30 January 2020
EMA/CHMP/70703/2020
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Givlaari

International non-proprietary name: givosiran

Procedure No. EMEA/H/C/004775/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AAR	Annualized attack rate
ADA	Anti-drug antibodies
ADP	ALAD-deficient porphyria
AE	Adverse events
Af	2'-fluoroadenosine
AHP	Acute hepatic porphyria
AIP	Acute intermittent porphyria
ALA	Aminolevulinic acid
ALAD	Aminolevulinic acid dehydratase
ALAS1	Aminolevulinate synthase 1
ALT	Alanine aminotransferase
Am	2'-O-methyladenosine
ANCOVA	Analysis of covariance
AS	Atomic Spectroscopy
ASGPR	Asialoglycoprotein receptor
AST	Aspartate aminotransferase
AX	Anion Exchange
BPI-SF	Brief Pain Inventory (Short Form)
BMI	Body mass index
cERD	circulating extracellular RNA detection
Cf	2'-fluorocytidine
CHE	Chronic high excreters
CKD	Chronic kidney disease
CLR	Clearance
Cm	2'-O-methylcytidine
CPP	Critical process parameter
CPV	Continuous process verification
CQA	Critical quality attribute
DMT	4,4'-dimethoxy trityl
DS	Drug substance
eGFR	Estimated glomular filtration rate
EMA	European Medicines Agency
FAS	Full analysis set
FASAIP	AIP patients in full analysis set
FID	Flame ionization detector
FLP	Full length product
FTIR	Fourier-transform infrared spectroscopy
GalNAc	Triantennary N-acetylgalactosamine

GC	Gas chromatography
GCP	Good clinical practice
Gf	2'-fluoroguanosine
GLP	Good laboratory practice
Gm	2'-O-methylguanosine
GMP	Good manufacturing practice
GTI	Genotoxic impurities
HCP	Hereditary coproporphyria
HMBS	Hydroxymethylbilane synthase
HPLC	High performance liquid chromatography
ICH	International council for harmonisation
ICP	Inductively coupled plasma
IC50	Half maximal inhibitory concentration
INR	International normalized ratio
IPRP	Ion pairing reversed phase
ISR	Injection site reaction
KF	Karl-Fisher
IV	Intravenous
LC	Liquid chromatography
LFT	Liver function test
LLoQ	Lower limit of quantitation
LOQ	Limit of quantitation
MMRM	Mixed model for repeated measures
mRNA	Messenger ribonucleic acid
MS	Mass Spectroscopy
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NMR	Nuclear magnetic resonance spectroscopy
NOR	Normal operating range
NSAID	Non-steroid anti-inflammatory drugs
OLE	Open label extension
PAR	proven acceptable range
PBG	Porphobilinogen
PCS	Hhysical component score
PD	Pharmacodynamics
PDCO	EMA paediatric committee
Ph.Eur.	European Pharmacopoeia
PIP	Paediatric investigation plan
PK	Pharmacokinetics
PPQ	Process performance qualification
PPS	Per protocol set

PRO	Patient reported outcome
PS or ps	Phosphorothioate (linkage sodium salt)
PTFE	Polytetrafluorethylen
QM	Once monthly
REC	Recommendation
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
SAE	Serious adverse events
SC	Subcutaneous
SF-12	Short form 12 health survey
siRNA	Small interfering ribonucleic acid
SNP	Single nucleotide polymorphisms
Tg-rasH2	Hemizygous rasH2 [CByB6F1-Tg(HRAS)2Jic Hemizygous]
Tm	Melting temperature
Uf	2'-fluorouridine
UF	Ultrafiltration
ULN	Upper limit of normal
Um	2'-O-methyluridine
UTI	Urinary tract infection
UV	Ultra violet
VP	Variegate porphyria

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Alnylam Netherlands B.V. submitted on 27 June 2019 an application for marketing authorisation to the European Medicines Agency (EMA) for Givlaari, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 23 February 2017.

Givlaari was designated as an orphan medicinal product EU/3/16/1731 on 29 August 2016 in the following condition: the treatment of acute hepatic porphyria.

Givlaari was granted eligibility to PRIME on 23 February 2017 in the following indication: prevention of acute attacks of hepatic porphyria.

Eligibility to PRIME was granted at the time in view of the following:

- There was an unmet medical need for the prevention of acute attacks of hepatic porphyria as no licensed treatments for the prevention of acute hepatic porphyria attacks were available; general prophylactic measures applied do not prevent long-term severe morbidity and mortality; liver transplantation provides only an option of last resort and is associated with substantial risk.
- The non-clinical and preliminary clinical data presented suggested that givosiran had the potential to significantly address the unmet medical need as data showed robust and consistent activity in reducing ALAS1 transcription thereby reducing levels of ALA/PBG as the key accumulating toxic metabolites in patients with AHP.
- In the two cohorts presented, a 74% and 58% decrease in mean annualised attack-rate could be shown (versus reductions of 23-34% with placebo). Improvements were also shown in terms of increase in the number of attack-free days, reduction in the number of hospitalisations and use of analgesics.

The applicant applied for the following indication: treatment of acute hepatic porphyria (AHP) in adults and adolescents aged 12 years and older.

The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Givlaari as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/givlaari.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0038/2019 on the granting of a product-specific waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request for consideration

Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

New active Substance status

The applicant requested the active substance givosiran contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

PRIME support

Upon granting of eligibility to PRIME, the Rapporteur was appointed by the CHMP.

A kick-off meeting was subsequently organised with the EMA, Rapporteur, assessors' team and experts from relevant scientific committees. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

Stereochemistry, level and types of impurities, proposed specification tests and limits for the drug substance, intermediates and drug product, initial risk assessment and QTTPs/CQAs, planned overall non-clinical programme and timing of submission of carcinogenicity data, dose selection, plans and timing of evaluations of patients with renal and hepatic impairment, correlation between the biomarker and clinical outcomes, interim analysis of phase 3 study, conditional marketing authorisation, safety, risk management plan and plans for long-term follow up.

Protocol assistance

The applicant received Protocol Assistance on 20 July 2017 (EMEA/H/SA/3587/1/2017/PA/PR/III) and on 22 March 2018 (EMEA/H/SA/3587/2/2018/PA/PR/I and EMEA/H/SA/3587/1/FU/1/2018/PA/PR/III) for the development programme in question. The Protocol Assistance pertained to the following Quality, Non-clinical and Clinical aspects:

- Acceptability of the proposed identification and qualification thresholds for individual oligonucleotide-related impurities
- Acceptability of the proposed starting materials for manufacture
- Acceptability of the submission strategy for the validation data package including a) the drug substance process validation plan and b) the drug product validation plan and sufficiency of presentation of validation data from 1 batch of drug product in the context of an envisaged conditional marketing authorisation
- Adequacy of the planned overall non-clinical programme to support a MAA for the treatment of adults and adolescents with AHP
- Timing of the submission of carcinogenicity data
- Adequacy not to conduct a thorough QT/QTc study
- Adequacy not to conduct a renal impairment study
- Adequacy not to conduct a hepatic impairment study
- Adequacy not to conduct a radiolabel PK study in humans
- Adequacy of the proposed strategy to evaluate whether a dedicated clinical drug-drug-interaction (DDI) study is warranted for CYP3A4 substrates
- Acceptability of the proposed SC dose regimen of 2.5 mg/kg monthly
- Acceptability of the proposed Phase 3 study design with a view to the choice of comparator, the inclusion and exclusion criteria to define the study population, the primary and secondary efficacy endpoints (in particular acceptability of 'reductions in ALA' as surrogate endpoint to predict clinical benefit), the randomisation and stratification scheme, the plans to exempt protocol-specified endpoints and disease related events from expedited reporting and the statistical analysis plan (including plans for interim analysis)
- Adequacy of the expected safety database to support MAA assessment
- Ability to support benefit/risk evaluation in patients suffering from other closely related, rare AHP's (VP, HCP and ADP) with data generated in the Phase 3 AIP study
- Adequacy of the expected overall data package to support a request for conditional marketing authorisation for the treatment of AIP
- Adequacy of the envisaged Sponsor's Specific Obligations and post-approval commitments to convert a potential conditional marketing authorisation to a full MA
- Adequacy of the evidence to be generated in the proposed Phase 3 study to demonstrate significant benefit

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Paula Boudewina van Hennik Co-Rapporteur: Fátima Ventura

The application was received by the EMA on	28 June 2019
Accelerated Assessment procedure was agreed-upon by CHMP on	29 May 2019
The procedure started on	18 July 2019
The following GCP inspection was requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	18 July 2019
– A GCP inspection at an investigator site, a Contract Research Organization site and a sponsor site in the United States and in Poland between 21/8/2019 and 18/09/2019. The outcome of the inspection carried out was issued on 10/10/2019	
The Rapporteur's first Assessment Report was circulated to all CHMP members on	20 September 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	23 September 2019
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	25 September 2019
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	3 October 2019
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	15 October 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	9 November 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	29 November 2019
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	10 December 2019
The applicant submitted the responses to the CHMP List of Outstanding Issues on	2 January 2020
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	16 January 2020
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Givlaari on	30 January 2020

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Givlaari has been developed for the treatment of acute hepatic porphyria.

2.1.2. Epidemiology and risk factors

Acute hepatic porphyria (AHP) is a family of rare, serious, and severely debilitating genetic disorders of the liver heme synthesis. An overall prevalence of AHP in Europe is estimated as 1.01 per 100,000 people, except in Sweden where AHP is somewhat more prevalent (2.3 per 100,000) due to a founder effect, with the majority of patients with the subtype acute intermittent porphyria (AIP) [Elder 2013]. AHP is an incompletely penetrant genetic disease; the frequency of loss of function mutations in the hydroxymethylbilane synthase (HMBS) gene is approximately 1 per 1600 to 1700 (approximately 60 per 100,000), but clinical penetrance of symptomatic disease is low due to the requirement of aminolevulinate synthase 1 (ALAS1) messenger ribonucleic acid (mRNA) induction in addition to a mutation in the heme synthetic pathway [Chen 2016; Elder 2013; Nordmann 1997]. Onset of disease symptoms typically starts after puberty, with women (approximately 80%) more commonly affected than men [Balwani 2012; Bissell 2017; Elder 2013].

Attacks are often triggered by exogenous factors such as medications (especially barbiturates, sulfonamides, and hydantoins), stress, hormones, caloric restriction, and infection. In women, attacks may occur around the menstrual cycle due to the significant hormonal changes at that time.

Many AHP patients remain undiagnosed for years. Mean time to diagnosis is 15 years [Bonkovsky 2014] because the disease is rare and is characterized by nonspecific symptoms (e.g., diffuse abdominal pain and nausea) that are not always accompanied by abnormalities on physical examination or routine laboratory testing [Siegesmund 2010].

2.1.3. Aetiology and pathogenesis

AHP is characterized by induced ALAS1 mRNA expression, increasing flux into the liver heme synthetic pathway and accumulation of neurotoxic aminolevulinic acid (ALA) and porphobilinogen (PBG) in the setting of a loss-of-function gene mutation in a downstream enzyme [Bissell and Wang 2015; Puy 2010; Siegesmund 2010].

There are 4 subtypes of AHP, each involving a defect in a distinct heme pathway enzyme: AIP caused by mutations in HMBS, also known as PBG deaminase; hereditary coproporphyria (HCP), caused by mutations in coproporphyrinogen oxidase; variegate porphyria (VP), caused by mutations in protoporphyrinogen oxidase; and aminolevulinic acid dehydratase (ALAD)-deficient porphyria (ADP), caused by mutations in ALAD [Anderson 2005; Bissell and Wang 2015]. While AIP is the most common AHP subtype, representing approximately 80% of all AHP cases, the pathophysiological mechanisms of all AHP subtypes are the same.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Patients with AHP can experience incapacitating, potentially life-threatening neurovisceral attacks and long-term comorbidities [Bissell 2015]. Most severe AHP patients (5-10%) have recurrent attacks (≥ 4 per year). In the EXPLORE natural history study, patients reported a mean of 9.5 attacks per year with a range of 0 to 54 attacks per year [Anderson 2016; Gouya 2017]. Porphyria attacks typically last 5 to 7 days, though some patients experience attacks lasting several weeks or longer [Stein 2013]. During attacks, patients experience severe incapacitating neurovisceral pain in the abdomen, back, and limbs usually accompanied by fatigue, nausea, and a constellation of clinical effects from injury to the nervous system, including motor weakness and mental status changes, autonomic instability (e.g., severe hypertension, tachycardia, diaphoresis), and in up to 20% of acute attacks, seizures [Albers 2004; Anderson 2016; Anderson 2005; APF_Transcript 2017; Balwani 2012; Bonkovsky 2014; Puy 2010; Simon 2018].The clinical manifestations and pain are often so severe during attacks that most patients typically require hospitalization for treatment [Kadish 2003]. Clinical presentations, including acute porphyria attacks, are similar between the AHP subtypes, owing to the common pathophysiologic basis of disease; however, patients with HCP and VP patients may also experience cutaneous symptoms (e.g., burning and blistering of the skin) independent of attacks due to accumulation of photosensitizing porphyrin precursors [Bissell 2015; Puy 2010].

There has also been increasing recognition that AHP patients have significant chronic disease manifestations that occur outside of the attack setting. Natural history studies performed in both the US (Porphyrias Consortium) and in Sweden with patients with AIP determined that 18% to 22% of patients with AIP experience chronic symptoms, most commonly pain (in the abdomen, back, or limbs) and fatigue [Andersson 2003; Bonkovsky 2014]. Data from the EXPLORE natural history study, which was conducted in a severely affected AHP patient population, determined that 64% of patients have chronic symptoms, most commonly pain, nausea, tiredness, and anxiety, with approximately 50% of patients experiencing daily symptoms. Chronic pain requires the regular administration of opioids in many patients. Porphyria disease activity negatively impacts quality of life and physical functioning. Due to unpredictability and severity of porphyria disease manifestations, many patients are unable to work or attend school, have decreased socialization, and increased rates of depression and anxiety [Neeleman 2018; Simon 2018]. Patients struggle with porphyria attacks, which cause severe, debilitating symptoms, extreme neuropathic pain, nausea, weakness to paralysis, confusion, hyponatremia and fatigue. Over time, these symptoms became chronic in most of the patients. Long term complications and comorbidities of AHP include chronic kidney disease, depression, anxiety, hypertension, irreversible neuropathy sometimes leading to quadriplegia, and liver disease.

2.1.5. Management

Current treatment options are limited for all AHP subtypes. There are no approved treatments that directly ameliorate or prevent chronic symptoms experienced by many AHP patients and no approved treatments to reduce the risk of attacks [Bonkovsky 2014; Deybach 2006; Stein 2013; Tollanes 2012]. Management of acute AHP attacks often requires urgent medical attention in a healthcare setting. Patients are initially treated with supportive care such as intravenous (IV) glucose, typically large doses of IV opioid analgesics, and anti-emetics along with the removal of known precipitating triggers, such as certain medications or fasting [Bissell 2017; Bonkovsky 2013; Stein 2013]. Intravenous hemin, a human blood-derived heme formulation, is the only therapy currently approved for the treatment of acute attacks; hemin is not approved as a chronic

treatment to prevent attacks [Bissell 2015]. While hemin infusion temporarily reduces production of ALA and PBG through feedback inhibition of *ALAS1* messenger ribonucleic acid (mRNA) expression, it has a short duration of action (elimination half-life of approximately 11 hours), which limits its effectiveness and contributes residual attack activity when used prophylactically. Hemin must be administered through a large peripheral or central venous catheter due to the risk of phlebitis. However, infection and thrombosis are common complications of indwelling IV catheter use. Frequent hemin administration can lead to increased risk of infections from indwelling venous catheters as well as hepatic iron build-up and subsequent injury due to iron overload. Despite these limitations, some patients are treated with regular, frequent prophylactic hemin infusions for lack of a suitable therapeutic alternative [Bissell 2015; Marsden 2015; Pischik 2015; Puy 2010; Schmitt 2018].

Additional treatments for AHP include chemically induced menopause with hormonal suppression therapy (e.g., gonadotropin-releasing hormone agonists) [Andersson 2003; Innala 2010], and liver transplantation for patients with refractory disease or those who no longer have adequate venous access [Soonawalla 2004; Stein 2013; Yasuda 2015]. However, the side effects of induction of menopause in a relatively young patient population make this treatment difficult to tolerate [Anderson 1990; Anderson 2005; Andersson 2003]. Liver transplant is a highly invasive treatment option not widely used due to limited availability of organs and risks associated with life-long immunosuppression.

Consequently, there is an unmet need for therapies that durably decrease the frequency of debilitating attacks, diminish chronic symptoms, and improve patients' physical functioning and quality of life.

About the product

Mode of action

Givosiran is a ribonucleic acid interference (RNAi) therapeutic that inhibits aminolevulinate synthase 1 (ALAS1) mRNA in the liver. Givosiran is designed to be selectively delivered to the liver via uptake by the Asialoglycoprotein Receptor (ASGPR). ASGPRs are primarily and highly (0.5-1 million per cell) expressed on the cell surface of hepatocytes and specifically bind to the glycoproteins with terminal galactose or Triantennary *N*-acetylgalactosamine (GalNAc) residues [Ashwell 1974; Baenziger 1980; Schwartz 1980]. Binding between the GalNAc ligand of givosiran and ASGPR triggers receptor-mediated endocytosis of the ligand-receptor complex, followed by release of the small interfering ribonucleic acid (siRNA) into the cytoplasm of the hepatocyte.

Upon delivery to the liver, givosiran uses the naturally occurring RNAi pathway to specifically target and silence *ALAS1* mRNA in the liver. This is accomplished by incorporation of givosiran siRNA into the cellular multiprotein enzyme cleavage complex known as the RNA-induced silencing complex, followed by separation of the 2 strands of the siRNA and binding of the antisense strand (the guide strand) to the complementary sequence in the *ALAS1* mRNA. Pairing of *ALAS1* mRNA with the antisense strand within the RNA-induced Silencing Complex (RISC)/siRNA complex results in specific and highly efficient cleavage of the ALAS1 mRNA. This prevents the synthesis of the corresponding ALAS1 protein [Bumcrot 2006; Elbashir 2001; Soutschek 2004; Vaishnaw 2010].

The RNAi-mediated lowering of induced liver *ALAS1* mRNA levels and the consequent sustained decrease in the accumulation of toxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG) is expected to prevent or reduce the occurrence of serious neurovisceral attacks and ongoing symptoms in patients with AHP.

Proposed indication

Givlaari is indicated for the treatment of acute hepatic porphyria (AHP) in adults and adolescents aged 12 years and older.

Recommended dose

The recommended dose of Givlaari is 2.5 mg/kg once monthly, administered via subcutaneous (SC) injection. Dosing is based on actual body weight. A dose modification is proposed for patients with severe or clinically significant transaminase elevations, who have dose interruption and subsequent improvement, with dose resuming initially at 1.25 mg/kg once monthly with possible increase to the recommended 2.5 mg/kg dose if no recurrence of severe or clinically significant transaminase elevations occur.

Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the fact that the proposed treatment is a new strategy for the prevention of AHP attacks in an area of recognized high unmet need. Moreover, the results presented were suggestive of a substantial clinical benefit for the affected patients. Therefore, givosiran is considered of major public health interest and a major therapeutic innovation.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a solution for injection containing 189 mg givosiran. The product contains 200 mg of givosiran sodium salt.

Other ingredients are: sodium hydroxide, phosphoric acid and water for injections.

The product is available in a glass vial with a polytetrafluorethylen (PTFE)-coated chlorobutyl rubber stopper and a flip-off aluminium seal. Each vial contains 1 mL solution for injection, as described in section 6.5 of the SmPC.

2.2.2. Active Substance

General information

Givosiran is a new active substance, consisting of a double stranded synthetic chemically modified small interfering RNA (siRNA) and containing a combination of 2' F and 2' O-methyl nucleotides, conjugated to a triantennary N-acetyl galactosamine (GalNAc) ligand to facilitate delivery of the siRNA to the liver.

The two single strands that form the double stranded RNA molecule are A-122230, the sense strand, and A-122227, the antisense strand. The abbreviated chemical name for the sense strand is 5' Cm-**ps**-Am-**ps**-Gm-Am-Am-Am-Gf-Am-Gf-Um-Gf-Um-Cf-Um-Cf-Am-Um-Cm-Um-Um-Am-L96 3', which is base-paired with the antisense strand 5' Um-ps-Af-**ps**-Af-Gf-Am-Uf-Gm-Af-Gm-Af-Cm-Af-Cm-Uf-Cm-Uf-Um-Uf-Cm-Uf-Gm-ps-Gm-**ps**-Um- 3'.

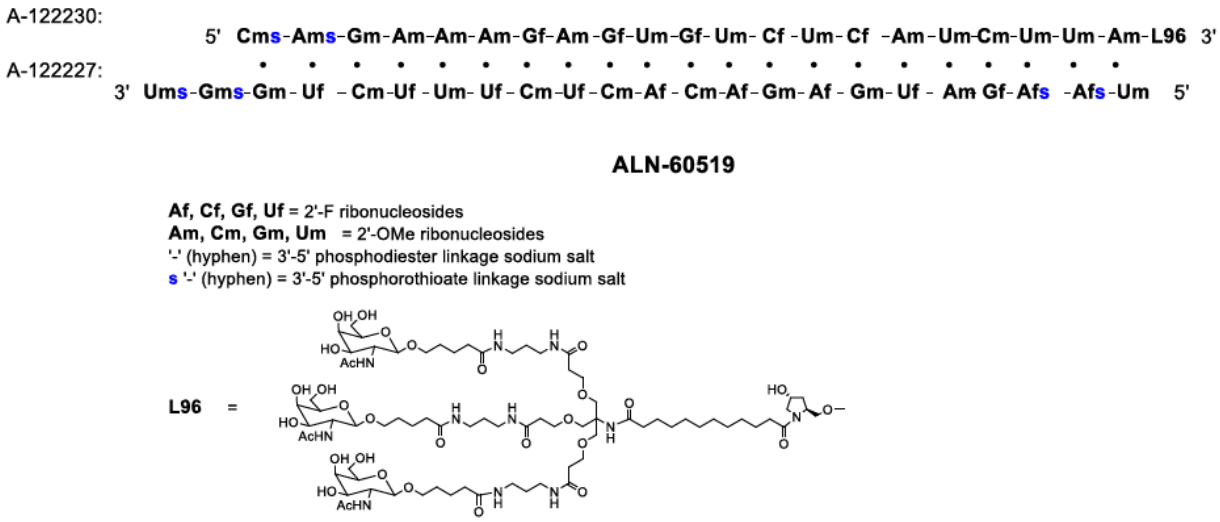
All phosphodiester groups are negatively charged with sodium as the counter ion.

Legend	
Af = 2'-fluoroadenosine	Am = 2'-O-methyladenosine
Cf = 2'-fluorocytidine	Cm = 2'-O-methylcytidine
Gf = 2'-fluoroguanosine	Gm = 2'-O-methylguanosine
Uf = 2'-fluorouridine	Um = 2'-O-methyluridine
'-' (hyphen) = 3'5' phosphodiester linkage sodium salt	
'-ps-' = 3'-5' phosphorothioate linkage sodium salt	

The molecular formula of the sodium salt is $C_{524}H_{694}F_{16}N_{173}$ $Na_{43}O_{316}P_{43}S_6$.

The sodium salt has a relative molecular mass of 17,245.56 Da g/mol and the following structure **(Figure 1).**

Figure 1: Givosiran structure



The chemical structure and solid state properties of the sense and antisense strand and of the active substance givosiran was elucidated by a combination of analytical methods commonly used for the characterisation of synthetic oligonucleotide active substances; namely: sense- and antisense strand (LC-MS, MS-MS sequence confirmation sodium by AS, UV spectroscopy, ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, ^{31}P-NMR, and FTIR) and active substance (LC-MS, melting (denaturation) temperature (Tm), non-denaturing ion pair reversed phase HPLC with UV detection, UV, sodium by AS, ^{1}H-NMR, imino-^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, ^{31}P-NMR, FTIR, circular dichroism, differential scanning calorimetry and thermogravimetric analysis.

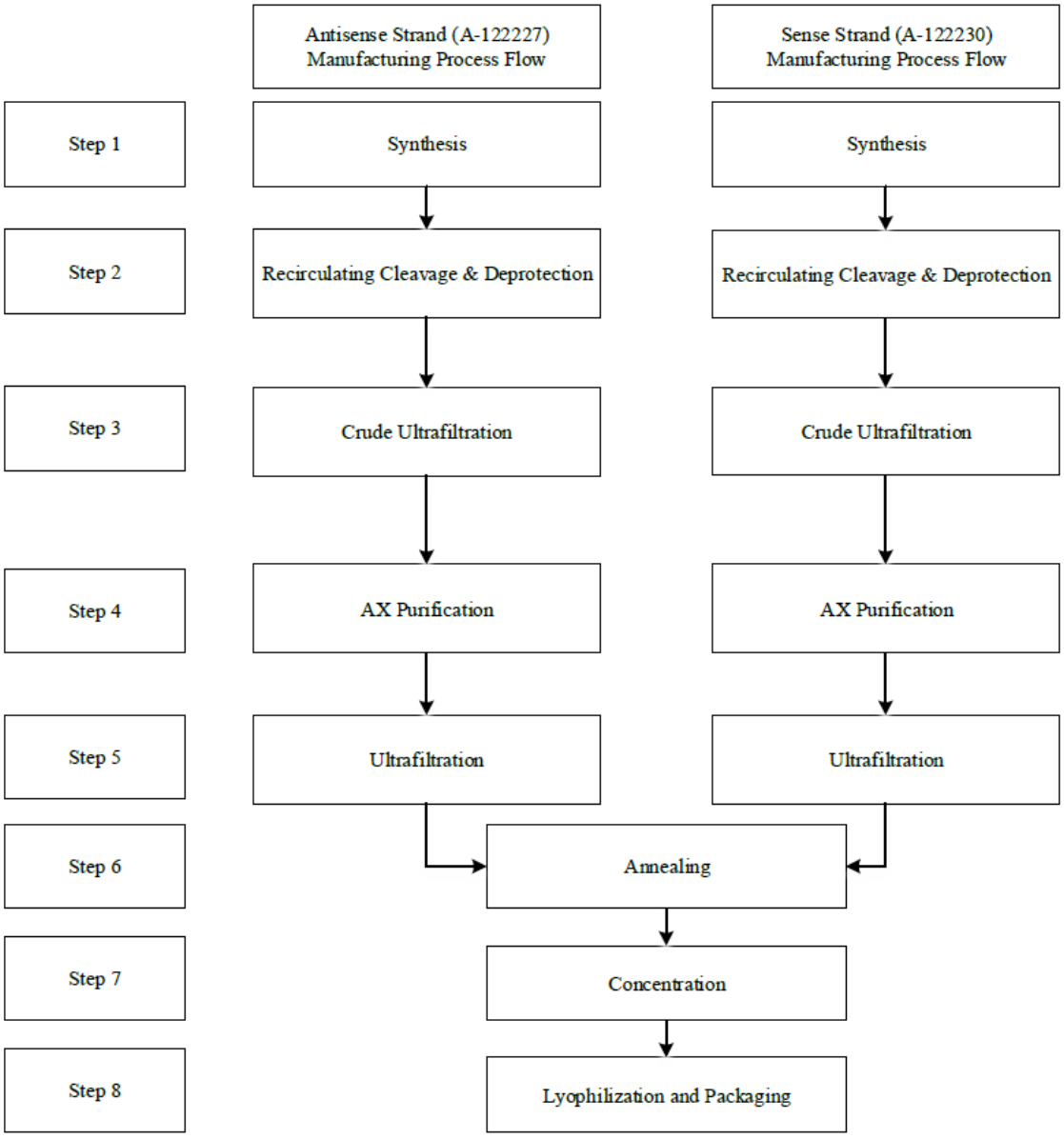
Givosiran is a white to pale yellow, hygroscopic powder, freely soluble in water. Since the active substance is dissolved in the finished product, particle size distribution and polymorphic form are not relevant.

All the pentose moieties of the nucleotides in the givosiran active substance are in the naturally occurring D-ribose form. The chirality of the D-ribose is maintained during the synthesis of the modified nucleotides. Since the ribose moieties in an RNA sequence are predominantly in the C-3'end conformation, RNA molecules adopt the classic A-form, as demonstrated by the spectrum of Circular Dichroism. The phosphorothioate

group (PS) is chiral, with either Rp or Sp absolute configuration at the phosphorus. The antisense strand contains four PS modifications, with two on 5'end and two at 3'end, resulting in the formation of sixteen (2^4=16) diastereomers. The sense strand contains two PS modification at the 5'end, corresponding to four (2^2=4) diastereomers. The diastereomer species of the antisense strand and of the sense strand resolve chromatographically by the anion exchange HPLC method with UV detection. Consistency of the stereochemical purity variation has been demonstrated. The applicant has confirmed that the consistency of the distribution of stereoisomers will be evaluated as part of any future changes to the synthesis process.

Manufacture, characterisation and process controls

Givosiran is manufactured by one manufacturer using a manufacturing process consisting of eight main steps using well defined starting materials with acceptable specifications.



Scheme 1: Givosiran active substance manufacturing process

The manufacturing process involves the synthesis of the single-strand oligonucleotide by solid-phase synthesis (Step 1), cleavage and deprotection of the nucleobase protecting groups (Step 2). The two strands are manufactured by solid phase flow-through synthesis process in synthesis columns comprising repetitive cycles of four sub-steps, detritylation, coupling, thiolation/oxidation, and capping. When the targeted single strand oligonucleotide is assembled, the step continues with final de-block, and ends with diethylamine washing.

Each strand is then individually purified and concentrated in Step 3 through Step 5; crude ultrafiltration (UF; Step 3), purification by anion exchange (Step 4); and a second UF (Step 5) for the sense and antisense strands. Step 1 to Step 5 are performed at a nominal scale of 250-mmol and 290-mmol for the sense and antisense strands, respectively. During Step 6, the two individual strands are annealed to form the duplex, which is concentrated at Step 7, then lyophilised and packaged in Step 8 to produce the givosiran active substance.

The sense single strand intermediate is synthesised on polymeric support loaded with the GalNAc containing moiety and the synthesis of the antisense single strand is carried out on CpG support loaded with 4,4'-dimethoxy trityl (DMT) protected 2'-OMe Uridine. Descriptions of relevant conditions, end of reaction determinations, and equipment (e.g. columns) have been given.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Critical quality attributes (CQAs, namely: microbial limits, physical attributes, pH, identity, assay, purity, elemental impurities, product and process related impurities) have been defined and, based on them, the control strategy has been identified. The control strategy, as proposed by the applicant, consists in the control of material attributes, control of the critical process parameters (CPPs), in process controls, control of the sense and antisense strands, and release testing. Normal operating ranges (NOR) and proven acceptable ranges (PAR) have been identified. The NORs and PARs are linear and in line with the results of the manufacturing process development. A design space is not claimed. The applicant is recommended to evaluate the appropriateness of tightening the acceptance criteria for active substance in-process control limits once data from a total of 30 batches become available. (REC 1)

Characterisation of the product related impurities in the givosiran active substance was performed by two orthogonal analytical techniques, including a two-step fractionation using denaturing AX-HPLC in the first dimension, and ion pairing reversed phase high performance liquid chromatography-mass spectrometry (IPRP HPLC-MS) in the second dimension, as well as by denaturing IPRP HPLC coupled with mass spectrometry. All impurity peaks with peak-area percent greater than 0.10% in AX-HPLC and IPRP-HPLC chromatograms were identified by structure, sequence, or by their mass. Clearance data clearly demonstrated that, following multiple washing and downstream purification steps, the levels of small molecule impurities, including residual solvents are reduced to levels below their respective limits of quantitation (LOQ), indicating consistent and efficient impurities removal during manufacturing steps.

Evaluation of potential genotoxic impurities in the synthesis of givosiran active substance has been performed in accordance with the principles stipulated in the ICH M7 guideline. An evaluation of the risk on the presence of nitrosamine impurities has been provided and indicates that N-nitrosamine impurities cannot be present in either the givosiran active substance or finished product.

The elemental impurities profiles throughout development and process validation demonstrate that the elemental impurities are adequately controlled and consistently low.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Changes introduced have been presented in sufficient detail and have been justified. The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The active substance is packaged in a 2 L, gamma-irradiated, highdensity polyethylene bottle closed with a polypropylene screw-top closure, which comply with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The active substance specification

includes tests for appearance, identity by chromatographic retention time and MS (non-denaturing IPRP-HPLC UV and IPRP-HPLC ESI-MS), sequence (MS-MS) and Tm (UV); assay (UV); purity and impurities with orthogonal HPLC methods (non-denaturing and denaturating IPRP-HPLC UV and denaturating AX-HPLC UV); sodium content (flame AAS); pH (Ph.Eur.); water content (KF); elemental impurities (ICP-MS), residual solvents (Headspace GC and FID); bacterial endotoxin and bioburden (Ph. Eur.).The tested parameters are appropriate for this type of active substance. The release specification includes confirmation of the sequence of both intermediates, sense strand and antisense strand, as in-process control (IPC), instead of identification by sequencing; this has been adequately justified and it is considered acceptable.

The convention of defining the impurities in the specification as groups rather than individual components has been satisfactorily justified.

It is acknowledged that oligonucleotides are excluded from the scope of the ICH guidance on impurities in active substances (Q3A (R2)). The applied thresholds are in line with the thresholds for synthetic peptides described in the Ph. Eur. Monograph 'Substance for pharmaceutical use' and is considered acceptable for givosiran as synthetic peptides are made by comparable processes (stepwise sequence growth on solid phase with protection and deprotection reagents and solvents), with the same limitations of impurity removal during the stepwise synthesis and similarly complex impurity profiles due to the iterative nature of the process with possible formation of many different product-related impurities. Additionally, the analytical methods applied to characterise and control product-related impurities of synthetic peptides and oligonucleotides are comparable. Therefore, the proposed limits for unidentified impurity peaks and unqualified impurity peaks are acceptable. In view of the results of the six clinical batches, limits for specified impurities by denaturing AX-HPLC UV and by denaturing IPRP-HPLC UV, as well as for other parameters could be tightened. The applicant is recommended to re-evaluate and tighten, when appropriate, the proposed acceptance criteria for specified impurities by denaturing AX-HPLC UV and by denaturing IPRP-HPLC UV, as well as for other parameters identified by the applicant, when 30 batches of the active substance have been manufactured. (REC 2)

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay, purity and identity were provided.

Batch analysis data on several batches of the active substance used for non-clinical, clinical, stability and process performance qualification are provided. Four of the eight batches were manufactured at commercial scale. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from six batches of active substance, four of which have been manufactured at commercial scale from the proposed manufacturer, stored in the intended commercial package for up to 48 months under long term conditions (-20°C) and for up to six months under accelerated conditions (25 ºC / 60% RH), according to the ICH guidelines, were provided.

The following parameters were tested: appearance, purity (non-denaturating and denaturating IPRP-HPLC, denaturating AX-HPLC), assay and water content.

All tested parameters were within the specification limits at long term and accelerated storage conditions, confirming that the total of impurities of givosiran active substance will remain within the registration specification limit (NMT 20.0 area %) during short-term storage at room temperature condition (~25°C) during the manufacture of the finished product.

Photostability testing, following the ICH guideline Q1B, was performed on two batches and demonstrated that givosiran active substance is not sensitive to light. Results on stress conditions (thermal, acidic, basic, oxidative, and photolytic stress) were also provided on one batch and confirmed that the chromatographic methods are stability indicating.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 30 months when stored at -20°C in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The medicinal product is a solution for subcutaneous injection that contains 200 mg givosiran sodium corresponding to 189 mg givosiran per ml. Each pack contains one single-use, 2 ml Type 1 glass vial with a Teflon-coated stopper and a flip-off aluminium seal containing 1 mL solution for injection. The product composition is provided below.

Table 1: composition of finished product

Component	Concentration (mg/mL)	Content per Vial (mg)	Function	Quality Standard
Givosiran drug substance (givosiran sodium)	189 givosiran (equivalent to 200 givosiran sodium)	189 givosiran (equivalent to 200 givosiran sodium)	Active ingredient	Manufacturer's specifications
Water for injection, sterile	qs to 1.0 ml	qs	Diluent	USP/NF, Ph. Eur., JP
Phosphoric Acid[a]	qs	qs	pH Adjustment	NF, Ph. Eur.
Sodium Hydroxide[a]	qs	qs	pH Adjustment	NF, Ph. Eur., JP

[a] Compendial grade 0.1 M phosphoric acid and/or sodium hydroxide solutions may be added in 5 ml increments as needed to adjust pH to 6.5 to 7.5 up to a maximum of 250 ml per batch. Refer to 3.2.P.3.3 Decription of Manufacturing Process and Process Controls for information on the pH titration step.
Abbreviations: JP=Japanese Pharmacopoeia, NF= National Formulary; Ph. Eur.=European Pharmacopoeia; qs=quantum sufficient; USP=United States Pharmacopoeia

Givosiran finished product is a solution of givosiran active substance dissolved in water for injection. The pH of the solution is adjusted to 6.5 to 7.5 with sodium hydroxide or phosphoric acid, as needed. Givosiran finished product is sterile and preservative-free and it is intended for single use. Bacterial endotoxins are controlled to a suitable limit.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

The formulation is simple and straightforward. Since the active substance is dissolved, particle size distribution and polymorphic form are not critical. The concentration (required dose volume), viscosity, pH and osmolality of the finished product are suitable for subcutaneous administration. In order to deliver 1.0 mL of extractable volume of finished product, the target fill volume for filling operation is set to 1.15 mL which represents a 0.15 mL overfill.

The manufacturing process development and control strategy followed a risk-based approach. The information provided in the pharmaceutical development is satisfactory. A summary of the givosiran finished product and quality attributes (QAs) along with their justification for criticality assignment is provided in **Table 2**.

Table 2: Quality attributes of givosiran finished product

Quality Attribute	**Critical/ Noncritical**	**Justification**	**Location of Controls**
Sterility	Critical	Impacts safety if limits are not met	Release test refer to 3.2.P.5.1, IPC during Step 2, refer to 3.2.P.3.4

Bacterial endotoxin	Critical	Impacts safety if limits are not met	Release test refer to 3.2.P.5.1, IPC during Step 2, refer to 3.2.P.3.4
Appearance	Critical	Unknown impact to safety, efficacy, and PK. Due to high uncertainty, this is considered a critical quality attribute.	Release test refer to 3.2.P.5.1
Particulate matter	Critical	Potential impact to patient safety. Minimal impact on efficacy. Conformance with regulatory expectations	Release test refer to 3.2.P.5.1
Volume in container	Critical	Impacts efficacy and PK if volume in container is insufficient. Conformance with regulatory expectations.	Release test refer to 3.2.P.5.1, refer to IPC during Step 3 refer to 3.2.P.3.4
pH	Critical	High pH is known to cause degradation of the 2'F species and may give rise to M-2 and M-20 degradants, impacting safety and efficacy	Release test refer to 3.2.P.5.1, IPC during Step 1, refer to 3.2.P.3.4
Osmolality	Critical	Osmolality is correlated with concentration (3.2.P.2.2). At the target concentration of 189 mg/mL, osmolality matches the reference range for serum (275-295 mOsm/kg).	Release test refer to 3.2.P.5.1, IPC during Step 1, refer to 3.2.P.3.4
Identity	Critical	Possible off-target effects if identity is not confirmed. Efficacy of drug and delivery to target organ may be compromised if identity is not confirmed.	DS Release test refer to 3.2.S.4.1, DP Release test refer to 3.2.P.5.1
Assay	Critical	Potential effects on safety if label claim is exceeded. Efficacy and PK of drug may be compromised if label claim is not met.	Release test refer to 3.2.S.4.1, IPC during Step 1, refer to 3.2.P.3.4, DP Release test refer to 3.2.P.5.1
Purity	Critical	Decreased purity will lead to increased impurities. It may result in off-target effects and have impact to safety. Efficacy and PK of drug may be compromised if purity is below target.	DS Release test refer to 3.2.S.4.1, DP Release test refer to 3.2.P.5.1
Impurities and degradants	Critical	Increased impurities may result in off-target effects and have impact to safety. Efficacy and PK of drug compromised if impurities are above target.	DS Release test refer to 3.2.S.4.1, DP Release test refer to 3.2.P.5.1

Process development studies were specifically performed to assess mixing, filter compatibility and fill volume.

Sterile filtration was chosen as the sterilisation method for siRNA products based on the following justifications: dry and steam heat have been demonstrated to impact the impurities profile of common chemically modified RNA used in givosiran manufacture, such as 2'-fluoro; temperatures required for thermal sterilization exceed

the melting temperature of siRNA duplex which would result in denaturation of the active substance; γ-Irradiation has been shown to cause oxidative degradation in nucleotides. Suitability of the filters and hold times has been demonstrated.

The formulation used during clinical studies is the same as that intended for marketing and has been manufactured using the proposed commercial manufacturing method.

The primary packaging is a glass vial with a PTFE-coated chlorobutyl rubber stopper and a flip-off aluminium seal. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability and extractable and leachable data and is adequate for the intended use of the product.

A syringe for SC administration is not provided with the product. Taking into consideration the lowest dose for a 12 years old child and the dose for a patient with bodyweight over 80 kg, it can be concluded that the finished product meets the requirements of the QWP Q&A on graduation of measuring devices for liquid dosage forms and hence it can be administered with syringes commonly available in hospitals. Compatibility with polycarbonate and polypropylene, the materials commonly used for the manufacture of syringes for subcutaneous use, has been demonstrated.

Manufacture of the product and process controls

The manufacturing process consists of three main steps: compounding (active substance dissolution, pH and concentration adjustment), sterile filtration (using two 0.2 µm filters connected in series) into a single-use bag (stored at 2°C-8°C for up to 72 hours) and aseptic filling (total fill duration is not more than 12 hours). Sterile filtration is completed within 120 minutes. Glass vials are washed, rinsed and depyrogenated via dry heat. The stoppers are steam sterilized prior to use. The process is considered to be a non-standard manufacturing process.

Aseptic filtration as method for sterilisation has been adequately justified. Suitability of the filters has been demonstrated. The filters have adequately been validated and evaluated for extractables and chemical compatibility (product exposure).

Results of process validation have been provided of three consecutive batches manufactured at the proposed site and using the commercial scale process. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Product specification

The finished product release specifications shown include appropriate tests for this kind of dosage form: appearance, identification by chromatographic retention and MS (non-denaturing IPRP-HPLC UV and IPRP-HPLC ESI-MS), assay (UV), purity and impurities with orthogonal HPLC methods (non-denaturing and denaturating IPRP-HPLC UV and denaturating AX-HPLC UV), pH, osmolality, particulate matter, bacterial endotoxins, sterility, extractable volume (all Ph.Eur.) and container closure integrity (dye ingress).

Givosiran finished product uses the same impurity evaluation strategy and chromatographic separation parameters used for givosiran active substance and its constituent single strands. The analysis confirms that givosiran active substance remains predominantly in its native duplex form in the finished product. The result is expressed as percentage of the main duplex peak based on the total area of the chromatogram. Some of the

proposed limits for impurities are slightly wider than the corresponding active substance specification limits. However, this can be acceptable as these limits are qualified by the non-clinical studies (see non-clinical report). The proposed limits for assay (by UV), purity, total impurities and specified (groups of) impurities (by denaturing IPRP-HPLC UV and denaturing AX-HPLC UV) are wide and could be tightened. The applicant is recommended to re-evaluate and tighten, when appropriate, the proposed acceptance criteria for assay (by UV), purity, total impurities and specified (groups of) impurities (by denaturing IPRP-HPLC UV and denaturing AX-HPLC UV) when 30 batches of finished product have been manufactured. (REC 3)

The proposed limits for unidentified impurity peaks (NMT 0.5%) and unqualified impurity peaks (NMT 1.0%) are acceptable as discussed in the active substance section.

The proposed limit for Bacterial Endotoxins (100 EU/ml) is acceptable. Dosing 2.5 mg/kg bodyweight which corresponds with 0.0125 mL/kg bodyweight, allows a limit of 5 EU/kg / 0.0125 mL/kg = 400 EU/mL.

The other proposed specification acceptance criteria are acceptable.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

Since the solution is slightly coloured, the applicant has been recommended to add a specific quality control 'Degree of coloration' (Ph Eur 2.2.2) in the finished product specification by Q2, 2020. (REC 4)

The chromatographic methods are in line with the methods applied for the control of the active substance. The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines.

The same reference standards used in the active substance testing are also used in the finished product. Satisfactory information regarding the reference standards has been presented.

Batch analysis data on eight batches of the finished product used for non-clinical, clinical, stability and process performance qualification are provided. Some batches were manufactured at commercial scale. The results confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data from 6 batches of finished product, some at full commercial scale, stored for up to 48 months under long term conditions (2-8 ºC) and (25 ºC/60% RH) and for up to 6 months under accelerated conditions (40 ºC/75% RH) according to the ICH guidelines were provided. Samples were stored in the up-right and inverted position to increase the contact time with the elastomer stopper. The registration stability batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for the parameters listed in the release specifications, with the exception of identity; its omission is acceptable as it is not affected by storage. The analytical procedures used are stability indicating. All results were within specification for the samples stored under long-term conditions. No significant changes have been observed under the named conditions.

In addition, two batches were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Only minor changes in impurity levels (<5%) were observed which are of no concern in reference to the transient exposure to light during administration of the product. The finished product is susceptible to oxidative stress but not to thermal stress. The analytical methods are stability indicating.

Based on the available stability data, the proposed shelf-life of 36 months at the storage conditions "Do not store above 25C. Keep vial in the outer carton to protect from light." as stated in the SmPC (section 6.3) is acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The manufacturing process of the finished product is straightforward. The results of the tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. In view of the few batches available at the time of this application, the applicant has been recommended to tightened the IPC and release specification limits of the active substance and finished product when more data is available; the addition of a test for degree of coloration in the finished product specification has also been recommended, see below list of recommendations for future quality development.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The applicant is recommended to evaluate the appropriateness of tightening the acceptance criteria for active substance in-process control limits once data from a total of 30 batches of the active substance become available.
2. The applicant is recommended to re-evaluate and tighten, when appropriate, the proposed acceptance criteria for specified impurities by denaturing AX-HPLC UV and by denaturing IPRP-HPLC UV, as well as for other parameters identified by the applicant, when 30 batches of the active substance have been manufactured.

3. The applicant is recommended to re-evaluate and tighten, when appropriate, the proposed acceptance criteria for assay (by UV), purity, total impurities and specified (groups of) impurities (by denaturing IPRP-HPLC UV and denaturing AX-HPLC UV) when 30 batches of finished product have been manufactured.
4. Since the solution is slightly coloured, the applicant has been recommended to add a specific quality control 'Degree of coloration' (Ph Eur 2.2.2) in the finished product specification by Q2, 2020.

2.3. Non-clinical aspects

2.3.1. Introduction

The pharmacology, safety pharmacology, pharmacokinetics, and toxicology of givosiran were evaluated in a series of *in vitro* and *in vivo* nonclinical studies. According to the Applicant, all pivotal studies were carried out in accordance with good laboratory practice (GLP).

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro studies

Identification of Givosiran: A Potent and Specific siRNA Targeting ALAS1 (Study BIO14037)

A series of 36 siRNAs were identified and synthesized, containing antisense strands with perfect complementarity to monkey and human ALAS1 mRNAs, and in some cases, perfect complementarity or 1 to 2 mismatches to mouse and/or rat ALAS1 mRNAs. *In vitro* activity of these chemically modified siRNAs with triantennary GalNAc ligands conjugated to the 3' end of the sense strand was evaluated by transient transfection in human liver carcinoma cell line-3B (Hep3B) liver cells.

From the most potent siRNAs identified, one was selected for additional lead optimization and modifications. This parent duplex, with complete sequence homology to human and monkey ALAS1, and is therefore expected to be pharmacologically active in each of the indicated species. From a series of chemical analogues of this compound that were analysed, based on a minimum reduction of ALAS1 mRNA in Hep3B cells of approximately 80% and 60% at 10 and 0.1 nM, respectively, ALN-60519 (givosiran) was selected as the lead development candidate based on an IC50 value of approximately 26 pM.

Polymorphism analysis (Study BIO14024)

Given the sequence specific nature of RNAi, a single mismatch between the antisense strand of the ALAS1 targeting siRNA and its target mRNA sequence can lead to a reduction or loss of activity. Naturally occurring single nucleotide polymorphisms (SNPs) in the siRNA target site could impact activity, an effect that could be exacerbated if the SNP was enriched in individuals from a specific geographic/ethnic background. A search of the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), build 141 of May 21, 2014, was performed to identify any potential SNPs that might occur in the givosiran binding site. Results demonstrated 100% sequence conservation for the givosiran target site in all humans whose ALAS1 gene has been sequenced to

date (source: NCBI dbSNP as of 16 June 2014). There are no SNPs, or any other type of polymorphism found in the givosiran target region of ALAS1 mRNA.

Activity of metabolites (Study BIO18001)

An evaluation of this AS(N-1)3' truncation metabolite by transfection in Hep3B cells demonstrated retention of pharmacological activity *in vitro*. The inhibition potential was similar for givosiran and the metabolite when tested at 10 nM, but was reduced for the metabolite when tested at the lower concentration of 0.1 nM (52% mRNA remaining after treatment for the metabolite, versus 16% remaining for givosiran).

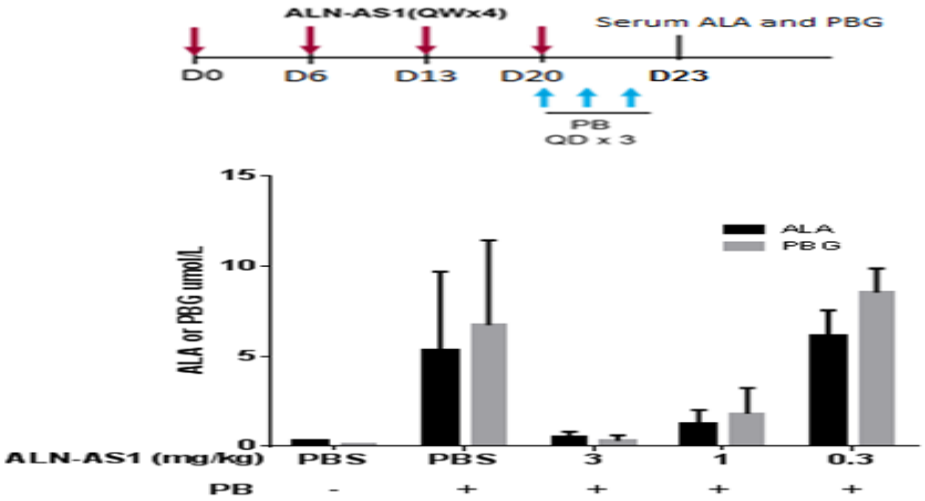
***In vivo* studies**

Studies in rat and mouse disease models

A mouse model of Acute intermittent porphyria (AIP) exists that is a compound heterozygote for porphobilinogen deaminase (PBGD) mutations and manifests similar biochemical features of human AIP such as decreased PBGD activity in the liver. Treatment of these mice with the cytochrome P450 (CYP)-inducing drug phenobarbital (PB), resulted in 3- to 5-fold induction of *ALAS1* mRNA and 50- to 200-fold induction of ALA and PBG, mimicking the increases in ALA and PBG plasma levels that occur coincidently with acute attacks in AIP patients.

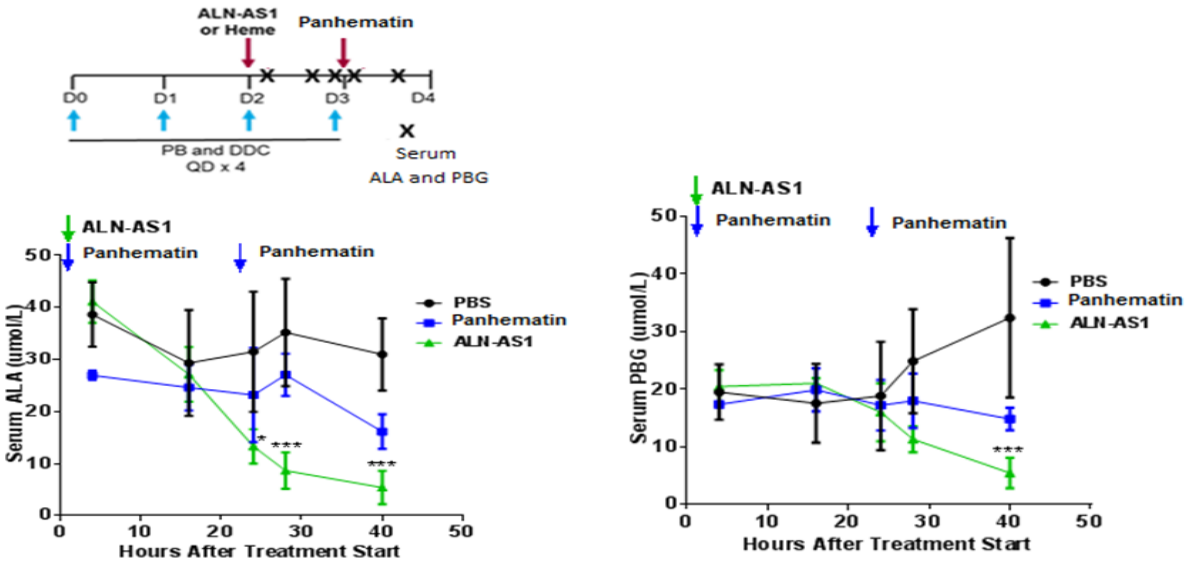
The potential for givosiran to lower ALA and PBG intermediates was evaluated in a prophylaxis setting (*Study BIO14012*), in which mice were administered 4 weekly doses of givosiran between 0.3-3 mg/kg near prior to PB administration (**Figure 2**).

Figure 2.*Serum ALA and PBG Levels After Multiple Subcutaneous Doses of Givosiran in a Mouse AIP Model. Bars represent the mean of 3 animals; error bars depict SD.*



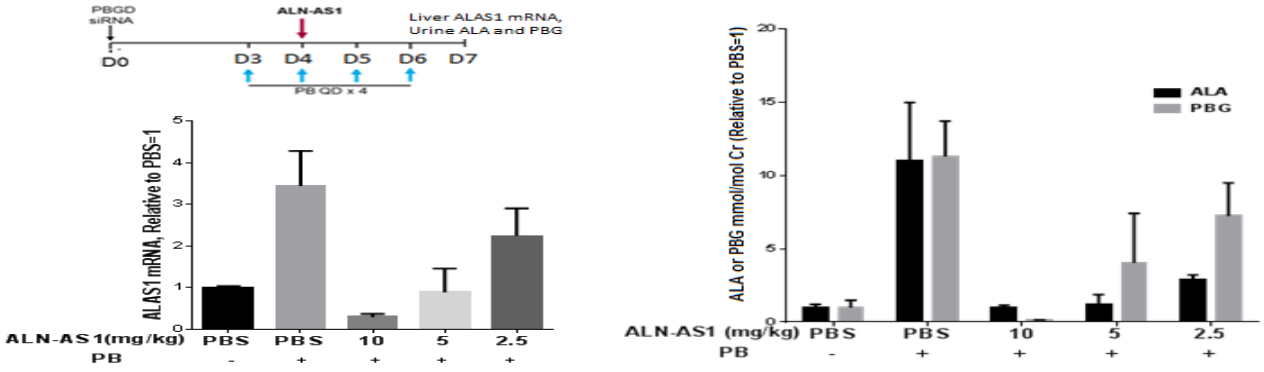
Using an acute treatment regimen (*Study BIO14050*), AIP mice were given daily intraperitoneal (IP) injections of PB and a second CYP) inducer, diethyldithiocarbamate (DDC), to achieve more sustained ALA and PBG increases. These animals were then treated with hemin (Panhematin, the current standard of care for patients with AIP) or givosiran (**Figure 3**).

Figure 3. ***Givosiran Decreases Serum ALA and PBG Faster Than Heme in a Mouse AIP Model. Bars represent the mean of 2-5 animals; error bars depict standard deviation (SD).***



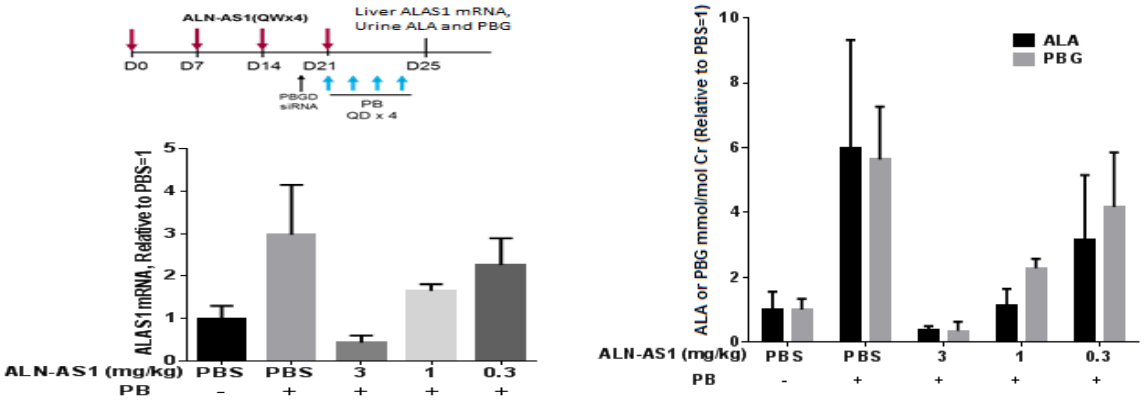
A rat AIP model was developed in which *PBGD* mRNA is reduced by approximately 80% in rat liver, by IV dosing a *PBGD*-specific siRNA in a lipid nanoparticle. In an acute treatment study (Study BIO14010), a single givosiran dose of 5 or 10 mg/kg SC administered on Day 2 of the PB induction phase prevented the induction of the *ALAS1* mRNA in rat liver. Furthermore, the givosiran 10 mg/kg SC dose nearly completely blocked any stimulation of ALA and PBG production (**Figure 4**).

Figure 4. ***ALAS1 mRNA and Urinary ALA and PBG Levels After a Single Subcutaneous Dose Acute Treatment With Givosiran in a Rat AIP Model. Bars represent the mean of 3 animals; error bars depicts standard deviation (SD).***



In a multiple dose study in the rat AIP model (Study BIO14011), 4 doses of ALN-AS1 at 3 mg/kg once weekly (QW) prevented any PB induced increase in liver *ALAS1* mRNA (**Figure 5**)**.** In addition, the 4 weekly doses of 3 mg/kg givosiran prevented any overproduction of urinary ALA and PBG. In both studies in the rat model, the level of *ALAS1* mRNA in liver tightly correlated with urinary ALA and PBG levels.

Figure 5. ***ALAS1 mRNA and Urinary ALA and PBG Levels After Multiple Subcutaneous Doses of Givosiran in a Rat AIP Model. Bars represent the mean of 3 animals; error bars depict standard deviation (SD).***



Studies in wild-type rats

WT rats (Sprague Dawley) were administered increasing doses of givosiran via SC injection. Dose-dependent lowering of liver *ALAS1* mRNA was observed following single-dose administration of givosiran in Sprague Dawley rats with approximately 50% reduction observed at 10 mg/kg givosiran. At the 2.5 mg/kg dose, liver *ALAS1* levels were still reduced by greater than 30%.

Repeat dosing in rats also resulted in dose-dependent *ALAS1* target mRNA lowering (Study BIO14009). A repeat-dose regimen of once weekly SC doses for 4 weeks resulted in more significant target reduction than occurred with a single dose. Whereas a single, 5-mg/kg dose resulted in approximately 40% reduction of *ALAS1* mRNA, 4 weekly 5 mg/kg doses resulted in greater than 60% reduction

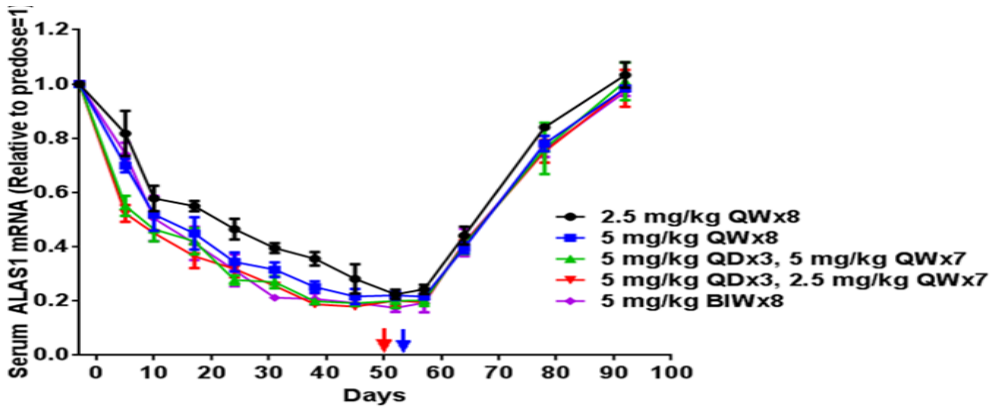
An additional study in rats with 8 weekly 2.5 mg/kg doses or a loading dose of 5 mg/kg followed by 7 weekly 1 mg/kg doses resulted in a maximum of 75% and 70% *ALAS1* mRNA silencing, respectively (*Study BIO18002*).

Studies in monkeys

ALAS1 is not a secreted serum protein, thus limiting the ability to monitor kinetics of drug activity and liver *ALAS1* mRNA recovery in the absence of serial liver biopsies. As such, a circulating extracellular RNA detection (cERD) assay was developed in preclinical species as a less invasive approach to monitor *ALAS1* mRNA levels in which exosomal RNA is isolated from serum and urine. The results exhibited statistically significant correlations and good consistency between serum and urine cERD *ALAS1* mRNA levels and intra-hepatic levels, at the same time points (4h) post-givosiran dosing (data not shown). Therefore, the cERD method was used to assess the impact of givosiran on *ALAS1* mRNA reduction in the repeat-dose studies in monkeys.

In monkeys, a single SC administration of givosiran at 1 or 10 mg/kg resulted in detectable reduction of serum *ALAS1* mRNA levels monitored with the cERD assay. Several different repeat-dose regimens were explored in monkeys. These included an 8-week study w to further assess *ALAS1* lowering with different dosing regimens (Study BIO14027, **Figure 6**).

Figure 6. Kinetics of Serum ALAS1 mRNA Reduction After 8 Weeks of Givosiran Dosing in Monkeys. Each graphical data point represents the remaining ALAS1 mRNA for the group average of 3 animal samples ± standard deviation (SD).



Secondary pharmacodynamic studies

An *in silico* search (BIO14038) was performed to identify any potential off-target sequences in the human genome, to which givosiran might bind resulting in reduced expression of that target. There are no sites in the genome with identical sequences to givosiran antisense strand or with only one mismatch. The closest matches contained at least two mismatches. Those target sequences with the highest potential for binding to givosiran were further assessed in an *in vitro* assay in HepG2 cells. None of the investigated target sequences showed any reduction of mRNA levels when the cells were treated with up to 10 nM givosiran, compared to the IC50 value of 88 pM for ALAS1 in this study.

Safety pharmacology programme

Cardiovascular and respiratory systems

A cardiovascular and respiratory safety pharmacology study (Study AS1-NCD14-019)_was conducted in conscious monkeys using telemetry. Using a cross-over study design, animals (n=5 males) were administered vehicle control (0.9% sterile saline) and givosiran at 150 mg/kg via single SC injection at a dose volume of 0.75 mL/kg. There were no mortalities or clinical observations during the course of the study. Therefore, the no observed effect level (NOEL) for cardiovascular and respiratory functional effects was ≥150 mg/kg givosiran.

Central nervous system

Neurological assessments were conducted as part of the 13-week (Study AS1-NCD14-011) and 39-week (Study AS1-GLP15-018) repeat-dose GLP toxicity study in monkeys. The NOEL for neurological functional effects was ≥150 mg/kg givosiran as no neurological findings with weekly administration of givosiran up to that dose were observed.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been performed.

2.3.3. Pharmacokinetics

Non-clinical pharmacokinetic studies were performed in mice (distribution), rats (absorption, distribution, metabolism and excretion) and monkeys (absorption, distribution, metabolism and excretion). In all studies, givosiran was administered subcutaneously, which is the intended clinical route.

Absorption

The plasma PK profiles of givosiran were evaluated after administration of a single IV dose or single and multiple SC doses in rats and monkeys. There were no apparent sex differences in the PK parameters in both species, therefore, the PK parameters presented are based on overall mean values generated by combining sexes, unless otherwise noted.

Studies in rats

The plasma PK of givosiran were evaluated after a single IV dose or single SC administration in rats. The results are shown in **Table 3**.

Table 3. *Overall Mean Givosiran Rat Plasma Pharmacokinetic Parameters After a Single Intravenous or Subcutaneous Administration (study AS1 NCD14 003)*

Route	**IV**	**SC**[a]		
Dose (mg/kg)	10	1	5	10
t_{max} (h)	0.08	0.3	0.4	1.1
C_0 (µg/mL)	148	N/A	N/A	N/A
C_{max} (µg/mL)	N/A	0.11	0.43	1.07
AUC_{last} (h*µg/mL)	11.8	0.15	1.29	2.79
AUC_{0-1} (IV) or **AUC_{0-2} (SC) (h*µg/mL)**	11.8	0.15	0.72	1.48
$t_{½}$ (h)	0.2	2.1	2.7	2.7
V_{ss} (mL/kg)	181	N/A	N/A	N/A
CL (mL/h/kg)	870	N/A	N/A	N/A
MRT_{0-t} (h)	0.1	1.0	1.7	1.8
F (%)	N/A	N/A	N/A	24

• Abbreviations: AUC=area under the concentration-time curve; AUC_{last}=area under the concentration-time curve from the time of dosing to the last measurable concentration; C_0=concentration at 0; CL=total body clearance; C_{max}=maximum observed concentration occurring at t_{max}; F=bioavailability; IV=intravenous; N/A=not applicable; SC=subcutaneous; $t_{½}$=elimination half-life; t_{max}=time to reach maximum concentration; V_{ss}=volume of distribution at steady state.

[a] All time points for the 0.1 mg/kg SC dose group were below the limit of quantitation.

The PK profiles of givosiran were also evaluated in rats after multiple SC doses with various dosing regimens (8 weekly doses of 1 or 2.5 mg/kg; and after a 5 mg/kg loading dose followed by 7 weekly doses of 1 mg/kg. The results are shown in **Table 4**.

Table 4. ***Mean Givosiran Plasma Pharmacokinetic Parameters in Rats After Multiple Subcutaneous Doses (study AS1 NCD14 003)***

Route	**SC**		
Dose (mg/kg)	**1**	**2.5**	**5/1[a]**
t_{max} (h)	2	2	2
C_{max} (µg/mL)	0.11	0.16	0.08
AUC_{last} (h*µg/mL)	NR	0.51	0.12
t½ (h)	NR	NR	NR

• Abbreviations: AUC_{last}=area under the concentration-time curve from the time of dosing to the last measurable concentration; CL=total body clearance; C_{max}=maximum observed concentration occurring at t_{max}; NR=not reportable; SC=subcutaneous; $t_{½}$=elimination half-life; t_{max}=time to reach maximum concentration.
Note: Values represent the overall combined (male+female) mean.
a A 5 mg/kg loading dose followed by 7 weekly doses of 1 mg/kg (5/1 mg/kg).

Exposure to givosiran and metabolite AS(N-1)3' after a single dose

A separate PK study in rats was conducted to determine the relative plasma exposure and PK profile of the major metabolite, AS(N-1)3'-givosiran after a single SC dose of givosiran at 10 mg/kg (study AS1-DSM18-010).

C_{max} of givosiran and AS(N-1)3'-givosiran in plasma were 1.06 and 0.190 µg/mL, respectively. Plasma AUC_{last} of givosiran and AS(N-1)3'-givosiran were 3.00 and 0.626 h*µg/mL, respectively. Plasma exposure of AS(N-1)3'-givosiran as assessed by AUC_{last} was approximately 21% of exposure of givosiran. After reaching C_{max}, givosiran and AS(N-1)3'-givosiran concentrations declined with the $t_{½}$ value of 3.0 and 8.2 hours, respectively.

Studies in monkeys

Single dose

The plasma PK of givosiran was evaluated after a single IV dose (10 mg/kg) or single SC administration (0.1, 1, 5 or 10 mg/kg) in cynomolgus monkeys (study AS1-NCD14-007) using a LC/MS method.

Givosiran plasma concentrations declined in a multiphasic manner. The mean CL and V_{ss} values were 340 mL/h/kg and 104 mL/kg, respectively, suggesting distribution of givosiran beyond the vasculature. After a single SC administration, givosiran was not detectable at the 0.1 mg/kg dose.

Multiple doses

The PK profiles of givosiran were evaluated in cynomolgus monkeys after multiple SC doses with various dosing regimens and analysed using a LC/MS method (**Table 5**).

Table 5. Overall Mean Givosiran Plasma Pharmacokinetic Parameters in Monkeys After Multiple Subcutaneous Doses (study AS1-NCD14-007)

Dose (mg/kg)	**2.5**		**5/1**[a]		**1**	
Dose No.	1	8	1	8	1	8
t_{max} (h)	1.6	1.8	1.8	1.4	1.7	1.5
C_{max} (µg/mL)	0.291	0.305	0.870	0.135	0.109	0.136
AUC_{last} (h*µg/mL)	1.17	1.35	3.80	0.340	0.292	0.379
$t_{1/2}$ (h)	4.2	3.7	2.9	2.2	2.6	2.8

Abbreviations: AUC_{last}=area under the plasma concentration-time curve from the time of dosing to the last measurable concentration; C_{max}=maximum observed concentration occurring at t_{max}; No.=number; $t_{½}$=elimination half-life; t_{max}=time to reach maximum concentration.
[a] Dosing regimen 5/1: 5 mg/kg loading dose followed by 7 weekly doses of 1 mg/kg.

Exposure to givosiran and metabolite AS(N-1)3' after a single dose

A separate PK study in cynomolgus monkeys was conducted to determine the relative plasma exposure and the PK of the major metabolite, AS(N-1)3'-givosiran after a single SC dose of givosiran at 30 mg/kg (study AS1-DSM18-009).

The C_{max} of givosiran and AS(N-1)3'-givosiran in plasma were 2.42 and 1.56 µg/mL, respectively. Plasma AUC_{last} of givosiran and AS(N-1)3'-givosiran were 26.4 and 19.4 h*µg/mL, respectively. Plasma exposure of AS(N-1)3'-givosiran as assessed by AUC_{last} was approximately 74% of exposure of givosiran. After reaching C_{max}, givosiran and AS(N-1)3'-givosiran concentrations declined with the $t_{½}$ values of 5.5 and 5.1 hours, respectively. Givosiran and AS(N-1)3'-givosiran were measurable in plasma up to 24 h after administration.

Distribution

Plasma protein binding

Plasma protein binding of givosiran in mouse, rat, monkey, and human plasma was measured using EMSA. The results are shown in **Table 6**.

Table 6. Plasma protein binding of givosiran (study AS1 DSM18 008)

	Mean Percent Plasma Protein Binding					
Concentrations (µg/mL)	**0.5**	**1.0**	**5.0**	**10**	**25**	**50**
Mouse	ND	91.3	79.8	61.8	38.1	10.1
Rat	ND	93.1	89.0	78.6	51.6	27.5
Monkey	ND	89.5	82.7	69.9	43.9	25.9
Human	ND	91.8	83.4	71.4	39.2	21.1[a]

Abbreviations: ND=not determined due to insufficient staining of sample lane.
a One replicate masked due to insufficient quantity of sample.
Source: AS1-DSM18-008.

Distribution in mice

Distribution in liver and kidneys in toxicokinetic studies

Tissue distribution of givosiran in transgenic Tg-rasH2 hemizygous BALB/cBy × C57BL/6 F1 mice was evaluated in an investigational toxicology study. Mice were administered once weekly SC injections of givosiran for 8 weeks and samples of liver and kidney were analysed for givosiran using a LC-MS/HRAM method (**Table 7**).

Table 7. Toxicokinetic parameters in liver and kidney of mice after multiple doses (8 doses, weekly) (study AS1 DSM16 003)

Tissue	**Liver**	**Kidney**
Dose (mg/kg)	**100**	**100**
t_{max}(h)	6	0.5
C_{max} (µg/g)	283	116
AUC_{last} (h*µg/g)	5530	1310

Distribution of givosiran and its active metabolite, AS(N-1)3'-givosiran, was also evaluated using a LC-MS/MS method in wild-type Tg-rasH2 mice after SC administration of 3 monthly givosiran and AS(N-1)3'-givosiran. Liver exposure of givosiran and AS(N-1)3'-givosiran increased with the increase in dose level from 500 to 1500 mg/kg, but the increases were less than dose proportional. The concentration of AS(N-1)3'-givosiran in the liver was approximately 30% of givosiran

Distribution of givosiran following 5 once weekly SC doses administration in male, CD-1 mice were evaluated in the liver and kidney after the last dose. Givosiran concentrations in tissues were determined using a HPLC Atto-probe method with fluorescence detection. Liver concentrations of givosiran were measurable for up to 336 to 672 hours (last sampling point) following the fifth weekly SC dose. The mean liver TK parameters are shown in **Table 8**.

Table 8. Mean Givosiran Liver and Kidney Toxicokinetic Parameters in Mice After Multiple Doses (5 Doses, Once Weekly) (study AS1 NCD14 005)

Tissue	**Liver**			**Kidney**		
Dose (mg/kg)	**30**	**100**	**300**	**30**	**100**	**300**
t_{max}(h)	8.0	24.0	24.0	2.0	0.5	1.0
C_{max} (µg/g)	202	285	595	19	165	1039
AUC_{last} (h*µg/g)	15,900	33,400	64,800	1400	9660	39,100
t½ (h)	37	43	61	125	NR	347

Abbreviations: AUC_{last}=area under the tissue concentration-time curve from the time of dosing to the last measurable concentration; C_{max}=maximum observed tissue concentration occurring at t_{max}; NR=not reportable; t½=elimination half-life.

Distribution in rats

Distribution of givosiran to the liver and kidney in rats was evaluated after a single SC dose of 10 mg/kg up to 1344 hours post-dose (study AS1-NCD14-003). Distribution of givosiran to the liver and kidney was also compared after administration of a single IV or SC dose of 10 mg/kg up to 1344 hours post-dose (**Table 9**). Givosiran concentrations in tissues were determined using a HPLC Atto-probe method with fluorescence detection. There were no apparent sex-related differences in the distribution of givosiran.

Table 9. Overall Mean Givosiran Liver and Kidney Pharmacokinetics in Rats After a Single Intravenous or Subcutaneous Dose (10 mg/kg) (study AS1-NCD14-003)

Tissue	**Liver**		**Kidney**	
Route	**IV**	**SC**	**IV**	**SC**
t_{max} (h)	2.1	4.0	0.3	6.0
C_{max} (µg/g)	102	208	81.0	19.0
AUC_{last} (h*µg/g)	5390	12,600	5440	3190
$t_{½}$ (h)	55	120	119	172

Abbreviations: AUC_{last}=area under the tissue concentration-time curve from the time of dosing to the last measurable concentration; C_{max}=maximum observed tissue concentration occurring at t_{max}; IV=intravenous; SC=subcutaneous; $t_{½}$=elimination half-life; t_{max}=time to reach maximum concentration.
Note: Values represent the overall combined (male+female) mean.
Source: AS1-NCD14-003.

Markedly lower concentrations of givosiran (100- to 800-fold <liver; 10- to 70-fold <kidney) were observed in adrenal, heart, lung, spleen, thyroid, thymus, pancreas, jejunum, and testes (data not shown). In most tissues, givosiran was measurable up to 24 h after administration. Givosiran was not detected in the brain.

Liver and kidney concentrations of givosiran were also assessed in rats after administration of 3 different SC multiple dosing regimens (8 weekly doses at 1 or 2.5 mg/kg; or after a 5 mg/kg loading dose followed by 7 weekly doses of 1 mg/kg, at time points that included 168 hours following each of the first 7 weekly doses, and up to 672 hours following the eighth dose (study AS1-NCD14-003).

After repeat SC dosing, C_{max} and AUC_{last} of givosiran in the liver and kidney increased approximately dose proportionally from 1 to 2.5 mg/kg, and values were similar after eight 1- or 5/1-mg/kg doses. Compared to the dose normalized C_{max} (20.8 and 1.90 µg/g in liver and kidney, respectively at 1 mg/kg) and AUC (1259 and 319 h*µg/g in liver and kidney, respectively at 1 mg/kg) after a single SC dose (10 mg/kg) in rats, there was no evidence of accumulation in the liver after repeated weekly SC doses (1 mg/kg) of givosiran. However, givosiran exposure in the kidney was 3- to 4-times higher, indicating that givosiran accumulated in the kidney after repeated weekly SC doses.

Distribution in rats by quantitative whole-body autoradiography

A tissue distribution study using quantitative whole-body autoradiography (QWBA) (study AS1-DSM17-018) was conducted in rats with [^{3}H]-givosiran (10 mg/kg SC) radiolabeled with adenosine. Blood to plasma concentrations ratios of total radioactivity after SC dosing to rats were generally less than one, indicating that the distribution of givosiran to red blood cells was minimal.

The tissues showing the highest maximum concentrations of radioactivity included liver, lymph nodes, urinary bladder, thoracic duct, and kidneys, with values of 162, 38.2, 28.4, 17.7, and 15.2 µg equivalents [^{3}H]-

givosiran/g, respectively. The tissues with the lowest C_{max} values were prostate gland, large intestine, and spleen, with concentrations of 0.691, 0.782, and 0.877 µg equivalents [^{3}H]-givosiran/g, respectively. Radioactivity was cleared from all tissues by 672 hours post-dose, with the exception of liver, kidneys, and dose site. At 1344 hours post-dose, radioactivity was still quantifiable in the dose site.

Distribution in liver and kidneys in toxicokinetic studies

As part of several of the toxicology studies, toxicokinetic analyses were performed in liver and kidneys of rats. The results are shown in **Table 10** (liver) and **Table 11** (kidney). Treatment was SC in all studies.

Table 10. Overall mean givosiran liver toxicokinetic parameters in rats after SC administration

Matrix	Study	Dose mg/kg	tmax (h)	Cmax (µg/g)	AUClast (µg.h/g)	t1/2 (h)
Liver	AS1-NCD14-018	3	8.0	75.6	4440	39.1
		10	4.0	379	29200	60.3
		30	5.0	634	39700	67.3
		100	8.0	2237	161000	47.4
	AS1-NCD14-001	10	24	94	8750	66
		30	24	362	37000	70
		100	24	597	77100	74
		300	24	1550	211000	107
	AS1-NCD14-010	3	4	72.1	3360	25
		10	8	226	22000	NR
		30	4	356	18800	426
	AS1-GLP16-022	10	8	297	16600	
		50	24	1130	101000	
		150	24	3930	293000	
	AS1-GLP15-022	3	8	41.4	777	
		10	8	208	4140	
		30	16	489	9500	

NR=not reportable

Table 11. Overall mean givosiran kidney toxicokinetic parameters in rats after SC administration

Matrix	**Study**	**tmax (h)**	**Cmax (µg/g)**	**AUClast (µg.h/g)**	**t1/2 (h)**
Kidney	AS1-NCD14-018	12.1	8.27	715	119
		14.0	13.7	1670	66.9
		49.0	24.2	2510	NR
		6.0	522	48700	81.1
	AS1-NCD14-001	24	14.7	2840	204
		24	72.5	11100	162
		24	443	59000	82
		24	1082	248000	NR
	AS1-NCD14-010	8	19.5	7370	274
		8	24.4	16000	493
		4	126	40800	492
	AS1-GLP16-022	24	24.8	3110	
		8	352	37200	
		8	1230	152000	
	AS1-GLP15-022	24	28.7	516	
		5	91.6	1910	
		24	931	15900	

NR=not reportable

Distribution in pregnant rats

In study AS1-DSM15-041, female rats were administered SC dose of givosiran 4 times (once weekly) on 22 days, 15 days, 8 days and 1 day before cohabitation at 10, 30 or 100 mg/kg (study AS1-DSM15-041). Mean (±SD) givosiran maternal liver concentrations are summarized in **Table 12**. Givosiran concentrations were not measurable in any fetus from any dose group.

Table 12. Mean (±SD) Givosiran Maternal Liver Concentrations (µg/g) After Multiple Subcutaneous Administration in Pregnant Sprague Dawley Rats (4 Doses, Once Weekly, and 12 Doses, Daily) (study AS1 DSM15 041)

Study Day	Treatment Group (Pre-Gestation; Post-Gestation)		
	10 mg/kg/wk; 1.5 mg/kg/day	**30 mg/kg/wk; 5 mg/kg/day**	**100 mg/kg/wk; 16.5 mg/kg/day**
DG 0	63.9±48.7	164±46.5	421±32.4
DG 18	32.5±3.91	149±69.5	512±32.4

Abbreviations: DG=day of presumed gestation; SD=standard deviation.

In the pivotal EFD study (study AS1-GLP16-011), the same dosing regimen was used. Mean givosiran maternal liver concentrations increased in a greater than dose proportional exposure (approximate 6-fold increase with a 3-fold increase in dose) for the low to middle dose groups, and in a dose proportional manner between the mid and high dose groups. Givosiran placentae concentrations were measurable only in the animals in the highest dose group (30 mg/kg/wk; 5 mg/kg/day), and 50% of placentae showed measurable levels. Mean placentae concentration of givosiran (0.388 µg/g) was significantly lower (~890-fold) than corresponding maternal liver concentration (347 µg/g). Givosiran concentrations were not measurable in any fetus from any dose groups.

Distribution in pregnant rabbits

In study AS1-DSM16-012, female rabbits were administered once daily SC injections of givosiran on DG 7 through DG 19 at doses ranging from 3 to 30 mg/kg. Givosiran maternal liver concentration increased greater than dose proportionally over the dose range of 3 to 30 mg/kg/day. Givosiran placentae concentrations were not measurable in the two lowest dose groups (3 mg/kg/day, 10 mg/kg/day). In the 30 mg/kg/day givosiran dose group, only 30% of all placentae had measurable concentrations, and the mean concentration was 442 ng/g. Concentrations in fetuses were not measured in this study.

In the definitive rabbit EFD study (study AS1-GLP16-018), pregnant rabbits were administered once daily SC injections from DG 7 through DG 19 at doses ranging from 0.5 to 5 mg/kg. One group received a single 20 mg/kg SC dose of givosiran on DG 7. Givosiran maternal liver concentrations increased greater than dose proportionally over the dose range of 0.5 to 5 mg/kg/day. No measurable concentrations of givosiran were found in any of the placentae or fetal samples, indicating that givosiran is not transferred to the fetus at the dose range evaluated in the study.

Distribution in monkeys

Pharmacokinetics in liver

Distribution of givosiran in male and female cynomolgus monkey liver was determined after a single IV or SC doses up to 2016 hours post-dose and analysed using a HPLC Atto-probe method with fluorescence detection. The AUC_{last} in the liver was approximately 7-fold higher after a single SC dose of 10 mg/kg than after the same dose administered IV indicating that liver uptake is more efficient after SC administration compared with IV administration. The liver to plasma AUC ratio was approximately 2500, and the $t_{1/2}$ in the liver was significantly longer (~146 hours) than that in plasma.

Mean C_{max} and AUC_{last} values increased approximately dose proportionally across single and multiple SC doses. Relative to a single 1-mg/kg SC dose, there was no apparent significant increase in liver exposure after multiple once weekly dosing (8 doses) at 1 mg/kg or after a loading dose of 5 mg/kg followed by 7 weekly doses of 1 mg/kg suggesting minimal accumulation in liver with repeat dosing. The $t_{½}$ was consistent across doses and regimens, indicating no dose- or time-dependent PK. After multiple doses, givosiran was measurable up to 1344 h after the last dose.

Metabolism

In vitro metabolism

The *in vitro* metabolic stability of givosiran was evaluated in pooled serum and liver S9 fractions obtained from C57BL/6 mouse, rat, monkey, and human, at a concentration of 5 µM (study BA14010).

Following 24 hours of incubation of givosiran (5 µM) in mouse, rat, monkey, or human serum, the percentage of antisense strand remaining was approximately 75%, 59%, 63%, and 89%, respectively; the percentage of sense strand remaining was approximately 95%, 95%, 100%, and 95%, respectively.

When mouse, rat, monkey, or human liver S9 fraction was incubated with givosiran (5 µM), there was a range of stability profiles for the 4 species with the rank order from most to least stable being mouse>monkey>human>rat, for both strands.

A separate in vitro study (study AS1-DSM18-015) was conducted in human liver S9 fraction with and without nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) to determine if givosiran was metabolized by drug metabolizing enzymes requiring NADPH as a cofactor (eg, cytochrome P450, CYPs). Both sense and antisense strands of givosiran were stable, and no change was observed with and without NADPH.

Metabolite profiling

Metabolism of antisense strand

Monkey plasma, liver and urine samples from study AS1-NCD14-007 and in selected human plasma and urine samples confirmed that that the major metabolite was AS(N-1)3'-givosiran (study BA17014). In monkey urine, AS(N-1)3'-givosiran was the major metabolite, with concentrations higher than givosiran at all time points (study BA17014).

AS(N-1)3'-givosiran was the major circulating pharmacologically active metabolite after SC administration of givosiran in humans. Relative to givosiran, the steady-state AUC exposure of AS(N-1)3'-givosiran is approximately 46% after administration of 2.5 mg/kg every month (refer to ALN-AS1-001). The systemic exposure of AS(N-1)3'-givosiran after SC administration of givosiran was 21% and 73% relative to givosiran

exposure in rats (10 mg/kg, study AS1-DSM18-010,) and monkeys (30 mg/kg, study AS1-DSM18-009,) respectively.

An evaluation of this AS(N-1)3'-givosiran metabolite by transfection in human hepatocellular carcinoma cell line 3B (Hep3B) cells demonstrated retention of pharmacological activity in vitro (study BIO18001, see section 2.1).

Metabolism of sense strand

Either in serum (mouse, rat, monkey, and human) or plasma (rat and monkey), the givosiran sense strand was minimally metabolized primarily generating a metabolite corresponding to the loss of one GalNAc group from the triantennary ligand at the 3' end (study BA14010). Similar to the finding in rat and monkey plasma, givosiran sense strand with the loss of 1 or 3 GalNAc groups were also detected in plasma and urine from two human patients.

Metabolic profiling of *in vitro* liver S9 fractions (mouse, rat, monkey, and human) and *in vivo* rat and monkey liver samples showed that the major putative metabolites of the givosiran sense strand were generated by the loss of 1, 2, or all 3 GalNAc moieties at the 3' end. Loss of GalNAc was evident at the earliest time point of 2 hours. Remaining full length by 24 h in vitro was 66% in rat, 19% in monkey and 56% in human liver S9 fractions. *In vivo*, decline of full-length sense strand in the liver was early, with 10% at 2 h and no full length at 8 h in rats, and 1% full length at 8 h in monkeys.

In vivo metabolism in intact and bile-duct cannulated rats

The *in vivo* metabolic profile of givosiran was determined after a single SC administration (10 mg/kg) of [^{3}H]-givosiran in both intact and bile-duct cannulated (BDC) rats (study AS1-DSM17-018).

The profile of an AUC-pooled plasma sample (plasma samples between 0.5 and 48 h) showed that the majority of the radioactivity was associated with unchanged givosiran and accounted for approximately 54% of the total radioactivity exposure through 48 hours (AUC_{0-48}). AS(N-1)3'-givosiran was a major circulating metabolite contributing 13.0% of the total radioactivity exposure from 0.5 to 48 h. At 48 h, givosiran and AS(N-1)3'-givosiran were not detectable. Other peaks (comprising up to 14% of total radioactivity) were shown to be dose formulation impurities (these peaks were also present in dose formulation samples).

The majority of the radioactivity in urine was eluted as an unknown peak of radioactivity, indicated as M1. Attempts were made to identify the M1 using different methodologies such as SPE clean up and different HPLC columns, but were not successful. M1 is thought to consist of a mixture of degraded drug-related components, which are considered likely polar and smaller oligonucleotides. M1 accounted for 38.7% and 18.8% of the dose in intact and BDC rats, respectively.

Unchanged givosiran was recovered in urine from both intact and BDC rats, accounted for 3.38% and 3.90% of the dose, respectively. AS(N-1)3'-givosiran accounted for 0.971% and 1.96% of the dose in urine from intact and BDC rats, respectively. In bile, approximately 6% and 5% of the dose was recovered as unchanged givosiran and AS(N-1)3'-givosiran, respectively. No unchanged givosiran was recovered in feces.

Excretion

A mass balance study was conducted in rats after administration of a single SC dose givosiran to male intact and BDC/jugular vein-cannulated (JVC) rats (study AS1-DSM17-018). Givosiran was also quantitated in

pooled urine and fecal samples after a single SC in rats (study AS1-NCD14-003) and monkeys ((study AS1-NCD14-007). Results from these studies re summarized in **Table 13**.

Table 13.Excretion of radioactivity after administration of [^{3}H]-givosiran or givosiran to rats (% of dose) (studies AS1 DSM17 018, AS1-NCD14-003, AS1 NCD14-007)

Species	N	Dose (mg/kg)	Route	Anal.	Urine (% dose)	Faeces (% dose)	Bile (% dose)	Recovery (% dose)	Time (h)
Rat	4M	10	SC	Radioactivity	37.3±5.08	9.45±4.46	NA	51.7	168
Rat	4M	10	SC	Radioactivity	57.8±10.7	14.3±4.69	NA	84.1±3.68	1344
Rat BDC	6M	10	SC	Radioactivity	30.4±4.11	1.42±0.46	27.4±8.61	86.6±2.05	168
Rat	6M, 6F	10	SC	Givosiran	10.4	0.09	NA	ND	168
Monkey	3M, 3F	10	SC	Givosiran	15.9±5.36	0	NA	ND	168

Excretion in milk of rats

Excretion of givosiran in the milk from female rats was evaluated in the developmental and perinatal/postnatal reproduction study (study AS1-GLP17-009). Givosiran was administered for a total of 6 doses between 3 and 30 mg/kg. Givosiran concentration in the milk was not measurable in 3 and 10 mg/kg groups. Only one dam out of 4 in 30 mg/kg group had a measurable concentration of 229 ng/mL (LLOQ: 200 ng/mL) in the milk. Concentration in plasma in the 30 mg/kg group on LD 12, at 2 h post-dose was 2010-3650 ng/mL.

Excretion in rabbit seminal fluid

Givosiran concentrations in seminal fluid were evaluated in male rabbits after a single SC administration of 2.5 or 20 mg/kg (study INV-DSM16-057). Givosiran was detectable at all time points (8-216 hours post-dose) in both dose groups with the C_{max} occurring at 8 hours post-dose. The mean C_{max} in seminal fluid were 0.499 and 68.9 ng/mL after a single SC dose of 2.5 and 20 mg/kg, respectively, indicating that the C_{max} increased greater than dose proportionally.

2.3.4. Toxicology

Single dose toxicity

The toxicity of givosiran following a single dose was evaluated in rats as part of the pivotal 13-week repeat-dose toxicity study (see section below).

Repeat dose toxicity

A summary of the repeat dose studies submitted for givosiran is presented in **Table 14**.

Table 14. Overview of repeat-dose toxicity studies with givosiran and the main findings

Study ID	Species/Sex/ Number/Group	Dose/Route	Duration	NOEL/ NOAEL (mg/kg /wk)	Major findings
AS1-NCD14-010	Rat, 10/s/g	3-10-30-100, SC	13wk +13 wk recov	ND	<u>Haematology</u> =30: ↓PLT <u>Clinical chemistry</u> =30 ↑ALP, ↑AAT, ↑Alb <u>Pathology</u> ≥10: Pale liver, dark foci at injection site. ↓Adrenal weight, ↓Liver weight, ↑Kidney weight <u>Histopathology</u> ≥3: ↑Hepatocellular vacuolation (minimal-marked), ↑Injection site macrophage vacuolation (minimal-mild) ≥10: ↑Adrenal vacuolation (minimal-> mild), ↑Liver single cell necrosis (minimal-mild), ↑Liver mitoses (minimal-mild), ↑Liver Kupffer cell pigmentation (minimal-mild), ↑Liver hepatocellular vacuolation (minimal-moderate) ↑Injection site haemorrhage (minimal-moderate), ↑injection site cell infiltrate (minimal-moderate) =30:↑ Kidney basophilic tubular granules <u>Recovery</u> ≥10: minimal to moderate hepatocellular vacuolation, minimal to mild eosinophilic cellular alteration =30: binucleated hepatocytes and karyomegaly

					Haematology =150F: ↓PLT, ↓Ret, ↑WBC, ↑Mono Clinical chemistry =150: ↑t-bili =150F: ↓t-prot, ↑ALP, ↑AST, ↑ALT Pathology ≥50: Liver discoloration (pale) ≥50M: ↓liver weight =150F: ↓Adrenal weight
AS1-GLP16-022	Rat 10/s/g	0-10-50-150, SC	13 wk	ND	Histopathology Liver ≥10 minimal to marked hepatocellular vacuolation =150: Minimal single cell necrosis, minimal to mild increased mitosis, minimal to mild increased pigment Kidney =150: minimal to mild tubular basophilic granules =150F: minimal tubular vacuolation Injection site ≥10: minimal to mild mixed cell infiltrates

AS1-GLP15-022	Rat 20/s/g	0-3-10-30	26 wk +13 wk recov	ND	<u>Clinical observation</u> ≥3: slight erythema <u>Haematology</u> =30: ↓APTT =30F: ↓PLT <u>Clinical chemistry</u> ≥3: ↑Tbili =30F: ↑ALP, ↑Trig, ↑Tprot, ↑Alb (Alb/Glob) <u>Pathology</u> ≥3: Liver discoloration (pale) 10F: ↑Kidney weight ≥10M: ↓liver weight <u>Histopathology</u> Kidney ≥10: minimal to mild basophilic granules tubular cells Liver ≥3: Minimal to marked hepatocellular vacuolation, minimal to mild increased mitoses ≥10:minimal to mild single cell necrosis, minimal to mild pigmentation Kupffer cells, minimal to mild eosinic cellular alteration =30: minimal to mild basophilic granules in Kupffer cells =30F: minimal hepatocellular karyomegaly Pancreas ≥10: minimal to mild angiectasis in islet of Langerhans Injection site ≥3: minimal to mild macrophage vacuolation ≥10: mild to moderate degeneration of cutaneous muscle <u>Recovery</u> ≥10: minimal to marked hepatocellular vacuolation, minimal to mild Kupffer cell pigmentation =30: focus of cellular alteration eosinophilic, minimal angiectasis in islet of Langerhans

					Clinical chemistry =150M:↑ASP, ↑ALT Histopathology Liver ≥50: mild-moderate basophilic granules in Kupffer cells =150: basophilic granules hepatocytes Injection site ≥15: minimal to mild macrophage vacuolation ≥50:minimal-moderate mononuclear infiltration Lymph nodes ≥15: minimal to mild macrophage vacuolation Recovery ≥50: liver basophilic vacuolation Kupffer cells, basophilic granules hepatocytes, injection site mononuclear infiltration, lymph node macrophage vacuolation
AS1-NCD14-011	Cynomolgus, 6/s/g	0-15-50-150	13 wk+13 wk recov	ND	
AS1-GLP15-018	Cynomolgus (juv), 3/s/g	0-10-30-100, SC	39 wk +13 week recov	10	Clinical chemistry ≥30: ↑ALP, =100: ↑ALT, ↑GGT =100M: ↑Chol =100F: ↑Creatinine Histopathology ≥30: Hepatocellular basophilic granules, basophilic granules Kupffer cells, lymph node vacuolation, lymph node plasmacytosis, injection site macrophage vacuolation, injection site mononuclear cell infiltrates =100: Hepatocellular single cell necrosis =100M: Kupffer cell pigmentation (minimal) Recovery ≥10F: ↑ALP ≥30f:↑ALT, minimal to moderate macrophage vacuolation in lymph nodes, minimal to mild basophilic granules Kupffer cells, minimal to moderate hepatocellular basophilic granules =100: ↑GGT 100F: minimal hepatocellular single cell necrosis

Genotoxicity

An overview of the genotoxicity studies conducted with givosiran is summarised in **Table 15**.

Table 15. Overview of genotoxicity studies with givosiran

Type of test/study ID/GLP	Test system	Concentrations/ Concentration range/ Metabolising system	Results Positive/negative/equivocal
Bacterial reverse mutation / AS1-NCD14-015 / GLP	S. typhimurium TA100, TA98, TA1535, TA1537 and E. coli WP2 uvrA	1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000 µg/plate, +/- S9	Negative

Mammalian cell chromosomal aberration / AS1-NCD14-016 / GLP	cultured human peripheral blood lymphocytes	128, 256, and 500 µg/mL +/- S9	Negative
Chromosomal aberrations in vivo / AS1-NCD14-017 / GLP	Rat, micronuclei in bone marrow	0, 500, 1000, and 2000 mg/kg	Negative

Carcinogenicity

The carcinogenic potential of givosiran when given by SC injection once every 28 days for 26 weeks to Tg-rasH2 hemizygous mouse was evaluated in study AS1-GLP17-004. Mice were administered doses of up to 1500 mg/kg/month without any evidence of treatment-related neoplastic changes at any dose levels.

Reproduction Toxicity

Findings from the reproductive and developmental studies with givosiran are summarised in **Table 16**.

Table 16. Reproductive and developmental studies with givosiran

Study type/ Study ID / GLP	**Species; Number Female/ group**	**Route & dose**	**Dosing period**	**Major findings**	**NOAEL (mg/kg &AUC)**
Fertility and Embryo-fœtal development **AS1-DSM16-011** GLP	SD Rat, 24F/g	Pre-gestation: 0, 3, 10, 30 mg/kg QW Post-gestation: 0, 0.5, 1.5, 5 mg/kg QD s.c.	GD -22-17	F0 Clinical chemistry ≥10/1.5: ↑AST, ↑Trig, ↑Pot ≥30/5: ↑ALT, ↑Phos, ↓Alb, ↓Alb/Glob F1 Skeletal anomalies =30/1.5: slight increase in incompletely ossified pubes	F0: 3/0.5 F1: 10/1.5
Embryo-fœtal development **AS1-DSM16-018** GLP	NZW Rabbit, 20F/g	0, 0.5, 1.5, 5 mg/kg/day 20 mg/kg (single dose) s.c.	GD 7-19 GD7 (single dose	F0 ≥0.5: ↓BW GD7-GD20, ↓FC GD7-GD20, ↓RBC, ↓HB, ↓HCT, trend ↑PLT ≥1.5: ↑Mono, ↑liver discoloration =5: ↑PLT, ↑Ret, ↑Fib, ↑AST, ↑ALT F1: =1.5: trend ↑resorptions ≥5: ↓live foetuses, ↑resorptions, ↑%postimplantation loss =20: ↑skeletal anomalies	F0 ND F1 0.5
Peri & postnatal development **AS1-GLP17-009** GLP	SD Rat, 22F/g	0, 3, 10, 30 QW s.c.	GD 7 – LD 18	F1 males: ≥10: ↑prepuital separation (~2 days)	30

Toxicokinetic data

Toxicokinetics were derived from the repeated dose studies in mice (AS1-DSM16-003, AS1-GLP17-006, AS1-NCD14-005), in rats (AS1-NCD14-001, AS1-NCD14-010, AS1-GLP16-022 and AS1-GLP15-022) and in monkeys (AS1-NCD14-002, AS1-NCD14-011, AS1-GLP15-018).

Givosiran was quickly absorbed after subcutaneous administration, with Tmax 0.5-1.0 h in mice, 0.5-2.0 h in rats and 1-4 h in monkeys. The exposure increased approximately dose-proportionally in mice and monkeys and dose-proportionally or more than dose-proportionally (at lower doses only) in rats. No relevant accumulation was observed in mice and monkeys. In rats, no significant accumulation or only slight accumulation (maximally 2-fold) was observed. Givosiran was removed fast from plasma with elimination half-life of 0.5-2.4 h in mice, 1.1-4.2 h in rats and 1.8-6.1 h in monkeys. The exposure was adequate in all species. It should be noted, however, that the compound had not left the body yet after one day in the rat (5x T1/2) but was still retained in the tissues, mainly liver, as about one third of the administered dose was still excreted between 168h and 1344h after dosing.

Anti-drug antibodies (ADAs) were analysed in the 26-week rat study (AS1-GLP15-022) and in the 39-week monkey study (AS1-GLP15-018). All samples in the rat were negative. In the monkey study one sample was positive at 30 mg/kg/week (on day 169) and 3 samples were positive at 100 mg/kg/week, all from one animal (on days 85, 169 and 253).

After subcutaneous administration to pregnant animals, T_{max} (0.5-2.0 h) was comparable to non-pregnant animals. Exposure increased approximately dose-proportionally in pregnant rats and pregnant rabbits at lower dose, but slightly greater than dose-proportionally at higher doses. No accumulation was observed in pregnant rabbits. Elimination half-life was slightly higher in pregnant rats (4.0-6.6 h) than in non-pregnant rats (1.1-4.2 h). Elimination half-life in pregnant rabbits was 16.7 h. Maximal exposure was low in pregnant rats (maximally 1.7x human exposure based on AUC) and slightly higher in rabbits (maximally 5x human exposure).

Local Tolerance

Dedicated local tolerance studies with givosiran were not submitted.

Other toxicity studies

No other toxicity studies with givosiran were submitted.

2.3.5. Ecotoxicity/environmental risk assessment

Table 17. Summary of main study results

Substance (INN/Invented Name): givosiran			
CAS-number (if available): N/A			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	log K_{ow} < 8.7 x 10^{-3}	Potential PBT: N
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**

Bioaccumulation	log K_{ow}		N/A
	BCF		N/A
Persistence	DT50 or ready biodegradability		N/A
Toxicity	NOEC or CMR		N/A
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, refined (prevalence, literature)	0.000025	µg/L	> 0.01 threshold N
Other concerns (e.g. chemical class)			N

2.3.6. Discussion on non-clinical aspects

The pharmacodynamic studies support the proof of principle that givosiran can reduce liver ALAS1 mRNA and subsequently serum ALA and PGB levels. However, the intended clinical treatment regimen of once per month has not been tested in animals. Monkey data show a mRNA reduction of only 20% after 4 weeks post-dose, suggesting that monthly dosing would not be sufficiently efficacious in monkeys. The half-life of givosiran in the liver is substantially longer in humans than in monkeys, which is presumably caused by a faster metabolism in monkeys than in humans.

No dedicated studies have been conducted to evaluate pharmacodynamic drug interactions. The potential for pharmacodynamic drug interactions with givosiran in humans is expected to be low since there are no other ALAS1 suppressive agents or other agents that affect ALAS1 production.

A bioinformatic and *in vitro* analysis conducted to determine the potential for hybridization-based off-target effects of givosiran revealed a >100 fold difference between the "on-target" reduction of ALAS1 by givosiran and the reduction of any of the 6 nearest predicted off-target transcripts by transfection in human liver carcinoma cell line-G2 (HepG2) cells, confirming the specificity of givosiran for ALAS1. Potential off-target effects for the metabolite AS(N-1)3' givosiran were not evaluated but the CHMP agreed that the off-target analysis carried out for the full length 23mer is representative of the potential off-targets of the AS(N-1)3′ givosiran metabolite and additional analysis was not considered necessary.

The PK and ADME of givosiran have been characterized *in vitro* and *in vivo*. Givosiran shows similar patterns of PK and ADME properties across the nonclinical species tested in vivo and across human and animals in vitro. Collective data demonstrated that the SC administration of givosiran results in adequate exposure of the siRNA to the intended target organ (liver). Overall, PK and ADME studies provide support for the interpretation of toxicology studies and help characterize the disposition of givosiran in humans at the dosing regimen of 2.5 mg/kg once monthly.

The givosiran metabolite, AS(N-3)5' givosiran, was found in liver S9 fractions from mouse, rat, monkey and human, but in vivo (in plasma and liver of rats and monkeys and plasma of 2 human patients) this metabolite was not found, or at least not in substantial amounts. *In vivo*, only AS(N-1)3' givosiran metabolite was detectable in relevant amounts.

AS(N-1)3' givosiran was the only circulating active metabolite in the plasma of rats, monkeys, and humans after SC administration. The collective data characterizing the metabolism of the antisense and sense strands demonstrated that the in vitro metabolic profiles for givosiran were comparable to those profiles observed

from the in vivo study samples, and the overall metabolic profile of givosiran was similar across all species tested, including human.

Toxicity of givosiran has been studied adequately. In the repeat-dose toxicity studies conducted in rats and monkeys, the rat was identified as the most sensitive species to givosiran-related effects, with the liver being identified as the primary target organ of toxicity in both the rat and monkey. No adverse findings were associated with chronic, weekly administration of givosiran to rats and monkeys at doses that achieved exposure multiples of 3.5- and 26.3 fold, respectively when compared to exposures achieved in patients receiving the maximum recommended human dose. In the 26-week rat study, angiectasis in the islets of Langerhans of the pancreas was observed. These findings did not occur after monthly dosing in rats and were not observed in monkeys. The effect is considered species specific with no evidence of relevance in humans.

There was no evidence of carcinogenicity in the 26-week conducted in ǂ Tg-rasH2 mice. A longer, 2-year carcinogenicity study in Sprague Dawley rats (AS1-GLP18-007), currently ongoing, will be submitted upon its completion.

Givosiran had no genotoxic potential when assessed in a standard battery of genotoxicity assays. Chronic toxicity studies in both rats and cynomolgus monkeys did not reveal proliferative or inductive effects.

Reproductive toxicity studies in rats did not suggest a direct cause for concern: a single skeletal variation was observed in foetuses and was not considered adverse. This finding was likely due to maternal hepatotoxicity and exposures were within the clinical range. Givosiran showed marked maternal toxicity in rabbits (including mean maternal body weight loss) and resulted in increased post-implantation loss as a result of increased early resorptions and a low incidence of skeletal variations. These findings are considered an indirect effect, secondary to maternal toxicity. No adverse developmental effects were observed in rats administered the maternally toxic dose of approximately 9 times the normalised maximum recommended human dose.

In a post-natal development study in rats, there was no effect on growth and development of the offspring.

No adverse effects were observed in the fertility of male and female rats when administered with givosiran.

Dedicated studies were not conducted in juvenile animals. In the chronic monkey GLP toxicology study, givosiran was administered once weekly at dose levels between 10 and 100 mg/kg for 39 weeks, followed by a 13-week recovery phase. At initiation of the treatment, monkeys were 13 to 17 months old. Monkeys at 24 to 36 months old are generally considered the corresponding development age of adolescent up to 12 years of age in human. Therefore, the available toxicology data in juvenile monkeys supports the administration of givosiran to adolescents, ≥12 years of age.

Dedicated antigenicity studies were not conducted. Such studies were considered unnecessary based on absence of structural similarity to known antigenic compounds, distribution of givosiran, and nonclinical data.

Givosiran PEC-surface water value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5. Givosiran is therefore not expected to pose a risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

There are no objections to the marketing authorization of Givlaari from a non-clinical perspective.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Study, Status, Data Cut-off	**Study Design, Objectives, Location**	**Dose(s)**	**N, Patient Type**
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)	Part A: 0.035 mg/kg to 2.5 mg/kg single SC dose Part B: 0.35 mg/kg and 1 mg/kg, monthly (x 2)	Part A and B: N=23; CHE subjects
	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 C: 2.5 mg/kg and 5 mg/kg once monthly (x 4) and quarterly (x 2)	Part C: N=17; AIP patients
ALN-AS1-002 (Study 002) *Ongoing* *Data cut-off:* *19 April 2019*[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] Dosing up to 3 years	N=16; AIP patients who completed Study 001C
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 Dosing for 6 months in the double-blind treatment period	N=94, AHP patients N=48 on givosiran N=46 on placebo

Ongoing; *Open-label extension* *Data cut-off:* *23 July 2019*[d]		1.25 mg/kg or 2.5 mg/kg SC once monthly	N=93[e], AHP patients N=56 on 2.5 mg/kg once monthly N=37 on 1.25 mg/kg once monthly
ALN-AS1-004 (Study 004) *Completed;* *Data lock:* *25 Jan 2019*	Phase 1, open-label study to evaluate the drug-drug interaction of givosiran with midazolam, caffeine, losartan, omeprazole, and dextromethorphan 1 clinical study center in Sweden	2.5 mg/kg single SC dose of givosiran Single oral dose of midazolam (5 mg), caffeine (200 mg), losartan (50 mg), omeprazole (40 mg), and dextromethorphan (30 mg)	N=10, CHE subjects

Abbreviations: ADA=anti-drug antibodies; AHP=acute hepatic porphyria; AIP=acute intermittent porphyria; ALA=aminolevulinic acid; ALAS1=aminolevulinate synthase 1; CHE=chronic high excreters; CYP=cytochrome P450; DB=double-blind; EU=European Union; MAD=multiple ascending dose; mRNA=messenger ribonucleic acid; OLE=open-label extension; PBG= porphobilinogen; PD=pharmacodynamic; PK=pharmacokinetic; 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 19 April 2019
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 23 July 2019 was used in analysis
e 1 patient discontinued treatment during the DB period of 003 and did not participate in the OLE

2.4.2. Pharmacokinetics

Pharmacokinetics and pharmacodynamics effects of givosiran has been evaluated in all 4 clinical studies (see Section 2.4.1). The studies addressed single and multiple ascending doses, the metabolite profile and potential for drug-drug interactions with givosiran. Covariates affecting the pharmacokinetics of givosiran were evaluated by population pharmacokinetics. Pharmacokinetics of the primary active metabolite AS(N-1)3' givosiran was also evaluated in most studies. Further, *in vitro* studies evaluating the drug-drug interaction potential of givosiran and exposure-response analyses have been performed.

No radiolabeled mass balance study, thorough QT prolongation study, and dedicated studies in subjects with dedicated hepatic and renal impairment have been conducted but patients with renal and hepatic impairment were allowed to be enrolled in study 003 as was agreed in three protocol assistance meetings with the European Medicines Agency (EMA) in July 2017 and March 2018 (EMEA/H/SA/3587/1/2017/PA/PR/III, EMEA/H/SA/3587/1/FU/1/2018/PA/PR/III, and EMEA/H/SA/3587/2/2018/PA/PR/I). Similarly, in agreement with the recommendations in the PIP adolescents could enrol in study 003. Immunogenicity of givosiran treatment was evaluated in all studies.

The recommended dose of givosiran is 2.5 mg/kg once monthly, administered by subcutaneous (SC) injection in abdomen, thigh or upper arm.

Bioanalytical methods

Givosiran and AS(N-1)3'givosiran were quantitated in human plasma and urine using validated LC-MS/HRAM and LC-MS/MS assays and are considered to have acceptable reproducibility. The pharmacodynamic markers ALA and PBG were determined in plasma and urine by LC-MS/MS assays validated in line with Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 02).

Population PK analysis

Plasma pharmacokinetics of givosiran and AS(N-1)3'givosiran in the target population was evaluated by popPK analysis. Plasma PK data of givosiran and its active metabolite, AS(N-1)3' givosiran, were described by simultaneous modelling of both analytes in a nonlinear mixed-effects modelling framework.

Absorption of givosiran is described by a first-order process. Body weight was found to have a significant effect on K_a, with the model predicting a prolonged t_{max} and a slower absorption in higher body-weight patients receiving a higher absolute dose, and shorter t_{max} and faster absorption in lower body-weight patients receiving a lower absolute dose. The conversion of givosiran to its primary metabolite, AS(N-1)3' givosiran, was incorporated as a first-order process in the model. Elimination from the central compartments for both givosiran and AS(N-1)3' givosiran consisted of hepatic and renal clearances. Hepatic clearance for both analytes was considered equal and incorporated in the model. The renal clearance for both givosiran and AS(N-1)3' givosiran (CLR) were fixed to individual patient's baseline eGFR value. A dose effect on hepatic clearance of givosiran and AS(N-1)3' givosiran was included to explain the greater than dose proportional increase in exposure observed at the 5.0 mg/kg dose in Study 001.

Population PKPD analysis

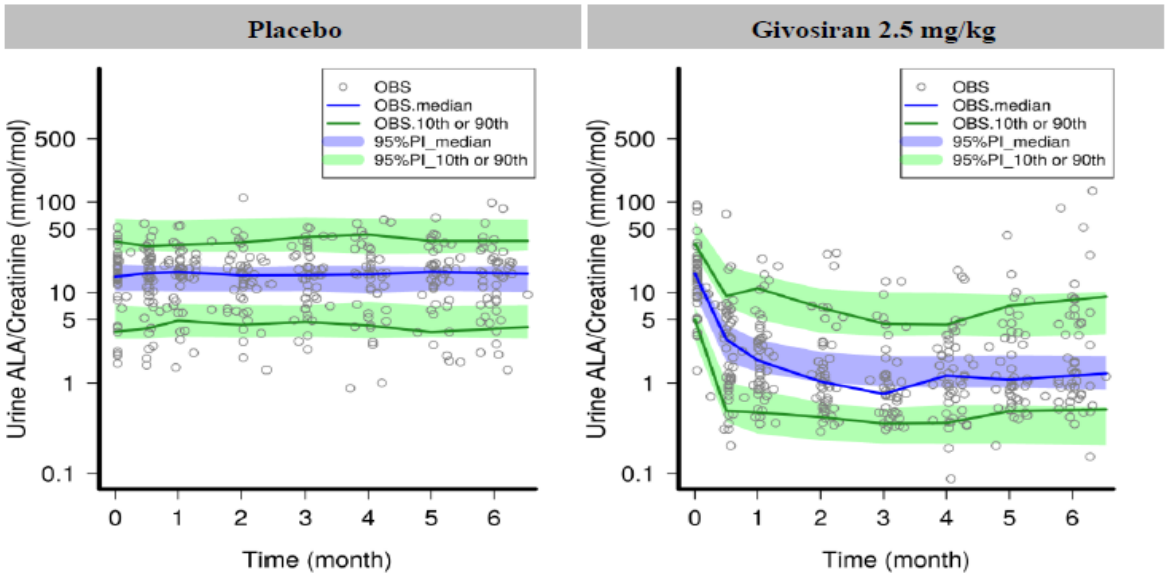
A population PK/PD model was developed to describe the time-course and estimate inter-individual variability of urinary ALA levels from pooled data following placebo and givosiran administration in CHE subjects and AHP patients. The model was used to support the dose and dosing interval and to support dosing in patients.

This concerned semi-mechanistic model using active siRNA (givosiran and AS(N-1)3'givosiran concentrations over time in liver and RISC compartments in rat as input for PK, which were fixed and allometrically scaled to human, and an estimated PD. Hence variability observed in PK in subjects with CHE or AIP is not incorporated in the model, only variability in subjects with CHE or AIP in PD parameters is estimated.

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 (CHE subjects versus AHP patients) on IC50 for givosiran and Synthesis rate constant of ALA (Kin, ALA), mild hepatic impairment on Kin,ALA, and baseline ALA on maximum inhibitory effect of givosiran on kin,ALA. The first-order rate constant between plasma and effect compartment was estimated to be 0.00761 hr-1 (half-life=91 hours, 3.79 days).

The Visual Predictive Check (VPC) plots for the final PK/PD model are presented in **Figure 7**.

Figure 7. Visual Predictive Check in AHP Patients Receiving Placebo or Givosiran 2.5 mg/kg Once Monthly Doses in Study 003 (Double-blind Period Only) (PKPD model)



Abbreviations: AHP=acute hepatic porphyria; ALA=aminolevulinic acid; OBS=observed data; PI=prediction interval.
Note: Gray open circles denote observed data; blue lines denote the observed median; green lines denote 10th or 90th percentile of observed data; blue shaded regions represent the 95% PIs of predicted median; green shaded regions represent 95% PIs of 10th and 90th predicted percentiles.

Population ALA-Attack Analysis

The objective of this analysis was to develop population models to describe the relationship between urine ALA levels and clinical activity, as measured by incidence of porphyria attacks (AAR). Separate models for ALA absolute model and ALA percent reduction were developed. The models were used to predict the AAR in patients and support the dose and dosing interval.

Predicted AAR values from both models were similar; prediction of AAR for a Median Risk AHP patient treated with placebo (ALA Absolute Model: mean 9.29 attacks/year; ALA Percent Reduction model: mean 10.4 attacks/year) and predicted AAR for a Median Risk AHP patient treated with 2.5 mg/kg QM givosiran (ALA AbsoluteModel: mean 1.87 attacks/year; ALA Percent Reduction Model: mean 1.88 attacks/year). However, there was high uncertainty in estimation of the ALA parameters especially for the absolute model.

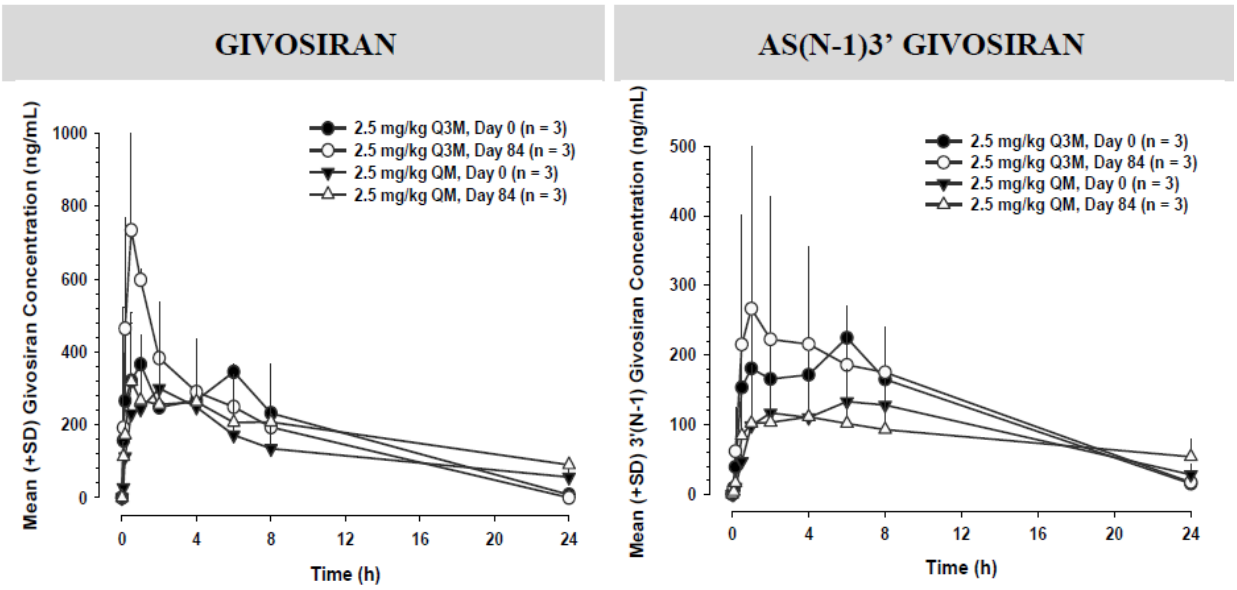
Historical AAR, prior hemin prophylaxis and baseline ALA (absolute model) were significant covariates, baseline age and gender not. AAR data were weighted by the number of observation days, which enables pooling of various studies since they had different treatment duration. Hemin was regularly used to recover from an attack and this influences ALA levels. Sensitivity analysis by excluding ALA data up to 12 days after hemin administration showed minimal impact on parameters from the original model where exclusion up to 3 days was used.

The model individual predictions followed the observed data reasonably well though there was some underestimation, converging to the mean. Both models seemed fit to predict the efficacy of givosiran in AHP patients there is considerable variability, nevertheless, the model estimated the ALA reductions from OLE extension study using 2.5 mg/kg and 1.25 mg/kg not used for model development, sufficiently well.

Absorption

Mean plasma concentration-time profiles for givosiran and its active metabolite, AS(N-1)3' givosiran in AIP subjects, after single and multiple dosing are shown in **Figure 8**.

Figure 8. Plasma concentration-time profiles for givosiran and AS(N-1)3'givosiran after single and multiple subcutaneous injections with 2.5 mg/kg givosiran in AIP subjects (Mean ± SD, study 001C)



Givosiran was rapidly absorbed from the SC injection site into plasma, the time to maximum plasma concentration (t_{max}) was 0.5 to 2 hours at the 2.5 mg/kg once monthly dose. At the 2.5 mg/kg once monthly dose, the steady state peak plasma concentrations of givosiran (Cmax) and area under the curve from time of dosing up to 24 hours after dosing (AUC24) were 321 ± 163 ng/mL and 4130 ± 1780 ng·h/mL, respectively, and corresponding values for the active metabolite were 123 ± 79.0 ng/mL and 1930 ± 1210 ng·h/mL, respectively. Effect of site of injection (abdomen, thigh, upper arm) has not been reported.

Absolute bioavailability has not been determined for givosiran but in rats the absorption of givosiran following SC administration was high >84% (study AS1-DSM17-018).

Distribution

The population estimate for the apparent central volume of distribution (V_d/F) was 10.4 L for both givosiran and AS(N-1)3' givosiran.

Givosiran is designed to be preferentially distributed to liver through the ASGPR-mediated hepatic uptake. This is reflected by the high liver to plasma AUC ratio of 4500 and 2500 in rats and monkeys, respectively, after SC dosing.

PopPK model was used to estimate the fraction of givosiran taken up in the liver. The apparent fraction of givosiran dose in liver was 80.7% (90%CI 69%-90%), with 51.6% available as givosiran and 29.1% available as AS(N-1)3'givosiran.

Elimination

At 2.5 mg/kg, the half-life of givosiran and AS(N-1)3' givosiran was estimated to be approximately 5 hours. Apparent clearance (CL/F) of givosiran and AS(N-1)3' givosiran was 36.6 L/h and 23.4 L/h, respectively.

Givosiran is cleared from the plasma compartment by conversion to AS(N-1)3'givosiran, uptake in the liver, and urinary excretion. Contribution of the three pathways to the total clearance was estimated 36% (metabolism), 52% (liver uptake), and 12% (urine).

After single and multiple givosiran dosing 5-14% and 4-13% of the dose were recovered in the urine over 24 hours as unchanged givosiran and AS(N-1)3' givosiran, respectively. The renal clearance ranged from 1.22 to 9.19 L/h for givosiran and 1.40 to 12.34 L/h for the active metabolite. The fraction of dose excreted in urine as unchanged givosiran or AS(N-1)3' givosiran was consistent across doses.

Excretion of givosiran in urine was investigated only for 24 hours post-dosing, no mass balance study was conducted because of the slow elimination of givosiran related material observed in rat and monkey.

Metabolism

Givosiran is metabolised by exo- and endonucleases. AS(N-1)3' givosiran is the main metabolite in plasma and urine with only traces of sense strand metabolites and AS(N-3) givosiran. In plasma the concentrations of AS(N-1)3' givosiran were approximately half the concentrations of givosiran.

Dose proportionality and time dependencies

Mean AUC and C_{max} of givosiran and AS(N-1)3' givosiran increased approximately dose-proportionally across a dose range of 0.35 mg/kg to 2.5 mg/kg following single or multiple dosing. In study 001C and 002 (sparse sampling), it seemed that at doses greater than 2.5 mg/kg, givosiran exposure increased slightly greater than dose-proportionally suggesting saturation of liver uptake of givosiran, in popPK analysis dose was a covariate on hepatic clearance.

There was no accumulation of givosiran and AS(N-1)3' givosiran after multiple dosing.

Special populations

Special populations were evaluated by popPK analysis. A total of 125 subjects, 14 (11.2%) CHE subjects and 111 (88.8%) AHP patients were included in the population PK modeling. The median age of subjects in the pooled dataset was 38 years with a range of 19 to 65 years. Body weight ranged from 39.5 to 131 kg, with median of 66.2 kg. Majority of subjects were female (88%) and white (80%). There were 30 (24.0%) subjects with normal renal function, 59 (47.2%) subjects with mild renal impairment, 35 (28%) subjects with moderate renal impairment, and 1 (0.8%) subject with severe renal impairment at baseline. There were 112 (89.6%) subjects with normal hepatic function, and 11 (8.8%) subjects with mild hepatic impairment, 1 (0.8%) subject each with moderate and severe hepatic impairment at baseline.

Body weight, renal function, dose and race (East Asian) were covariates in the popPK model, the differences were <30% compared to typical patient. Covariate effect of baseline age, patient population (CHE subjects versus AHP patients), sex, and hepatic function (normal versus mild hepatic impairment) was not statistically significant.

Although adolescents were allowed to be enrolled in study 003, none was because of the rarity of a high attack rate in adolescents. PopPK analysis predicted 22-40% lower exposure in adolescents compared to adults for givosiran and AS(N-1)3'givosiran

Pharmacokinetic interaction studies

The potential of givosiran for drug drug interaction has been investigated by *in vitro* studies. *In vitro* studies indicated a low interaction profile for givosiran as givosiran was not a substrate, inhibitor or inducer of CYP enzymes and transporters *in vitro*.

Indirect inhibition of CYP enzymes by givosiran could not be excluded based on ex vivo and in vivo studies in rat and monkey and a DDI (cocktail) study was conducted in CHE patients. The effect of givosiran on the CYP enzymes was evaluated 28 days after subcutaneous injection of 2.5 mg/kg givosiran, which was approximately maximal PD effect. Givosiran treatment resulted in the following:

- 1.3-fold increase in C_{max} and approximately 3.1-fold increase in $AUC_{0\text{-}inf}$ of caffeine (CYP1A2 substrate)

- 2.00-fold increase in C_{max} and 2.4-fold increase in $AUC_{0\text{-}inf}$ of dextromethorphan (CYP2D6 substrate)

- 1.1-fold increase in C_{max} and 1.6-fold increase in $AUC_{0\text{-}inf}$ of omeprazole (CYP2C19 substrate)

- 1.2-fold increase in C_{max} and 1.5-fold increase in $AUC_{0\text{-}inf}$ of midazolam (CYP3A4 substrate)

- No effect on the exposure of losartan as geometric mean ratio was close to 1 (CYP2C9 substrate)

2.4.3. Pharmacodynamics

Mechanism of action

Givosiran is designed to be selectively delivered to the liver using the binding between the GalNAc ligand of givosiran and ASGPR receptor expressed on the liver. Upon delivery to the liver, givosiran uses the naturally occurring RNAi pathway to specifically target and silence ALAS1 mRNA in the liver. The RNAi-mediated lowering of induced liver ALAS1 mRNA levels and the consequent sustained decrease in the accumulation of toxic heme intermediates ALA and PBG is expected to prevent or reduce the occurrence of serious neurovisceral attacks and ongoing symptoms in patients with AHP.

Primary pharmacology

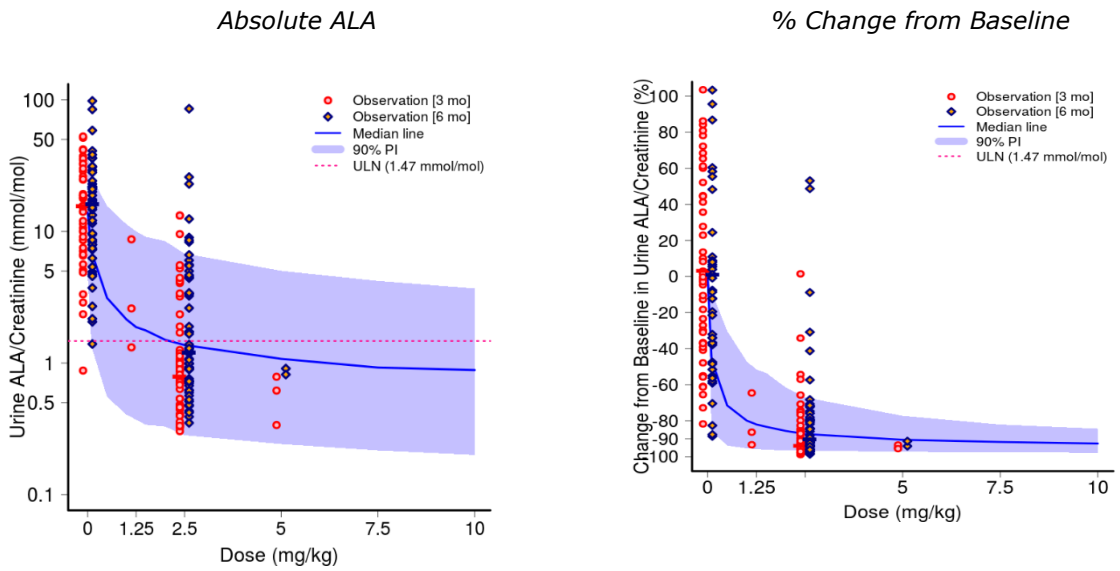
The primary action of givosiran sodium is the reduction of the plasma levels of toxic metabolites ALA and PBG. Plasma ALA and PBG levels were shown to be highly correlated with corresponding urinary levels.

The relationship between the dose and PD effect (ALA and PBG lowering) was investigated in the exploratory Study 001 with chronic high excreters (CHE) subjects as well as AIP patients. Givosiran sodium resulted in a rapid dose-dependent decrease in urinary ALAS1 mRNA levels, ALA and PGB levels.

A modelling and simulation approach was employed to further quantitatively characterize the dose-response relationship for PD and clinical activity. According to the model, givosiran dosing resulted in a dose dependent

increase in ALA lowering with increasing givosiran doses leading to greater ALA lowering and lower interpatient variability in response (**Figure 9**).

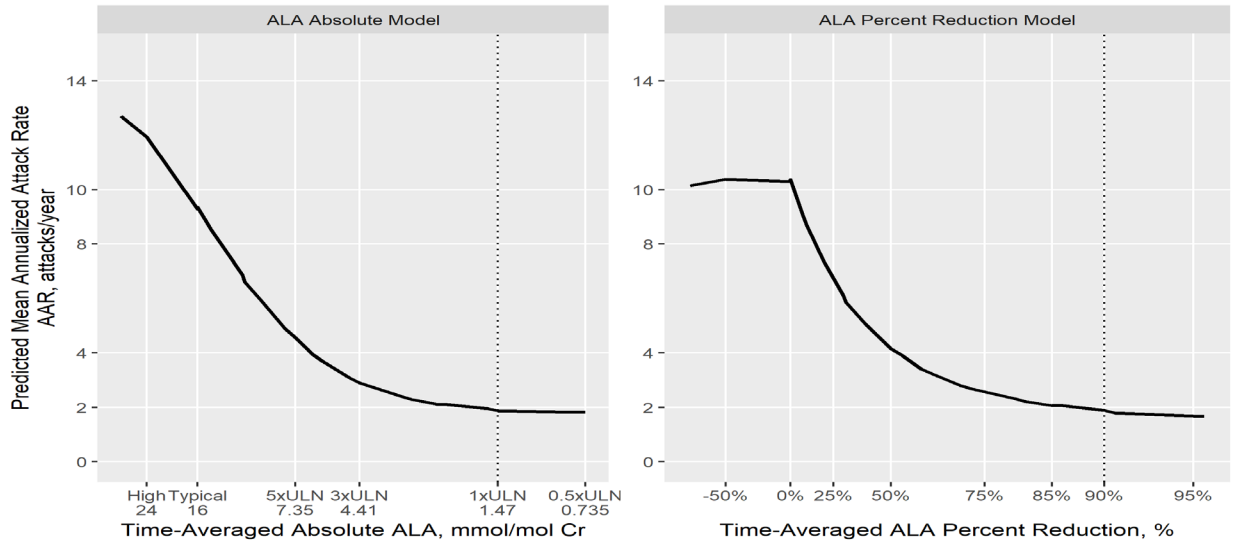
Figure 9. Steady-State Dose-Response Relationship for Urinary ALA After Once Monthly Doses of Givosiran



Abbreviations: AHP=acute hepatic porphyria; ALA=aminolevulinic acid; PI=prediction interval; ULN=upper limit of normal.
Note: Red circles=observed data at Month 3; blue diamonds=observed data at Month 6.

Reduction in these PD biomarkers was also shown to correlate with the reduction in annualized attack rate (AAR) in the clinical studies as well as ALA/AAR modelling (**Figure 10).**

Figure 10. Model-predicted AAR from ALA Absolute Model and ALA Percent Reduction Model in AHP Patients



Abbreviations: AHP=acute hepatic porphyria; AAR=annualized attack rate; ALA= aminolevulinic acid; Cr=creatinine; ULN=upper limit of normal.
Note: Predicted AAR for the median risk population are shown

Secondary pharmacology

No dedicated QT study was performed.

Immunogenicity

Across the 4 clinical studies, there was only 1 case of treatment-induced ADA in 131 subjects (AHP patients + CHE subjects) who received givosiran.

2.4.4. Discussion on clinical pharmacology

The pathophysiology of AHP is thought to be primarily attributed to the accumulation of toxic intermediate metabolites in heme synthesis, ALA and PBG. Givosiran aims to reduce the levels of these toxic metabolites by silencing the ALAS1 mRNA in the liver that is responsible for their synthesis. This is expected to prevent or reduce the occurrence of serious neurovisceral attacks as well as chronic symptoms and disability in patients with AHP. The choice of two main PD biomarkers in the givosiran clinical development program, urinary ALA and PBG, is therefore acceptable.

Givosiran is designed to preferentially distribute to the liver by ASGPR-mediated uptake. This results in a short plasma half-life of approximately 6 hours after subcutaneous dosing with plasma concentrations declining to the lower limit of quantitation (LLOQ) within 24 hours post-dose. This contrasts with the sustained reductions in ALA and PBG in urine for months. Hence, the long duration of PD effect does not directly correlate with the plasma concentrations. This finding is in line with the non-clinical data that has been used to support the 2.5 mg/kg once monthly dosing regimen and demonstrated that the pharmacokinetics of givosiran in the target organ (liver) drives the long duration of PD effect

Due to the increase in the liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in 7 out of 48 patients on 2.5 mg/kg givosiran dose observed in the DB period of the pivotal study 003 (see Section 2.5) a lower 1.25 mg/kg was introduced in the OLE period of this study. However, the study design, not randomized dose assignment and the limited number of patients in every group does not allow a proper assessment of efficacy and safety of this dose. There seems to be no correlation between plasma exposure of givosiran and ALT elevations. However, the number of patients in the dose-finding studies was very small and does not allow to draw firm conclusions. From the data presented, a lower efficacy of this dose may be expected.

In addition to the clinical studies, the adequacy of the selected 2.5 mg/kg once monthly dosing regimen was further supported by the population PK/PD modelling and simulations using data from Studies 001, 002, and 003. In general, the model supported that the 2.5 mg/kg dose once monthly results in near maximum PD/clinical effects (for both dose-ALA and dose-AAR).

It is proposed to dose givosiran as mg/kg. Body weight, renal function, dose and race (East Asian) were covariates in the popPK model, however, the differences were <30% compared to typical patient. No clinically relevant effect of age, sex, race, or body weight on the PK and PD of givosiran was observed or predicted by the PK/PD modelling. Similarly, PKPD was comparable across BMI ranges (<18.5, 18.5-25, >25 kg/m^2). Therefore, dosing based on body weight is agreed. No dose adjustment for adolescents >12 years old or elderly is considered necessary.

Given the targeted delivery and uptake of givosiran to the liver via the ASGPR receptor (the expression of which in the cardiac tissue is negligible), as well as its physicochemical properties as a siRNA compound, it is not expected that givosiran might affect QT interval. Moreover, the data from the pre-clinical and clinical studies do not indicate any clinically relevant changes in QTc interval. No dedicated studies have been performed by the applicant to investigate pharmacodynamic interactions of givosiran with other medicinal products or substances. However, given the mechanism of action, no such interactions are expected. Hemin infusions lower ALA levels in blood and, therefore, have an effect on the ALA lowering by givosiran. Hemin was included as covariate in the population PKPD model and in the ALA-AAR analysis, urinary ALA data sampled within 3 days of hemin co-administration were excluded from analysis. Overall the effects of hemin are adequately accounted for and are not expected to have an impact on clinical efficacy or safety of the product.

An *in vivo* interaction study showed that givosiran moderately reduced the activity of CYP1A2 and CYP2D6, weakly reduced the activity of CYP3A4 and CYP2C19, but had no impact on activity of CYP2C9. This is probably due to reduction of de novo heme synthesis in the liver as result of down regulation of ALAS1. The Applicant presented the possible impact of givosiran on use of concomitant medications in management of acute hepatic porphyria. Caution is recommended in the SmPC and dose adjustments may be considered when administering medicinal products that are substrates of CYP2D6 or CYP1A2 while on treatment with givosiran.

2.4.5. Conclusions on clinical pharmacology

Pharmacokinetics of givosiran in the target organ (liver) drives the long duration of PD effect. Givosiran was causes a rapid dose-dependent decrease in urinary ALAS1 mRNA levels, ALA and PGB levels. The selected 2.5 mg/kg once monthly dosing regimen was chosen based on the exploratory studies with CHE and AHP patients and is further supported by the population PK/PD modelling and simulations.

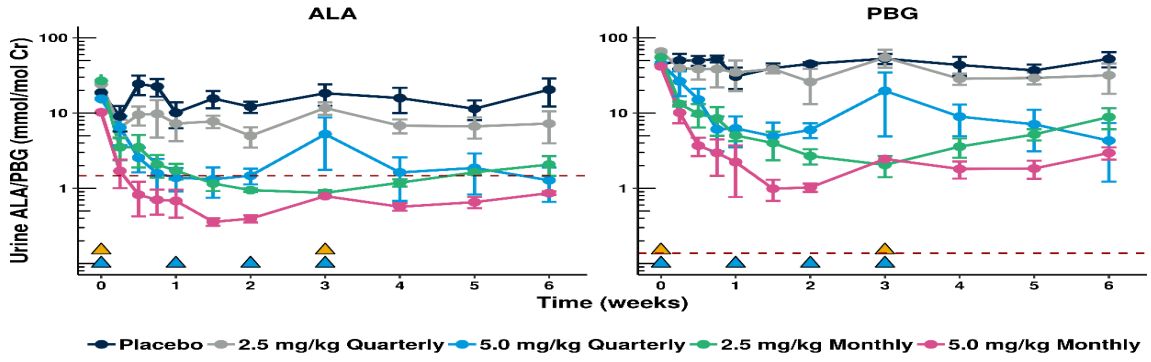
2.5. Clinical efficacy

2.5.1. Dose response studies

The selection of the dose and dosing frequency was supported by a Phase 1 single, placebo-controlled multiple-dose Study 001 in CHE subjects (Study 001a and b) and AIP patients (Study 001c) and an open-label extension long-term dosing study in AIP patients (Study 002).

In Study 001, CHE subjects givosiran caused a rapid dose-dependent decrease in urinary ALAS1 mRNA levels, ALA and PGB levels. The lowering effect, however, did not reach a plateau over the tested range (up to 2.5mg/kg). Due to this reason, as well as the fact that AIP patients have higher baseline levels of ALA and PBG compared to CHE subjects, higher doses were chosen for further studies with AIP patients: 2.5 mg/kg and 5 mg/kg every month or every 3 months. In AIP patients, monthly dosing interval was shown to be superior to the quarterly dosing interval in reducing urine ALA and PBG levels, as well as reducing AAR and hemin use. The 5 mg/kg dose did not seem to result in a much larger reduction in ALAS1 mRNA compared to the 2.5 mg/kg dose (**Figure 11**).

Figure 11. Study 001C: Mean (±SEM) Urinary ALA and PBG Levels After Once Monthly and Once Quarterly Doses of Givosiran in AIP Patients



Abbreviations: AIP=acute intermittent porphyria; ALA=aminolevulinic acid; Cr=creatinine; PBG=porphobilinogen; SEM=standard error of the mean.
Note: Yellow triangles denote time of once quarterly doses, blue triangles denote time of once monthly doses. Dashed red line denotes the ULN for ALA and PBG

Reduction in ALA and PBG also seems to be comparable between the two groups. However, the sample size in every group was very small (3 and 2 patients in 2.5 and 5 mg/kg monthly dose groups respectively).

The optimal dose established in the Study 001 was confirmed in the OLE Study 002, as well as pivotal efficacy and safety Study 003. These studies indicate the sustained suppression of ALA and PBG levels after givosiran treatment at the 2.5 mg/kg once monthly dose for a long period of time (up to 35 months in the Study 002).

Adequacy of the selected 2.5 mg/kg once monthly dosing regimen was further supported by the population PK/PD modelling and simulations using data from Studies 001, 002, and 003. In general, the model supported that the 2.5 mg/kg dose once monthly seems to be near maximum PD/clinical effects for both dose-ALA and dose-AAR. Based on the simulations described, it is, therefore, expected that the lower dose of 1.25 mg/kg will result in a lower ALA and AAR reductions. PKPD modelling also supported the once monthly dosing interval; a quarterly dosing interval was predicted to result in less reduction in ALA and AAR. This is in agreement with less reduction in ALA observed in study 001C in AIP patients.

No clinically relevant effect of age, sex, race, or body weight on the PK and PD of givosiran was observed in the clinical studies or predicted in the pop PK/PD model. No dose adjustment for adolescents >12 years old or elderly are considered needed.

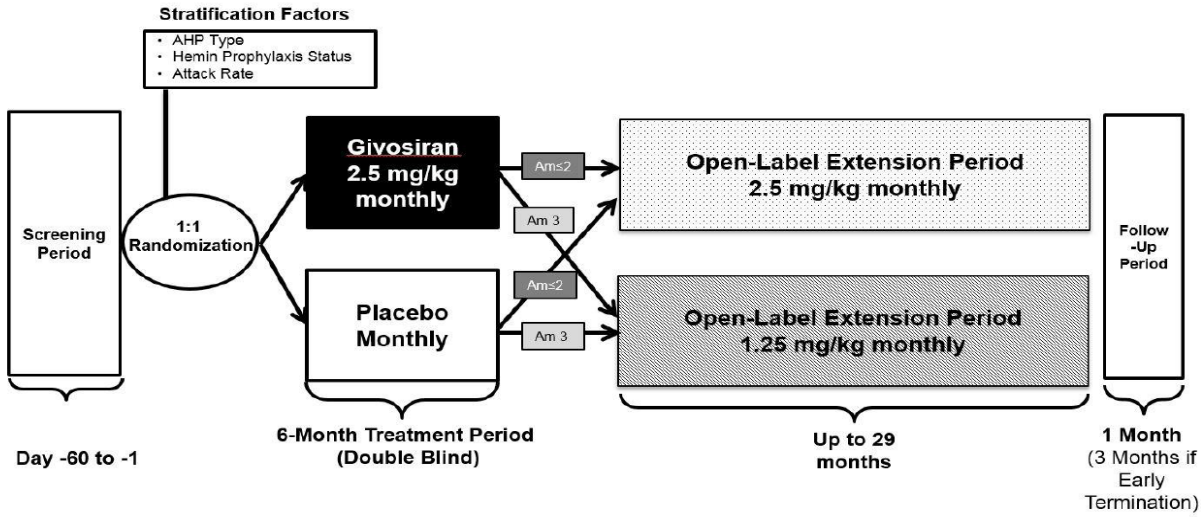
2.5.2. Main study

ALN-AS1-003 (ENVISION): A Phase 3 randomized, double-blind, placebo-controlled, multi-centre study with an open-label extension to evaluate the efficacy and safety of givosiran in patients with acute hepatic porphyrias.

Methods

Figure 12 provides a schematic of the overall study design.

Figure 12. ALN-AS1-003 study schema



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

Study Participants

Inclusion criteria (selection):

1. Age ≥12 years

2. Documented diagnosis of AIP, HCP, VP, or ADP based on clinical features (e.g. acute attacks of abdominal, back, chest, extremities, and/or limb pain), at least 1 documented urinary or plasma PBG or ALA value ≥4×ULN within the past year prior to or during screening, AND one of the following:

- Documented genetic evidence of mutation in a porphyria-related gene, defined as ANY of the following:
  - AIP: mutation in the HMBS gene (also referred to as the PBGD gene)
  - HCP: mutation in the CPOX gene
  - VP: mutation in the PPOX gene
  - ADP: mutation in the ALAD homozygous or compound heterozygous genes
- OR if the results of a patient's genetic testing did not identify a mutation in a porphyria-related gene (<5% of cases), a patient may have been eligible for the study if they had both clinical features and diagnostic biochemical criteria consistent with AHP

3.Had 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

4. Willing to discontinue and/or not initiate use of prophylactic hemin at the time of screening and for the duration of the study 5. Had adequate venous access for study sample collection as judged by the Investigator

Exclusion criteria (selection):

1. Any of the following laboratory parameter assessments at screening:
   - ALT >2×ULN
   - Total bilirubin (TBL) >1.5×ULN. Patients with elevated TBL that is secondary to documented Gilbert's syndrome were eligible if the TBL was <2xULN
   - International normalized ratio (INR) >1.5 (patients on an anti-coagulant [eg, warfarin] with an INR<3.5 are allowed
2. Estimated glomerular filtration rate (eGFR) < 30mL/min/1.73 m^2 using the Modification of Diet in Renal Disease (MDRD) formula
3. On an active liver transplantation waiting list or anticipated to undergo liver transplantation during the blinded study treatment period
4. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or to GalNAc
5. Known active HIV infection or evidence of current or chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection
6. Females who were pregnant, breast-feeding, or planning to become pregnant during the study
7. History of recurrent pancreatitis, or acute pancreatitis with disease activity within the past 12 months prior to screening
8. History of serious infection within 1 month prior to screening.

9. 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 had been successfully treated.

Treatments

Eligible patients were randomized to receive either givosiran (2.5 mg/kg dose) or placebo (1:1) once monthly for up to 6 months. In light of liver transaminase elevations observed in some patients during this study, a lower givosiran dose of 1.25 mg/kg administered once monthly (QM) was introduced as a down-titration dose in patients who had study drug withheld due to transaminase elevations per protocol-specified dosing rules with protocol Amendment 2.

After completion of the 6-month DB Period, patients were given the option of continuing into the OLE period and receiving treatment with givosiran for up to 29 months. Patients who enrolled in the OLE before protocol amendment 3 was implemented received 2.5 mg/kg givosiran once monthly. Patients who enrolled in the OLE after implementation of protocol amendment 3 received 1.25 mg/kg givosiran once monthly. Patients who were assigned to 1.25 mg/kg were permitted to have givosiran increased after month 13 to 2.5 mg/kg if they had inadequate disease control, as pre-specified in the study protocol.

Use of hemin for the treatment of acute or ongoing porphyria attacks was allowed during the study and was recorded as a concomitant medication in the eCRF. Analgesic medications, including opioids (synthetic and non-synthetic substances [narcotics]) or non-opioids, such as non-steroid anti-inflammatory drugs (NSAIDs), acetaminophen, or neuropathy medications (e.g., anti-depressants and anti-seizure medications), were permitted for the management of porphyria and for porphyria attacks, based on clinical judgment.

Objectives

The primary objective was to assess the effect of givosiran versus placebo on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in AIP patients.

Secondary objectives (selection):

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

• Evaluate the effects of givosiran, compared to placebo, on hemin usage in patients with AIP

• Evaluate the effects of givosiran, compared to placebo, on the rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with any AHP

Outcomes/endpoints

Primary

Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6-month DB period (porphyria attack composite endpoint in AIP patients)

Secondary

- AAR in AHP patients

- Levels of urinary ALA and PBG in AIP patients
- Annualized days of hemin use in AIP patients
- Patient reported outcomes in AIP patients (physical component score (PCS) of the short form 12 health survey (SF-12), pain, nausea, fatigue)

Exploratory (selection)

- Rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home (porphyria attack composite endpoint in AIP/AHP patients)
- Rate of all porphyria attacks
- Rate of administered hemin doses
- Daily worst pain, daily worst nausea, and daily worst fatigue scores over 12 months
- PCS of the SF-12
- PGIC
- Analgesic usage (opioid and non-opioid)

Sample size

The planned total enrolment for the study was approximately 74 patients, including approximately 70 AIP patients. Seventy patients would have yielded at least 90% power to detect a 45% reduction in the AAR of the porphyria attack composite endpoint at a 2-sided 5% significance level assuming a mean AAR of 8, a standard deviation (SD) of 5 in the placebo arm (a mean of 4 and a SD of 2.9 for 6 months to preserve the over-dispersion of 3.8), and a mean AAR of 4.4 with SD of 3 in the givosiran arm (a mean of 2.2 and a SD of 1.8 for 6 months to preserve the over-dispersion of 4.2), using a negative binomial model. This study design still had at least 80% power even if the dropout rate was as high as 15% under the same assumptions.

Randomisation

Subjects were randomised in a 1:1 ratio to 2.5 mg/kg givosiran or placebo SC injection QM for 6 months. Patients were stratified based on their disease severity (use of hemin prophylaxis regimen at the time of screening and by each patient's historical AAR) and AHP type. As very few non-AIP patients were anticipated to be enrolled in the study, no additional stratification factors were considered for these patients.

Allocations to the two doses (1.25 and 2.5 mg/kg) in the OLE period were not randomised.

Blinding (masking)

The main 6-months study period was double-blinded.

Statistical methods

The primary analysis of the primary endpoint was performed in AIP patients in the Full Analysis Set using a negative binomial regression model to compare AAR of composite attacks between treatment arms, including fixed effects of the treatment arms and the stratification factors and the logarithm of the amount of time as an offset variable.

Sensitivity analyses were performed to evaluate the robustness of the primary analysis from the following aspects: impact of potential under-counting of attacks; impact of potential under- or over-counting of attacks due to the 1-day window; per protocol analysis set; considering composite attacks as recurrent events.

Secondary endpoints of ALA, PBG levels and PCS of SF-12 score in AIP patients were analysed using a mixed model for repeated measures (MMRM) model. The annualized days of hemin use in AIP patients and AAR of AHP were analysed using a negative binomial regression model similar to the one used for the primary endpoint. For daily worst scores in pain, nausea and fatigue, the area under the curve (AUC) of change from baseline and average change from baseline over 6 months in weekly mean scores in AIP patients were analysed using an analysis of covariance (ANCOVA) model.

An unblinded interim analysis will be conducted when approximately 30 AIP patients have completed at least 3 months of the treatment period. The endpoint for this interim analysis is the ALA level at 3 months. Stopping for efficacy or futility is not planned in this study.

For the final analyses of the 6 month-double blind primary treatment period, a significance level of 0.049 will be used to test the efficacy endpoints, reflecting a penalty of 0.001 for the unblinded interim analysis. A fixed-sequence testing strategy for the primary and secondary endpoints will be implemented to control the overall type I error rate.

After database lock, it was observed that the data for ALA, PBG, pain, and nausea violated the assumptions of normality for the MMRM/ANCOVA models based on Q-Q plots and the Shapiro-Wilk test of normality. Therefore, a non-parametric Wilcoxon test was conducted to re-analyse these endpoints.

Results

Participant flow

All patients (100%) completed the DB period of the study. Only one patient discontinued the treatment due to elevation in the liver enzymes (>8xULN). This patient completed the 6-month DB period and the 6-month Visit; however, the patient then withdrew from the study after the 6-month Visit and did not enter the OLE period. A total of 93 patients (98.9%) continued into the OLE period, where one more patient discontinued the treatment due to pregnancy and three patients discontinued because they no longer wanted to participate in the study.

Recruitment

Study Start: 07 December 2017

Interim data cut-off: 31 January 2019 (open label extension phase is ongoing)

Conduct of the study

A total of 19 major protocol deviations were reported among 6 patients. These included:

• The syringes were not masked with the blinding strip prior to study drug administration during the 6-month DB period (4 patients with a total of 17 instances at 1 site over a period of 6 months and the site was retrained when this deviation was discovered)

• One patient did not meet an inclusion criterion and was enrolled in the study (patient did not experience 2 porphyria attacks requiring hospitalization, urgent healthcare visit or treatment with IV hemin at home within 6 months prior to screening) (1 patient with 1 instance).

• One patient did not meet an inclusion criterion and was enrolled in the study (willing to comply with the contraceptive requirements during the study period) (1 patient with 1 instance)

Only the patients not meeting the minimum threshold of porphyria attacks was excluded from the Per Protocol Analysis Set as the other major protocol deviations were considered to not have an effect on the interpretation of efficacy results. In addition, none of the protocol deviations were considered to have an effect on the safety results.

Three major global protocol amendments took place during the study:

Amendment 1 (4 May 2018), was issued as part of the response to a single case of anaphylactic reaction reported in Study ALN-AS1-002 and provided guidance on monitoring, diagnosing and management of potential anaphylactic reactions.

Amendment 2 (26 July 2018), was generated in response to liver transaminase elevations observed during the study and implemented a standard hepatic assessment panel if patients develop significant ALT elevation and provided 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.

Amendment 3 (21 September 2018), in light of liver transaminase elevations observed in the study, a lower givosiran dose of 1.25 mg/kg once QM was introduced in Amendment 2 as a re-challenge dose for patients who resume dosing after resolution of liver transaminase elevations. In order to generate additional data at this dose level, evaluation of the 1.25 mg/kg once QM dose was proposed for patients crossing over to the OLE period under this amendment, after their completion of the 6-month DB period.

Baseline data

Demographics and disease characteristics of the patients included in the double-blind phase of the study are summarised in **Tables 18** and **19**.

Table 18. Demographics for the 6-month DB Period, study ALN-AS1-003, AHP and AIP Patients (Safety Analysis Set)

Parameter	AHP Patients			AIP Patients		
	Placebo (N=46)	Givosiran (N=48)	Overall (N=94)	Placebo (N=43)	Givosiran (N=46)	Overall (N=89)
Age at Screening (years)						
Mean (SD)	37.4 (10.5)	40.1 (12.1)	38.8 (11.4)	37.3 (10.5)	40.7 (12.0)	39.0 (11.4)
Min, Max	20, 60	19, 65	19, 65	20, 60	19, 65	19, 65
Age category in years, n (%)						
12 to <18	0	0	0	0	0	0
18 to 64	46 (100.0)	47 (97.9)	93 (98.9)	43 (100.0)	45 (97.8)	88 (98.9)
≥65	0	1 (2.1)	1 (1.1)	0	1 (2.2)	1 (1.1)
Gender, n (%)						
Male	5 (10.9)	5 (10.4)	10 (10.6)	4 (9.3)	5 (10.9)	9 (10.1)
Female	41 (89.1)	43 (89.6)	84 (89.4)	39 (90.7)	41 (89.1)	80 (89.9)
Body Weight (kg)						
Mean (SD)	67.88 (16.82)	65.85 (15.63)	66.84 (16.17)	68.50 (16.69)	65.71 (15.91)	67.06 (16.26)
Min, Max	41.5, 115.7	39.5, 131.3	39.5, 131.3	41.5, 115.7	39.5, 131.3	39.5, 131.3
BMI (kg/m^2)						
Mean (SD)	25.49 (6.38)	24.31 (5.15)	24.89 (5.78)	25.66 (6.34)	24.27 (5.24)	24.94 (5.80)
Min, Max	16.6, 49.7	16.4, 44.9	16.4, 49.7	17.8, 49.7	16.4, 44.9	16.4, 49.7
Race, n (%)						
White	34 (73.9)	39 (81.3)	73 (77.7)	33 (76.7)	37 (80.4)	70 (78.7)
Black or African American	1 (2.2)	0	1 (1.1)	0	0	0
Asian	7 (15.2)	8 (16.7)	15 (16.0)	6 (14.0)	8 (17.4)	14 (15.7)
Ethnicity, n (%)						
Not Hispanic or Latino	42 (91.3)	42 (87.5)	84 (89.4)	40 (93.0)	40 (87.0)	80 (89.9)
Not reported	1 (2.2)	0	1 (1.1)	1 (2.3)	0	1 (1.1)
Region, n (%)						
North America[a]	18 (39.1)	16 (33.3)	34 (36.2)	17 (39.5)	16 (34.8)	33 (37.1)
Europe	19 (41.3)	23 (47.9)	42 (44.7)	18 (41.9)	22 (47.8)	40 (44.9)
Other (Asia, Australia, Mexico)	9 (19.6)	9 (18.8)	18 (19.1)	8 (18.6)	8 (17.4)	16 (18.0)
Min, Max	0, 46	4, 34	0, 46	0, 46	4, 34	0, 46

Table 19. Demographics and Baseline Disease Characteristics, study ALN-AS1-003; AHP Patients (All Givosiran Treated Set)

	Placebo/Givosiran			Givosiran/Givosiran		
	Pbo/ Givo 2.5 n=29	**Pbo/ Givo 1.25 n=17**	**All Pbo/ Givo n=46**	**Givo 2.5/ Givo 2.5 n=27**	**Givo 2.5/ Givo 1.25 n=20**	**All Givo/ Givo n=48**
Age at Screening (yrs), Median (min, max)	38.0 (20, 60)	35.0 (20, 57)	36.0 (20, 60)	44.0 (23, 65)	37.5 (19, 58)	42.0 (19, 65)
Female, n (%)	26 (89.7)	15 (88.2)	41 (89.1)	24 (88.9)	18 (90.0)	43 (89.6)
Years Since Diagnosis, Median (min, max)	8.31 (0.2, 30.3)	2.39 (0.1, 38.5)	6.46 (0.1, 38.5)	9.10 (0.4, 43.3)	5.30 (0.2, 31.3)	6.98 (0.2, 43.3)
Prior Hemin Prophylaxis Regimen, n (%)	14 (48.3)	4 (23.5)	18 (39.1)	13 (48.1)	7 (35.0)	20 (41.7)
Composite Porphyria Attacks[a] in 6 Months Prior to Randomization, Median (min, max)	4.0 (0, 23)	3.0 (1, 19)	3.5 (0, 23)	5.0 (2, 17)	4.0 (2, 17)	4.0 (2, 17)
Historical AAR, Median (min, max)	8.0 (0, 46)	6.0 (2, 38)	7.0 (0, 46)	10.0 (4, 34)	8.0 (4, 34)	8.0 (4, 34)
Prior Chronic Symptoms, n (%)	14 (48.3)	12 (70.6)	26 (56.5)	12 (44.4)	11 (55.0)	23 (47.9)
Prior Chronic Opioid Use, n (%)	7 (24.1)	6 (35.3)	13 (28.3)	8 (29.6)	6 (30.0)	14 (29.2)
Transaminases increased[a]	11 (37.9)	7 (41.2)	18 (39.1)	11 (40.7)	5 (25.0)	17 (35.4)
Renal Failure and Impairment, HLT (including CKD; renal failure, impairment or injury, and AKI)	11 (37.9)	3 (17.6)	14 (30.4)	6 (22.2)	3 (15.0)	10 (20.8)
CKD	8 (27.6)	1 (5.9)	9 (19.6)	5 (18.5)	3 (15.0)	8 (16.7)
Diagnosed with Neuropathy	9 (31.0)	7 (41.2)	16 (34.8)	12 (44.4)	8 (40.0)	20 (41.7)
Diagnosed with Iron Overload	10 (34.5)	5 (29.4)	15 (32.6)	10 (37.0)	6 (30.0)	16 (33.3)
Central Venous Catheterization	21 (72.4)	11 (64.7)	32 (69.6)	17 (63.0)	17 (85.0)	35 (72.9)
Complications Related to Central Venous Access	11 (37.9)	5 (29.4)	16 (34.8)	10 (37.0)	5 (25.0)	15 (31.3)
Hypertension	7 (24.1)	4 (23.5)	11 (23.9)	9 (33.3)	5 (25.0)	14 (29.2)

Numbers analysed

The populations (analysis sets) for the 6-month DB period and the OLE period were defined as follows:

• Full Analysis Set (FAS): All randomized patients who received at least 1 dose of study drug. Patients are grouped by their randomly assigned treatment group (ie, as randomized).

• AIP patients in the Full Analysis Set (FASAIP): All randomized AIP patients (with identified mutation in the HMBS gene) who received at least 1 dose of study drug. Patients are grouped by their randomly assigned treatment group (ie, as randomized).

• Per Protocol Analysis Set (PPS): All randomized AIP patients (with identified mutation in the HMBS gene) who received at least 4 doses (>60%) of study drug during the 6-month DB period, were followed for collection of attack data through 6 months (≥162 days) and did not experience major protocol deviations that may impact the primary efficacy results (eg, not meeting the key inclusion/exclusion criteria). Patients are analyzed according to their randomly assigned treatment group.

•Safety Analysis Set: All patients who received at least 1 dose of study drug, grouped according to the treatment actually received. Patients who received at least 1 dose of givosiran during the 6-month DB period are included in the givosiran arm.

• PK Analysis Set: All patients who received at least 1 dose of study drug and have evaluable PK data contributing to the estimation of PK parameter.

• PD Analysis Set: All patients who received at least 1 dose of study drug and who have at least 1 post-dose urine sample for the determination of ALA or PBG are included in the PD analyses.

• All Givosiran Treated Set: All patients who received at least 1 dose of givosiran, including patients who took givosiran during the 6-month DB period and patients who first took placebo during the 6-month DB period and switched to givosiran during the OLE period.

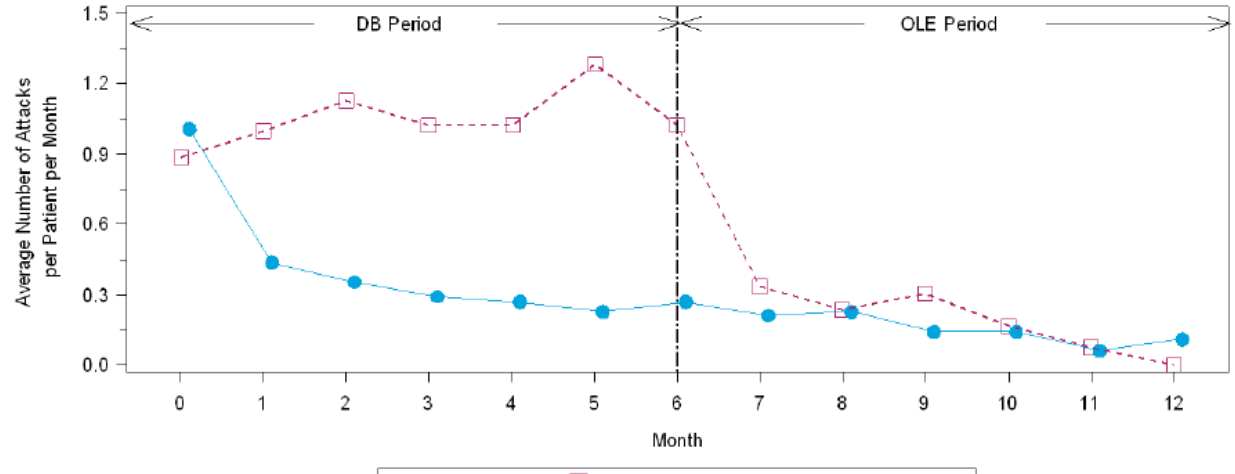
Outcomes and estimation

Primary endpoint

Annualized Rate of Porphyria Attack Composite Endpoint

The primary efficacy outcome of of annualized rate of porphyria attack composite endpoint is shown in **Figure 13**, demonstrating that 2.5 mg/kg once monthly givosiran treatment led to statistically significant reduction of 74% in the AAR of composite attacks compared to placebo in AIP patients (rate ratio=0.26, p<0.0001).

Figure 13. Average Number of Attacks per Patient Per Month During the DB and OLE Periods; Porphyria Attack Composite Endpoint in AHP patients (FAS), study ALN-AS1-003



No. of Patients:													
Placebo	46	46	46	46	46	46	46	45	38	26	18	13	7
Givosiran	48	48	48	48	48	48	48	47	35	28	21	16	9

Abbreviations: AHP=acute hepatic porphyria; DB=double-blind; FASAIP=AIP patients in the full analysis set; IV=intravenous; OLE=open-label extension.
Note: Month 0 represents the average rate per month from the 6 months prior to randomization, and the estimate was calculated as total number of attacks/total duration in months. Month 1 and beyond were categorized relative to the first dose of study drug, and the estimate was calculated as total number of attacks/total number of patients reached that month. One month=28 days was used in categorization.

The results of all sensitivity analyses were consistent with the primary analysis. The proportion of attacks with median pain score >7 was lower for givosiran patients (21.1%) compared to placebo patients (32.0%).

The effect of givosiran was sustained in the OLE study: the composite AAR remained reduced for patients continuing on givosiran treatment and got reduced for patients switching from placebo to givosiran. Specifically, placebo patients crossing to the 2.5 mg/kg QM givosiran dose during the OLE period demonstrated a trend towards a larger reduction in composite AAR: 88% reduction in AAR for placebo patients who crossed over to the 2.5 mg/kg QM givosiran dose in the OLE and 76% reduction for placebo patients crossing over to the 1.25 mg/kg QM dose group through Month 12 based on intra-patient comparisons.

Secondary endpoints

Urinary ALA and PBG Levels

Givosiran produced a substantial decrease in these toxic metabolites in both DB and OLE period: the median reduction for ALA was 86 and 84% in the DB and OLE period respectively and the median reduction for PBG was 91 and 78% in the DB and OLE period respectively. Similar results were seen in the overall AHP population. The 1.25 mg/kg givosiran dose was shown to produce somewhat lesser reduction in ALA and PBG compared to the 2.5 mg/kg dose.

Hemin Use

Givosiran treatment led to a similar decrease in hemin use: 77% decrease in annualized days of hemin use with around 50% of patients having no need to use hemin at all. In the placebo group, 23.3% of patients had 0 days of hemin use. Continued treatment with givosiran in the 6-month DB and OLE period (givosiran/givosiran patients) led to a maintenance of the effect observed in the 6-month DB period. Placebo group patients who crossed over to the 2.5 mg/kg QM givosiran dose in the OLE had a greater reduction in hemin use (>99% reduction), compared with the 1.25 mg/kg QM dose (54%) through Month 12, based on intra-patient comparisons.

Pain Numerical Rating Score

The study was not enriched for the baseline levels of pain. Therefore, the patients enrolled had mostly mild to moderate pain levels (with patients in the givosiran group having a somewhat lower score at baseline). Givosiran was shown to significantly reduce the pain levels and the treatment separation was greater for the patients with a baseline pain score ≥ 2, with no difference between treatments for patients with a baseline pain level <2.

The treatment with givosiran led to a statistically significant difference in the AUC of change from baseline in weekly mean score of daily worst pain and average change from baseline in weekly mean score in AIP patients compared with placebo (p=0.0455 and 0.0493 respectively as per post-hoc Wilcoxon analysis). The absolute difference change in the median pain score between the groups was 0.5.

Patients in the placebo/givosiran group had a median weekly mean pain score of daily worst pain of 3.50 at baseline. Following 6 months of placebo treatment, the median score increased by 0.1 of a point. At Month12 (after 6 months of givosiran treatment), the median score dropped by 0.54 points, similar to the decrease in pain seen in patients who received givosiran initially in the 6-month DB period.

The decreases in pain with givosiran treatment were also reported in the context of lower analgesic use (opioid and non-opioid) during and between attacks.

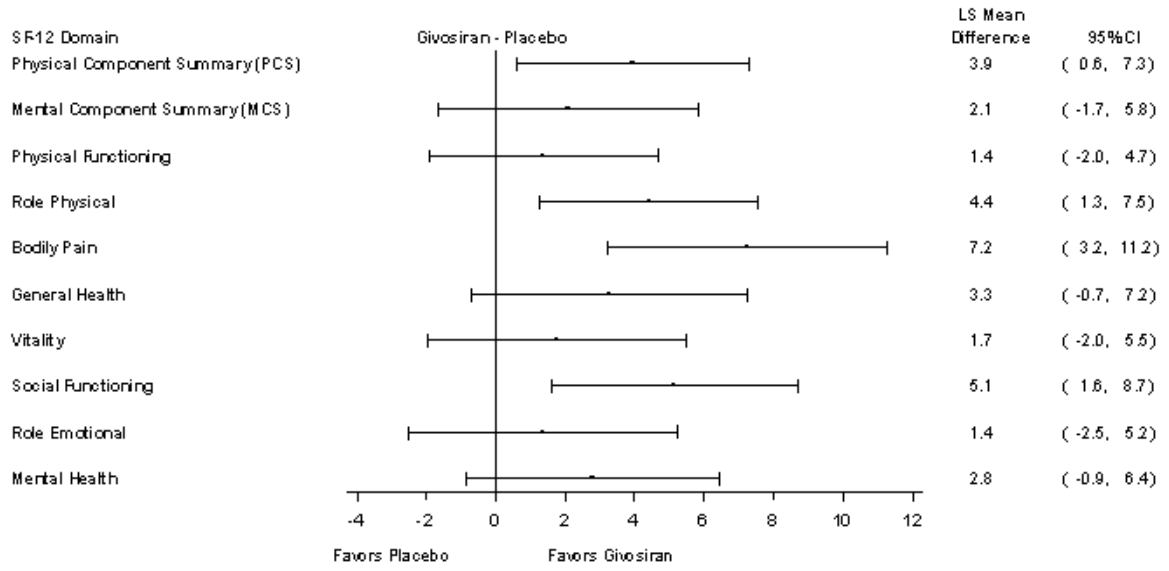
Fatigue and Nausea Numerical Rating Score

Givosiran treatment did not result in a significant change in the daily worst fatigue and daily worst nausea score.

PCS of SF-12

PCS score of SF-12 was shown to be significantly improved in patients on givosiran treatment (**Figure 14**). Placebo crossover patients had similar improvements in PCS scores in the SF-12 domain after 6 months of givosiran treatment in the OLE period as patients in the givosiran group had after 6 months in the DB period. Patients on givosiran 2.5 mg/kg QM treatment continuously through the Month 12 data-cut, demonstrated maintenance of improvement in PCS score in the SF-12 domain at Month 12.

Figure 14: Forest Plot of Change from Baseline to Month 6 in SF-12 Domain Scores; AIP Patients, study ALN-AS1-003



Abbreviations: AIP=Acute intermittent porphyria; CI=confidence interval; FAS_{AIP}=AIP patients in the full analysis set; LS Mean=Least square mean; MCS=mental component summary; PCS=Physical Component Summary; SF-12=the 12-item Short-Form Health Survey version 2.

Exploratory Endpoints

All Porphyria Attacks:

Consistent with the results observed for the porphyria attack composite endpoint, givosiran led to a reduction in the AAR of all attacks compared to placebo for both AIP patients (median AAR of 2.14 for givosiran and 10.74 for placebo; 68.3% reduction) and AHP patients (median AAR of 2.14 for givosiran and 11.74 for placebo; 67.4% reduction)

Analgesic Usage:

Givosiran treatment led to a lower proportion of days with opioid use and nonopioid use over the 6-month DB period compared with placebo. The number of patients with at least 1 analgesic medication during the 6-month DB period was lower in patients treated with givosiran compared with placebo over the 6-month DB period. Similar results were observed in AHP patients

Quality of life

Quality of life and patient experience measures were improved with givosiran compared to placebo.

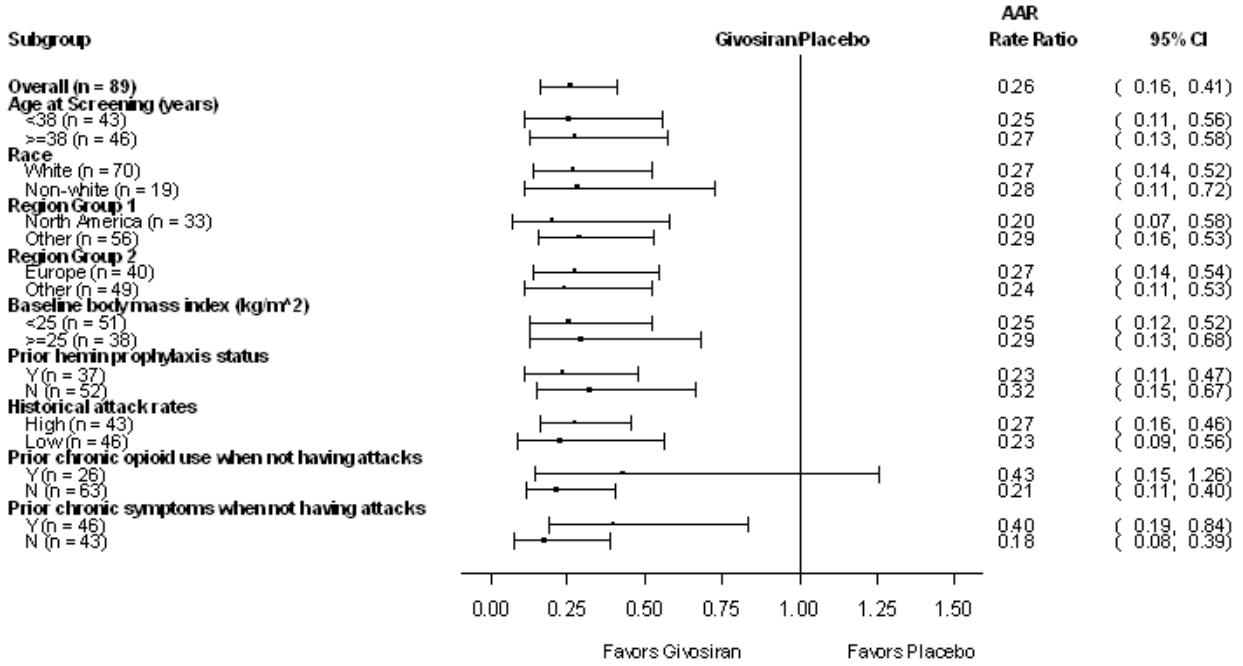
- A larger proportion of givosiran AIP patients rated themselves as "very much improved" or "much improved" since the start of the study at Month 6 compared with placebo (61.1% and 20.0%, respectively), on the PGIC.

The overall patient experience was improved for givosiran compared to placebo, as demonstrated by "much better" ability since before the study on functional impacts, activities of daily living, and treatment satisfaction on the PPEQ in AIP patients

Ancillary analyses

The subgroup analysis performed for the primary endpoint of composite AAR showed consistency in the efficacy of givosiran between different groups. Age, race, region, gender, body mass index (BMI) or medical history of the disease did not have an influence on the givosiran efficacy (**Figure 15**).

Figure 15. Forest Plot of Annualized Rate of Porphyria Attack Composite Endpoint During the 6-Month DB Period; Subgroup Analysis; AIP patients, study ALN-AS1-003



Abbreviations: AIP=Acute intermittent porphyria; CI=Confidence interval; DB=double-blind; FASAIP =AIP Patients in Full Analysis Set; IV=Intravenous.
Rate ratio and corresponding CIs were derived using negative binomial regression model with the logarithm of the follow-up time as an offset variable.
Note: Sex was not included as a subgroup because <10 patients were male.

In addition to reducing the frequency of attacks, givosiran led to a reduction in the severity of composite attacks when they did occur. Severe attacks were defined as attacks accompanied by severe pain (median pain score ≥7 on a 0 to 10 numerical rating scale) (Oldemanger 2013).

The proportion of severe attacks is shown in **Table 20**.

Table 20. Composite Porphyria Attacks with Median Pain Score ⩾7 During the 6-month DB Period; AHP Patients (Full Analysis Set)

	Placebo (N=46)	**Givosiran (N=48)**
Total number of attacks	297	90
Total number of attacks with median pain scores ≥7, n (%)	95 (32.0)	19 (21.1)
Number of patients with at least 1 attack	38	24

	Placebo (N=46)	Givosiran (N=48)
Number of patients with at least 1 attack with median pain score ≥7; n/N (%)	24/38 (63.2)	10/24 (41.7)

Abbreviations: AHP=acute hepatic porphyria; DB=double-blind.
Median pain scores of attacks were calculated based on pain scores collected during each composite attack.
Source: Appendix 1, Study 003, Table 60.1.40.4.

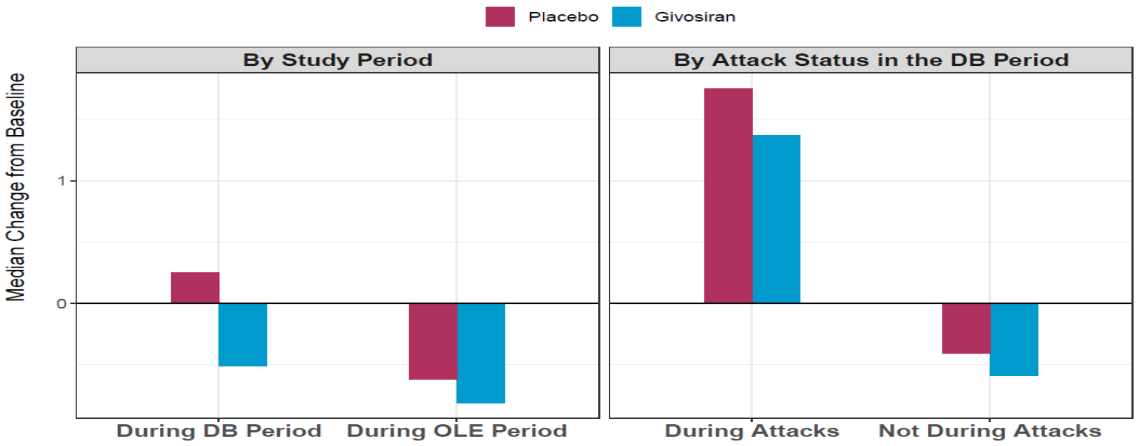
Moreover, even among those patients who experienced at least 1 attack, a lower proportion of givosiran patients (41.7%) compared with placebo patients (63.2%) had a severe attack.

The decrease in severe attacks on givosiran was accompanied by a lower proportion of givosiran patients compared to placebo using analgesics (opioids [75.0% vs 84.2%], IV opioids [37.5% vs 52.6%], and non-opioids [58.3% vs 71.1%]) during composite attacks (refer to Appendix 1, Study 003, Table 60.1.40.2). In addition, while hospitalization for attacks may be determined by local healthcare treatment practices, hospitalization rates may also be reflective of attack severity. Givosiran led to a 43% reduction in attacks requiring hospitalization compared to placebo (refer to Study 003 CSR1 Table 19).

Impact on Pain and Analgesic Use During and Between Attacks

Consistent reduction in pain scores was found with givosiran whether the analyses included data from during or between attacks (also referred to as "not during attacks"). For these analyses, "during attacks" included all Investigator-adjudicated porphyria attacks (the porphyria attack composite endpoint plus attacks at home not treated with hemin), which is the most appropriate way to conduct these analyses given that all attacks during the study, regardless of treatment location or treatment type, were characterized by worsening porphyria symptoms beyond the patient's normal day-to-day variability and required treatment beyond their usual daily management. The median of change from baseline in daily worst pain between attacks (ie, not during attacks) was lower (more improvement) for givosiran than placebo (**Figure 16,** right panel).

Figure 16. Median Change from Baseline in Pain Score by Study Period and All Attack Status in AIP Patients (AIP Patients in Full Analysis Set)

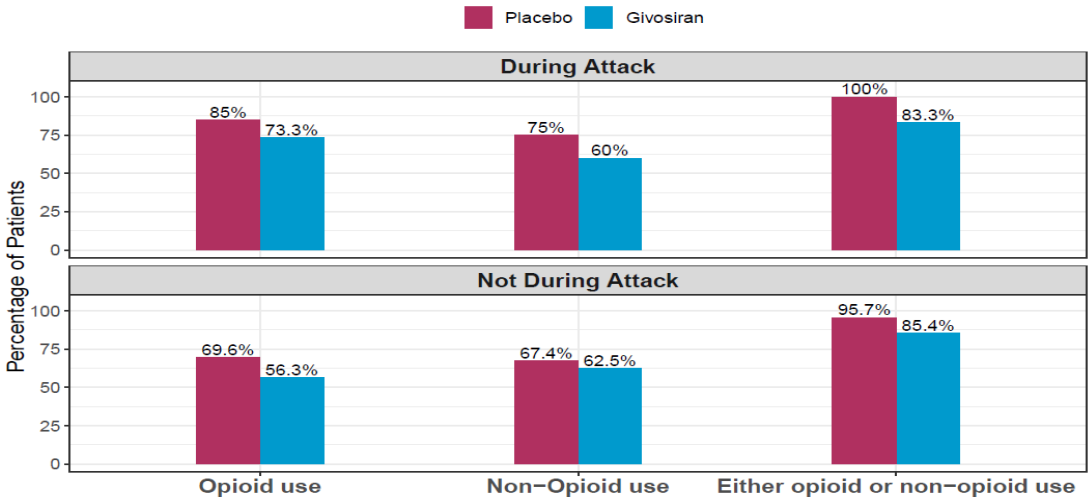


Abbreviations: AIP=acute intermittent porphyria; DB=double-blind; OLE=open-label extension.
Changes <0 indicate improvement.
Placebo patients received placebo during the DB period and crossed over to givosiran during the OLE period.
Note: All Investigator-adjudicated attacks are included.

In addition, on days between attacks, givosiran consistently demonstrated a lower proportion of days with daily pain scores across a range of ≥2 to ≥7 as well as a lower proportion of days with a daily pain score worse than baseline compared to placebo (data not shown).

The reduction in pain with givosiran was not attributable to increased analgesic use as it was accompanied by a lower proportion of patients requiring analgesic use during and between all attacks **(Figure 17**).

Figure 17. Summary of Analgesic Usage by All Attack Status During the 6−month DB Period in AHP Patients (Full Analysis Set)



Abbreviations: AHP=acute hepatic porphyria; DB=double-blind.
Note: Includes all Investigator-adjudicated attacks.

Similar improvements in pain and reduced analgesic use between attacks were observed for composite attacks (data not shown). No significant changes were observed in daily worst fatigue or nausea scores during the 6-month randomized DB period of the study.

Efficacy in non-AIP patients

Acute intermittent porphyria (AIP) is the most common AHP subtype, and therefore 5 non-AIP patients o were included in the study. Efficacy results for these patients is summarized in **Table 21**.

Table 21. Key Efficacy and Safety Results in Non-AIP Patients in Study 003

	Placebo/Givosiran			Givosiran/Givosiran	
	VP	AHP Without Identified Mutation	AHP Without Identified Mutation	VP	HCP
Exposure					
Number of doses of givosiran received	10	2	9	3	14
Total follow-up on givosiran (days)	258	56	233	162	398
Efficacy					
ALA, mmol/mol Cr					
Baseline	2.19	23.10	15.68	21.25	3.31
Month 6	2.17	12.12	17.07	0.62	0.72
Last value	0.59 (Month 15)	3.93 (Month 7)	1.73 (Month 14)	-	0.86 (Month 14)
PBG, mmol/mol Cr					
Baseline	1.51	42.52	15.68	35.26	0.44
Month 6	1.04	32.96	17.07	0.10	0.06
Last value	0.04 (Month 15)	11.45 (Month 7)	3.38 (Month 14)	-	0.06 (Month 14)
Composite AAR					
Historical AAR	6	10	4	6	16
6-month DB period	6.8	21.6	0	0	15.2
During givosiran treatment	2.8	1[(i)]	0	0	11.0
Days of hemin use					
6-month DB period	4	0	0	0	32
During givosiran treatment	4	0	0	-	37

Abbreviations: AAR=annualized attack rate; AE=adverse event; AIP=acute intermittent porphyria; ALA=aminolevulinic acid; Cr=creatinine; DB=double-blind; HCP=hereditary coproporphyria; PBG=porphobilinogen; SAE=serious adverse event; VP=variegate porphyria.
Data presented as of the data cutoff date of 23 July 2019.
Note: One of the patients with AHP without identified mutation discontinued study drug on Day 197 (Day 29 on givosiran) and withdrew from the study on Day 224 (Day 56 on givosiran).
[1)] AAR was calculated for patients who had at least 85 days of follow-up during the OLE period. Patient 405-3003 withdrew from the study before 85 days of follow-up; therefore, number of attacks is presented.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 22. Summary of Efficacy for trial ALN-AS1-003

Title: ENVISION: A Phase 3 Randomized, Double-blind, Placebo-Controlled, Multi-center Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias			
Study identifier	ALN-AS1-003 , EudraCT Number: 2017-002432-17 , IND number: 126094		
Design	A Phase 3 Randomized, Doubleblind, Placebo-Controlled, Multicenter Study with an Open-label Extension to Evaluate the Efficacy and Safety of Givosiran in Patients with Acute Hepatic Porphyrias		
	Duration of main phase:		6 months
	Duration of Run-in phase:		6 months
	Duration of Extension phase:		Up to 29 months
Hypothesis	Superiority		
Treatment groups	Placebo		sodium chloride 0.9% w/v for SC administration, 6 months, 46 AHP patients
	Givosiran		200 mg/mL givosiran sodium for SC administration, 6 months, 48 AHP patients.
Endpoints and definitions	Primary endpoint	Porphyria attack composite endpoint in AIP patients	Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6-month DB period (porphyria attack composite endpoint in AIP patients)
	Secondary	Porphyria attack composite endpoint in AHP patients	Porphyria attack composite endpoint in AHP patients
		Urinary ALA levels at 3 months	Urinary ALA levels in patients with AIP at 3 months
		Urinary ALA levels at 6 months	Urinary ALA levels in patients with AIP at 6 months
		Urinary PBG levels at 6 months	Urinary PBG levels in patients with AIP at 6 months
		Annualized days of hemin use	Evaluated by annualized days of hemin use in patients with AIP over the 6-month DB period
		Porphyria attack composite endpoint in AHP patients	Annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6-month DB period (porphyria attack composite endpoint in AIP patients)
		AUC of change from baseline in daily worst pain	Daily worst pain score as measured by BPI-SF NRS in patients with AIP over the 6-month DB period

		AUC of change from baseline in daily worst fatigue	Daily worst fatigue score as measured by Brief Fatigue Inventory-Short Form (BFISF) NRS in patients with AIP over the 6-month DB period
		AUC of change from baseline in daily worst nausea	Daily worst nausea score as measured by NRS in patients with AIP over the 6-month DB period
		Change from baseline in PCS of SF-12 at 6 months	Change from baseline in the PCS of the SF-12 in patients with AIP at 6 months.
Database lock	31 January 2019		

Primary Analysis

Analysis population	AIP patients in Full Analysis Set (FASAIP): All randomized AIP patients (with identified mutation in the HMBS gene) who received at least 1 dose of study drug. Patients were grouped by their randomly assigned treatment group (ie, as randomized).		
time point	3 or 6 months		
Descriptive statistics and estimate variability	Treatment group	**Placebo**	**Givosiran, 2.5 mg/kg**
	Number of subjects	43	46
	Porphyria attack composite endpoint in AIP patients		
	Mean 95% CI	12.52 9.35, 16.76	3.22 2.25, 4.59
	Urinary ALA levels at 3 months, mmol/mol Cr		
	Mean 95% CI	19.965 17.032, 22.898	1.756 -1.053, 4.566
	Urinary ALA levels at 6 months, mmol/mol Cr		
	Mean 95% CI	23.150 18.089, 28.21	4.013 -0.690, 8.715
	Urinary PBG levels at 6 months, mmol/mol Cr		
	Mean 95% CI	49.110 39.243, 58.976	12.906 3.663, 22.149
	Annualized days of hemin use		
	Mean 95% CI	29.71 18.41, 47.94	6.77 4.20, 10.92
	Porphyria attack composite endpoint in AHP patients		
	Mean 95% CI	12.26 9.22, 16.29	3.35 2.37, 4.74
	AUC of change from baseline in daily worst pain		
	Mean 95% CI	-0.196 -9.468, 9.077	-12.876 -21.776, -3.976
	Median (post-hoc) Q1, Q3	5.286 -23.05, 11.14	-11.514 -29.181, 3.040
	AUC of change from baseline in daily worst fatigue		

	Mean 95% CI	-4.208 -13.534, 5.117	-11.148 -20.100, -2.197
	AUC of change from baseline in daily worst nausea		
	Mean 95% CI	-4.011 -10.878, 2.856	1.481 -5.102, 8.063
	Change from baseline in PCS of SF-12 at 6 months		
	Mean 95% CI	1.431 -0.995, 3.856	5.369 3.046, 7.693
Effect estimate per comparison	**Primary endpoint**		
			Givosiran / Placebo
	Porphyria attack composite endpoint in AIP patients	Rate ratio 95% CI P-value	0.26 0.16, 0.41 p<0.0001
	Secondary endpoint		
			Givosiran - Placebo
	Urinary ALA levels at 3 months	Difference in LS Mean 95% CI P-value	-18.209 -22.260, -14.158 p<0.0001
	Urinary ALA levels at 6 months	Difference in LS Mean 95% CI P-value	-19.137 -26.039, -12.235 p<0.0001
	Urinary PBG levels at 6 months	Difference in LS Mean 95% CI P-value	-36.204 -49.708, -22.699 p<0.0001
			Givosiran / Placebo
	Annualized days of hemin use	Ratio 95% CI P-value	0.23 0.11, 0.45 p<0.0001
	Porphyria attack composite endpoint in AHP patients	Rate ratio 95% CI P-value	0.27 0.17, 0.43 p<0.0001
			Givosiran - Placebo
	AUC of change from baseline in daily worst pain	Difference in Mean 95% CI P-value	-12.680 -25.526, 0.166 0.0530
		Median of treatment difference 95% CI P-value (post-hoc)	-10.067 -22.833, 0.936 0.0455
	AUC of change from baseline in daily worst fatigue	Difference in LS Mean 95% CI P-value	-6.940 -19.837, 5.957 0.270*

	AUC of change from baseline in daily worst nausea	Difference in LS Mean 95% CI P-value	5.492 -4.000, 14.984 0.246*
	Change from baseline in PCS of SF-12 at 6 months	Difference in LS Mean 95% CI P-value	3.939 0.592, 7.285 0.0216*

*not significant

Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

Clinical studies in special populations

The maximum age of the patients included into the givosiran clinical program was 65 (one patient).

The influence of renal or hepatic impairment on givosiran PD was assessed across the studies. Only 5 patients with mild hepatic impairment and none with moderate or severe hepatic impairment were included in the study. Also, 25 patients with mild and 10 patients with moderate renal impairment were enrolled. Even though the results are variable due to the low number of patients in every group, the PD response of givosiran did not seem to be affected.

Supportive studies

Study 001C was a randomized, placebo-controlled study conducted in AIP patients testing four dose regiments: 2.5 mg/kg givosiran once monthly, 2.5 mg/kg givosiran once quarterly, 5 mg/kg givosiran once monthly, and 5 mg/kg givosiran once quarterly.

Study 002 is an ongoing open-label, long-term extension study in AIP patients who have completed Study 001C. After completion of the evaluation period in Study 001, eligible patients from Part C transitioned into this study to receive givosiran treatment for an additional 3 years.

Both studies are small (16-17 patients), but overall support the beneficial effects of givosiran on various clinical parameters, including AAR, ALA & PBG levels, hemin use or quality of life.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The data contributing to the efficacy of givosiran in patients with AHP come from 3 clinical studies: one dose-finding study in the AHP patients (001C), an open-label extension of this study (002) and a single pivotal trial (003), which is acceptable given the rarity of the disease. The pivotal study is a phase 3 randomized double-blind, placebo-controlled multicentre study with an open-label extension to evaluate the efficacy and safety of givosiran in patients with AHP.

Since currently no treatment is available to manage AHP (apart from hemin which is indicated for the treatment of acute porphyria attacks, though not approved to reduce the risk of attacks), the use of placebo as a comparator is supported. The trial duration is considered sufficient to assess the primary endpoint and some secondary endpoints.

The goal of givosiran treatment is to prevent acute porphyria attacks by reducing the plasma levels of toxic metabolites, ALA and PBG, which are thought to be responsible for these attacks and other chronic disease manifestations. Therefore, the choice of a porphyria attack composite endpoint as a primary endpoint and urinary ALA/PBG levels as secondary endpoints is supported. The porphyria attack composite endpoint is a relevant clinical endpoint, which consists of the most common and severe type of attacks (attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home). Hemin infusions are a big burden on its own for the AHP patients and they also carry the risks of morbidities, such as iron overload or infections due to the catheter lines. Therefore, the reduction in need of such infusions is of benefit to the patient and is supported as a secondary endpoint. All other patient reported outcomes (e.g. daily worst pain, nausea, fatigue and quality of life) are also considered of importance since AHP is a chronic disease with manifestations of pain and low quality of life even between the attacks. The short period of time used to collect baseline data for these parameters and the differences in the collection of patient reported outcomes before and during the study, however, brings uncertainties to the data interpretation. The data on the cross-over patients from placebo (6 months data in the DB period) to givosiran (>6 months in the OLE period) can, however, be used as supportive in this case. In general, the objectives and efficacy endpoints of the pivotal and supportive studies are clearly defined and are considered clinically relevant.

The inclusion and exclusion criteria are acceptable. The study population is reflective of the population with AHP but was enriched for the attack rate: only patients with more than 2 attacks in the past 6 months were eligible to enrol in the study. Adolescents >12 years were allowed to enrol in the study. However, due to the rarity of these patients, none was eventually enrolled. Subjects with moderate or severe liver impairment were excluded from the study, which is understandable given the known liver safety toxicity profile for this class of drugs.

The randomization strategy is in general satisfactory. Patients were stratified based on their disease severity (historic attack rate) and AHP type. The characteristics of the patients in the placebo and givosiran groups were well balanced. An interim analysis in the DB of the Study 003 period was planned, but not used due to the fast study enrolment. All patients completed the 6-month DB period of the study and were included in the main analysis set.

The primary endpoint, annualised attack rate, was analysed using a negative binomial regression model. Sensitivity analyses tested the impact of attack data collection, model assumptions and missing data handling. Secondary rate endpoints (days of hemin use and AAR in AHP patients) were analysed using negative binomial regression. Change from baseline in pain, nausea or fatigue was analysed using ANCOVA and change from baseline in ALA, PGB, PCS and SF-12 by MMRM. A fixed-sequence testing strategy for the primary and secondary endpoints is used to control the overall type I error rate. These methods are considered acceptable.

The endpoints ALA, PBG, pain and nausea were re-analysed using a non-parametric Wilcoxon test, after a Shapiro-Wilk test indicated the assumption of normality was violated. Although this is a standard procedure, it was introduced post-hoc. Since the primary analysis models are relatively robust against a deviation, both the parametric and the non-parametric tests are considered of importance.

The selection of the dose tested in the pivotal study (2.5 mg/kg) as well as the monthly dosing interval can be considered reasonably characterized and justified based on the PK/PD data from the dose-finding and supportive studies. Due to the elevations in the liver enzymes observed in some of the patients following givosiran treatment, a lower dose (1.25 mg/kg) was introduced as per Protocol Amendment 2 and 3 as down-titration.

Efficacy data and additional analyses

Treatment with givosiran resulted in a rapid, clinically meaningful and statistically significant decrease (approximately 75%) in the composite AAR in both AIP and AHP populations, as indicated by the analysis of FASAIP and FASAHP. Importantly, 50% of patients in the givosiran group had 0 attacks. The number of non-AIP patients in the clinical development program is very low (5). Only five non-AIP patients received givosiran in the Study 003 and one patient was treated with givosiran in the Compassionate Use Program. One of these patients discontinued the dosing after the 3^{d} dose due to the ALT elevation 9.9 x ULN and another one discontinued the study after the 2^{d} dose because the patient had no wish to participate in the study any longer. Givosiran seems to have a beneficial effect on the ALA/PBG levels, as well as AAR in this patient population as well, even though the data is considered very limited. Nevertheless, extrapolation to all AHP subtypes was considered acceptable by the CHMP based on a common pathophysiology (e.g accumulation of toxic porphyrins ALA and PBG) and the PK/PD and safety data of givosiran.

No adolescents participated in the study. However, similar PD effect is expected between different body weight categories and therefore similar efficacy is expected in this population (please see PD section for more discussion). Therefore, the indication for adolescents is acceptable.

The effects of the proposed 2.5 mg/kg dose have been sufficiently demonstrated. The efficacy and safety data of the lower dose, 1.25 mg/kg, is, however, very limited. The study design, small number of patients in every group, the non-randomized assignment of the patients to two doses in the OLE period, do not allow for an adequate assessment of efficacy and safety of the 1.25 mg/kg dose. This is reflected as a warning in the SmPC. This dose is reserved only for resumption of treatment in patients who had to discontinue givosiran due to clinically relevant transaminase elevations.

The subgroup analysis performed for the primary endpoint of composite AAR showed consistency in the efficacy of givosiran between different groups. Age, race, region, gender, BMI or medical history of the disease did not have an influence on the givosiran efficacy. The mild hepatic impairment or mild to moderate renal impairment does not seem to influence PD of givosiran. The influence of moderate to severe hepatic impairment on givosiran effects was not assessed in the clinical program that brings some uncertainties to the safety of the product in this patient group. An appropriate warning is included in the SmPC indicating that the efficacy and safety of givosiran was not properly studied in the population with moderate or severe hepatic impairment. Additional efficacy and safety data in these patient populations should be collected post-marketing.

Available results indicate that the treatment meets a high medical need in AHP patients by significantly reducing the frequency of severe porphyria attacks. Chronic symptoms of the disease (mostly neurological pain) can originate from the chronic elevations of the ALA/PBG levels in some patients even between the attacks, but they are also long-lasting consequences on nerve (and other organs) damage during the attacks. The Applicant presented data on pain levels, as well as analgesics use during and between attacks. From the data it can be concluded that the pain levels decreased in patients on givosiran both during and between

attacks. Even though the absolute decrease in pain score is rather moderate in general, its relevance is supported by the reduction in the opioid and non-opioid analgesics use by approximately 10% between the attacks and 17% during the attacks. Moreover, the proportion of the attacks with a pain score >7 was lower in the givosiran group, suggesting the beneficial effects of givosiran on attack severity as well.

In addition, givosiran showed beneficial effects on the QoL and physical functioning. In the Patient Global Impression of Change (PGIC) a larger proportion of patients with AIP treated with givosiran (61.1%) than with placebo (20%) rated their overall status as "very much improved" or "much improved" since the start of the study. Importantly, bodily pain domain of the SF-12 during the attack-free recall period showed greater improvement on givosiran treatment compared to placebo, supporting the results on the general pain levels and reduced analgesics use between the attacks, even though the number of patients in every comparison group is limited. From the data presented, it can be assumed that the effects of givosiran on the patient reported outcomes improve further overtime, which is expected, given that chronic neurologic impairment requires a prolonged rehabilitation.

2.5.4. Conclusions on the clinical efficacy

Overall, the data presented show that givosiran, in addition to reducing the attack frequency, has an effect on various aspects of patient wellbeing, such as pain and analgesics use between attacks, QoL and social engagement of the patents and thus is an effective treatment for the treatment of acute hepatic porphyria (AHP) in adults and adolescents aged 12 years and older.

2.6. Clinical safety

Patient exposure

Overall patient exposure to givosiran is presented in **Table 23**.

Table 23. Overall exposure to givosiran (Pooled Safety Set)

Parameter	Studies 001C/002 (N=17)	Study 003 (N=94)	Total AHP (N=111)
Total duration of study drug exposure (months)[a]			
N	17	94	111
Mean (SD)	21.93 (8.01)	5.42 (3.40)	7.95 (7.40)
Median (min, max)	21.78 (2.2, 30.9)	5.58 (0.1, 13.8)	6.08 (0.1, 30.9)
Cumulative study drug exposure (person-years)[b]	31.07	42.45	73.52
Number of patients on study drug; n (%)			
≥1 day	17 (100.0)	94 (100.0)	111 (100.0)
≥3 months	16 (94.1)	65 (69.1)	81 (73.0)

Parameter	Studies 001C/002 (N=17)	Study 003 (N=94)	Total AHP (N=111)
≥6 months	16 (94.1)	42 (44.7)	58 (52.3)
≥9 months	15 (88.2)	15 (16.0)	30 (27.0)
≥12 months	15 (88.2)	3 (3.2)	18 (16.2)
≥15 months	15 (88.2)	0	15 (13.5)
≥18 months	14 (82.4)	0	14 (12.6)
≥24 months	8 (47.1)	0	8 (7.2)
≥30 months	2 (11.8)	0	2 (1.8)
≥36 months	0	0	0
Total number of doses received			
N	17	94	111
Mean (SD)	20.5 (7.3)	6.3 (3.6)	8.5 (6.8)
Median (min, max)	21.0 (5, 30)	6.5 (1, 15)	7.0 (1, 30)
Cumulative number of doses received	349	589	938
Total number of patients received at least; n (%)			
4 doses	17 (100.0)	66 (70.2)	83 (74.8)
7 doses	15 (88.2)	47 (50.0)	62 (55.9)
10 doses	15 (88.2)	18 (19.1)	33 (29.7)
13 doses	15 (88.2)	4 (4.3)	19 (17.1)
16 doses	14 (82.4)	0	14 (12.6)
19 doses	12 (70.6)	0	12 (10.8)
22 doses	6 (35.3)	0	6 (5.4)
25 doses	5 (29.4)	0	5 (4.5)
Number of patients with; n (%)			
No missing dose	15 (88.2)	87 (92.6)	102 (91.9)
1 missing dose	2 (11.8)	5 (5.3)	7 (6.3)
2 missing doses	0	1 (1.1)	1 (0.9)
3 missing doses	0	1 (1.1)	1 (0.9)
≥4 missing doses	0	0	0
Total drug exposure (mg)			
N	17	94	111
Mean (SD)	4599.91 (1951.63)	976.54 (669.04)	1531.47 (1628.10)
Median (min, max)	4019.07 (1499.0, 8502.6)	968.40 (59.0, 2715.1)	1110.11 (59.0, 8502.6)

Abbreviations: AHP=Acute hepatic porphyria; ISS=Integrated Summary of Safety; max=maximum; min=minimum; SD=standard deviation.

[a] Individual duration of exposure (months) = individual duration of treatment (days)/30.44.

[b] Individual duration of exposure (years) = individual duration of treatment (days)/365.25.

As the majority of the data are in patients who received 2.5 mg/kg QM givosiran, patients who are receiving 1.25 mg/kg QM givosiran in the OLE period are pooled with patients who are receiving the 2.5 mg/kg regimen. The median duration of exposure is limited for patients on the 1.25 mg/kg regimen (1.51 months [range 0.3 to 3.3] for patients who crossed over from placebo in the double-blind period and 1.18 months [range 0.1 to 3.3] in patients who crossed over from 2.5 mg/kg givosiran during the double-blind period.

Adverse events

A summary of adverse events (AE) during the placebo-controlled experience is presented in **Table 24**.

Table 24. Summary of Adverse Events During the 6-month Double-blind Period; AHP Patients (Study 003 Safety Analysis Set)

Category	Placebo (N=46) n (%)/No. Events	Givosiran (N=48) n (%)/No. Events
At least 1 AE	37 (80.4)/202	43 (89.6)/228
At least 1 drug-related AE	12 (26.1)/23	22 (45.8)/75
At least 1 severe AE	5 (10.9)/6	8 (16.7)/14
At least 1 severe AE related to study drug	1 (2.2)/1	3 (6.3)/3
At least 1 SAE	4 (8.7)/7	10 (20.8)/10
At least 1 SAE related to study drug	0	3 (6.3)/3
At least 1 AE leading to treatment discontinuation	0	1 (2.1)/1
At least 1 study drug-related AE leading to treatment discontinuation	0	1 (2.1)/1
At least 1 AE leading to study withdrawal before Month 6 visit	0	0
At least 1 study drug related AE leading to study withdrawal before Month 6 visit	0	0
At least 1 AE leading to study withdrawal after Month 6 visit	0	1 (2.1)/1
At least 1 study drug related AE leading to study withdrawal after Month 6 visit[a]	0	1 (2.1)/1
Death	0	0

Abbreviations: AE=adverse event; AHP=Acute hepatic porphyria; LFT=liver function test; OLE=open-label extension; SAE=serious adverse event.

Notes: If a patient experienced more than 1 event in a given category, that patient was counted only once in that category. A patient can contribute to multiple events in the number of events. Related AEs are AEs with a missing relationship or those considered "definitely related" or "possibly related" to study drug by the investigators. Severe AEs include both severe events and events with missing severity.

[a] This patient discontinued study treatment during the double-blind period due to prespecified protocol stopping rules for LFT elevations. As she was not eligible to receive givosiran during the subsequent OLE period, she withdrew from the study after completing the Month 6 visit.

Differences, in the incidence of adverse events between the two treatment groups are summarised in **Table 25**.

Table 25. Adverse Events with a Higher Frequency (⩾5 Percentage Point Difference Between Treatment Groups) in Either Treatment Group During the 6 month Double-blind Period by Preferred Term; AHP Patients (Study 003 Safety Analysis Set)

Category Preferred Term	Placebo (N=46) n (%)/ No. Events	Givosiran (N=48) n (%)/ No. Events	Difference in %
AEs with Higher Frequency in the Givosiran Group			
Injection site reaction	0	8 (16.7)/15	16.7
Nausea	5 (10.9)/6	13 (27.1)/15	16.2
Chronic kidney disease	0	5 (10.4)/5	10.4
Glomerular filtration rate decreased	0	3 (6.3)/3	6.3
Rash	0	3 (6.3)/3	6.3
Alanine aminotransferase increased	1 (2.2)/1	4 (8.3)/6	6.1
Fatigue	2 (4.3)/2	5 (10.4)/6	6.1
AEs with Higher Frequency in the Placebo Group			
Pyrexia	6 (13.0)/7	1 (2.1)/3	10.9
Hypoaesthesia	4 (8.7)/5	0	8.7
Dyspepsia	4 (8.7)/4	0	8.7
Vomiting	5 (10.9)/5	2 (4.2)/5	6.7
Urinary tract infection	6 (13.0)/6	3 (6.3)/4	6.7
Back pain	4 (8.7)/4	1 (2.1)/1	6.6

Abbreviations: AE=adverse event; AHP=Acute hepatic porphyria.
Notes: If a patient experienced more than 1 event in a given category, that patient was counted only once in that category. A patient can contribute to multiple events in the number of events. Includes AEs occurring or worsening on or after the first dose of study drug and before the first dose of the OLE period for patients who entered into OLE or through 28 days after the last dose or any study drug related AEs for patients who discontinued treatment during the double-blind period. Preferred terms are sorted by decreasing frequency in the Difference column.

In the Overall Pooled Experience, AEs were reported in 94.6% of patients. AEs reported in ≥15% of patients receiving givosiran included events coding to the PTs of nausea (32.4%), ISR (24.3%), fatigue (22.5%), nasopharyngitis (22.5%), headache (19.8%), and abdominal pain (18.0%). AEs considered related to givosiran treatment by the Investigator were reported in 65.8% of patients. Related AEs occurring in ≥5% of patients were: ISR (24.3%), nausea (18.0%), fatigue (9.9%), injection site erythema (7.2%), asthenia (6.3%), ALT increased (6.3%), AST increase (6.3%), and headache and vomiting (5.4% each). AEs over time in patients treated with givosiran in the Overall Pooled Experience tended to remain stable over the course of exposure.

Serious adverse event/deaths/other significant events

SAE

Overall, more patients in the givosiran group reported serious adverse events (SAE) compared to placebo (**Table 26**). Most SAEs occurred in 1 patient each.

Table 26. Serious Adverse Events During the 6-Month double-blind Period by System Organ Class and Preferred Term; AHP Patients (Study 003 Safety Analysis Set)

System Organ Class Preferred Term	Placebo (N=46) n (%)	Givosiran (N=48) n (%)
At least 1 SAE	4 (8.7)	10 (20.8)
General disorders and administration site conditions	1 (2.2)	1 (2.1)
Pyrexia	1 (2.2)	1 (2.1)
Infections and infestations	3 (6.5)	2 (4.2)
Device related infection	2 (4.3)	1 (2.1)
Escherichia urinary tract infection	1 (2.2)	0
Gastroenteritis	0	1 (2.1)
Sepsis	1 (2.2)	0
Septic shock	1 (2.2)	0
Injury, poisoning and procedural complications	1 (2.2)	0
Fractured sacrum	1 (2.2)	0
Investigations	0	1 (2.1)
Liver function test abnormal	0	1 (2.1)
Metabolism and nutrition disorders	0	1 (2.1)
Hypoglycaemia	0	1 (2.1)
Psychiatric disorders	0	1 (2.1)
Major depression	0	1 (2.1)
Renal and urinary disorders	0	2 (4.2)
Chronic kidney disease	0	2 (4.2)
Respiratory, thoracic and mediastinal disorders	0	1 (2.1)
Asthma	0	1 (2.1)
Surgical and medical procedures	0	1 (2.1)
Pain management	0	1 (2.1)

Abbreviations: AE=adverse event; AHP=Acute hepatic porphyria; MedDRA=Medical Dictionary for Regulatory Activities; OLE=open-label extension; PT=preferred term; SAE=serious adverse event; SOC=System Organ Class.
Notes: Based on MedDRA version 21.0. If a patient experienced more than 1 events in a given category, that patient was counted only once in that category. A patient can contribute to multiple events in the number of events. Includes AEs occurring or worsening on or after the first dose of study drug and before the first dose of the OLE period for patients who entered into OLE or through 28 days after the last dose or any study drug related AEs for patients who discontinued treatment during the double-blind period. System Organ Class and PT within an SOC are sorted alphabetically.

In the Overall Pooled Experience, SAEs were reported in 28 (25.2%) patients treated with givosiran. The SAEs reported in 2 or more patients were abdominal pain, pyrexia, influenza, UTI, and chronic kidney disease; these events occurred in 2 patients each (1.8%). There was a total of 4 SAEs related to study drug (1 event of anaphylaxis, 1 event of LFT abnormal, 1 event of CKD, and 1 event of transaminases increased).

Death

The only fatal event (haemorrhagic pancreatitis) during the givosiran treatment occurred in the Study 001 in a patient receiving a 5mg/kg monthly dose. The patient had a complex medical history and a gallbladder sludge at the time of presentation.

Laboratory findings

No significant changes in haematology or serum chemistry parameters were observed during the DB period of the Study 003. A higher proportion of patients in the placebo group reported lipase or amylase elevations compared to the givosiran group. On the other hand, more patients in the givosiran group experienced ALT/AST elevation, creatinine elevation and decrease in eGFR. Most of these elevations were transient and resolved during the study.

A trend in a transient decrease in the diastolic blood pressure was observed on givosiran treatment, with no apparent change in the systolic blood pressure. Electrocardiograms did not reveal any abnormalities on givosiran treatment.

Safety in special populations

Only one patient of 65 years old was included to the clinical development program. No patients above that age were studied.

No clear influence of gender, race, BMI or geographic region on the givosiran safety profile was observed. A somewhat higher frequency of AEs was reported in the older patient group (≥38 years of age) in both placebo and givosiran treatment groups. More patients with prior hemin prophylaxis reported AEs (84.8%) compared to the patients with no prior hemin prophylaxis (76.9%). However, more patients with no prior hemin prophylaxis reported SAE (20%) compared to the patients with prior hemin prophylaxis (15.2%).

The AEs associated with hepatic or renal events were usually reported in individuals with pre-existing hepatic/renal condition. Three patients who had moderate renal function at baseline (eGFR ≥30 to <60 ml/min/1.73 m^2) progressed to severe renal impairment during the study (eGFR <30 ml/min/1.73 m^2).

No data is available on the use of givosiran in pregnant women and it is unknown whether givosiran is excreted in human milk.

Immunological events

Anti-drug antibodies were assessed over the course of each study. Overall, the incidence of treatment-emergent ADA in givosiran-treated patients was low (one patient). The presence of ADA did not affect the safety profile of givosiran.

Safety related to drug-drug interactions and other interactions

In the Study 004 DDI study with CHE patients, givosiran resulted in a moderate reduction (≤3.07-fold) in activity of cytochrome P450 (CYP) 1A2 and CYP2D6, weak reduction (≤1.59-fold) in activity of CYP3A4 and CYP2C19 and had no effect on CYP2C9 activity. No additional concerns were raised with respect to the safety of givosiran.

Discontinuation due to adverse events

Overall, four patients on givosiran discontinued the studies 001/002 or 003. Three of them discontinued due to the development of a SAE (ALT elevation, anaphylactic reaction or haemorrhagic pancreatitis) and one patient discontinued the OLE period of the Study 003 due to pregnancy.

In the overall pooled givosiran experience, AEs leading to treatment interruption occurred in 8.1% of patients; of the patients with dose interruptions, 7 were in Study 003 and 2 occurred in Study 001C/002. AEs leading to dose interruption in more than 1 patient are CKD (2 patients) and ALT/AST elevations (2 patients).

Post marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

Data from one pivotal study and two supportive studies comprise the safety database. Overall exposure to givosiran is limited but this is considered acceptable, considering the rarity of the disease.

In general, the trial population is representative of the target population. Data on adolescents (≥ 12 year of age and ≤18) is absent (due to the extreme rarity of the condition in those age groups) and almost absent in the elderly. However, pharmacokinetic data suggest that exposure to givosiran is not age-dependent and therefore the safety profile in those age groups is expected to be similar to that observed in the development programme of givosiran. Similarly data in the elderly or in patients with various stages hepatic impairment and severe renal impairment is either absent or limited and use of givosiran in such patients is included as missing information in the RMP.

The frequency of total adverse events and SAE related to the study drug was higher in the givosiran treatment group compared to placebo in the pivotal study. In the overall pooled experience, 65.8% patients experienced treatment-related AE and 3.6% experienced SAE. One death occurred in the givosiran treatment group (due to haemorrhagic pancreatitis). Three out of four patients discontinued the studies because of SAE, and nine patients had a dose interruption due to the AE.

Adverse events that were most frequently reported in the givosiran group are injection site reaction, nausea, fatigue, ALT elevation, rash, eGFR decrease and CKD. Most of the AEs were mild or moderate in severity and have resolved over the course of the study, with the exception of several events of CKD or fatigue. A recall phenomenon in the place of a previous injection brings some uncertainties to whether the drug might have an effect on the immune system systemically. One case of anaphylactic reaction was reported following givosiran use (in a patient with a history of various allergies and asthma). Overall, the adverse events observed in the study are listed in the SmPC.

The elevation in liver function test is dose-dependent and consistent with the toxicological data and known to be associated with siRNA use. Currently, the SmPC includes a warning and recommendations on the monitoring of serum transaminases (ALT and AST) and total bilirubin. Moreover, a warning on the dose resumption after the treatment interruption is included in the SmPC due to the reported significant transaminase elevations. The transaminase elevations following givosiran treatment were more frequently reported in patients with a history of hepatic disorders or history of ALT elevations compared to patients with no history of hepatic disorders or ALT elevations. A similar situation was observed with AEs associated with

renal events that were usually reported in individuals with pre-existing CKD and reduced eGFR. Therefore, patients with the history of hepatic or renal disorders require an additional safety warning in the SmPC to highlight an increased risk of givosiran side effects in these vulnerable patient populations. Hepatic and renal effects are also included in the RMP as important potential risks.

Pancreatic dysfunction in patients with AHP including elevations in amylase or lipase, acute and chronic pancreatitis have been reported in the literature and supported in a natural history study in AHP patients and claim database studies. In clinical studies, two cases of pancreatitis were reported, including one fatal case in Study 001, that was considered unlikely related to givosiran in the context of the patient's medical history, the presence of significant comorbidities. The other case was from the open-label extension phase of Study 003. The event was also considered unlikely related to the drug by the investigator, given the alternative aetiology of cholelithiasis in the setting of elevated alkaline phosphatase levels. Nevertheless, pancreatitis has been added as an important potential risk in the RMP.

Longer-term safety as well as further characterisation of the effect of givosiran on hepatic and renal effects, pancreatitis and in patients with moderate or severe hepatic impairment and with ESRD or on dialysis will be collected in a planned AHP Registry. Additional information on these safety concerns is also expected from the ongoing open-label extension phase of studies ALN-AS1-002 and 003.

Givosiran does not seem to result in changes in haematology or serum chemistry parameters. No clear influence of intrinsic or extrinsic factors on the givosiran safety profile was observed, aside the renal or hepatic impairment. Due to the lack of data, givosiran should only be considered in pregnant or lactating women if the benefits for the mother outweigh the risks for the foetus. Use in pregnant or lactating women and effects on pregnancy outcomes are included in the RMP as missing information and will be also investigated in the planned disease Registry.

Finally, the carcinogenic potential for givosiran is being investigated in an ongoing 104-Week subcutaneous carcinogenicity study in Sprague Dawley rats which will be submitted once completed.

2.6.2. Conclusions on the clinical safety

Despite the limited size of the safety database, due to the rarity of AHP, the overall safety profile of givosiran is considered acceptable. The main safety concerns are the potential effects of treatment on the liver and the kidney which are addressed adequately through appropriate routine risk minimisation measures. Further information is also expected through the planned post-authorisation studies.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns

Important identified risk	None
Important potential risk	• Hepatic effects • Renal effects

	• Pancreatitis
Missing information	• Longer-term safety (>3 years) • Use in patients with moderate or severe hepatic impairment • Use in patients with end stage renal disease or on dialysis • Use in pregnant or lactating women and effects on pregnancy outcomes • Carcinogenicity

Pharmacovigilance plan

Summary of Additional Pharmacovigilance Activities

Study Number and Title	**Rationale and Objectives**	**Study Design**	**Study Population**	**Study Status**	**Milestones**
AS1-GLP18-007 A 104-Week Subcutaneous Carcinogenicity Study in Sprague Dawley Rats	To determine the carcinogenic potential of ALN-AS1 when given by subcutaneous (SC) injection once every 28 days for 104 weeks	Non-clinical	NA	Ongoing	Final Report (planned): Aug 2021
ALN-AS1-002 OLE study of givosiran for patients with AIP who completed Study 001 Part C	To evaluate the long-term safety and clinical activity of givosiran in patients with AIP who have completed a previous clinical study with givosiran	Phase 1/2 OLE in patients who completed Study 001C	Patients with AIP	Ongoing	First patient first visit: 24 Oct 2016
					Last patient last visit (planned): Oct 2021
					Interim study report: 09 Apr 2019
					Final study report (planned): Feb 2022
ALN-AS1-003 OLE study to the multicenter,	To evaluate the long-term effect of givosiran	Phase 3 OLE	Patients with AHP	Ongoing	First patient first visit: 16 Nov 2017

Study Number and Title	Rationale and Objectives	Study Design	Study Population	Study Status	Milestones
double-blind placebo-controlled phase 3 Study (Study 003)					Last patient last visit (planned): May 2021
					Interim study report (double-blind period): 14 May 2019
					Interim study report OLE: *TBD*
					Final study report OLE (planned): Sep 2021
Company Sponsored AHP Registry	To characterize the longer-term safety and effectiveness of givosiran in a real-world cohort of AHP patients. To collect and evaluate information on pregnancy complications, birth outcomes, breast feeding and infant outcomes in women exposed to givosiran during pregnancy.	Prospective observational longitudinal cohort study	Adult and adolescent patients with AHP	Planned	Planned protocol submission: 3 months after positive EC decision
					Planned final protocol: Q3 2020
					Study progress reports will be provided with each PSUR. Interim analysis (As agreed with PRAC)
					Data collection end date and Final study report planned due date: (As agreed with PRAC)

Abbreviations: AHP=acute hepatic porphyria; AIP=acute intermittent porphyria; EC=European Commission; OLE=open-label extension; PRAC=Pharmacovigilance Risk Assessment Committee; PSUR=Periodic Safety Update Report; TBD=to be determined

Risk minimisation measures

Summary of Risk Minimisation Measures

Safety concern	Risk Minimisation Measure
Important identified risks	
None	
Important potential risks	
Hepatic Effects	Routine risk communication: • The effect of givosiran on serum transaminases are described in the Special warnings and precautions for use Section 4.4 and Undesirable effects (Section 4.8) of the SmPC and in Section 2 and Section 4 of the Package Leaflet. Routine risk minimization activities recommending specific clinical measures to address the risk: • Liver function tests should be monitored prior to initiating treatment, monthly for the first 6 months, and as clinically indicated thereafter as described in Special warnings and precautions for use Section 4.4 of the SmPC. • Interruption or discontinuation should be considered for clinically relevant transaminase elevations as per Special warnings and precautions for use Section 4.4 of the SmPC. • In patients with clinically relevant transaminase elevations who have dose interruption and subsequent improvement in transaminase levels, dose resumption at 1.25 mg/kg once monthly could be considered, as described in Posology and method of administration (Section 4.2) of the SmPC. Other routine risk minimization measures beyond the Product Information: • Legal status: Prescription-only medication Additional risk minimization measures: • None

Renal Effects	Routine risk communication: • The effect of givosiran on renal function is described in the Special warnings and precautions for use Section 4.4 and Undesirable effects (Section 4.8) of the SmPC and in Section 2 and Section 4 of the Package Leaflet. Routine risk minimization activities recommending specific clinical measures to address the risk: • Monitoring of renal function during treatment is required in patients with pre-existing renal disease as described in the Special warnings and precautions for use Section 4.4 of the SmPC. Other routine risk minimization measures beyond the Product Information: • Legal status: Prescription only medication Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • Evaluation of data from the ongoing OLE Study 002 and Study 003 • Post-authorization observational study
Pancreatitis	Routine risk communication: • Not Applicable Routine risk minimization activities recommending specific clinical measures to address the risk: • Not Applicable Other routine risk minimization measures beyond the Product Information: • Legal status: Prescription only medication
Missing information	
Longer-term safety (>3 years)	Routine risk communication: • A summary of the safety profile of givosiran in the clinical development program is provided in the Undesirable effects (Section 4.8) of the SmPC. Additional risk minimization measures:

	• None
Use in patients with moderate or severe hepatic impairment	Routine risk communication: • Information on the absence of data in patients with moderate and severe hepatic impairment is included in the Posology and method of administration section 4.2 and Pharmacokinetic properties Section 5.2 of the SmPC. Additional risk minimization measures: • None
Use in patients with end-stage renal disease or on dialysis	Routine risk communication: • Information on the absence of data in patients with ESRD and patients on dialysis is included in the Posology and method of administration section 4.2 and Pharmacokinetic properties Section 5.2 of the SmPC. Additional risk minimization measures: • None
Use in pregnant or lactating women and effects on pregnancy outcomes	Routine risk communication: • Information on the limited clinical data in pregnant women and no clinical data in lactating women is included in the Fertility, pregnancy and lactation (Section 4.6) of the SmPC, with a cross-reference to nonclinical data on embryo-foetal development, lactation, and fertility in the Preclinical safety data (Section 5.3) sections of the SmPC. Routine risk minimization activities recommending specific clinical measures to address the risk: • Advice is provided to evaluate the benefits and risks of treatment with givosiran during pregnancy and breastfeeding for the mother and infant, and the mother's clinical need for givosiran in the Fertility, pregnancy and lactation (Section 4.6) of the SmPC and section 2 of the Package Leaflet. Additional risk minimization measures:

	• None
Carcinogenicity	Routine risk communication: • Information is provided in the Preclinical safety data (Section 5.3), of the SmPC that givosiran did not exhibit a genotoxic potential in vitro and in vivo, and that animal studies have not been conducted to evaluate the carcinogenic potential of givosiran. Routine risk minimization activities recommending specific clinical measures to address the risk: • NA

Abbreviations: ESRD=end-stage renal disease; NA=not applicable; OLE=open-label extension; SmPC=Summary of Product Characteristics.

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.5 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 20 November 2019. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant compared the structure of givosiran with active substances contained in authorised medicinal products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

The CHMP, based on the available data, considers givosiran to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

2.10. Product information

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Givlaari (givosiran) is included in the additional monitoring list as:

- It contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU;

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Givlaari has been developed for "the treatment of acute hepatic porphyria (AHP) in adults and adolescents aged 12 years and older".

Acute hepatic porphyria (AHP) is a family of rare, serious, and severely debilitating genetic disorders of liver heme synthesis. Symptoms arise due to accumulation of the toxic heme intermediates aminolevulinic acid (ALA) and porphobilinogen (PBG). In these patients, expression of delta-aminolevulinate synthase 1 (ALAS1) is induced in the presence of a pathogenic loss of function gene mutation in a downstream heme synthesis enzyme. There are 4 subtypes of AHP, each involving a defect in a distinct heme pathway enzyme, with acute intermittent porphyria (AIP) being the most common AHP subtype representing approximately 80% of all AHP cases.

Most severe AHP patients (5-10%) have recurrent attacks (≥ 4 per year). Each porphyria attack is serious, highly morbid, and carries potential for permanent disability, and where specific treatment is delayed or not available, attacks can be life-threatening. Due to unpredictability and severity of porphyria disease manifestations, many patients are unable to work or attend school, have decreased socialization, and increased rates of depression and anxiety.

3.1.2. Available therapies and unmet medical need

Current treatment options for AHP are limited. Patients are initially treated with supportive care during the attacks such as intravenous (IV) glucose, typically large doses of IV opioid analgesics, and antiemetics along with the removal of known precipitating triggers, such as certain medications or fasting [Bissell 2017; Bonkovsky 2013; Stein 2013]. Intravenous hemin is the only therapy currently approved for the treatment of acute attacks; hemin is not approved as a chronic treatment to prevent attacks [Bissell 2015]. While hemin infusion temporarily reduces production of ALA and PBG through feedback inhibition of ALAS1 messenger ribonucleic acid (mRNA) expression, it has a short duration of action (elimination half-life of approximately 11 hours), which limits its effectiveness and permits residual attack activity when used prophylactically. Moreover, they are a big burden for AHP patients as they carry the risk of morbidity, such as iron overload or infections due to the catheter lines. The repeated use of hemin commonly causes venous access problems in AHP patients.

Additional treatments for AHP include chemically induced menopause with hormonal suppression therapy (e.g. gonadotropin releasing hormone agonists) and liver transplantation for patients with refractory disease or those who no longer have adequate venous access.

Consequently, there is a clear unmet need for therapies that durably decrease the frequency of debilitating attacks, diminish chronic symptoms, and improve patients' physical functioning and quality of life. In addition, therapeutics that reduce the need for opioid analgesics or drugs requiring chronic indwelling central venous catheters would reduce morbidity associated with these interventions.

3.1.3. Main clinical studies

The main evidence of efficacy comes from a single phase III randomized double-blind, placebo-controlled multicentre study with an open-label extension to evaluate the efficacy and safety of givosiran in patients with AHP who experienced at least 2 attacks in the past 6 months (n=94). The 6-month double-blind (DB) period of this study, in which patients received 2.5 mg/kg givosiran or placebo SC once monthly, forms the primary demonstration of efficacy of givosiran.

The ongoing OLE period, in which patients receive 2.5 or 1.25 mg/kg givosiran SC once monthly, will provide additional information on efficacy and safety of givosiran and will continue for up to 29 months.

3.2. Favourable effects

The treatment with givosiran 2.5 mg/kg dose monthly resulted in a rapid and statistically significant decrease (approximately 75%) in the AAR in the AIP population (primary endpoint): rate ratio 0.26 (95%CI: 0.16, 0.41, p<0.001) in favour of givosiran. In total, 50% of patients in the givosiran group and 17.4% of patients in placebo group had 0 attacks.

The results of some secondary endpoints were in line with the primary endpoint: givosiran decreased ALA (86% from the baseline) and PBG (91% from the baseline) levels in AIP patients, while ALA and PBG levels in the placebo group remained relatively stable and elevated well above normal. The difference in LS Mean between givosiran and placebo was -18.2 mmol/mol Cr (95% CI: -17.97, -9.63, p<0.001) for ALA and -27.48 mmol/mol Cr (95% CI: -34.04, -20.99, p<0.001) for PBG. Also, the number of days of hemin use significantly decreased following givosiran treatment with a rate ratio of 0.23 (95% CI: 0.11 0.45, p<0.001).

Treatment with givosiran reduced the weekly mean score of daily worst pain compared with placebo in AIP patients (measured as mean change from baseline in the AUC) with median of treatment difference 0.5 (p=0.0455) on a 10-point VAS. Moreover, givosiran treatment led to the decrease in opioid and non-opioid analgesics use during and between attacks.

Subgroup analyses were performed for the primary endpoint of the composite AAR based on age, race, region, gender, BMI and medical history of the disease. These factors did not have an influence on givosiran efficacy.

These effects were also shown to be sustained in the OLE period of the Study 003, where patients who received placebo in the DB period also switched to the givosiran treatment.

3.3. Uncertainties and limitations about favourable effects

The applicant is applying for an indication in the overall AHP population. However, data on non-AIP patients is very limited. In total, only 5 non-AIP patients participated in the givosiran clinical trials. However, based on the common pathophysiology of all sub-types of AHP and the consistent results in the small number of non-AIP patients treated with givosiran, the proposed broad indication is considered acceptable.

Patients with moderate/severe hepatic impairment or severe renal impairment were excluded from the study. However, mild hepatic impairment and mild to moderate renal impairment did not seem to influence PD or efficacy of givosiran and the product information states the lack of data in these sub-group of patients.

No adolescents or elderly patients >65 years of age were enrolled to the study. The efficacy in these groups is therefore largely extrapolated from the adult population and pharmacokinetic data which suggest that age was not a significant covariate in the pharmacokinetics of givosiran.

The short study duration as well as the data collection approach does not allow for a full appreciation of the givosiran effects on all the studied chronic symptoms and patient reported outcomes. However, the data presented by the applicant suggest that: 1) givosiran positively affects not only attack frequency, but also severity (based on pain scores and analgesics use during the attacks); 2) givosiran has a positive effect on pain and analgesics use also between the attacks; and 3) the quality of life as well as patient social engagement improves substantially on givosiran treatment. Therefore, the indication "treatment of AHP" is acceptable.

3.4. Unfavourable effects

The frequency of total adverse events (AE) and serious adverse events (SAE) related to the study drug was higher in the givosiran treatment group compared to placebo in the pivotal study. One death occurred in the givosiran treatment group in the supportive OLE Study 002 (due to haemorrhagic pancreatitis). Three out of four patients who discontinued the studies did so because of an SAE (2.7%), and nine patients had a dose interruption due to the AE (8.1%).

Adverse events that were most frequently reported in the givosiran group compared to placebo are injection site reaction (25% vs 0%), nausea (27.1% vs 10.9%), fatigue (10.4% vs 4.3%), ALT elevation (8.3% vs 2.2%), rash (6.3% vs 0%), eGFR decrease (6.3% vs 0%) and CKD (10.4% vs 0%). Most of the AEs were mild or moderate in severity and resolved over the course of the study.

SAE were reported during the studies including one case of anaphylactic reaction (in a patient with a history of various allergies and asthma), one fatal case of haemorrhagic pancreatitis and one case of obstructive pancreatitis (in patients with a complex medical history).

The elevation in LFT is a known dose-dependent side effect of this type of siRNA compounds that target the liver and is consistent with the non-clinical data. The data from the pivotal study suggest that LFT elevations following givosiran treatment were more frequently observed in patients with a history of hepatic disorders or history of ALT elevations compared to patients with no history of hepatic disorders or ALT elevations. A similar situation was observed with AEs associated with renal events that were usually reported in individuals with pre-existing CKD and reduced eGFR. No clear influence of other intrinsic or extrinsic factors on the givosiran safety profile was observed. Furthermore, givosiran does not seem to result in significant changes in haematology or serum chemistry parameters.

3.5. Uncertainties and limitations about unfavourable effects

The main limitation of the current safety database is the low number of patients in the clinical program due to the rarity of the disease and the short duration of exposure. This rather short follow-up and small sample size bring some uncertainties to whether all the potential side effects of the treatment are captured within the study and if any of the adverse effects might re-occur or persist for a longer period of time.

Data on adolescents (12-18 years old) or patients >65 years of age is absent (due to the rarity of these patients) and the data on patients with various stages of renal or hepatic impairment is either absent or very limited. Therefore, this brings uncertainties to the safety of givosiran in these sub-populations and the data will need to be collected post-approval as part of the RMP, even though the safety profile of givosiran is expected to be similar across all age groups. Due to the concerns over potential hepatic toxicity, the SmPC includes a warning related to the monitoring of serum transaminases and total bilirubin and dose modification in specific clinical situations.

3.6. Effects Table

Table 27. Effects Table for Givlaari for the treatment of acute hepatic porphyria (AHP) in adults and adolescents aged 12 years and older. (data cut-off: 31st January 2019)

Effect	Short Description	Unit	Givosiran	Placebo	Uncertainties/ Strength of evidence
Favourable effects					
Porphyria attack composite endpoint	Mean annualized rate of porphyria attacks requiring hospitalization, urgent healthcare visit, or IV hemin administration at home in patients with AIP over the 6-month DB period	Number of attacks	3.22 (2.25, 4.59)	12.52 (9.35, 16.76)	p<0.0001 50% of patients in the givosiran group and 17.4% in the placebo group had 0 attacks. Urinary ALA and PBG levels were decreased by >85% with givosiran, supporting the proposed MoA.

Effect	Short Description	Unit	Givosiran	Placebo	Uncertainties/ Strength of evidence
AUC of change from baseline in daily worst pain	Daily worst pain score as measured by BPI-SF NRS in patients with AIP over the 6-month DB period	AUC of change	-12.876 (-21.776, -3.976)	-0.196 (-9.468, 9.077)	p=0.0455 (post-hoc) The use of opioid and non-opioid analgesics decreased with givosiran treatment by approximately 10% between the attacks and 17% during the attacks.
Physical Component Score of SF-12	Change from baseline in the PCS of the SF-12 in patients with AIP at 6 months	score	5.369 (3.046, 7.693)	1.431 (-0.995, 3.856)	p=0.02, not formally significant
Unfavourable effects					
Chronic kidney disease	Percent of patients experiencing chronic kidney disease	%	10.4	0	Two patients had givosiran interrupted due to the CKD in the OLE period.
ALT	Percent of patients experiencing alanine aminotransferase increased	%	8.3	2.2	One case of ALT elevation in the givosiran group led to the treatment discontinuation (9.9xULN) and two cases led to the treatment interruption (5.4×ULN and 4.9xULN).

Abbreviations: IV: intravenous, AIP: Acute intermittent porphyria, DB: double-blind, ALA: Aminolevulinic acid, AUC: Area under the curve, BPI-SF: Brief Pain Inventory (Short Form) NRS: Numerical Rating Scale, SF-12: Short form 12 health survey, PCS, ALT: Alanine aminotransferase, CKD: chronic kidney disease, OLE: open-label extension, ULN: upper level of normal

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Porphyria attacks are mostly very severe, with extreme pain and require hospitalisation or urgent health care visit. Hemin infusions used for treatment of attacks are a big burden for AHP patients as they carry the risk of morbidity, such as iron overload or infections due to the catheter lines. Therefore, the observed pronounced decrease in the attack rate and the reduction in need of hemin infusions following givosiran treatment are considered clinically meaningful and of high benefit for the patient.

Adverse events were more frequently reported in the givosiran treatment group compared to placebo. Most of the adverse events were mild or moderate in severity and most of them resolved within the study duration. Only few AE led to the treatment discontinuation or dose interruption.

Hepatic events are an important risk and may be a class effect of liver-targeting siRNA products. A warning and dose recommendations are provided in the SmPC to minimise these risks. The other main safety concern with givosiran use are renal events. It should be noted that AEs associated with renal events were usually reported in individuals with pre-existing renal conditions and this is emphasised in the product information which states that such patients may require careful monitoring of renal function during treatment.

3.7.2. Balance of benefits and risks

The clinical efficacy of givosiran has been convincingly demonstrated in terms not only of a pharmacodynamic effect but more importantly by significantly reducing the frequency of severe porphyria attacks. This is expected to have a significant impact on the quality of life of affected patients.

The overall safety profile of givosiran is considered acceptable and can be managed adequately by the proposed risk minimisation measures. Considering the nature of the disease and the lack of approved treatments, the demonstrated benefits of givosiran treatment outweigh the risks associated with its use.

The long-term effect of treatment on safety in AHP patients will be further evaluated in the planned observational study.

3.8. Conclusions

The overall B/R of Givlaari is positive.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Givlaari is favourable in the following indication:

treatment of acute hepatic porphyria (AHP) in adults and adolescents aged 12 years and older.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that givosiran is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.