32. Study 002/003: Correlation Between ALT Elevations and Model-Predicted Givosiran Liver Cmax During First 3 Months of Study for Patients Receiving Givosiran by Dose Regimen

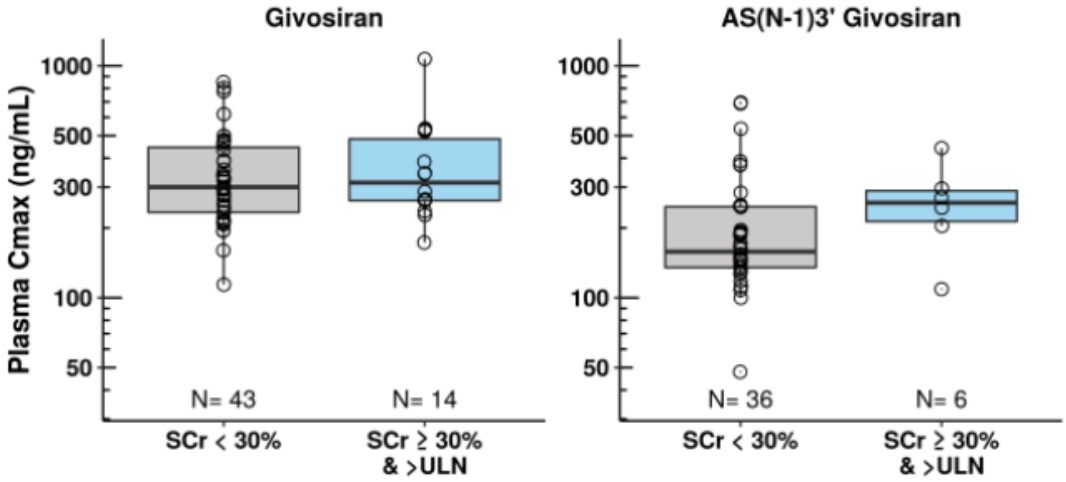


Abbreviations: ALT=alanine aminotranferase; C_{max}=maximum concentrations; ULN=upper limit of normal.
(Source: Applicant's PK/PD Report, Figure 28)

Evaluation of Relationship between SCr Elevations and Givosiran Plasma PK

The applicant concluded there is no correlation between the maximum postdose SCr levels and plasma Cmax for givosiran or its metabolite (Figure 33).

Figure 33. Study 002/003: Correlation Between SCr Elevations and Plasma Cmax for Givosiran and AS(N-1)3' Givosiran



Abbreviations: ALT=alanine aminotranferase; C_{max}=maximum concentrations; ULN=upper limit of normal.
Note: Concentration at 2 hours is considered as C_{max}. All measurable concentrations at Month 6 for Study 002 and Month 5 for Study 003 are shown. AS(N-1)3' givosiran was not measured in Study 002. Out of 16 patients with SCr ≥30% and >ULN, 14 had measurable givosiran concentrations, 2 were below the limit of quantitation, and 1 had a missing PK sample. Out of 48 patients with SCr <30%, 43 had measurable Givosiran concentrations, 4 had missing PK samples, and 1 patient discontinued.
(Source: Applicant's PK/PD Report, Figure 31)

Reviewer's Comments for the Applicant's E-R for Safety Analysis:

The applicant's E-R analysis for ALT elevation is certainly limited by the small number of ALT events >3x ULN (n=7). This number makes it hard to evaluate rates of safety across different concentrations. Instead the applicant has chosen to evaluate concentration as a function of event. The most assuring piece of information comes from Figure 32 which highlights that subjects with exposure 2-3 fold higher than the 2.5 mg/kg dose, who received the 5 mg/kg dose did not experience these ALT elevations.

19.4.6 Immunogenicity

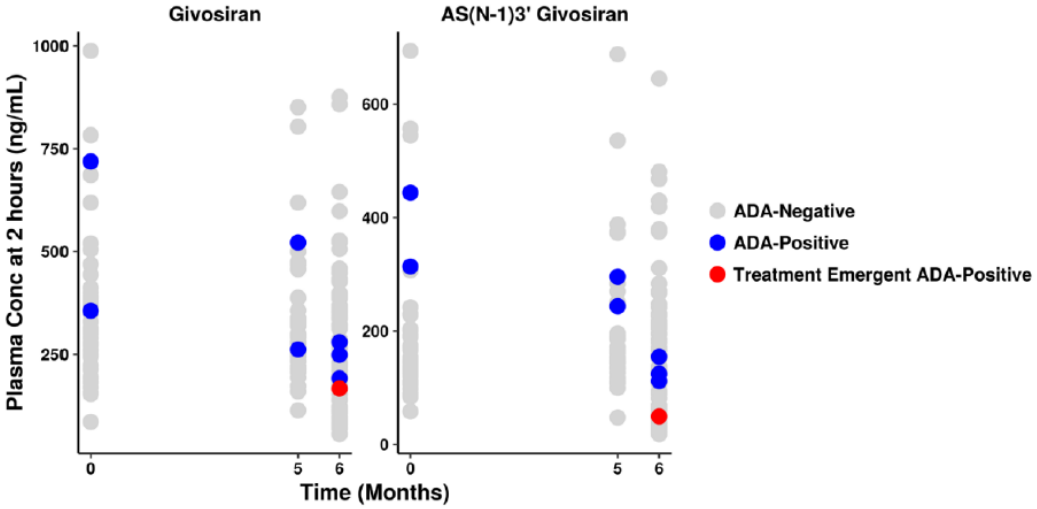
Immunogenicity was assessed in all 4 studies. For the registration Phase 3 trial Study ENVISION, ADA assessment were conducted at baseline, Day 29, Months 3, 6, 7, 12, 18, 24, 36. A validated enzyme linked immunosorbent assay (ELISA) with a LLOQ of 37.4 ng/mL and minimal required dilution (MRD) of 50-fold was used to assess the immunogenicity potential of givosiran by detecting serum immunoglobulin (Ig) G (IgG)/IgM antibodies against givosiran.

In the DB period, 1/46 (2.2%) patient in the placebo group and 2/48 (4.2%) patients in the givosiran group tested positive for ADA at baseline. The ADA titer in these 3 patients increased by less than 4-fold (two 2x serial dilutions) during the study (titer ≤100). In the combined DB

and OLE period, 1/94 (1.06%) patients treated with givosiran developed de novo ADA during the study.

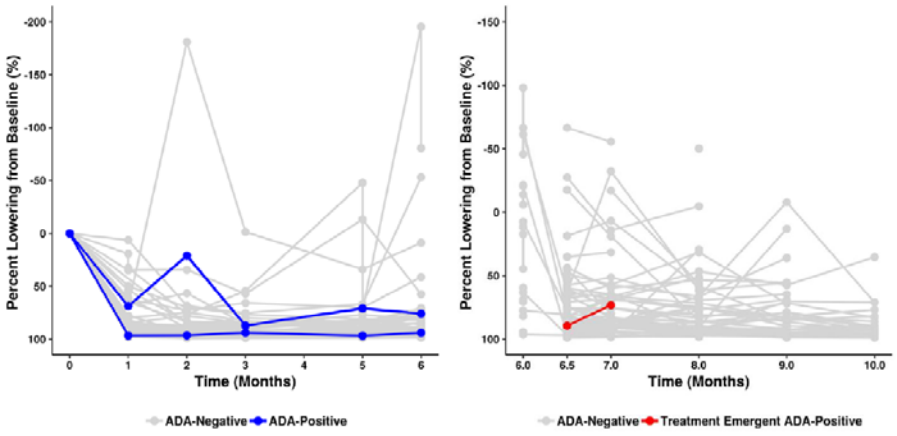
Presence of ADA appears to have no impact on the PK of givosiran. In the 2 patients randomized to givosiran in the DB period who were ADA-positive at baseline, and the 1 patient with treatment-induced ADA in the OLE period, concentrations of givosiran and AS(N-1)3' givosiran were comparable to those in ADA-negative patients (Figure 34).

Figure 34. Comparison of Givosiran and AS(N-1)3' Givosiran Concentrations in ADA-Positive and ADA-Negative Patients



Similarly, presence of ADA appears to have no impact on the PD of givosiran (Figure 35).

Figure 35. Comparison of Change from Baseline ALA Levels Between ADA-Positive and ADA-Negative Patients



19.5 Additional Clinical Outcome Assessment Analyses

See section 8.1. Review of Relevant Individual Trials Used to Support Efficacy.

NDA 212194 GIVLAARI (givosiran)
Multidisciplinary Review Signatures

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Regulatory Project Manager	Jennifer Lee, PharmD	ORO/DROOD	Sections: 3	**Select one:** ☑ Authored ☐ Approved
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Nonclinical Reviewer	Brenda Gehrke, PhD	OOD/DHOT	Sections: 5	**Select one:** ☑ Authored ☐ Approved
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Nonclinical Reviewer	Matthew Thompson, PhD, MPH	OOD/DHOT	Sections: 5	**Select one:** ☑ Authored ☐ Approved
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Deputy Division Director (DHOT)	Haleh Saber, PhD	OOD/DHOT	Sections: 5, 14	**Select one:** ☑ Authored ☑ Approved
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Clinical Pharmacology Reviewer	Huiming Xia, PhD	OCP/DCPV	Sections: 6	**Select one:** ☑ Authored ☐ Approved
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Clinical Pharmacology Team Leader	Jeanne Fourie Zirkelbach, PhD	OCP/DCPV	Sections: 6	**Select one:** ☑ Authored ☑ Approved
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Pharmacometrics Reviewer	Justin Earp, PhD	OCP/DPM	Sections: 6	**Select one:** ☑ Authored ☐ Approved
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NDA 212194 GIVLAARI (givosiran)
Multidisciplinary Review Signatures

Pharmacometrics Team Leader	Lian Ma, PhD	OCP/DPM	Sections: 6	**Select one:** ☑ Authored ☑ Approved
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Deputy Division Director (OCP)	Brian Booth, PhD	OCP/DCPV	Sections: 6, 15	**Select one:** ☑ Authored ☑ Approved
	Signature: Nam A. Rahman -S Digitally signed by Nam A. Rahman -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Nam A. Rahman -S, 0.9.2342.19200300.100.1.1=1300072597 Date: 2019.11.13 16:03:17 -05'00'			
Clinical Reviewer	Andrew Dmytrijuk, MD	ODE1/DHP	Sections: 1, 2, 4, 7, 8, 9, 10, 11, 12, 13, 19	**Select one:** ☑ Authored ☐ Approved
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Statistical Reviewer	Kunthel By, PhD	OB/DBIX	Sections: 7, 8	**Select one:** ☑ Authored ☐ Approved
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Statistical Team Leader	Yu-te Wu, PhD	OB/DBIX	Sections: 8	**Select one:** ☑ Authored ☑ Approved
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Division Director (OB)	Thomas Gwise, PhD	OB/DBIX	Sections: 8, 16	**Select one:** ☑ Authored ☑ Approved
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Cross-Disciplinary Team Leader (CDTL)	Kathy Robie Suh, MD, PhD	ODE1/DHP	Sections: All	**Select one:** ☑ Authored ☑ Approved
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Supervisory Associate Division Director (Clinical)	Albert Deisseroth, MD, PhD	ODE1/DHP	Sections: All	**Select one:** ☑ Authored ☑ Approved
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Acting Office Director/Signatory	Richard Pazdur, MD	OOD	Sections: All	**Select one:** ☑ Authored ☑ Approved
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