21 July 2022
EMA/CHMP/689555/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Amvuttra

International non-proprietary name: vutrisiran

Procedure No. EMEA/H/C/005852/0000

Note

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

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List of abbreviations

10-MWT	10-meter walk test
ADA	Antidrug antibodies
ADR	Adverse drug reactions
AE	Adverse Event
AL	Amyloid light-chain
ALT	Alanine transaminase
ANCOVA	Analysis of covariance
ANCOVA/MI	Analysis of covariance model incorporating multiple imputation
AS	Active substance
ASGPR	Asialoglycoprotein
ASO	Antisense oligonucleotide
AST	Aspartate transaminase
ATC	Anatomical Therapeutic Classification
ATTR	Transthyretin-mediated amyloidosis
AX-HPLC	Anion exchange HPLC
BMI	Body Mass Index
CCS	container closure system
CHMP	Committee for Human Medicinal Products
CI	Confidence interval
Cmax	Maximum observed concentration occurring at tmax
COVID-19	Coronavirus disease 2019
CPP	Critical process parameters
CQA	Critical quality attributes
CSR	Clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
CTCAE	Common Technology Criteria for Adverse Events
CV	Cardiovascular
CYP	Cytochrome P450
DDI	Drug-drug interaction
DMC	Data monitoring committee
FP	Finished product
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EOP2	End of Phase 2
EQ-5D-5L	EuroQoL 5 dimensions 5 levels
EQ-VAS	EuroQoL visual analogue scale
ESRD	End-stage renal disease

FAP	Familial Amyloid Polyneuropathy
FP	Finished product
GalNAc	*N*-acetylgalactosamine
GCP	Good Clinical Practice
GI	Gastrointestinal
hATTR	Hereditary transthyretin-mediated amyloidosis
hERG	Human ether-à-go-go-related gene
HGLT	High level group term
HLT	High level term
HPLC	High performance liquid chromatography
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
Ig	Immunoglobulin
IPC	In-process control
IPRP-HPLC	Ion pair-reversed phase HPLC
IRR	Infusion-related reaction
ISR	Injection-site reaction
IV	Intravenous(ly)
KPS	Karnofsky Performance Status
LFT	Liver function test
LNP	Lipid nanoparticles
LoOI	List of Outstanding Issues
LS	Least squares
LV	Left ventricular
M9	Month 9
M18	Month 18
MAR	Missing at random
mBMI	Modified body mass index
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed-effects model for repeated measures
MNAR	Missing not at random
mITT	Modified intent-to-treat
mNIS+7	Modified neuropathy impairment score +7
mRNA	Messenger RNA
NEC	Not elsewhere classified
NIS	Neuropathy impairment score
NIS-LL	Neuropathy Impairment Score-Lower Limbs
NIS-R	NIS-reflexes
NIS-S	NSI-sensation

NIS-W	NIS-weakness
NMT	Not more than
Norfolk QoL-DN	Norfolk Quality of Life-Diabetic Neuropathy
NT-proBNP	*N*-terminal pro b-type natriuretic peptide
NYHA	New York Heart Association
OD	Orphan designation
OLT	Orthotopic liver transplantation
PFS	Prefilled syringe
PFS-S	Prefilled syringe equipped with a passive needle safety system
Ph. Eur.	European Pharmacopoeia
PMDA	Japanese Pharmaceuticals and Medical Devices Agency
PMM	Pattern-Mixture Model
PND	Polyneuropathy disability
PP	Per Protocol
PT	Preferred term
q3M	Once every 3 months
q3w	Once every 3 weeks
QoL	Quality of Life
QST	Quantitative sensory testing
QTc	Corrected QT interval
QTPP	Quality Target Product Profile
RBP	Retinol binding protein
RH	Relative humidity
RISC	RNA-induced silencing complex
RNAi	RNA interference
RTE	Randomised Treatment Extension
R-ODS	Rasch-built Overall Disability Scale
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard Deviation
siRNA	Small interfering ribonucleic acid
SMQ	Standardized MedDRA query
SmpC	Summary of Product Characteristics
SOC	System organ class
T4	Thyroxine
Tmax	Time to reach maximum concentration
TQT	Thorough QT
TTR	Transthyretin
ULN	Upper limit of normal

USM	Urgetn Safety Measure
US	United States
USP	United States Pharmacopoeia
UV	Ultra Violet spectroscopy
V30M	Valine to methionine mutation at position 30
V122I	Isoleucine substitution for valine at position 122
wt	Wild type

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Alnylam Netherlands B.V. submitted on 10 September 2021 an application for a marketing authorisation to the European Medicines Agency (EMA) for Amvuttra (vutrisiran), 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 29 January 2021.

Amvuttra was designated as an orphan medicinal product (EU/3/18/2026) on 25 May 2018 in the following condition:

- Treatment of transthyretin-mediated amyloidosis (ATTR amyloidosis).

The applicant initially applied for the following indication:

- Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with polyneuropathy

The final indication for Amvuttra is for the

- treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with stage 1 or stage 2 polyneuropathy

1.2. Legal basis, dossier content

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 test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included the EMA Decision(s) EMEA-002425-PIP01-18 – P/0015/2019 on the granting of a (product-specific) waiver.

The waiver covers all subsets of the pediatric population (0 to 18 years) on the grounds that vutrisiran does not represent a significant therapeutic benefit as clinical studies are not feasible.

1.4. Information relating to orphan market exclusivity

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Amvuttra 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/amvuttra

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products Vyndaqel, Tegsedi and Onpattro.

1.5. Applicant's request for consideration

1.5.1. New active Substance status

The applicant requested the active substance vutrisiran, 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.

1.6. Protocol assistance

The applicant received the following protocol assistance on the development, relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
20 September 2018	EMEA/H/SA/3876/1/2018/PA/III	Peter Mol and Armando Magrelli

The Protocol assistance pertained to the following non-clinical, and clinical aspects:

- Adequacy of the completed and planned nonclinical studies to support MAA. Deferral of submission of data from the 2-year rat carcinogenicity study until after marketing approval.
- Acceptability not to conduct a thorough QT/QTc study, further in vitro or in vivo DDI studies, and a radiolabeled human ADME study. Assessing the influence of hepatic impairment or renal impairment on the PK, PD, and safety properties of ALN-TTRSC02 by using population a PK/PD approach in the planned Phase 3 studies enrolling patients with mild hepatic impairment or mild to moderate renal impairment.
- Design of an initially proposed single pivotal Phase 3 study (ALN-TTRSC02-002) including posology, single arm trial, external control, patient population, study duration, co-primary and other key endpoints, statistical considerations and sample size. During the procedure the applicant proposed a revised design including a within study patisiran-LNP comparator reference arm and change in the co-primary endpoints.

1.7. Steps taken for the assessment of the product

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

- Rapporteur: Martina Weise (DE)
- Co-Rapporteur: Bruno Sepodes (PT)

The application was received by the EMA on	10 September 2021
The procedure started on	30 September 2021
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	20 December 2021
The CHMP Co-Rapporteur's first critique was circulated to all CHMP and PRAC members on	30 December 2021
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	4 January 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	27 January 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	18 March 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	28 April 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	5 May 2022
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	19 May 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	6 June 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	6 July 2022
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 Amvuttra on	21 July 2022
The CHMP adopted a report on similarity of Amvuttra with Onpattro, Vyndaqel and Tegsedi on (see Appendix on similarity)	21 July 2022
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	21 July 2022

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis), also known as variant transthyretin-mediated amyloidosis, is a rare, autosomal dominant, rapidly progressive, multi systemic disease caused by variants in the *transthyretin* (*TTR*) gene that results in debilitating morbidity and high mortality. Amyloid deposits accumulate in multiple organs, particularly the peripheral nervous system, gastrointestinal tract, kidney, and heart, which manifests in progressive polyneuropathy including sensorimotor neuropathy and autonomic neuropathy. Cardiomyopathy, nephropathy, and gastrointestinal dysfunction frequently develop simultaneously. The phenotypic presentation of the disease is dependent on the pattern of affected organs. The most common manifestations of hATTR amyloidosis are polyneuropathy and cardiomyopathy.

2.1.2. Epidemiology

The worldwide prevalence of hATTR-PN has been estimated at approximately 10,000 patients. In Europe, the incidence is estimated from 0.003 to 0.10 cases per 10,000 per year (between 5000 to 6000 patients or 0.3 new cases per year per 1 million inhabitants), with the majority of cases in Portugal, France, Italy, and the United Kingdom. In Europe, the prevalence is highest in northern Portugal and northern Sweden (as high as 50 per 100,000 inhabitants).

2.1.3. Biologic features, Aetiology and pathogenesis

In hATTR amyloidosis, inherited variants in the *TTR* gene lead to destabilization of the tetrameric protein and disassociation of the TTR subunits into dimers and individual variant and wild-type (wt) monomers, which subsequently misfold.

There are over 120 reported *TTR* genetic variants associated with hATTR amyloidosis with heterogeneity in disease presentation from predominantly neuropathic, predominantly cardiac or mixed phenotypes. Worldwide, the most common disease-causing variant results in a valine to methionine mutation at position 30 in the TTR molecule, V30M (p. TTRV50M). V30M is predominantly associated with polyneuropathy and is found primarily in families with heritage from Portugal, Sweden, Japan, and Brazil. In the US, the isoleucine substitution for valine at position 122 in TTR, V122I (pV142I), is the most prevalent TTR-associated variant with a prevalence of approximately 4% in West Africans and African Americans. V122I is associated with predominantly cardiac manifestations but also can be associated with concurrent polyneuropathy.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Historically, due to incomplete understanding of aetiology and pathogenesis, two clinical syndromes of hATTR amyloidosis have been described in the medical literature: hATTR amyloidosis with polyneuropathy (previously known as familial amyloidotic polyneuropathy, or FAP) and hATTR amyloidosis with cardiomyopathy (previously known as familial amyloidotic cardiomyopathy, or FAC), both of which are characterized by amyloid deposits comprised of both mutant and wtTTR.

Many patients with hATTR amyloidosis are not diagnosed until their neuropathy is already at least moderate in severity, with sensorimotor and autonomic abnormalities starting to impact ambulation.

The main clinical manifestations of hATTR-PN are progressive peripheral sensorimotor and autonomic neuropathy. Non-specific and symmetrical numbness, pain, and temperature sensitivity typically begins in the lower extremities, progressing distal to proximal. In patients with hATTR amyloidosis, sensory abnormalities include painful dysesthesias in the feet and hands, as well as loss of sensation, which may lead to thermal burns in these areas and to joint damage in the lower limbs. Progressive muscle atrophy and motor weakness in both lower and upper limbs lead to impaired ambulation and inability to perform activities of daily living. Autonomic dysfunction results in debilitating orthostatic hypotension leading to loss of consciousness, severe gastrointestinal symptoms (including early satiety, chronic nausea/vomiting, malnutrition/weight loss, and both diarrhoea and constipation), and bladder dysfunction with recurrent urinary tract infections, as well as cardiac arrhythmias. The rate of neuropathy progression is influenced by *TTR* genotype, age at symptom onset, and extent of neurologic impairment at time of diagnosis.

In the heart, infiltration of cardiac tissue with amyloid leads to wall thickening and cardiomyopathy, manifested by heart failure due to diastolic and systolic dysfunction, as well as conduction disturbances and arrhythmias. Cardiac involvement has been estimated to occur in 80% of ATTR. Similar to polyneuropathy, patients with more severe cardiac disease at the time of diagnosis experience rapid progression with substantial worsening of echocardiographic and biomarker measures of cardiac function, ambulation, and quality of life, seen over a period of 18 months or less. Motor neuropathy follows within a few years, which affects ambulatory status.

Hereditary ATTR-PN is classified into 3 stages based on ambulatory status of the hATTR patient: in Stage 1, the patients present with weaknesses in the lower limbs and do not require assistance with ambulation, while they show gait dysfunctions, distal amyotrophies and hand involvement in Stage 2 and depend on assistance with ambulation, and are either wheel-chair bound or bedridden with generalised weakness and areflexia in Stage 3. This staging system was used to classify severity of disease in patients being considered for enrolment in the pivotal clinical study of inotersen (CS2). Disease severity can be also assessed using the Polyneuropathy Disability (PND) score, which is a 5-stage scoring system.

Given the severity of hATTR, there is a significant impact on patients' and caregivers' quality of life. Caregivers have moderate to high levels of fatigue and spend a significant amount of time caring for patients. Hereditary ATTR is associated with a substantial disruption in employment rates and work productivity. There is also a large mental health burden on both caregivers and patients.

The constellation of progressive morbidity from amyloid infiltration in patients with hATTR amyloidosis results in severe disability, wasting due to gastrointestinal malabsorption, malnutrition, and cardiac cachexia. Death usually results from heart failure (including sudden death caused by ventricular arrhythmias or electromechanical dissociation) or infection. The survival after diagnosis is dependent on time from first symptom to diagnosis and also on age of onset. The applicant refers to publications claiming a median survival after diagnosis of a mere 4.7 years (range 1.3 to 24.8 years) with a reduced survival (3.4 years) for patients presenting with cardiomyopathy.

2.1.5. Management

Before the approval of patisiran and inotersen, therapeutic strategies to treat hATTR included orthotopic liver transplant (OLT) or pharmacotherapy with tafamidis or off-label use of diflunisal, both of which are transthyretin (TTR) stabilizers that work by preventing dissociation of the tetramer into amyloid-forming monomers.

Tafamidis ("Vyndaqel") was approved across the EU for the treatment of ATTR in adult subjects with Stage 1 symptomatic polyneuropathy to delay peripheral neurological impairment and has also been licensed in Japan and several other countries. Diflunisal is a non-steroidal anti-inflammatory drug (NSAID) that is presently used off-label in subjects with Stage 1 and Stage 2 disease; however, the cardiovascular and renal side effects associated with the NSAIDs class limit the use of this drug in older patients with hATTR-PN or patients with hATTR CM.

There are currently three European Commission (EC) approved therapies available in the European Union (EU) for the treatment of hATTR amyloidosis in adults with polyneuropathy: ONPATTRO (patisiran), TEGSEDI (inotersen) and VYNDAQEL (tafamidis). Patisiran and inotersen act by targeting the production of TTR synthesis in the liver by acting on messenger RNA (mRNA): patisiran through ribonucleic acid interference (RNAi); and inotersen through RNAse H-mediated cleavage. Tafamidis acts by binding to the thyroxine-binding site on TTR to reduce its dissociation into misfolded amyloidogenic monomers. Both ONPATTRO (patisiran) and TEGSEDI (inotersen) are also approved in the United States (US) and Japan. VYNDAQEL (tafamidis), is also approved in Japan for the treatment of polyneuropathy in adults with transthyretin amyloidosis, however it is not approved in the US for this indication. Other treatment approaches currently used in clinical practice for hATTR amyloidosis include: orthotopic liver transplantation (OLT), which eliminates variant TTR from the circulation but does not negate the hepatic production of wtTTR, and another TTR tetramer stabilizer (diflunisal).

2.2. About the product

The initially claimed indication was :

- Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with polyneuropathy

and the final agreed indication is :

- Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with stage 1 or stage 2 polyneuropathy

Vutrisiran is an RNAi therapeutic comprised of a synthetic, chemically modified, double-stranded small interfering RNA (siRNA) that specifically targets variant and wtTTR messenger RNA (mRNA). Reduction of both variant and wtTTR production in the liver, which are the fundamental pathogenic proteins causing hATTR amyloidosis, will reduce ongoing deposition of amyloid deposits and potentially allow for clearance of existing deposits; thus, halting or reversing disease progression.

2.3. Type of Application and aspects on development

The legal basis for this application refers to:

- Article 8.3 of Directive 2001/83/EC - complete and independent application

The applicant did not request an accelerated assessment.

Proof of GMP compliance for all manufacturing and testing sites is available. No inspection was deemed required.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as a sterile solution for subcutaneous injection in a pre-filled syringe containing 25 mg vutrisiran as active substance. Each pre-filled syringe contains vutrisiran sodium equivalent to 25 mg vutrisiran in 0.5 mL solution.

Other ingredients are: sodium dihydrogen phosphate dihydrate, disodium phosphate dihydrate, sodium chloride, water for injections, sodium hydroxide (for pH adjustment), phosphoric acid (for pH adjustment).

The product is available as 0.5 mL solution in a pre-filled syringe (Type I glass) with stainless steel 29-gauge needle with a needle shield as described in section 6.5 of the SmPC.

2.4.2. Active Substance

2.4.2.1. General information

Vutrisiran is a chemically synthesised double-stranded ribonucleic acid (RNA) molecule containing a combination of 2'-F and 2'-OMe nucleotides. The two single strands that form the double stranded RNA molecule are the sense strand A-131354, and the antisense strand A-131359. The sense strand contains 21 and the antisense strand 23 nucleotides, respectively, which results upon hybridisation between the complementary strands in a two base overhang at the 3’ end of the antisense strand. The sense strand contains two phosphorothioate linkages at the 5’ end while the antisense strand contains four, two each at the 3’ and the 5’ end. The 3’ end of the antisense strand is conjugated to the triantennary N-acetylgalactosamine (GalNAc) moiety.

The chemical names of vutrisiran sense and antisense strands are:

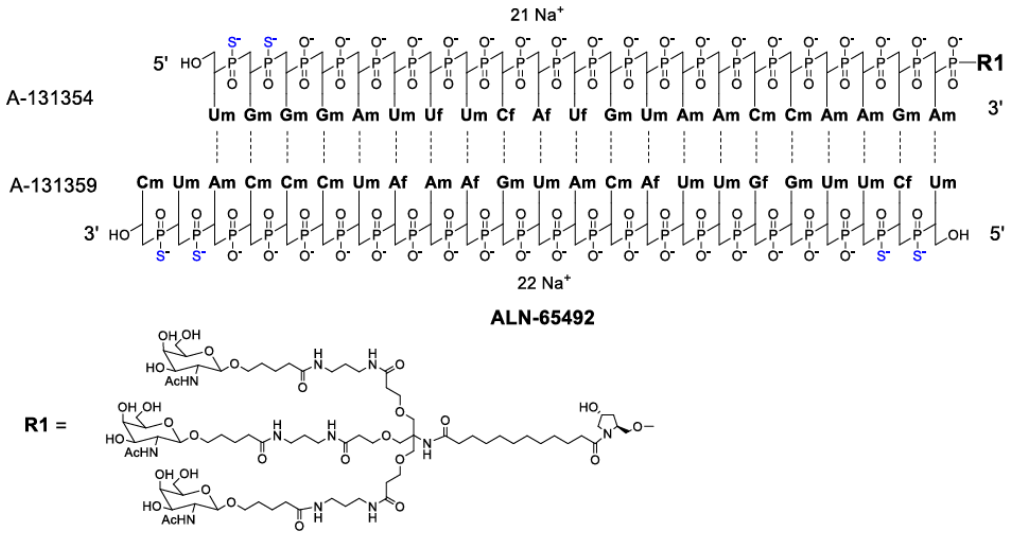
Sense Strand (A-131354): 2'-*O*-methyl-P-thiouridylyl-(3'-5')-2'-*O*-methyl-P-thioguanylyl-(3'-5')-2'-*O*-methylguanylyl-(3'-5')-2'-*O*-methylguanylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-deoxy-2'-fluorouridylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-deoxy-2'-fluorocytidylyl-(3'-5')-2'-deoxy-2'-fluoroadenylyl-(3'-5')-2'-deoxy-2'-fluorouridylyl-(3'-5')-2'-*O*-methylguanylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methylcytidylyl-(3'-5')-2'-*O*-methylcytidylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methylguanylyl-(3'-5')-2'-*O*-methyl-3'-adenylate-{(2S,4R)-1-{1-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)oxy]-16,16-bis-({3-[(3-{5-[(2-acetamido-2-deoxy-β-D-galactopyranosyl)oxy]pentanamido}propyl)amino]-3-oxopropoxy}methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oyl}-4-hydroxypyrrolidin-2-yl}methyl hydrogen, 21 sodium salt

base-paired with

Antisense Strand (A-131359): 2'-*O*-methyluridine-(3'-5')-2'-deoxy-2'-fluoro-P-thiocytidylyl-(3'-5')-2'-*O*-methyl-P-thiouridylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-*O*-methylguanylyl-(3'-5')-2'-deoxy-2'-fluoroguanylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-deoxy-2'-fluoroadenylyl-(3'-5')-2'-*O*-methylcytidylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-*O*-methylguanylyl-(3'-5')-2'-deoxy-2'-fluoroadenylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-deoxy-2'-fluoroadenylyl-(3'-5')-2'-*O*-methyluridylyl-(3'-5')-2'-*O*-methylcytidylyl-(3'-5')-2'-*O*-methylcytidylyl-(3'-5')-2'-*O*-methylcytidylyl-(3'-5')-2'-*O*-methyladenylyl-(3'-5')-2'-*O*-methyl-Pthiouridylyl-(3'-5')-2'-*O*-methyl-P-thiocytidylyl, 22 sodium salt

The structure of vutrisiran active substance (AS) can be represented using an expanded structural formula showing the phosphate backbone as shown in Figure 1. The bases involved in base pair formation are connected with a dotted line. All phosphodiester groups are negatively charged with sodium as the counter ion. The structure of R1, the N-acetylgalactosamine (GalNAc) containing moiety is also provided.

Figure 1: Expanded Structural Formula of Vutrisiran



Abbreviations: 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=uracil 2′-*O*-methyluridine; R1=triantennary GalNAc (*N*-acetylgalactosamine)

The molecular formula and mass of the vutrisiran and the two single strands are provided in Table 1.

Table 1: Molecular Formula and Molecular Mass

	Drug Substance	Sense Strand	Antisense Strand
Molecular formula of the free acid	$C_{530}H_{715}F_9N_{171}O_{323}P_{43}S_6$	$C_{296}H_{415}F_4N_{92}O_{171}P_{21}S_2$	$C_{234}H_{300}F_5N_{79}O_{152}P_{22}S_4$
Molecular formula of the sodium salt	$C_{530}H_{672}F_9N_{171}Na_{43}O_{323}P_{43}S_6$	$C_{296}H_{394}F_4N_{92}Na_{21}O_{171}P_{21}S_2$	$C_{234}H_{278}F_5N_{79}Na_{22}O_{152}P_{22}S_4$
Molecular weight of the free acid	16,344.55 Da	8788.55 Da	7556.00 Da
Molecular weight of the sodium salt	17,289.77 Da	9250.17 Da	8039.60 Da

The chemical structures of vutrisiran and the single strands were elucidated and confirmed by a combination of LC-MS, MS-MS sequencing, anion exchange HPLC, FAAS, UV absorption, UV spectroscopy, ^{1}H-/^{13}C-/^{19}F-/^{31}P-NMR, FTIR, thermal dependent UV absorbance, circular dichroism, differential scanning calorimetry and thermogravimetric analysis. The multitude of applied techniques allowed an in-depth characterization of the single and double strand samples. The results were consistent and according to expectations. The nucleotide sequence, including backbone structure and position of the GalNAc moiety, of the single strands was confirmed. The duplex structure of the AS was established.

The active substance is a pale yellow, hygroscopic powder, freely soluble in with a solubility of 361 mg/mL.

The chirality of the naturally occurring 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' endo conformation, RNA molecules adopt the classic A-form as demonstrated by the spectrum of Circular Dichroism.

The stereochemistry is determined by the synthetic method. 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 on the 5'-end, corresponding to four (2^2=4) diastereomers. The diastereomer species of antisense strand and sense strand can be resolved chromatographically by a method presented in the dossier.

2.4.2.1. Manufacture, characterisation and process controls

Vutrisiran AS manufacturing process consists of eight steps. Step 1 through Step 5 consist of the synthesis of the single-strand oligonucleotide by conventional solid-phase synthesis (Step 1), cleavage and deprotection (Step 2), crude ultrafiltration (CUF; Step 3), purification by anion exchange (Step 4), and a second UF (Step 5) for the sense and antisense strands. Each strand is then individually purified and concentrated in Step 3 through Step 5. 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 vutrisiran AS. The batch size has been clearly stated. No reprocessing steps are foreseen.

Protected phosphoramidites are considered suitable starting materials (SMs) for synthetic oligonucleotides. An appropriate justification for the classification of phosphoramidites as starting materials has been provided. Detailed information on the impurity profiles of the phosphoramidite starting materials and their classification into critical and non-critical impurities has been provided. The selection of all other SMs has been adequately justified and supported by data. In summary, the selection of all SMs has been carried out according to the principles of ICH Q11. The selection of the SMs is considered adequately justified taking into account also the manufacturing process which includes numerous cycles, including purification steps, which ensure the proper purge of starting materials' potential related and degradation impurities preventing their carry over to single strands or even final vutrisiran AS. The proposed specifications and analytical methods for the control of SMs are acceptable. Suppliers of the starting materials are mentioned in the dossier. It has been confirmed that the addition of an alternative vendor for the starting materials will be approved by a variation.

The quality of double-stranded oligonucleotides is pre-determined by the quality of the single strand precursors. Therefore, sufficient control of the single strands by adequate specifications is applied. In addition lists of all materials, solvents and reagents used in the AS synthesis were provided in 3.2.S.2.3, and adequate specifications have been provided for each of them. Stability data were provided to support the hold times and conditions proposed within the description of manufacturing process (3.2.S.2.2) and manufacturing process development (3.2.S.2.6) focusing not only on microbiological but also on degradation data.

Process characterization activities to develop the control strategy and commercial manufacturing have been sufficiently described. Risk assessments were performed utilizing historical process understanding from the applicant's manufacturing platform across multiple Alnylam products. Process parameter target set points, normal operating ranges (NOR) and proven acceptable ranges (PAR) were identified using DOE and one factor at a time (OFAT) approaches. For individual unit operations, the desired outcomes from characterization studies were minimization of impurities and maximization of purity and yield. Comprehensive information has been provided for each step of the manufacturing process.

Information on the process control strategy is provided in section 3.2.S.2.4. The critical process steps and associated CPPs for each unit operation and the non-critical process parameters of the manufacturing process were summarised. Proven acceptable ranges (PARs) have been defined. These PARs are acceptable and have been justified in the process development studies. The in-process (IPCs) tests performed at each step for the manufacture of the single strands were described and are considered acceptable.

A total of three full scale batches support the manufacturing process validation. Process validation runs have been conducted under individual protocols for each strand, and cover all unit operations, followed by one protocol for duplex formation, with each vutrisiran AS batch evaluated independently of one another. Protocols (including the protocol for continued process verification (CPV) program) and validation reports were provided.

Information on batch history has been provided and all changes were described in detail and the influence on AS quality has been sufficiently investigated and discussed.

The characterisation of the AS and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

As per ICH Q3A, impurities are classified into organic impurities, inorganic impurities and residual solvents. The organic impurities are further classified into process-related impurities and product-related impurities.

Product-related oligonucleotide impurities are formed during the manufacturing process or during storage, including degradants. These impurities are controlled in the manufacturing process and at long-term storage conditions by two orthogonal HPLC techniques (AX-HPLC and IPRP-HPLC) in the single strands and the final active substance.

Typical impurities are deletion (shortmers) and addition (longmers) impurities, partially deprotected oligonucleotide chains that are not fully deprotected or improperly deprotected during manufacture, phosphodiester (P=O) impurities, where a phosphodiester replaces the thiophosphate (P=S) in the sense and antisense strands, impurities carried over from parent starting material impurities and in particular those associated with the triantennary N-acetyl galactosamine (GalNAc) portion of the sense strand.

In general, a good understanding of the impurity profile in the single strands and the final AS has been demonstrated. Numerous impurities have been identified by LC-MS techniques. Degradation pathways and the impact of annealing on degradation have been sufficiently investigated and discussed. Impurity monitoring is performed on impurities grouped by adjusted RRT ranges which is acceptable for synthetic oligonucleotides with an extremely complex impurity profile. The qualification of impurities has been sufficiently described.

Process related organic impurities are low molecular weight organic impurities such as residual starting materials, reagents and by-products from the manufacturing process. These impurities have been discussed and are considered removed due to extensive washing, chromatographic and ultrafiltration steps.

Residual solvents have been adequately addressed and batch analysis data for 4 batches have been provided.

There are genotoxic substances formed during the manufacture of both single strands, all of which are associated with the synthesis, recirculating cleavage and deprotection steps. The evaluation of the presence of these genotoxic materials in the AS is performed in accordance with the principles stipulated in the ICH M7 guideline. The potential impurities, including potential genotoxic impurities,

were critically discussed. In addition, information on the *in silico* approach to evaluate the potential genotoxic impurities in the AS was included. The respective conclusion on the data obtained in the studies performed was provided.

The AS is packaged in 2 L, gamma-irradiated, high-density polyethylene (HDPE) bottle closed with a polypropylene (PP) screw-top, and a secondary foil laminate bag. The container closure complies with the EU food contact regulations including the Framework Regulation (EC) No. 1935/2004, Regulation (EC) No. 2023/2006 (GMP) and regulation (EU) No. 10/2011 as amended. The certificate of compliance with foodstuff legislation is provided in the dossier.

2.4.2.2. Specification

The active substance specification ncludes tests for appearance (visual), identification by duplex retention time (IPRP-HPLC UV), identification by molecular mass (IPRP-HPLC MS), identification by single strand molecular mass (IPRP-HPLC ESI-MS), identification by Tm (UV), sodium content (Flame AAS), purity ((non-denaturing) IPRP-HPLC UV), purity ((denaturing) IPRP-UPLC UV), impurities ((denaturing) AX-HPLC UV), assay (UV), pH (Ph. Eur.), water content (KF), elemental impurities (ICP-MS), residual solvents (GC), bacterial endotoxins (Ph. Eur.) and bioburden (Ph. Eur.).

All relevant AS attributes are covered in the specification. The proposed acceptance criteria of the AS specification are considered acceptable. Batch release and stability data from the four clinical batches were originally used as basis for setting acceptance criteria.

The specification limits for elemental impurities, residual solvents and bacterial endotoxins were determined according to relevant guidelines and pharmacopoeial requirements and deemed acceptable. Several orthogonal methods are applied to determine identity and purity/product-related impurities. The concept of grouping of impurities as proposed in the specification is acceptable. Process-related impurities are adequately controlled. The specification limits have been partly revised during the procedure. The CHMP recommended and the applicant has committed to re-assess and, if necessary, further tighten the AS specification limits for duplex purity, purity and impurities including specified impurities by denaturing AX-HPLC UV as well as IPRP-UPLC UV, melting temperature, sodium content, assay, pH and water content when there are data available from an additional 10 batches manufactured with the commercial process (REC).

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 and impurities testing has been presented.

Batch analytical data were provided for three nonclinical/research/stability batches, four clinical/stability batches and three commercial scale process performance qualification (PPQ)/clinical/stability batches and relevant CoAs have been presented. The batches showed compliance with the proposed specification.

2.4.2.3. Stability

Stability data on three commercial scale batches stored in the intended commercial packaging for up to 12 months under long term conditions (-20°C ± 5°C) and for 6 months under accelerated conditions (25°C ± 2°C / 60 ± 5%) according to the ICH guidelines were provided.

Samples were tested for assay, purity and impurities by non-denaturing IPRP-HPLC, denaturing AC-HPLC and denaturing IPRP-UPLC, assay and water content. Results obtained showed no relevant changes under any of the abovementioned conditions and no trends were observed.

Supportive stability data were presented for three developmental, and four clinical batches stored under long-term storage conditions (-20°C ± 5°C) for up to 60 months and under accelerated conditions (25°C ± 2°C / 60 ± 5%) for 6 months. Storage during stability testing was performed in a representative container closure system.

The clinical batches showed no relevant changes under any of the studied conditions. The developmental batches showed no changes apart from slight decreases/increases in purity/total impurities by denaturing IPRP-UPLC at 25°C±2°C/60%±5% RH after 6 months and an upward trend in water content by Karl Fischer titrimetry at both the long term an the accelerated storage conditions. Of note, the three developmental batches were intentionally manufactured with an exaggerated impurity profile, and as such the level of degradation could reach the specification limit earlier. These batches were considered to be a worst case to demonstrate product stability.

Photostability testing following the ICH guideline Q1B was performed on one commercial scale batch. Following the exposure, samples were tested for physical appearance, duplex purity by non-denaturing IPRP-HPLC, assay by UV, and purity by denaturing AX-HPLC and IPRP-UPLC, as well as water content and pH. At 1x exposure dose, little to no change was observed in quality attributes tested and the results remained within the specification requirement. At 8x exposure, moderate changes to the DS were observed, mainly resulting in the oxidation of phosphorothioate to phosphate linkages.

The following forced degradation conditions were tested on one of the developmental batches: thermal, acidic, basic, oxidative, and photolytic stress. Two different AS degradation studies were performed to demonstrate that the two denaturing chromatographic methods (AX-HPLC and IPRP-UPLC) are stability-indicating.

Based on the overall presented stability results, the proposed retest period of 36 months at the storage condition of -20°C ± 5°C is acceptable. Stability data obtained from the accelerated conditions also confirm that short excursions outside the recommended storage conditions will not negatively impact vutrisiran AS quality.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

The finished product (FP) is presented as a sterile solution preservative-free, clear, colourless to yellow solution for subcutaneous injection. Each single-use prefilled syringe with passive needle safety system is designed to deliver 0.5 mL vutrisiran solution. The FP is 50 mg/mL vutrisiran (equivalent to 53 mg/mL vutrisiran sodium) solution formulated in 10 mM sodium phosphate with 110 mM sodium chloride. The composition of the FP is presented in section 2.4.1 of this report and in section 6.1 of the SmPC. The excipients and packaging materials are widely used in comparable pharmaceutical products. The vutrisiran finished product (FP) was designed based on the therapeutic hypothesis that vutrisiran sodium, which is a small interfering RNA (siRNA), formulated in a buffered solution (10 mM phosphate buffer containing 110 mM NaCl), administered via subcutaneous (SC) injection will enable delivery of the siRNA to the liver. A comprehensive QTTP has been established. The formulation and the manufacturing process were developed based on the CQAs required for this type of product.

The properties of the active ingredient that may affect the FP manufacturing process are hygroscopicity and solubility. Regarding the solubility of the AS in the aqueous solution, there are no problems as vutrisiran is freely soluble in water. Hygroscopicity may affect the correct concentration of vutrisiran in the FP and for that reason, the concentration is assayed during and at the end of mixing of the bulk solution.

For subcutaneously administered FP, physiological pH, viscosity and osmolality are key parameters for smooth and complete application. Based on studies on the these properties and the impact on the impurity profile, the final formulation has been selected. The commercial formulation has already been used since the phase 3 clinical studies. The discussion on the composition development is considered sufficient.

The FP does not contain overages, however, an overfill is necessary to allow withdrawal of the nominal volume from the pre-filled syringe. Appropriate studies have been performed to justify the overfill and the respective IPC.

The manufacturing process is a common process for aqueous sterile FP that cannot be subjected to terminal sterilisation: buffer formulation and filtration, FP formulation and first filtration, sterile filtration and filling. Steam sterilisation of the FP is not feasible; this is acceptable due to the melting temperature of the vutrisiran duplex structure of 83 °C, which leads to denaturation into single strands.

Following a risk-based approach, manufacturing process development studies were conducted to evaluate mixing, filling, filter size, and compatibility of product contact materials. The overall control strategy was determined based on the results of these studies along with the results of the risk assessment. Detailed lists of critical and non-critical process parameters with limits (target, NOR, PAR) and reference to the respective CQA were presented. The control strategy described in the development section has been fully transferred to the commercial manufacturing process described in section P.3. The manufacturing process development is sufficiently described, and further development studies are not considered necessary for this straightforward process.

Compatibility of the FP with the manufacturing equipment and the CCS has been discussed. In addition, extractables/leachables from the manufacturing equipment materials in contact with the FP have sufficiently been evaluated.

The container closure system (CCS) for the FP consists a Neopak 1 mL, long type I borosilicate glass syringe with a ½", 29G staked needle and a pre-assembled rigid needle shield (RNS) and a bromobutyl rubber, fluoropolymer coated, threaded plunger stopper. The PFS is equipped with a needle guard and an add-on finger flange. The proposed primary packaging systems are standard for injectable formulations. The same CCS was also used in Phase 3 clinical trials.

Sufficient information has been provided for the CCS in terms of protection, security and compatibility. In section 3.2.R the Applicant sufficiently describes the medical device part of the FP with respect to device design, development, risk management and the assembly process and controls. In addition, the required Notified Body Opinion was provided confirming that the CCS is compliance with the applicable GSPRs.

Container closure integrity testing results demonstrate that the primary container closure components prevent microbial ingress under the foreseen storage conditions. The chemical compatibility of the CCS with the FP has been demonstrated in stability studies.

In a simulation study no organic leachables were detected, only traces of inorganic substances. To support the conclusion that the primary packaging materials do not pose a risk to the quality of the FP, the applicant initiated testing for leachables at 30°C/ 75% and 40°C/ 75% RH in accordance with the intended shelf life. Results for leachables testing at 30°C/ 75% for 9 months and 40°C/ 75% RH for 6 months were presented with no results above the reporting limit. The applied safety concern threshold for leachables in vutrisiran FP is in accordance with ICH M7 (4 doses per year with an assumed lifetime of 70 years results in 280 dosing days).

The design and development, performance requirements, design verification and validation for the passive needle safety system assembly process has been described in 3.2.R.2.The FP is designed to maintain sterility during assembly, storage, shipping, and distribution prior to use.

2.4.3.1. Manufacture of the product and process controls

FP manufacturing sites and other sites involved in product (QC) testing, assembly with the passive needle safety system, secondary packaging and labelling, physical importation and batch release for the EEA have been clearly stated. Relevant proof of GMP compliance for all sites as necessary has been provided.

The manufacturing process of the FP comprises three steps: buffer formulation and filtration; finished product formulation with bioburden reduction filtration; and sterile filtration and filling, resulting in the filled syringes followed by labelling, and assembly with needle guard and add-on finger flange and packaging in a thermoform tray to protect the prefilled syringes from damages during transport. The FP manufacturing process as well as the assembly process of the pre-filled syringe (PFS) are adequately described.

Process parameters with target, NOR and PAR as well as in-process controls were sufficiently described in accordance with the control strategy derived from development studies. Details regarding the holding times at various in-process stages during manufacturing of the finished product are provided.

The acceptance criteria for bioburden prior to filtration is in accordance with the Note for Guidance on Manufacture of the Finished Dosage Form (NMT 10 CFU/100 ml).

Both syringe components are received sterile, i.e. ready-to-use. The syringe is sterilised by ethylene oxide and the plunger stopper by gamma radiation. The validated sterilisation conditions applicable to both procedures have been included in the dossier.

Process validations studies on three batches were provided. No major or critical deviations occurred during the three PPQ runs that impacted CPPs, IPCs and the release testing results. All acceptance criteria for the three PPQ batches were met. The validation runs cover the pre-defined range for compounding. Filling of the maximum number of syringes is covered by media fill runs. Results of media fill runs sufficiently demonstrated the maximum filling time for the FP solution into the syringes. The holding times have been sufficiently validated in terms of microbiological and physico-chemical quality and relevant data has been presented. Validation studies for the filters used for bioburden reduction and sterile filtration were performed.

Overall, the manufacturing process is considered sufficiently validated and capable of producing the finished product of intended quality in a reproducible manner.

2.4.3.2. Product specification

The release and shelf-life specifications for the FP includes tests for appearance (visual), appearance: clarity, opalescence and colouration (visible spectrophotometry), identification by duplex retention time (IPRP-HPLC UV), identification by molecular weight (IPRP-HPLC MS), purity ((non-denaturing) IPRP-HPLC UV), purity ((denaturing) AX-HPLC UV), purity ((denaturing) IPRP-UPLC UV), assay (UV), pH (Ph. Eur.), osmolality (Ph. Eur.), particulate matter (Ph. Eur.), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), volume in container (Ph. Eur.), dose uniformity (Ph. Eur.), container closure integrity (dye ingress), mechanical syringe performance (in-house).

The FP specifications include all required tests for a sterile aqueous medicinal product containing double-stranded RNA supplied in a pre-filled syringe.

The specification limits for purity and impurities for release are identical to the limits for the AS. The limits for shelf life are partly higher than the release limits if the evaluation of the stability data has shown susceptibility to changes over time. The specification limits have been revised during the procedure. The CHMP recommended and the applicant has committed to re-evaluate and, if necessary, further tighten the specification limits of the finished product after approval when data from an additional 10 commercial batches are available (REC).

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Test results for the three PPQ batches with a validated test procedure have shown a total elemental impurity content of less than 30% of the PDE for the corresponding elements. Thus it has been concluded that no additional controls are required in the finished product specification.

A risk evaluation on the presence of nitrosamine impurities in the finished product was provided in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk of possible presence of nitrosamine impurities in the active substance or the related finished product was identified. Therefore, no additional control measures are deemed necessary. The nitrosamines risk evaluation report is considered acceptable.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. The finished product is released on the market based on the above release specifications through traditional finished product release testing.

Batch analysis data have been provided on seven commercial scale batches in vials and 8 batches in pre-filled syringes. The 8 batches in pre-filled syringes were manufactured by the commercial manufacturing site. All the test results were within the proposed specification limits demonstrating the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

2.4.3.3. Stability of the product

Stability data on three commercial scale batches stored in the intended commercial packaging for up to 9 months under long term conditions (2-8°C, 25°C / 60% RH, and 30°C / 75% RH) and for 6 months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines were provided. Samples were stored horizontally, with horizontal position being considered worst case due to maximal contact of the aqueous finished product with the plunger and tip cap.

Supportive stability data were presented for three clinical and two further supportive batches stored under the same long-term storage conditions (2-8°C, 25°C / 60% RH and 30°C / 75% RH) for up to 30 months and under accelerated conditions (40°C / 75% RH) for 6 months. Samples were packaged into the syringes proposed for routine storage (except for the needle safety system).

Samples were tested for appearance, purity ((non-denaturing) IPRP-HPLC UV), purity ((denaturing) IPRP-UPLC UV), purity ((denaturing) AX-HPLC UV), assay, pH, osmolality, particulate matter, volume in container, bacterial endotoxins, sterility, container closure integrity and mechanical syringe performance. The test methods used are the same as those for release and are stability indicating. No

significant changes were observed in any of the attributes at either long-term or accelerated conditions and the results met the specifications valid at the time.

A photostability study was conducted in accordance with ICH Q1B on one commercial scale batch. The study results indicate the FP is considered to be photostable.

In addition a study of exposure to thermal cycling for four times between -20°C and 40°C for three consecutive days at each condition showed no adverse effects on FP quality.

Based on the presented data, the proposed shelf life of 2 years and the storage conditions "Do not store above 30°C. Do not freeze.", as stated in the SmPC sections 6.3 and 6.4 are accepted.

2.4.3.4. Adventitious agents

No excipients of human or animal origin are used in the manufacture of the FP.

2.4.4. 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 results of 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.

At the time of the CHMP opinion, there were two minor unresolved quality issue having no impact on the benefit-risk balance of the product related to reassessing the specification limits for the active substance and finished product once additional commercial batches become available. These are proposed as Recommendations for future quality development (see below).

2.4.5. 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.

2.4.6. Recommendations 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:

- to re-assess the active substance specification limits for duplex purity, purity and impurities including specified impurities by denaturing AX-HPLC UV as well as IPRP-UPLC UV, melting temperature, sodium content, assay, pH and water content when there are available data from an additional 10 batches manufactured with the commercial process.

- to re-assess the finished product specification limits for purity and impurities when there are available data from an additional 10 batches manufactured with the commercial process.

2.5. Non-clinical aspects

2.5.1. Introduction

Vutrisiran is a second generation double stranded small interfering ribonucleic acid (siRNA) with several modifications including 2'-fluoro and 2'O-methylated ribose sugars as well as phosphorothioate- substitutions at the 5'-end of the sense strand and both 5'- and 3'-termini of the antisense strand, which have been established to enhance metabolic stability and may suppress activation of the innate immune system (reviewed by Hu B *et al*. Signal Transduct Target Ther. 2020; 5(1): 101). In addition, the 3'end of the sense strand is covalently linked to triantennary *N*-acetylgalactosamine (GalNAc) residues. The GalNAc- moiety overcomes the common inability of hydrophilic and macromolecular oligonucleotides to permeate cellular membranes by enabling specific delivery and endocytosis of the siRNA via the highly conserved and abundant asialoglycoprotein (ASGPR) receptors in the liver (Nair JK *et al*. J Am Chem Soc. 2014; 136(49): 16958-61). While ASGPR are subsequently recycled and available for further uptake of GalNAc-conjugates, vutrisiran is released into the cytoplasm to specifically mediate the degradation of wildtype (wt) and mutant transthyretin (TTR) mRNA by RNA interference. Consequently, the amount of circulating TTR protein is reduced, which inhibits further amyloid deposits thereby preventing deterioration of hereditary transthyretin amyloidosis (hATTR).

The strategy of effective liver targeting and RNA silencing has been earlier developed for the siRNA patisiran that is approved for hATTR as i.v. infusion in a lipid nanoparticle formulation (*patisiran*) and was also established for GalNAc-conjugated siRNA therapeutics, which were recently licensed for different indications (givosiran, lumasiran and inclisiran).

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Vutrisiran was initially identified in a screen for chemically optimized GalNAc-conjugated siRNAs that target the 3'-UTR of wt and mutant TTR transcripts. Bioinformatics and direct sequencing methodologies revealed only one single nucleotide polymorphism in the human TTR target sequence with a negligible allelic frequency of 0.0005 near the 3'-end of the antisense strand of vutrisiran, which unlikely impacts on the specificity or potency of vutrisiran. Accordingly, vutrisiran concentration-dependently inhibited TTR mRNA with IC_{50} values of 0.21 nM or 3.63 nM following either transfection, or free uptake of the siRNA into primary monkey hepatocytes.

As the binding region of vutrisiran in the TTR mRNA is identical between humans and monkeys, but is not conserved in mice, rats and rabbits, non-clinical *in vivo "proof-of-concept"* investigations were restricted to monkeys and transgenic mice expressing the most prevalent human valine to methionine variant of TTR at amino acid 30 (V30M). Following single or repeated once monthly s.c. injections of 0.3 to 3 mg/kg vutrisiran, TTR protein was dose- and time-dependently reduced between -60 % to -98 % of pre-dose levels within 7 to 14 days in TTR V30M transgenic mice and within 21 to 28 days in monkeys. Thereafter, TTR slowly recovered, but remained reduced at less than -20 % to -70 % of baseline values until study terminations on day 185 in mice and day 204 in monkeys. Of note, the TTR reductions after single vutrisiran doses of 10 mg/kg i.v. or 1 mg/kg s.c. in monkeys showed highly similar profiles. The peak TTR declines correlated with comparable C_{max} and AUC_{last} in liver tissue.

In contrast, the lack of pharmacological activity of vutrisiran was confirmed in toxicity studies in rats and rabbits. Conversely, s.c. administration of a rodent-specific vutrisiran orthologue showed effective reductions TTR protein by >95 % in rats.

2.5.2.2. Secondary pharmacodynamic studies

Potential off-target transcripts of vutrisiran had been identified *in silico*. In hepatoma cells *in vitro*, up to 10 nM vutrisiran did not silence five of these possible off-target transcripts with 5 to 8 mismatches compared to the TTR mRNA sequence, while inhibitions of three other mRNAs between 50 to 75 % were observed at higher vutrisiran concentrations up to 100 nM. As vutrisiran decreases TTR mRNA with an IC_{50} of approximately 0.1 nM, the interference with these off-target transcripts at more than 100-fold higher concentrations is of insignificant clinical relevance. Hence, pharmacodynamic interactions studies were not performed.

2.5.2.3. Safety pharmacology programme

Single s.c. vutrisiran doses up to 300 mg/kg, which corresponded to maximum plasma levels of 49.1±7.7 µg/ml, did not impact on cardiovascular function (heart rate, ECG, blood pressure), respiratory rate and body temperature in a combined GLP compliant study in telemetered Cynomolgus monkeys. Likewise, examinations of neurological functions (behaviour, reflexes) at s.c. vutrisiran doses up to 300 mg/kg within repeated-dose toxicity studies in monkeys did not show any abnormalities and followed ICH M3(R2) recommendations and European 3R principles (EMA/CPMP/ICH/286/1995; EMA/CHMP/CVMP/3Rs/742466/2015).

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were performed with vutrisiran due to its specificity for human TTR mRNA that renders the interaction with the expression of other transcripts unlikely.

2.5.3. Pharmacokinetics

The pharmacokinetic properties of vutrisiran have been analysed *in vitro* and after single s.c. compared to i.v. doses in rats and Cynomolgus monkeys *in vivo*. These investigations were complemented by toxicokinetic determinations as indicated below.

Vutrisiran was rapidly absorbed after s.c. administration reaching peak plasma concentrations between 0.5 and 1 h in rats and 1.9 and 2.8 h in monkeys. In both species, the vutrisiran plasma exposure increased dose-proportionally up to 3 mg/kg and greater than dose-proportionally at higher dosages, which was attributed to saturation of ASGPR-mediated uptake of vutrisiran into the liver. No significant accumulation or sex difference was noted upon repeated once monthly s.c. injections in both species. Distinct elimination phases could not be reliably determined in the two species, but estimated plasma half-lives ranged from 2 to 3.3 h in rats and 3.3 to 4.7 h in monkeys.

Vutrisiran showed concentration-dependent plasma protein binding between 75 to 87 % in animals, which is comparable to humans (82 %). No preferential distribution into blood cells was found.

The more than 40-fold higher distribution volume of vutrisiran compared to the total blood volume in rats indicated extensive tissue distribution. In fact, QWBA analysis following s.c. administration of 3 mg/kg ^{3}H-labelled vutrisiran in rats revealed the predominant distribution of radioactivity into the liver, injection site, lymph nodes, thoracic lymphatic duct, and kidneys, but also into the

gastrointestinal tract. By day 56, radioactivity was cleared from all tissues except lymph nodes, kidney cortex and injection site.

The effective hepatic delivery of s.c. dosed vutrisiran was further evident by the consistently higher liver concentrations of vutrisiran compared to plasma and kidneys across mice, rats and monkeys, which lacked any gross differences between sexes. Maximum liver concentrations after s.c. dosing were reached each by 8 and 24 h in rats and monkeys. Thereafter, vutrisiran was eliminated more slowly from liver tissue with half-lives of approximately 4 to 6 days in rats and 20 to 30 days in monkeys.

Vutrisiran was largely stable in plasma of mice, rats, monkeys and humans *in vitro*. In plasma and liver samples of rats and monkeys *in vivo*, vutrisiran metabolites were characterized by either truncation of one or three nucleotides at the 3'-terminus of the antisense strand (= AS(N-1)3' and AS(N-3)3' each) or by sequential cleavage of GalNAc-groups followed by loss of the linker from the 3'-end of the sense strand. At later time points, consecutive exonucleolytic degradation from the 3'-termini of both strands of vutrisiran was additionally observed. Significant cleavage by endonucleases did not occur. Unchanged vutrisiran remained the predominant drug-related material in the liver of rats and monkeys at 14 days and 15 days post dosing, respectively, and was also the main component in urine and bile of intact and bile duct-cannulated rats.

Renal and biliary excretion were identified as predominant routes of elimination of vutrisiran in a mass balance study in intact and bile duct-cannulated rats after single s.c. dosing of ^{3}H-labelled vutrisiran. While 52.5 % of the radioactive vutrisiran dose was detected in urine of intact rats, about 25.8 % and 24 % were recovered in bile and urine of bile duct-cannulated rats. The majority of the urinary radioactivity appeared to be mainly associated with truncated metabolites of vutrisiran, but approximately 3.71 % and 5.75 % of the dose were confirmed as unchanged parent compound in intact and bile duct-cannulated rats. In bile, approximately 9 % and 3 % of the radioactivity were each unveiled as unchanged vutrisiran and the AS(N-1)3'-metabolite. In monkeys, renal excretion was also determined as primary elimination route of vutrisiran and about 11 % and 24 % of the s.c. administered 1 or 30 mg/kg doses were recovered as unchanged vutrisiran in urine. In both rats and monkeys, faecal excretion was negligible.

Vutrisiran was neither a substrate, nor an inhibitor of major cytochrome P450 enzymes. In pregnant rats and rabbits, vutrisiran was detected in the placenta. In foetal liver or the remainder of foetal carcasses of both species, vutrisiran levels were below the level of quantification indicating that vutrisiran was not transferred to the foetuses. A rodent vutrisiran orthologue was also not quantifiable in the placenta of pregnant rats and in rat foetal tissues. The milk transfer of vutrisiran was not investigated.

2.5.4. Toxicology

The toxicity of vutrisiran was investigated in compliance with GLP and prevailing ICH requirements in repeat-dose toxicity studies with multiple once monthly s.c. administrations for 13 weeks and 6 months in rats as well as 13 weeks and 9 months in monkeys to evaluate exaggerated effects compared to the quarterly therapeutic regimen proposed for hATTR patients. Monkeys were selected as pharmacologically responsive species, whereas rats served to unravel possible off-target effects. Nevertheless, a rodent-specific orthologue of vutrisiran was also tested in a combined dose-range finding (DRF) fertility and developmental toxicity study in rats. In addition, a standard battery of genotoxicity studies and reproductive and developmental toxicity investigations in rats and rabbits were performed. Given the generally mild effects, recovery periods did not follow the dosing phases of the 6 and 9 months toxicity studies. Non-GLP compliant DRF experiments preceded the pivotal

investigations including Tg-rasH2 WT, C57Bl/6 and CD-1 mice to support future carcinogenicity evaluations. In addition, quarterly s.c. injections were compared with more frequent monthly doses in a 6 months exploratory toxicity study in rats to support the intended clinical treatment schedule. Control animals concomitantly received 0.9 % NaCl.

2.5.4.1. Single dose toxicity

Single dose toxicity studies have not been conducted with vutrisiran, which is endorsed in accordance with prevailing ICH and European guidance (EMA/CPMP/ICH/286/1995; EMA/CHMP/SWP/81714/2010).

2.5.4.2. Repeat dose toxicity

In all repeat-dose toxicity studies, no clinical signs, altered coagulation or most clinical chemistry parameters, abnormal organ weights, ophthalmological or macroscopic findings were evident. A relationship of the low mortality incidences in rats with vutrisiran could either be excluded or rated improbable.

All s.c. vutrisiran doses in the toxicity studies in monkeys (≥30 mg/kg/month) reduced TTR serum protein levels as expected without any dose-dependency. TTR decreases between -38.7 to -50.1 % were already evident after the first vutrisiran injection with maximum declines up to -99 % after the third to fourth monthly dose. TTR reductions were maintained in the respective dosing phases and throughout the 12 weeks recovery period of the subchronic toxicity study.

It should be noted that the TTR protein normally interacts with the retinol binding protein 4 (RBP4)-retinol complex to prevent renal clearance of RBP4 and to enable recycling of RBP4 after intracellular retinol release, which is the major pathway for retinoid transport (Li Y *et al*. Hepatobiliary Surg Nutr. 2014; 3(3): 126-39). In addition, TTR protein is known for the limited transport of about 15 % of total human thyroxine T_4 (Buxbaum JN and Reixach N. *Cell Mol Life Sci*. 2009; 66(19): 3095-101). Accordingly, the TTR protein reductions by vutrisiran concomitantly produced sustained decreases of vitamin A by up to -86 to -89 % and thyroxine T_4 (up to -45 %) in monkeys. Nevertheless, no histological abnormalities in retinol-dependent tissues (eyes, thyroid and pituitary) were eminent.

In the liver, mild to moderate centrilobular and multifocal basophilic hepatocellular vacuolation manifested at ≥15 mg/kg in rats, while minimal basophilic granules in Kupffer cells and hepatocytes were identified at ≥30 mg/kg in monkeys. Mitotic figures and karyomegaly were also slightly increased in rat hepatocytes at ≥10 mg/kg. In the 6 months exploratory toxicity study in rats, these liver findings were more pronounced at the end of the recovery period compared to termination of dosing with higher incidence after monthly compared to quarterly s.c. administrations.

Moreover, vacuolated macrophages were detected at minimal incidences in the inguinal lymph nodes at ≥12 mg/kg in rats, whereas mild to moderate amounts were present at ≥30 mg/kg in mesenteric lymph nodes and at ≥100 mg/kg in axillary inguinal and mandibular lymph nodes of monkeys. Vacuolated macrophages partially mixed with inflammatory cells were also enriched at the injection sites as minimal to slight infiltrates at ≥12 mg/kg in rats and ≥30 mg/kg in monkeys.

In accordance with the primary excretion of vutrisiran, minimal to moderate basophilic granules were found in proximal renal tubule epithelia at ≥40 mg/kg in rats, but not in monkeys. Except liver changes, the histological alterations in kidneys, lymph nodes and injections sites were reversible upon cessation of dosing.

No anti-drug-antibodies (ADAs) were discovered in the 6 months toxicity study in rats, whereas eight of the 32 monkeys in the 9 months chronic toxicity study developed ADAs that did not impact on the

pharmacology, toxicokinetic or toxicity of vutrisiran. Of note, two monkeys of the vutrisiran groups and one control monkey even had cross-reactive antibodies prior to first administrations. Only one of these monkeys administered 100 mg/kg vutrisiran showed ADAs until day 29, but not beyond. In the other five monkeys, ADAs were confirmed at the end of dosing in 2 of 8 monkeys of the 30 mg/kg group and 5 of 8 animals of the 100 mg/kg group. As no ADAs were detected in the 300 mg/kg qM high dose group, their development obviously showed no dose relationship. Vutrisiran did not stimulate various cytokines in human whole blood samples *in vitro* and in a 13 weeks DRF toxicity study in monkeys *in vivo*.

Among clinical chemistry parameters, alkaline phosphatase levels were moderately increased upon long-term quarterly dosing of vutrisiran ≥10 mg/kg in rats and in all repeat-dose toxicity studies with monthly injections ≥100 mg/kg in monkeys.

Based on these findings in long-term toxicity studies, the respective high dose levels of 150 mg/kg in rats and 300 mg/kg in monkeys were established as NOAELs.

2.5.4.3. Genotoxicity

Vutrisiran was not genotoxic in an Ames test and a mammalian cell assay in human lymphocytes with and without metabolic activation *in vitro*. *In vivo*, vultrisiran did not induce micronuclei in bone marrow of rats with C_{max} and $AUC_{0-24\ h}$ based exposure margins of 2978x and 4618x for males and 2449x and 3526x for females, respectively. No toxicity occurred up to the highest vutrisiran dose. Hence, vutrisiran is not expected to exert any genotoxic potential.

2.5.4.4. Carcinogenicity

Carcinogenicity investigations were not provided, but 2-year bioassays in rats and CD-1 mice have been initiated. Submission of the results in rats is envisaged by the end of 2021 and in mice at the beginning of 2024.

In preparation for the originally planned 6-month short-term carcinogenicity study, two DRF studies in Tg-rasH2 WT and C57BL/6 mice were conducted. The dose selection followed ICH S1C(R2) recommendations (≤1500 mg/kg). Given the dose-dependent hepatotoxicity in both mouse strains, which might exceed the clearance capacity of the liver and could secondarily promote clinically irrelevant neoplasia, this plan was abandoned.

Therefore, a third DRF study in CD-1 mice was performed to support a life-time carcinogenicity investigation in this strain at doses that provide at least 25× exposure multiples to the anticipated human AUC. The toxicity profile of CD-1 mice was consistent with that of other mouse strains. With respect to the higher dose frequency in mice compared to humans and scaling for species differences based on body surface area, the high dose (MTD 50 mg/kg/monthly) corresponds to 29.2x the clinical dose (25 mg/quarterly). C_{max} and AUC based multiples of exposures were 255x and 64x in males as well as 240x and 50x in females, respectively.

2.5.4.5. Reproductive and developmental toxicity

The reproductive and/or developmental toxicity of s.c. administered vutrisiran was investigated in studies on fertility and early embryonic development in rats, embryo-foetal development in rats and rabbits and on pre-postnatal development in rats.

In the pivotal fertility and early embryonic development study, weekly administration of vutrisiran decreased prostate gland weights and prostate gland secretions, but no effects on sperm parameters

were observed. Hepatotoxicity was noticed in both genders. The paternal NOAEL was established at 15 mg/kg qw and the maternal NOAEL at 30 mg/kg qw. However, vutrisiran did not impact on reproductive performance in either sex or on early embryonic development. The NOAEL for these parameters was the highest dose studied of 70 mg/kg qw (323-fold the MRHD normalised to 0.035 mg/kg/week). Of note, treatment with a single dose of the rodent-specific vutrisiran orthologue induced the anticipated reductions of thyroxine and vitamin A in a combined DRF study on fertility and development in rats. Low levels of this orthologue were determined in maternal kidney samples, but not in maternal liver, placenta or foetal tissues. Although vutrisiran was quantifiable in maternal placenta, it was also not found in foetal livers or the remainder of the carcasses indicating that vutrisiran was not transferred to the foetuses.

In the embryo-foetal development study in rats, vutrisiran elicited maternal toxicity. Macroscopic and microscopic findings were observed in different organs (liver, pancreas and uterus) as well as adverse effects on body weight gain and food consumption. Increased premature delivery, increased post-implantation loss, decreases in viable foetuses and a reduction in foetal body weights were noticed during Caesarean and foetal evaluations. The incidence of skeletal variations was increased in high dose foetuses, but no gross external or visceral anomalies were observed. The NOAEL for maternal toxicity was established at 10 mg/kg/day (323-fold the MRHD normalised to 0.005 mg/kg/day) and for embryo-foetal development at 3 mg/kg/day (97-fold the MRHD normalised to 0.005 mg/kg/day).

In the study on embryo-foetal development in rabbits, vutrisiran did neither affect maternal parameters, nor embryo-foetal development. The NOAEL for maternal toxicity and embryo-foetal development was therefore the high dose of 30 mg/kg/day (1935-fold the MRHD normalised to 0.005 mg/kg/day). Vutrisiran was quantifiable in maternal liver and kidney of pregnant rabbits, whereas low concentrations were found in the placenta. Vutrisiran levels were below the level of quantification in all foetal tissues, indicating that maternal exposure did not result in the transfer of vutrisiran to foetuses. As expected, treatment with vutrisiran did not have any effects on hepatic TTR transcripts confirming that vutrisiran is not pharmacologically active in rabbits.

In a pre-postnatal development study in rats, vutrisiran administered every 6th day showed no effects on the dams as well as on growth and development of the offspring. The NOAEL for the F0 and F1 generation was established at 20 mg/kg (approximately 92-fold the MRHD normalised to 0.035 mg/kg/week). Vutrisiran was not detected in plasma samples of F1 pups exposed to vutrisiran via milk. However, since there was a time gap between maternal vutrisiran administration and plasma sampling in the offspring, the milk transfer as previously shown for other GalNAc-conjugated siRNAs cannot *per se* be excluded for vutrisiran.

2.5.4.6. Toxicokinetic data

Toxicokinetic plasma exposure was determined in plasma following single or repeated s.c. administrations of vutrisiran in repeated-dose toxicity studies in male and female mice, rats and monkeys as well as in pregnant rats and rabbits of the reproduction toxicity program. The vutrisiran levels were also evaluated in the liver and kidneys of mice, rats, rabbits and monkeys.

The toxicokinetic plasma exposure of vutrisiran commonly increased in a greater than dose-proportional manner in mice, rats and monkeys. In all three animal species, vutrisiran did not accumulate in plasma. Differences between sexes were less than 2-fold.

Vutrisiran exposure was generally higher in liver compared to kidneys or plasma. Vutrisiran did not enrich in the liver of rats, whereas mild liver accumulation was observed at elevated doses in mice and monkeys. Across the three species, the kidney exposure of vutrisiran increased more than dose proportionally and was found to accumulate upon prolonged monthly administrations by about 1.5- to

3.2-fold in mice and 3- to 4-fold in rats, respectively. Albeit not specifically determined, kidney accumulation can also be reasonably expected for monkeys, given the 1.6- to 2.6-fold higher vutrisiran liver concentrations upon multiple administrations.

After quarterly compared to once monthly s.c. administrations of the same 50 mg/kg vutrisiran dose in rats, the liver and kidney concentrations were slightly lower but remained detectable throughout the treatment-free recovery period.

2.5.4.7. Local Tolerance

The local tolerance of s.c. administered vutrisiran was evaluated as part of repeat-dose toxicity studies. No dermal irritation was seen in rats. Apart from transient occasional signs of minor erythema and/or oedema in toxicity studies in monkeys, which were apparently related to the administration procedure, no other local intolerabilities or dermal irritation was noted including modified Draize scoring.

2.5.4.8. Other toxicity studies

No other toxicity studies including immunotoxicity, dependence or phototoxic potential were conducted or are deemed necessary. In exploratory investigations, vutrisiran did not induce pro-inflammatory cytokines in plasma samples from monkeys or healthy human donors.

2.5.5. Ecotoxicity/environmental risk assessment

The Applicant provided an environmental risk assessment (ERA) in accordance with the Guideline on the environmental risk assessment of medicinal products for human use (EMEA/CHMP/SWP/4447/00 corr. 2). Vutrisiran is not a PBT substance as log Kow does not exceed 4.5.

The PEC surface water has been refined with prevalence data from the Orphan Designation EU/3/18/2026. The PEC surface water value is below the action limit of 0.01 µg/L. A Phase II environmental fate and effects analysis is not required.

Considering the above data, vutrisiran is not expected to pose a risk to the environment.

Table 2: Summary of main study results

Substance (INN/Invented Name): Vutrisiran			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	< -2.9 at pH 7	Potential PBT N
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	< -2.9 at pH 7	not B
PBT-statement:	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$ refined with prevalence	0.0000027	µg/L	>0.01 threshold N
Other concerns (e.g. chemical class)			N

2.5.6. Discussion on non-clinical aspects

Vutrisiran is a siRNA that specifically decreases hepatic TTR transcript levels by RNA interference. As a consequence, the systemic amounts of TTR protein are reduced, which inhibits the accumulation of amyloid deposits within tissues known to cause hATTR. Due to interspecies differences in the TTR mRNA sequence, vutrisiran is pharmacologically cross-reactive in monkeys, but not in rodents or rabbits. Pharmacodynamic investigations including human cell lines, primary monkey hepatocytes and *in vivo* studies in transgenic mice carrying the most prevalent human V30M TTR variant or in monkeys demonstrated the specific dose- and time-dependent reductions of TTR protein. The sequence specificity of vutrisiran was confirmed *in silico* and *in vitro*, which did not reveal any binding of vutrisiran to transcripts with fewer than three mismatches, while the interaction with mRNAs harbouring 5 to 8 mismatches was only detected at clinically irrelevant concentrations. In addition, the PK/PD relationship of vutrisiran supports the suitability of the s.c. administration route and was verified at elevated doses in toxicity studies in monkeys.

Vutrisiran did not affect cardiovascular, respiratory and neurological functions in monkeys and rats of the toxicology program, which coincides with safety pharmacological results of other GalNAc-conjugated siRNAs (Sutherland JE *et al.* Nucleic Acid Ther. 2020; 30(1): 33-49; EPARs of givosiran (EMEA/H/C/4775), lumasiran (EMEA/H/C/5040) and inclisiran (EMEA/H/C/5333)). The potential inhibition of hERG currents was not analysed *in vitro*, which is accepted, because the large molecular size of oligonucleotide drugs likely precludes their interaction with the pore of cardiac ion channels (Berman CL *et al*. Nucleic Acid Ther. 2014; 24(4): 291-301).

The absorption, distribution and plasma protein binding capabilities, metabolism as well as excretion profiles of vutrisiran corroborate its effective liver targeting and enhanced metabolic stability against nucleolytic degradation. These pharmacokinetic characteristics of vutrisiran essentially coincide with those of other licensed GalNAc-conjugated siRNAs, which contain similar chemical modifications (see Agarwal S *et al*. Nucleic Acid Ther. 2021; 31(4): 309-315; EPARs of givosiran (EMEA/H/C/4775), lumasiran (EMEA/H/C/5040) and inclisiran (EMEA/H/C/5333)). The lack of any interaction with major cytochrome P450 enzymes is also supported by *in vitro* data obtained for these siRNA-GalNAc conjugates suggesting that this class of agents does not act as substrate, inhibitor or inducer of major CYPs or drug transporters (Ramsden D *et al*. Drug Metab Dispos. 2019; 47(10): 1183-1194). The suitability of quarterly compared to former monthly injections has been further demonstrated in an exploratory study in rats, which, hence, supports the clinical s.c. injection schedule once every 3 months proposed for human therapy.

In all repeat-dose toxicity studies in rats and monkeys, mild but widely consistent non-adverse histological changes in the liver, kidneys, lymph nodes and injection sites were identified, which match the principal distribution and accumulation sites of vutrisiran. The mild temporary injection site reactions coincide with observations in clinical trials of vutrisiran (pain, erythema, bruising, pruritus, warmth; see section 4.8 of the SmPC). Thus, no particular concerns arise from non-clinical studies with respect to the local tolerability of vutrisiran.

The comparison of monthly and quarterly s.c. vutrisiran administrations in a 6 months exploratory toxicity study in rats also indicated that the dosing frequency impacts on the incidences of liver alterations, which correlated with higher vutrisiran liver concentrations following monthly vs. quarterly injections. No prominent off-target toxicities were solely restricted to rats. Nonetheless, rats were generally more sensitive than monkeys.

No antigenicity studies were conducted with vutrisiran, but ADAs were evaluated using a validated method in the rat 6 month and monkey 9 month repeat-dose toxicity studies. ADAs did not emerge in rats, but developed in the 9 months toxicity study in monkeys independently of the administered

vutrisiran dose. The ADAs are apparently not neutralising, because they did not impact on the pharmacodynamic, pharmacokinetic or toxicological properties of vutrisiran and were also determined at low incidences in the clinical program of vutrisiran without any effect on TTR reductions (see section 3.3 below). The possible development of ADAs has been considered in section 4.8 of the proposed SmPC.

Long-term quarterly s.c. vutrisiran doses ≥10 mg/kg in rats and monthly injections ≥100 mg/kg in all repeat-dose toxicity studies in monkeys moderately increased alkaline phosphatase levels. As the elimination of alkaline phosphatase was earlier correlated with hepatic galactose receptors (Blom E *et al*. Clin Chim Acta. 1998; 270(2): 125-37), these elevations might be possibly related to the slower clearance of alkaline phosphatase from the circulation because of its competition with vutrisiran in binding to hepatic ASGPR.

Given the overall mild toxicity findings, it is agreed that the respective vutrisiran high doses of the 6 months and 9 months toxicity studies in rats and monkeys can be considered as NOAELs. These NOAELs provide huge safety margins >1000-fold in rats and >3000-fold in monkeys when the respective exposure is normalized to quarterly dosing with regard to the anticipated levels at the maximum recommended human dose (MRHD). The safety margins remain even sizeable at the respective low dose levels in rats and monkeys or when dose normalisations would be omitted.

The observed effects of vutrisiran in repeat-dose toxicity investigations and the lack of any genotoxic potential in an ICH S2(R1) compliant standard battery of investigations (EMA/CHMP/ICH/126642/2008) highly correlate with former results obtained for other members of the class of GalNAc-conjugated siRNAs including the approved givosiran, lumasiran and inclisiran (Janas *et al*. Toxicol Pathol. 2018; 46(7): 735-745; Sutherland JE *et al.* Nucleic Acid Ther. 2020; 30(1): 33-49; EPARs of *"Givlaari"* (EMEA/H/C/4775), *"Oxlumo"* (EMEA/H/C/5040) and *"Leqvio"* (EMEA/H/C/5333)).

Carcinogenicity studies were not submitted and are presently ongoing in rats and mice. Considering the proposed vutrisiran treatment of a serious indication with limited life-expectancy, the delayed completion of carcinogenicity studies post approval has been previously accepted (EMEA/H/SA/3876/1/2018/PA/III, 2018). Nevertheless, it was suggested to reconsider the need for carcinogenicity testing at all using the weight-of-evidence gained from other siRNA-based therapeutics with the same target and/or similar backbone modifications. This discussion was, however, not provided. The lack of information concerning carcinogenicity testing has been included in section 5.3 of the SmPC, while the schedule proposed by the Applicant for submission of the corresponding reports of both carcinogenicity studies has been considered as post-authorisation recommendation (see section 8.2 below).

In reproductive and developmental toxicity studies, vutrisiran did not affect male and female fertility and early embryonic development at more than 300-fold higher exposures in rats when normalized to human levels at the MRHD. Embryo-foetal and pre/postnatal development was not impaired at >90-fold higher levels in rats and at >1900-fold in rabbits compared to normalized exposures at the MRHD. However, vutrisiran is not pharmacologically active in rats and rabbits. Consequently, no effects on TTR or vitamin A serum levels could be observed, except in one dose group of a combined fertility and early embryonic development DRF study in rats treated with a rodent-specific orthologue. This limits the predictive value of these reproduction toxicity studies in terms of human safety. The TTR/RBP4 complex constitutes the major carrier for retinoids in humans (~95 %; Li Y *et al*. Hepatobiliary Surg Nutr. 2014; 3(3): 126-39). Retinoids are fundamental for normal embryonic development and require balanced levels, because both excess dietary and insufficient vitamin A may cause similar patterns of teratogenic malformations (reviewed by Zile MH. J Nutr. 1998; 128 (2 Suppl): 455S-458S; Collins MD and Mao GE. Annu. Rev. Pharmacol. Toxicol. 1999; 39: 399–430; Ross SA *et al*. Phys. Rev. 2000; 80(3): 1021-1054). There may be additionally different species sensitivities towards retinoids because of altered

pharmacokinetics in humans and monkeys compared to rodents and rabbits (Nau H. J Am Acad Dermatol. 2001; 45: S183-7).

Vutrisiran PEC surfacewater 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. Therefore, vutrisiran is not expected to pose a risk to the environment.

In accordance with the specific mechanism of action of vutrisiran and the weight-of-evidence approaches recommended by pertinent ICH and European guidelines (CHMP/167235/2004; EMEA/CHMP/SWP/94227/2004; EMA/CHMP/ICH/752211/2012), it is accepted that no other toxicity studies including evaluations of the immunotoxicity, dependence or phototoxic potential have been performed. Consistent with the outcome of repeat-dose toxicity studies, no immunostimulatory potential of vutrisiran was evident in exploratory investigations.

2.5.7. Conclusion on the non-clinical aspects

Vutrisiran effectively silenced TTR transcript levels in monkeys and human transgenic mice. The general pharmacokinetic and toxicological properties of vutrisiran coincide with earlier experience gained with other members of this class of GalNAc-conjugated siRNAs.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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**

Table 3 (modified from Module 5.2 Tabular Listing of All Clinical Studies, Module 2.5 Clinical Overview and Module 2.7.2 Summary of Clinical Pharmacology): Listing of Clinical Studies

Study Identifier	Study Title and design	Study population	Duration	Vutrisiran and Patisiran Presentation(s)[a]/ Formulation[b] and Dose
ALN-TTRSC02-001 *Completed* *Data lock: 13 February 2018*	A Phase 1, Randomized, Single-Blind, Placebo Controlled, Single-Ascending Dose, Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Study of Subcutaneously Administered ALN-TTRSC02 in Healthy Subjects	N=80, healthy subjects N=60 on vutrisiran N=20 on placebo	Up to 4.7 months (including screening) with maximum 8 months after last postdose follow-up if necessary	Solution for injection, 0.5 mL in 2 mL glass vial administered with syringe Formulated in water for injection Single SC injection of 5, 25, 50, 100, 200, and 300 mg

Study Identifier	Study Title and design	Study population	Duration	Vutrisiran and Patisiran Presentation(s)[a]/ Formulation[b] and Dose
	1 clinical study center in the United Kingdom			
ALN-TTRSC02-002 *Ongoing* *Primary analysis (18-month Treatment Period) completed* *Data cut-off:* *26 August 2021*	HELIOS-A: A Phase 3 Global, Randomized, Open-label Study to Evaluate the Efficacy and Safety of ALN-TTRSC02 in Patients with Hereditary Transthyretin Amyloidosis (hATTR Amyloidosis) 57 centers across 22 countries	N=164, patients with hATTR amyloidosis N=122 on vutrisiran N=42 on patisiran **vs** external control, the placebo group of the APOLLO study (N=77)	Duration of main phase: 18 months Treatment Period (ongoing) Duration of Run-in phase: not applicable Duration of Extension phase: 18 months Treatment Extension Period (ongoing)	Vutrisiran: 25 mg SC injection administered q3M Solution for injection, 0.5 mL in 2 mL glass vial administered with syringe; Solution for injection, 0.5 mL in prefilled syringes with passive needle safety system. Formulated in 10 mM sodium phosphate and 110 mM sodium chloride, pH 7 Patisiran (reference comparator): 0.3 mg/kg IV infusion administered q3w
External Control/Reference Population ALN-TTR02-004 Completed (17 August 2017)	APOLLO: Phase 3, randomized (2:1), DB, placebo controlled study. Primary analysis at M18. In 19 countries	225 patients: 148 patisiran and 77 placebo patients	Duration of main phase: 18 months Treatment Period	q3w IV dose of patisiran 0.3 mg/kg (patisiran group) or placebo (placebo group). All patients received premedication with corticosteroid, anti-histamines and paracetamol 60 minutes prior to the infusion

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Vutrisiran pharmacokinetics were investigated after single ascending doses in Study 001 in healthy subjects and after multiple-dose administration in HELIOS-A in patients with hATTR amyloidosis.

Analytical methods

The PK of vutrisiran was evaluated by monitoring the antisense strand of vutrisiran in plasma and urine (Study 001) and in plasma (HELIOS-A) using validated liquid chromatography/tandem mass

spectrometry-high resolution accurate mass (LC/MS-HRAM) assays. Vutrisiran concentrations were reported as the full-length double-stranded siRNA based on antisense concentrations. It was clarified that the assay measures the concentration of the sense strand with covalently linked GalNAc moiety. An assay range of 10 ng/mL to 1000 ng/mL was validated and QC were used according to the relevant guidelines. Adequate accuracy and precision were observed for calibrators and controls in the validation. The assays were also validated for extraction recovery, dilution linearity, matrix effects, processed sample stability, hemolysis and lipemic interference (plasma only), whole blood stability, batch size, and injection carryover. Long term storage stability in plasma was demonstrated for 472 days when stored at -20°C and 540 days when stored at -70°C. Long term storage stability in urine treated with 0.1% CHAPS was 21 days when stored at -20°C and 314 days when stored at-70°C and for urine treated with 1.0% CHAPS was 21 days when stored at -20°C and 320 days when stored at-70°C. Incurred sample re-analysis was performed on 11.4% of plasma samples of study 001, and 95.0% of the samples met the prespecified criteria. All studies plasma samples were analysed within the validated storage stability. Regarding urine samples, ISR was performed on 10.4% of study samples, and 82.1% of the samples met the pre-specified criteria. All study samples were analysed within 598 days of collection. The 600-day urine frozen stability was established in amendment 2 of validation report TSLR15-252. Vutrisiran is stable in urine at -70 °C for 600 days.

A validated ELISA was used to assess the immunogenicity of vutrisiran by detecting serum immunoglobulin (Ig) G (IgG)/IgM anti-drug antibodies (ADA) against vutrisiran. The assay detects ADA against the entire molecule, ie, vutrisiran drug substance including the GalNAc moiety, and the linker. Since full length vutrisiran siRNA is used as the capturing target antigen, this ELISA method is expected to detect ADA against vutrisiran, as well as shorter oligonucleotides, linker, and GalNAc moieties. Phosphorylated vutrisiran was covalently linked to the assay plate without blocking the antigenic epitopes of vutrisiran. Captured ADA in human serum (or positive control serum) was detected using goat anti-human IgG/IgM (or anti-rabbit IgG) detection antibodies conjugated to horseradish peroxidase. A substrate (3,3’,5,5’-tetramethylbenzidine [TMB]) was added to generate a chromophore and the absorbance (A450nm) was measured to determine the level of human anti-vutrisiran IgG and IgM antibodies present in the serum samples. Samples were first analyzed with a screening assay and samples that showed positive ADA based on screening assays were further evaluated in a confirmatory assay. Only samples that were positive in a confirmatory assay were deemed to be ADA-positive. The method showed a sensitivity of 90.6 ng/mL and a precision with a %CV <25%.

Methods for assessing the PK of patisiran were identical to those that were previously submitted to support the marketing approval of patisiran.

Absorption

In humans, vutrisiran was rapidly absorbed from the SC injection site into plasma with a median t_{max} ranging from 2 to 6.6 hours. There was low-to-moderate variability in plasma exposure of vutrisiran after SC dosing. There was no accumulation of vutrisiran in plasma after q3M dosing of 25 mg. Absolute bioavailability of vutrisiran after SC dosing in humans has not been determined. Based on a non-clinical mass balance study, 100% bioavailability is expected given the near complete absorption of radioactivity from the injection site into the systemic circulation within 24 hours.

Distribution

More than 80% of vutrisiran is bound to human plasma proteins in vitro, at concentrations close to those observed with the 25 mg dose in clinic (mean C_{max} of approximately 0.1 µg/mL). In vitro protein

binding was concentration-dependent and decreased with increasing vutrisiran concentrations (77.9% at 0.5 µg/mL to 19.0% at 50 µg/mL). The population estimate for the apparent central compartment volume of distribution (Vc) of vutrisiran in humans was 10.07 L (RSE=5.76%).

Vutrisiran is expected to predominantly distribute to the liver in humans, as was observed in rats and monkeys. Indirect evidence of targeted liver distribution in humans is evident from the significant PD effect observed at doses as low as 5 mg/kg. A human ADME study of vutrisiran was not conducted due to concerns of prolonged exposure to radioactivity in the liver and the associated potential health hazard.

Elimination

Metabolite profiling in human plasma samples indicates that there are no detectable circulating metabolites of vutrisiran. In plasma, full-length vutrisiran was the only drug-related material observed. In urine, full-length vutrisiran represented 98% of the total drug recovered while AS(N-3)3' vutrisiran was determined to be a minor metabolite (2% of total administered dose) excreted in urine. Within hepatocytes, vutrisiran is primarily metabolized by endo- and exonucleases to short nucleotide fragments of varying sizes within the liver. In vitro studies indicate that vutrisiran is not a substrate of CYP enzymes.

In Study 001 the mean $t_{½}$ ranged from 4 to 7.5 hours. After reaching C_{max}, vutrisiran concentrations declined rapidly to the LLOQ within 24 to 48 hours. The mean apparent total body clearance (CL/F) ranged from 17.8 to 31.0 L/h across doses. The mean fraction of unchanged drug eliminated in urine over 24 hours (Fe_{0-24}) ranged from 15.4 to 25.4%. Mean CL/F ranged from 17.8 to 31.0 L/h and the mean renal clearance (CL_R) ranged from 4.45 to 5.74 L/h across the dose levels tested (25 to 300 mg), indicating that CL_R accounts for 15.7 to 27.5% of total clearance. These data indicate that in humans renal clearance is not a major route of elimination of vutrisiran.

In HELIOS-A individual t_{max} values ranged from 2 to 6.6 hours. After reaching C_{max} plasma concentrations declined rapidly, reaching LLOQ by 24 hours in a majority of patients. At the recommended dose regimen of 25 mg q3M in humans, the population estimate for mean $t_{½}$ was 6.29 hours and CL/F was 21.6 L/h. For a 70 kg patient with normal renal function, model-estimated hepatic uptake clearance (CL_H) and renal clearance (CL_R) were 14.6 and 5.4 L/h, respectively.

Dose proportionality and time dependencies

Within the single ascending dose study a dose proportional increase in C_{max} was found while AUC_{last} and AUC_{inf} showed a slightly greater than dose-proportional increase across the dose range studied. Vutrisiran plasma concentrations over time were similar on Days 1 and 253, consequently, C_{max} and area under the concentration-time curve from 0 to 24 hours (AUC_{0-24}) values at steady state (Day 253) were similar to their respective first dose values, indicating an absence of accumulation of vutrisiran in plasma after q3M dosing of 25 mg.

Special populations

Population PK Analysis

The PK of vutrisiran after single and multiple doses of vutrisiran in healthy subjects and patients was analyzed within a modelling framework using pooled data from Study 001 and HELIOS-A. Covariate effects of age, health status (healthy subjects versus hATTR amyloidosis patients), sex, race, presence of ADA, Hispanic or Japanese ethnicity, and mild hepatic impairment did not impact PK of vutrisiran.

There was no significant impact of impaired renal function, with <25% higher predicted C_{max} and AUC_{0-24} in patients with mild or moderate renal impairment compared to normal renal function. Body weight and vutrisiran presentation (PFS-S versus vial with syringe) were significant covariates on Ka. Lower body weight and PFS-S administration were both associated with faster absorption and a higher C_{max}. However, the range of individual C_{max} values across body weights, with either vutrisiran presentation, overlapped with no distinct separation of individual C_{max} values.

None of the evaluated covariates were deemed to be clinically relevant; therefore, no dose adjustments are recommended based on demographics or vutrisiran presentation (PFS-S versus vial with syringe). The population PK analysis supports the adequacy of the proposed dosing regimen of 25 mg q3M for all subgroups of patients with ATTR amyloidosis.

Intra- and inter-individual variability

In the population PK analysis the inter-individual variability of hepatic clearance, which represented 73% of total clearance, was 33.5%. The absorption rate constant Ka varied about 39.7% inter-individually.

Pharmacokinetics in target population

Vutrisiran PK profiles and exposures were similar in healthy subjects and patients with hATTR amyloidosis. Across studies and dose levels, the median t_{max} ranged from 2 to 6.6 hours post-dose and mean t½ ranged from 4 to 7.5 hours. From the population PK analysis, the following PK parameters were derived: For a 70-kg patient with normal renal function (eGFR of 90 mL/min/1.73 m2), the model-estimated total plasma clearance was 20 L/h, with a portion of hepatic clearance of 14.6 L/h, representing 73% of total clearance, and renal clearance of 5.4 L/h (equal to eGFR), representing 27% of total clearance. The estimated K_a was 0.1423 h^{-1} for doses administered with vial with syringe and 0.2346 h^{-1} for doses administered with PFS-S.

Pharmacokinetic interaction studies

In-vitro studies showed that vutrisiran was not a substrate and did not inhibit any of the major cytochromes nor was it a substrate of any major drug transporters. Overall, the data suggest a low potential for DDI with CYPs or transporters; therefore, no clinical interaction studies were warranted.

2.6.2.2. Pharmacodynamics

Pharmacodynamic effects of vutrisiran have been investigated in single-ascending dose FIH trial as well as the pivotal study in patients. The overall clinical development rational for vutrisiran was to find a dosing schedule that shows PD activity that is comparable to patisiran but where the drug is given less frequent. Pronounced and prolonged primary and secondary pharmacodynamic effects can be observed even after single doses as low as 5 mg. The biomarkers of pharmacodynamic activity confirmed, that a stable and durable reduction of TTR comparable to patisiran is possible with 25 mg vutrisiran given subcutaneously once every three months.

Mechanism of action

Vutrisiran is an RNAi therapeutic comprised of a synthetic, chemically modified, double-stranded siRNA. It contains an N-acetylgalactosamine (GalNAc) ligand allowing rapid and specific delivery to hepatocytes via uptake by the asialoglycoprotein receptor (ASGPR). Upon delivery to the liver, vutrisiran uses the naturally occurring RNAi pathway to specifically target and silence TTR mRNA.

Reduction of both variant and wt TTR production in the liver will reduce ongoing deposition of amyloid deposits and potentially allow for clearance of existing deposits, thus halting or reversing disease progression. The therapeutic hypothesis for vutrisiran is supported by clinical data from the patisiran APOLLO study.

Primary and Secondary pharmacology

Primary pharmacology

Single SC doses of vutrisiran (5 to 300 mg) in healthy adult subjects reduced serum TTR in a dose-dependent manner; higher doses achieved a faster onset and more durable suppression of serum TTR levels. The PD effect of 25 mg vutrisiran was comparable across Study 001 and HELIOS-A, with a similar magnitude of TTR reduction from baseline at 90 days post-dose (median of 81.8% in Study 001 versus 73.6% in HELIOS-A). Upon repeat q3M dosing in HELIOS-A, further TTR reductions were achieved. At the time of the endpoint mNIS+7 (9 months, are not formally the primary endpoint for EU) about 95% of predicted steady-state reduction had been achieved. This increased further to 99% at Month 18. Peak, through and time-average values also appear more consistent at the later time points.

Only a very limited number of patients (3 in the vutrisiran group in HELIOS-A) showed insufficient pharmacodynamic response and overall inconsistent values. The reasons for this are not known. In healthy volunteers TTR reductions were comparable between Japanese and Non-Japanese subjects.

Secondary pharmacology

Since TTR serves as a carrier for vitamin A, as a secondary effect dose-dependent decreases in serum vitamin A levels were observed. Serum vitamin A profiles paralleled changes in serum TTR and a significant correlation was found between TTR and vitamin A reduction (correlation coefficient of 0.9388; $p<0.0001$). Vitamin A reductions in Japanese subjects mirrored those in the Non-Japanese population.

Across Study 001 and HELIOS-A, the incidence of treatment-emergent ADA due to vutrisiran was 2.2% (4/179). The presence of ADA had no impact on the PK, PD, efficacy, or safety of vutrisiran.

Based on Study 001 results, vutrisiran has no effect on QTc interval at supratherapeutic plasma concentrations approximately 12-fold higher than the mean C_{max} of vutrisiran at the clinically relevant dose of 25 mg. No relationship was observed between plasma concentration and QTcF in time-matched samples, indicating a lack of effect of vutrisiran on QTc.

Dose-response relationship

Single SC doses of vutrisiran reduced serum TTR in a dose-dependent manner, with higher doses achieving faster, greater magnitude, and more durable suppression of serum TTR levels with reduced inter-patient variability. A semi-mechanistic population PK/PD model was developed with data from healthy subjects to quantify the relationship between vutrisiran dose and serum TTR reduction. With repeat dosing, at steady state the 25 mg q3M regimen was predicted to achieve median trough TTR reduction of 85.1% at Month 18, which was similar to the observed median trough TTR reductions of 81% with patisiran at Months 9 and 18. In HELIOS-A, the observed median trough TTR percent reduction at Month 9 was 84.7 %, consistent with the model-predicted magnitude of TTR reduction of 84.3% with vutrisiran 25 mg administered q3M.

PK/PD and PD-Efficacy Relationship

Population PK/PD analyses in healthy subjects and hATTR amyloidosis patients (n=202) demonstrated a relationship between predicted vutrisiran liver concentrations and reductions in serum TTR. Covariate analyses showed similar TTR reductions in patients with mild to moderate renal impairment or mild hepatic impairment, as well as by sex, race, prior use of TTR stabilizers, TTR genotype, ADA status, delay in dosing due to COVID-19 pandemic, vutrisiran presentation (vial with syringe versus prefilled syringe with passive needle safety system [PFS-S]), and across a wide age and body weight range. The population PK/PD model supports the use of vutrisiran 25 mg administered q3M in all patients with ATTR amyloidosis.

The disease progression model was developed to quantitatively describe mNIS+7 change over time as a function of serum TTR levels in patients with hATTR amyloidosis. Over 9 months, a median +12.9 change in mNIS+7 is predicted in placebo treated patients and a median -0.654 change in mNIS+7 is predicted in vutrisiran-treated patients (median TTR reduction 85.4%). Over 18 months, a median +27.4 change in mNIS+7 is predicted in placebo-treated patients and a median -2.15 change in mNIS+7 is predicted in vutrisiran-treated patients (median TTR reduction 88.7%).

Vutrisiran significantly reduced disease progression across all subgroups evaluated in the model, which included sex, race, TTR genotype, prior TTR stabilizers use, symptom onset age at less than 50 years, treatment group (vutrisiran versus patisiran), delay in dosing due to COVID-19 pandemic, age, body weight, baseline serum TTR, and baseline mNIS+7. The disease progression model supports the use of 25 mg vutrisiran administered q3M in all patients with hATTR amyloidosis.

2.6.3. Discussion on clinical pharmacology

Vutrisiran has been developed with the expressed goal of replicating the pharmacodynamic effect of patisiran, but with a less burdensome treatment regime. Therefore, the clinical pharmacology program was rather compact and consisted of only two studies overall. In the First-In-Human study (Study 001) single ascending doses were given that exceeded the clinically relevant range. Modelling was then used to determine the dosing regimen for the single pivotal trial (HELIOS-A).

The PK analysis was based on analytical determinations using validated methods that included liquid chromatography/tandem mass spectrometry-high resolution accurate mass (LC/MS-HRAM) assays for the plasma and urine samples. The assay measures the concentration of the sense strand with covalently linked GalNAc moiety and the antisense strand of vutrisiran. Therefore, the existence of the GalNAc moiety was included in the sense strand assay.

Furthermore, regarding the analysis of the urine samples, stability of human urine QC samples after storage at -70°C for 600 days was established, but initially not reported. Amendment 2 of validation report TSLR15-252 confirmed that Vutrisiran is stable in urine at -70 °C for 600 days.

The pharmacokinetic profile was well characterized in Study 001. Vutrisiran is rapidly absorbed after SC injection and cleared from plasma primarily by distribution to the liver. With a half-life of about 4 to 7.5 hours concentrations decline rapidly to below LLOQ with 24 to 48 hours. PK profiles on Day 1 as well as on repeat dosing days when given once every three months are practically identical.

As is common with RNA-based drug products vutrisiran is not a substrate of cytochromes or common drug transporters, therefore there is a very low potential for any drug-drug-interaction. Metabolism is through unspecific endo- and exonucleases to short nucleotide fragments.

No covariates were identified that had clinically relevant effects on vutrisiran PK. These data support a single fixed dose regimen for all patients.

The primary pharmacodynamic effect is the reduction of TTR plasma levels. This has been established as the key predictor of clinical efficacy. Although vutrisiran is cleared rapidly from plasma it shows a prolonged pharmacodynamic effect which is expected to be due to the drug being rather stable within hepatocytes and a resulting extended residence time. The estimated half-life within the liver is about 55 days.

Significant reductions of TTR are found a single dose with peek reductions reached around week 7. With 3-monthly dosing further reductions are achieved with mean time averaged reductions of 83.6 %. Overall peak-to-through fluctuations were low within the observed time frame. Preliminary data post month 9 point to a potentially even further reduction, which was confirmed with additional Month 18 data. Further increases in TTR reduction however seem to have no additional effect on either efficacy or safety values. As TTR is the main transport mechanism for vitamin A corresponding reductions were found with a high correlation between TTR reduction and vitamin A reduction compared to baseline.

The relationship between pharmacodynamics effects and clinical efficacy as well as disease progression as a function of serum TTR level has also been further described with appropriate models.

2.6.4. Conclusions on clinical pharmacology

The pharmacokinetic profile of vutrisiran has been sufficiently characterized after single ascending doses from 5 to 300 mg in healthy volunteers and after 25 mg q3m dosing in patients.

Primary and secondary pharmacodynamic effects of vutrisiran have been investigated in two clinical trials. A dose dependent reduction of TTR with a prolonged effect even after single doses was found. The clinical development rational that was used for vutrisiran was to achieve similar TTR reduction as found with patisiran. Overall a durable and stable reduction of TTR has been established with corresponding secondary reductions of vitamin A, with 99% of predicted steady-state values reached by Month 18.

Overall, the pharmacodynamic effects as well as the dose-response relationship have been well characterized and support use of vutrisiran at the 25 mg 3qM dose.

2.6.5. Clinical efficacy

Vutrisiran (also referred to as ALN-65492 or ALN-TTRSC02) is a second-generation ribonucleic acid interference (RNAi) therapeutic comprised of a synthetic, chemically modified, double-stranded, small interfering RNA (siRNA) that specifically targets transthyretin [TTR] messenger RNA (mRNA) in the liver and is being developed for the treatment of patients with TTR-mediated amyloidosis (ATTR amyloidosis), with this application focusing on patients with hereditary ATTR amyloidosis (hATTR amyloidosis) with polyneuropathy.

Vutrisiran injection for subcutaneous use contains 50 mg/mL vutrisiran (free acid; equivalent to 53 mg/mL vutrisiran sodium, ALN-65492), formulated in a buffered solution (10 mM phosphate buffer contained 110 mM sodium chloride). The clinical dose is a fixed dose of 25 mg administered as a subcutaneous (SC) injection once every 3 months (q3M).

The target indication has been finally agreed as:

- *Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with stage 1 or stage 2 polyneuropathy*

The Applicant (Alnylam) has been recently granted (27/08/2018) an approval for Onpattro (patisiran) containing another double-stranded small interfering ribonucleic acid (siRNA), patisiran. The Applicant

developed the RNA silencing molecule vutrisiran with the same mechanism of action as patisiran for the reduction of both variant and wild-type TTR production in the liver. The formulation of vutrisiran allows an infrequent and easy to administer subcutaneous (SC) injection once every 3 months (q3M) via a prefilled syringe with passive needle safety system (PFS-S). Such a dosing regimen minimises the need for healthcare encounters, and it is certainly less burdensome to patients compared to an IV administration.

The safety and efficacy of vutrisiran for the treatment of ATTR amyloidosis across the majority of the spectrum of disease was evaluated in two studies: a Phase 1, randomized, single-blind, placebo-controlled, single-ascending dose study and a Phase 3, randomized, open-label study in adult patients with hATTR amyloidosis with polyneuropathy (ALN-TTRSC02-002, HELIOS-A). Data from an external placebo group from the patisiran program (APOLLO study) were also used.

2.6.5.1. Dose response study(ies)

Vutrisiran drug product (DP) is a sterile, preservative-free, colourless to yellow solution of 50 mg/mL vutrisiran (free acid; equivalent to 53 mg/mL vutrisiran sodium, ALN-65492) formulated in a buffered solution of 10 mM sodium phosphate with 110 mM sodium chloride. The DP formulation has been developed for the SC route of administration and was supplied in 2 primary container closure systems during Phase 3 clinical development:

The clinical dose is a fixed dose of 25 mg administered as a subcutaneous (SC) injection once every 3 months (q3M).

Vial: a single-use Type I glass vial with bromobutyl fluoropolymer stopper and flip-off seal. This presentation was used in the Phase 1 Study 001 and the 9-month primary efficacy period of HELIOS-A, the Phase 3 study.

Prefilled syringe (PFS): a single-use Type I glass PFS equipped with a passive needle safety system (PFS-S). This PFS-S presentation was introduced in HELIOS-A after completion of the 9-month efficacy period and is being used in the ongoing HELIOS-B study.

Both the vial with syringe and a passive needle safety system (PFS-S) presentations contain the same DP formulation and are designed to deliver 0.5 mL vutrisiran DP solution (equivalent to 25 mg of vutrisiran). The PFS-S presentation is the intended to-be-marketed presentation.

Over the course of the clinical development of vutrisiran, there was a change in dosage strength and formulation to accommodate the Phase 3 dose of 25 mg. This involved a decrease in the concentration of the drug substance from 165 mg/mL (Phase 1) to 50 mg/mL with the concomitant addition of excipients as buffers to maintain neutral pH. Importantly, the changes in dosage strength and formulation did not impact the quality and stability of vutrisiran DP. In clinical studies (Study 001 and HELIOS-A), plasma exposure following administration of a 25 mg vutrisiran dose on Day 1 were comparable, confirming no impact of the change in dosage strength and formulation on the PK of vutrisiran. Thus, a dedicated bioequivalence or bioavailability study was not considered necessary by the Applicant and has not been conducted. Furthermore, the PK and PD of vutrisiran were not impacted by the change in DP presentation (vial with syringe versus PFS-S).

The dose and dosing frequency for vutrisiran (25 mg q3M) were selected to achieve TTR reduction with minimal peak-to-trough fluctuation over the dosing interval. There was a need for identifying a dosing regimen that would yield similar magnitude of TTR reduction, a similar PD effect, as observed with patisiran in the APOLLO study, and consequently expected to have similar clinical efficacy as patisiran.

Selection of the dosing regimen for vutrisiran was supported by TTR reduction data from the Phase 1 Study 001 in healthy subjects. Additionally, a PK/PD modeling approach was employed by the Applicant to characterize the dose-TTR reduction property of vutrisiran and determine the optimal dosing regimen for the Phase 3 studies in patients. Adequacy of the selected Phase 3 dosing regimen was confirmed in the HELIOS-A study in patients with hATTR amyloidosis with polyneuropathy, where median steady-state trough TTR reduction of 85% was observed together with improvement of neurological disease manifestations at Month 9 (please see relevant Clinical Efficacy sections).

The process for selecting the appropriate dosing regimen was based on identifying similar PD effects between vutrisiran and patisiran, which is considered appropriate. A similar magnitude of TTR reduction, a similar PD effect, as observed with patisiran in the APOLLO study, had to be identified which consequently was expected to have similar clinical efficacy as patisiran. Similar TTR reduction as patisiran was observed for vutrisiran in the phase 1 Study 001, in which dose dependent median maximum serum TTR reductions from baseline were shown. 25 mg vutrisiran administered q3M subcutaneously appeared to be a regimen that could yield similar TTR reduction as patisiran with acceptable safety, and was further evaluated in the Phase 3 study (HELIOS-A). A semi-mechanistic population PK/PD model was also developed. Based on this PK/PD modelling, the 25 mg q3M regimen of vutrisiran was predicted to provide sustained TTR reduction over the 3-month dosing interval similar to the observed TTR reduction profiles with intravenous 0.3 mg/kg q3w patisiran in the APOLLO study and it was well-tolerated. Intrinsic and extrinsic factors did not appear to influence the recommended fixed dose regimen of 25 mg q3M.

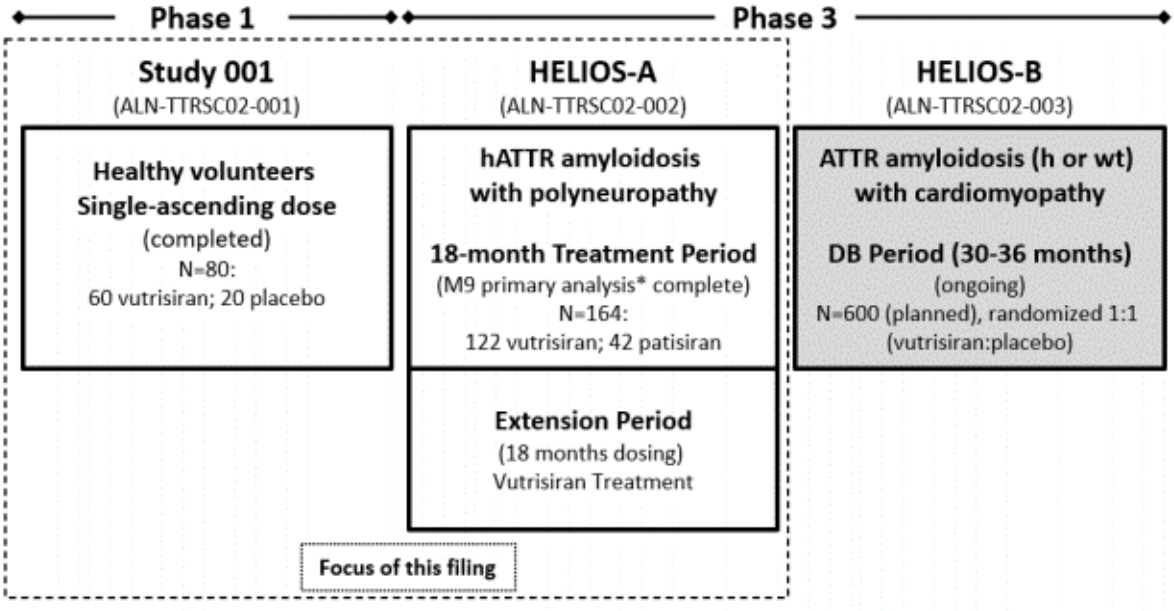
It is noted, however, that a prefilled syringe equipped with a passive needle safety system (PFS-S) was used in HELIOS-A and it is intended for marketing. The phrase in section 4.2 of the SmPC "*Therapy should be initiated under the supervision of a physician knowledgeable in the management of amyloidosis*" is acknowledged. It has been confirmed by the Applicant that dedicated human factor (HF) studies for self-administration have not yet been conducted with vutrisiran and therefore, the Notified Body Opinion included in the initial MAA a statement that 'the device was designed for professional use only'. It is also confirmed that vutrisiran will be administered by a healthcare professional (HCP) only, and an appropriate statement has been included in the SmPC.

2.6.5.2. Main study

The pivotal phase 3 study supporting vutrisiran was an open-label study with patisiran as the comparator (as a reference comparator) **(HELIOS-A or ALN-TTRSC02-002)**. The results from this study were compared to an external placebo group from a previous patisiran **Study ALN-TTR02-004 (APOLLO) which was a Phase 3, multicenter, multinational, randomized, double-bind, placebo controlled** study in hATTR amyloidosis patients with polyneuropathy. APOLLO study was the main study supporting the approval of Onpattro (patisiran).

The clinical development program for vutrisiran is outlined in the following Figure 2:

Figure 2 (from 2.5 Clinical Overview): Vutrisiran Clinical Development Program



Of note, for the regulatory submission in the US, the predefined primary analysis was performed at month 9, whereas for the EU, the primary analysis was performed at month 18. In the EU, during the scientific advice procedure, EMA/CHMP/SAWP indicated a preference for a marketing authorization application based upon 18 months data, as outlined later in the report. The first CSR for ALN-TTRSC02-002 (Study 002 CSR1) presented the efficacy analysis at Month 9 and summarized available safety and efficacy data from the ongoing Treatment Period and Treatment Extension Period, with a data cut-off date of 10 November 2020 (refer to Study 002 CSR1).

The second CSR for ALN-TTRSC02-002 (Study 002 CSR2) presents the Month 18 analysis of efficacy and summarizes available safety and efficacy data from the completed Treatment Period and ongoing Treatment Extension Period (which includes both the Legacy Treatment Extension Period and the Randomized Treatment Extension [RTE] Period described in Section 9.1), with a data cut-off date of 26 August 2021.

The Applicant did not provide an updated version of Modules 2.5 and 2.7.

ALN-TTRSC02-002 – (HELIOS-A)

Table 4: Study details of the pivotal study supporting the proposed indication

Study ID	No. of study centres / locations	Design	Study Posology	Study Objective	Subjs by arm entered/ compl.	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
ALN-TTRSC02-002 (HELIOS-A)	57 study centers in 22 countries: Argentina, Australia, Belgium, Brazil, Bulgaria, Canada, Cyprus, France, Germany, Greece, Italy, Japan, Korea, Malaysia, Mexico, Netherlands, Portugal, Spain, Sweden, Taiwan, UK, US	ongoing, global, Phase 3, randomized, open-label	Vutrisiran 25 mg q3M (SC) or patisiran 0.3 mg/kg q3w (IV). Premedication with corticosteroid, anti-histamines and paracetamol 60 minutes prior to the infusion (patisiran group only)	Efficacy of vutrisiran on mNIS+7 (neuropathy impairment)	164 patients: 122 vutrisiran and 42 patisiran Measurements at Month 18 of primary endpoint for 118 patients on vutrisiran and 38 patients on patisiran	The estimated duration of treatment for each patient is approximately 3 years. The study is being conducted in 2 parts: an 18-month Treatment Period, with the primary efficacy analysis at Month 9 and additional efficacy analyses at Month 18, followed by an 18-month Treatment Extension Period, in which all patients are treated with vutrisiran.	Vutrisiran F 43 (35.2%), M 79 (64.8%), Patisiran: F 15 (35.7%), M 27 (64.3%) Median age 60.0 years for both vutrisiran and patisiran	- Diagnosis of hATTR amyloidosis with documented TTR variant - NIS 5-130, inclusive - PND score of ≤3b NYHA HF classification ≤II - KPS of ≥60%	Change from baseline in the Modified mNIS+7 compared to the placebo arm of the APOLLO study at Month 18

Abbreviations: hATTR=hereditary transthyretin-mediated (amyloidosis); HF=heart failure; IV=intravenous; KPS=Karnofsky Performance Status; M=month; mNIS+7=modified neuropathy impairment score +7; NIS=neuropathy impairment score; No.=number; NYHA=New York Heart Association; OL=open label; PND=polyneuropathy disability; TTR=transthyretin; UK=United Kingdom; US=United States.

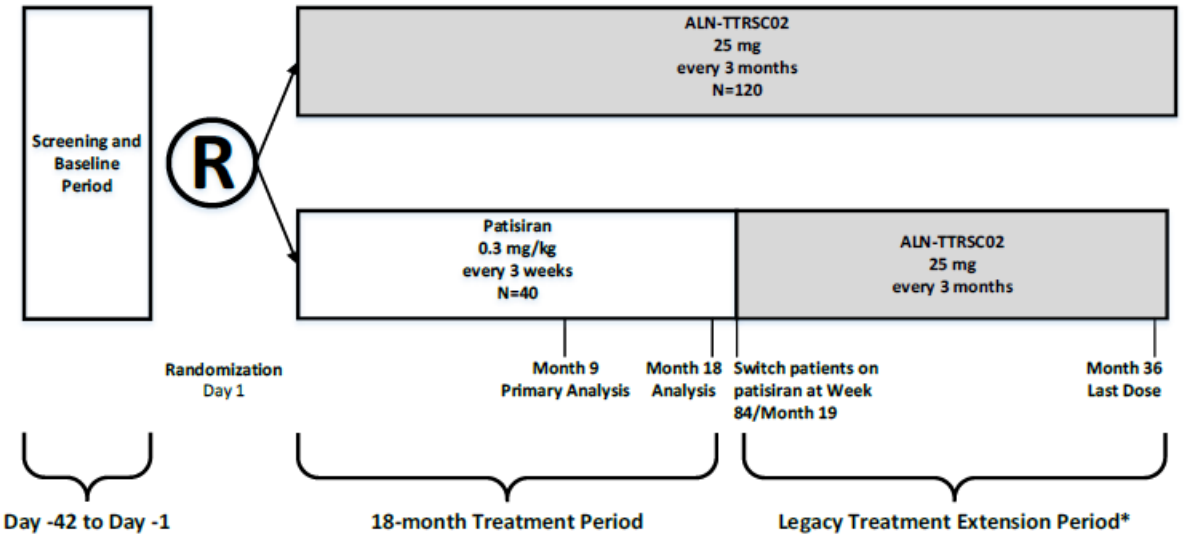
Methods

ALN-TTRSC02-002 (HELIOS-A) is an ongoing, global, Phase 3, randomized, open-label study designed to evaluate efficacy, safety, PK, and PD of vutrisiran in adult patients with hATTR amyloidosis with polyneuropathy. The study is being conducted in 2 parts:

- 18-month Treatment Period (hereafter referred to as "Treatment Period"), with the early efficacy analysis at Month 9; the Month 9 early analysis period is complete. Data for 164 patients (122 vutrisiran; 42 patisiran) are available as of the Month 9 data cut-off date (10 November 2020). For Month 18, data for 164 patients (122 vutrisiran; 42 patisiran) are also available as of the data cut-off date 26 August 2021
- 18-month Treatment Extension Period (which includes the Legacy Treatment Extension Period and the Randomised Treatment Extension Period), in which all patients will receive vutrisiran 25 mg q3M or 50 q6M; as of the Month 9 data cut-off date, no patisiran-treated patients have entered the Treatment Extension Period. As of the Month 18 data cut-off date, 33 patisiran-treated patients entered the Treatment Extension Period.

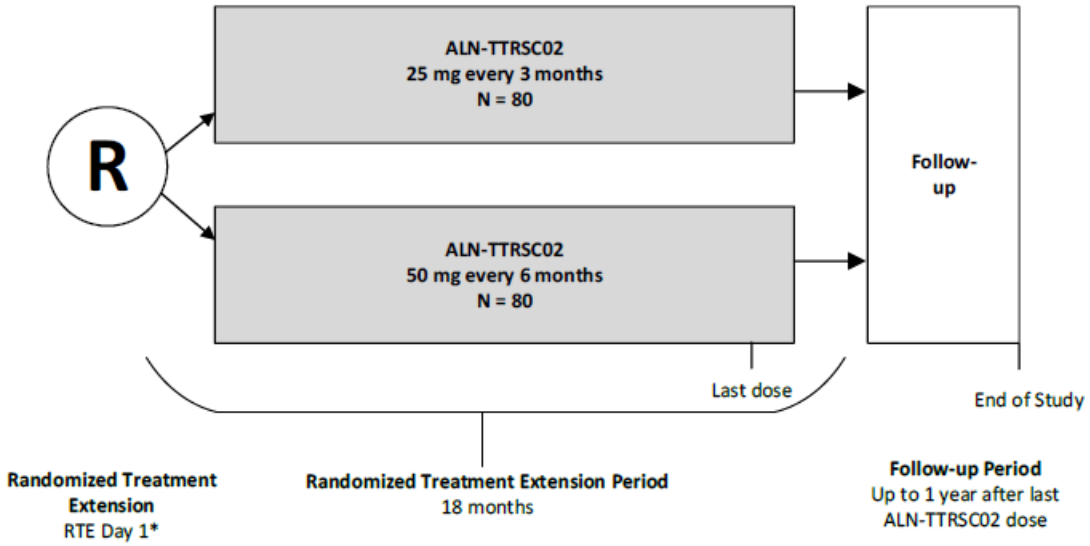
The following Figure 3 represents the HELIOS-A Study Design.

Figure 3 (from HELIOS-A CSR2): HELIOS-A Study Design



Abbreviations: ALN-TTRSC02=vutrisiran; RTE=Randomized Treatment Extension.
* Previously referred to as the 18-month Treatment Extension Period (per protocol Amendment 3 and earlier); the Legacy Treatment Extension Period, as of Amendment 4, was replaced with the RTE Period (Figure 2). Patients transition into the RTE Period either after completion of the 18-month Treatment Period or at their next vutrisiran dosing visit in the Legacy Treatment Extension Period, depending on the timing of amendment approval and completion of the Month 18 efficacy visit. Patients complete the RTE Period in lieu of the Legacy Treatment Extension Period.

Figure 4 (from HELIOS-A CSR2): HELIOS-A Study Design



Abbreviations: ALN-TTRSC02=vutrisiran; RTE=Randomized Treatment Extension.
*RTE Day 1 in lieu of the Legacy Treatment Extension Period Study Week 84 visit, or later.

Study Participants

HELIOS-A included adults age 18 (or age of legal consent, whichever is older) to 85 years of age, with a documented TTR mutation, and a confirmed diagnosis of symptomatic hATTR amyloidosis with a Neuropathy Impairment Score (NIS) of 5 to 130 (inclusive), Polyneuropathy Disability (PND) score of ≤3b and Karnofsky Performance Status (KPS) of ≥60%.

The same key inclusion criteria were used in the APOLLO study, as well. It is noted that patients whose neuropathy may be too advanced to permit completion of the study were excluded from the study.

Treatments

Vutrisiran 25 mg SC injection is administered q3M (12 weeks ±3 days during the Treatment Period and ±7 days during the Treatment Extension Period). Vutrisiran is supplied for this study in two formats for SC administration: a vial for SC injection and, with the implementation of Amendment 1, a prefilled syringe with passive needle safety system (PFS-S).

Patisiran 0.3 mg/kg IV infusion is administered q3w±3 days. The amount (in mg) of study drug to be administered is determined based on the patient's weight (kg). Dosing is based on actual body weight. For patients weighing ≥100 kg, the maximum recommended dose is 30 mg. All patients receive premedication with a corticosteroid (dexamethasone or equivalent), paracetamol, an H1 antagonist (diphenhydramine or equivalent), and an H2 antagonist (ranitidine or equivalent) prior to patisiran administration to reduce the risk of injection-site reactions. Details on the required premedication are provided in the study protocol (refer to Study 002 CSR2 Appendix 16.1.1 Section 5.2.2.2).

At Week 84, patients in the patisiran group are switched to vutrisiran treatment and receive the first vutrisiran dose. The last dose of patisiran is at Week 81, and patients should receive treatment with vutrisiran 3 weeks later at Week 84 and thereafter q3M (12 weeks ±7 days) for the remainder of the study. If a patient receiving patisiran is unable to complete the Month 18 efficacy visit at the study center due to COVID-19 before Week 84, they may transition to treatment with vutrisiran at Week 84 or later.

Objectives

HELIOS-A was designed to assess the efficacy of 25 mg vutrisiran administered q3M on neurologic impairment and other key clinical measures relevant to patients with hATTR amyloidosis with polyneuropathy, including quality of life, ambulatory ability, nutritional status, and disability.

Primary:

• To determine the efficacy of vutrisiran in patients with hATTR amyloidosis by evaluating the effect on neurologic impairment

Secondary:

• To determine the efficacy of vutrisiran on quality of life, gait speed, neurologic impairment, nutritional status, and disability

• To demonstrate the non-inferiority of vutrisiran compared to patisiran with respect to serum transthyretin (TTR) levels

Exploratory:

• To determine the effect of vutrisiran on:

– Disability and nutritional status

– Manifestations of cardiac amyloid involvement

– Other assessment of neurologic impairment

– Other assessments of quality of life

– Disease stage

– Performance of daily activities

• To characterize the pharmacodynamic (PD) effect of vutrisiran and patisiran on serum TTR and vitamin A levels

• To characterize plasma pharmacokinetic (PK) of vutrisiran and patisiran

• To assess presence of antidrug antibodies (ADAs) to vutrisiran and patisiran

Safety:

• To determine the safety and tolerability of vutrisiran in patients with hATTR amyloidosis

The primary objective was demonstration of superiority on neurological impairment to an external placebo group that was used in a previous study of the other siRNA molecule, patisiran, developed by the same Applicant.

Outcomes/endpoints

The primary endpoint of the study was the difference between vutrisiran and external placebo in the change from baseline to Month 18 (formally for EU) of the composite neuropathy impairment score (mNIS+7), a disease-specific composite measure of polyneuropathy. mNIS+7 is considered a sensitive and reproducible measure of neuropathy progression which has been used as a primary endpoint in multiple clinical studies in hATTR amyloidosis including APOLLO.

The key secondary endpoint was the change from baseline in Norfolk QoL-DN total score compared to placebo at Month 18 for EU.

For the US submission the change from baseline at Month 9 in mNIS+7 and in Norfolk QoL-DN total score, are key primary and secondary endpoint, respectively. Norfolk QoL-DN was included to support the clinical relevance of observed changes in mNIS+7.

An additional endpoint 10-MWT at Month 9 and Month 18 assesses ambulatory ability and was chosen to further support the clinical benefit of vutrisiran.

Exploratory endpoints addressed the effect of vutrisiran on nutritional status, disability, and cardiac disease manifestations, as well as the effect on additional clinical measures at Month 9. At Month 18, nutritional status and disability endpoints were secondary.

TTR reduction with vutrisiran compared to patisiran was evaluated and compared descriptively to the within study patisiran reference comparator; a formal non-inferiority comparison to patisiran was performed at Month 18 only.

Table 5 (modified from Module 2.7.3. Summary of Clinical Efficacy): Summary of Efficacy Assessments, Endpoints and Collection Schedule for the Month 18 Efficacy Analysis in HELIOS-A

Assessment	Brief Description	Interpretation of the Score	Treatment Period Schedule of Assessments[a]	Endpoints Vutrisiran (HELIOS-A) vs Placebo (APOLLO)
Primary and Key Secondary (Statistically significant changes in both endpoints must be observed to declare a positive trial)				
mNIS+7, Primary	Comprehensive composite neuropathy impairment score that assesses motor, sensory and autonomic neurologic impairment.	Score range: 0 (no impairment) to 304 points (maximum impairment) Higher score=greater severity of disease Decrease from baseline=improvement in neuropathy; Increase from baseline=worsening of disease	Baseline, Month 9, and Month 18	CFB Month 18

Assessment	Brief Description	Interpretation of the Score	Treatment Period Schedule of Assessments[a]	Endpoints Vutrisiran (HELIOS-A) vs Placebo (APOLLO)
Norfolk QoL-DN, Key Secondary	Standardized QoL questionnaire designed to measure the perception of the effects of polyneuropathy by the patient.	Score range: -4 (best possible QoL) to 136 (worst QoL) Lower score=higher QoL Decrease from baseline=improvement in QoL; Increase from baseline=worsening in QoL	Baseline, Month 9, and Month 18	CFB Month 18
Secondary				
10-MWT	Measure of ambulation that assesses how fast a patient can walk a distance of 10 meters	Gait speed reported in meters/second. Higher speed = faster gait speed/ambulation Increase from baseline=improvement in gait speed/ambulation; Decrease form baseline=worsening of gait speed/ambulation	Baseline, Month 9, and Month 18	CFB Month 18
mBMI	Measure of nutritional status.	Reported as BMI (kg/m^2) x albumin (g/L) Higher mBMI=better nutritional status Increase from baseline=improvement in nutritional status; Decrease from baseline=worsening of nutritional status	Baseline, Day 85, Day 169, Month 9, Day 337, Day 421, Day 505, Month 18	CFB Month 18

Assessment	Brief Description	Interpretation of the Score	Treatment Period Schedule of Assessments[a]	Endpoints Vutrisiran (HELIOS-A) vs Placebo (APOLLO)
R-ODS	Patient-reported disability scale that assesses activity and social participation limitations and specific activities of daily living.	Score range: 0 (maximal disability) to 48 points (no disability) Higher score=less disability Increase from baseline=improvement in disability; Decrease from baseline=worsening of disability	Baseline, Month 9, and Month 18	CFB Month 18
TTR levels	A measure of the pharmacodynamic activity of vutrisiran.	Lower hepatic TTR production = Lower circulating serum TTR levels	Baseline, Day 22, Day 43, Day 85, Day 169, Day 253, Month 9, Day 337, Day 421, Day 505, Day 547, Month 18	Percent reduction over time through Month 18
Exploratory				
NT-proBNP	A cardiac biomarker used to assess cardiac stress and degree of heart failure.	Elevated levels are associated with increased cardiac stress.	Baseline, Day 85, Day 169, Month 9, Day 337, Day 421, Day 505 and Month 18	CFB over time

Assessment	Brief Description	Interpretation of the Score	Treatment Period Schedule of Assessments[a]	Endpoints Vutrisiran (HELIOS-A) vs Placebo (APOLLO)
NIS	A composite neurologic impairment score that assesses motor strength and weakness motor weakness, sensation, and reflexes; scored based on physical exam findings	Score range: 0 to 244 points Higher score=greater severity of disease Decrease from baseline=improvement in neuropathy; Increase from baseline=worsening of disease	Baseline, Month 9, and Month 18	CFB over time
EQ-5D and EQ-VAS	General patient-reported QoL questionnaires	EQ-5D: score range 0 to 1 EQ-VAS: score range 0 to 100 Higher scores=better quality of life Increase from baseline=improvement in quality of life; Decrease form baseline=worsening quality of life	Baseline, Month 9, and Month 18	CFB over time

Assessment	Brief Description	Interpretation of the Score	Treatment Period Schedule of Assessments[a]	Endpoints Vutrisiran (HELIOS-A) vs Placebo (APOLLO)
FAP Stage and PND Score	Measures polyneuropathy severity; based largely on ambulatory ability including need of walking aids.	FAP stage: • 0: No symptoms • I: Unimpaired ambulation • II: Assistance with ambulation required • III: Wheelchair-bound or bedridden PND stage: • 0: No symptoms • I: Sensory disturbances but preserved walking capability • II: Impaired walking capacity but ability to walk without a stick or crutches • IIIA: Walking with the help of one stick or crutch • IIIB: Walking with the help of two sticks or crutches. • IV: Confined to a wheelchair or bedridden Lower scores=greater ambulatory function	Baseline, Month 9, and Month 18	CFB over time
KPS	Method of assessing functional status	11-point scale correlating to percentage values ranging from 100% (Normal no complaints; no evidence of disease) to 0% (death) Higher percentage score indicates greater ambulatory function	Baseline, Month 9, and Month 18	CFB over time

Assessment	Brief Description	Interpretation of the Score	Treatment Period Schedule of Assessments[a]	Endpoints Vutrisiran (HELIOS-A) vs Placebo (APOLLO)
TTR levels	A measure of the pharmacodynamic activity of vutrisiran.	Lower hepatic TTR production = Lower circulating serum TTR levels	Baseline, Day 22, Day 43, Day 85, Day 169, Day 253, Month 9, Day 337, Day 421, Day 505, Day 547, Month 18	Percent reduction over time

Abbreviations: 10-MWT=10-meter walk test; BMI=body mass index; CFB=change from baseline; mBMI=modified body mass index; EQ-5D-5L=EuroQol-5 Dimensions 5-Levels; EQ-VAS=EuroQol-Visual Analog Scale; FAP=Familial Amyloid Polyneuropathy; KPS=Karnofsky Performance Status; mNIS+7=modified neuropathy impairment score+7; NIS=neuropathy impairment score; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; NT-proBNP=N-terminal prohormone B-type natriuretic peptide; PND=Polyneuropathy disability; QoL=quality of life; R ODS=Rasch-built Overall Disability Scale; TTR=transthyretin.

aThe schedule of assessments for the Treatment Extension Period is provided in the study protocol (Study 002 CSR2 Appendix 16.1.1).

The Modified Neuropathy Impairment Score +7 (mNIS+7) is a validated comprehensive composite score that quantitates the wide range of motor, sensory, and autonomic neurologic impairment due to injury of large and small nerves in patients with hATTR amyloidosis with polyneuropathy (a minimum score of 0 points, representing no impairment and maximum of 304 points, representing maximal impairment). The higher the score the worse the condition of the patient. The mNIS+7 was developed by the Mayo Clinic group for use specifically in patients with hATTR amyloidosis with polyneuropathy and it has been used in all the recent clinical studies performed in hATTR with polyneuropathy.

Norfolk Quality of Life-Diabetic Neuropathy is another validated tool used in all of the studies in hATTR with polyneuropathy. The Norfolk QoL-DN is a comprehensive, patient-reported, health-related quality of life questionnaire that includes multiple domains pertinent to all aspects of the polyneuropathy in hATTR (with the lowest score -4 being the best possible QoL up to 136 being the worst QoL). Also in the case of Norfolk QoL-DN, the higher the score the worse the QoL of the patient.

For both the primary endpoint and key secondary endpoint the results at month 18 were also provided within the timeframe of this MAA procedure (please see Statistical methods and Results sections below).

The 10-meter walk test (10-MWT) measures the time it takes a patient to walk 10 meters and is expressed as gait speed (meters/second). Gait speed is indicative of vitality, with higher values being the better condition of the patient.

Sample size

Enrolment was planned for approximately 160 patients. The sample size of 160 patients is considered sufficient, taking into consideration prevalence and epidemiology of this orphan condition.

Randomisation and blinding (masking)

164 adult patients with hATTR amyloidosis with polyneuropathy were finally randomized 3:1 to vutrisiran or patisiran (122 vutrisiran and 42 patisiran). Patients were randomized at 57 study centers in 22 countries ranging from North America, South America, Europe, Asia, and Australia. Randomization was stratified by TTR genotype (V30M vs. non-V30M) and baseline NIS score (<50 vs. ≥50).

The randomisation 3:1 allowed more patients in the vutrisiran group and, at the same time, sufficient number of patients in the patisiran group to be able to perform meaningful comparisons.

Statistical methods

Study hypotheses

The HELIOS-A study uses the placebo group of the APOLLO study as an external control for the primary, most secondary, and most exploratory efficacy analyses (at Month 18, formally for EU).

For most inferentially-evaluated efficacy endpoints, the null hypothesis for the superiority comparison of vutrisiran vs placebo (APOLLO) is defined as follows:

- H_0: No difference between vutrisiran and placebo (APOLLO): difference (vutrisiran – placebo) = 0

For the TTR percent reduction endpoint, the null hypothesis for the noninferiority comparison of vutrisiran vs patisiran (HELIOS-A) is defined as follows:

- H_0: Vutrisiran is inferior to patisiran (HELIOS-A): difference in median TTR reduction (vutrisiran – patisiran) ≤ -10%

Multiple comparisons procedure

Two different multiple comparisons procedures are specified in the Statistical Analysis Plan (SAP):

- In the US, Japan, and Brazil, the overall family-wise error rate is controlled at α=0.05 for the primary and secondary endpoint hypothesis tests as follows:

Table 6 (from Table 1 of the Statistical Analysis Plan): Multiple Comparisons Procedure (US/Japan/Brazil)

MCP Step[a]	Endpoint	Comparison Group vs Vutrisiran	MCP Criteria
Evaluated at the Month 9 analysis timepoint			
1	Modified Neuropathy Impairment Score +7 (mNIS+7) change from baseline at Month 9	Placebo (APOLLO)	Nominal P value ≤ α
2	Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) total score change from baseline at Month 9	Placebo (APOLLO)	Nominal P value ≤ α
3	10-MWT gait speed change from baseline at Month 9	Placebo (APOLLO)	Nominal P value ≤ α
Evaluated at the Month 18 analysis timepoint			
4	Modified Neuropathy Impairment Score +7 (mNIS+7) change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
5	Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) total score change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
6	10-MWT gait speed change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
7	mBMI (BMI [kg/m2] multiplied by serum albumin level [g/L]) change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
8	R-ODS change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
9	TTR percent reduction through Month 18	Patisiran (HELIOS-A)	2-sided 95% LCB for treatment difference > -10%

[a] Per serial gatekeeping MCP, if the MCP criterion is satisfied in a given step, the hypothesis test is deemed statistically significant and the next step will be evaluated; otherwise all hypotheses in the given and subsequent steps are deemed not statistically significant.
LCB=lower confidence bound; MCP=multiple comparisons procedure.

For the US filing, results for both the primary endpoint, mNIS+7 change from baseline at Month 9, and key secondary endpoint, Norfolk QoL-DN total score change from baseline at Month 9, must be statistically significant to declare a positive trial.

- In the EU, during its scientific advice procedure, the EMA/CHMP/SAWP indicated a preference for a marketing authorization application based upon 18 months data. Therefore, the Month 9 endpoints included in the US/Japan/Brazil multiple comparisons procedure (MCP) are not included in the MCP for the EU and other regions, where instead mNIS+7 change from baseline at Month 18 is considered the primary endpoint. The overall familywise error rate in the EU and other regions is controlled at α=0.05 for the primary and secondary endpoint hypothesis tests as follows:

Table 7 (from Table 2 of the Statistical Analysis Plan): Multiple Comparisons Procedure (EU/Other Regions)

MCP Step[a]	Endpoint	Comparison group vs Vutrisiran	MCP Criteria
Evaluated at the Month 18 analysis timepoint			
1	Modified Neuropathy Impairment Score +7 (mNIS+7) change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
2	Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) total score change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
3	10-MWT gait speed change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
4	mBMI (BMI [kg/m2] multiplied by serum albumin level [g/L]) change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
5	R-ODS change from baseline at Month 18	Placebo (APOLLO)	Nominal P value ≤ α
6	TTR percent reduction through Month 18	Patisiran (HELIOS-A)	2-sided 95% LCB for treatment difference > -10%

[a] Per serial gatekeeping MCP, if the MCP criterion is satisfied in a given step, the hypothesis test is deemed statistically significant and the next step will be evaluated; otherwise all hypotheses in the given and subsequent steps are deemed not statistically significant.
LCB=lower confidence bound; MCP=multiple comparisons procedure.

For filings in the EU and other regions, results for the primary endpoint, mNIS+7 change from baseline at Month 18, must be statistically significant to declare a positive trial.

Clinical Study Report 1 (Study 002 CSR1) provided the results of the primary efficacy analysis at Month 9 and summarized safety and efficacy data from the ongoing Treatment Period and Treatment Extension Period, with a data cut-off date of 10 November 2020. The second CSR for ALN-TTRSC02-002 (Study 002 CSR2) presented the Month 18 analysis of efficacy, and summarized available safety and efficacy data from the completed Treatment Period, and ongoing Treatment Extension Period (which includes both the Legacy Treatment Extension Period and the Randomized Treatment Extension [RTE] Period described in Section 9.1), with a data cut-off date of 26 August 2021.

Efficacy evaluation at Month 18

Analysis populations

Modified Intent-to-Treat (mITT) Population: All randomized patients who received any amount of study drug. Patients were analyzed according to the treatment to which they were randomized.

TTR Per-protocol (PP) Population: All mITT Population patients with a nonmissing TTR assessment at baseline and ≥1 trough TTR assessment between Months 6 (Week 24) and Month 18 (Week 72) that met the postbaseline TTR assessment requirements. Patients were analysed according to the treatment to which they were randomized.

Table 8 (from Table 3 of the Statistical Analysis Plan): Post-baseline TTR Assessment Requirements by Treatment Group

Treatment Group	Post-baseline TTR Assessment Requirements
Vutrisiran or Patisiran	• Assessment must be before administration of study drug at the current visit • Assessment after initiation of local standard treatment for hATTR amyloidosis excluded (Section 3.5)
Vutrisiran	• Patient must receive planned, complete administration of study drug at the planned treatment visit approximately 12 weeks before the TTR assessment • Patient must receive planned, complete administration of study drug at 2 consecutive planned treatment visits at any time before the TTR assessment visit to ensure steady state
Patisiran	• Patient must receive planned, complete administration of study drug at the planned treatment visit approximately 3 weeks before the TTR assessment

- Month 18 Efficacy PP Population: All mITT Population patients treated with vutrisiran or placebo meeting the following criteria:
- Month 18 efficacy visit date within 3 calendar months of protocol-planned Month 18 efficacy visit window
- No serious or severe COVID 19 custom query AE terms reported on or before Month 18 efficacy visit date
- For vutrisiran-treated patients, received all planned vutrisiran doses up to and including Week 72 with ≤ 28-day delay
- Patients will be analyzed according to the treatment to which they were randomized.

Analysis of change from baseline in mNIS+7 and change from baseline in Norfolk QoL-DN total score at Month 18 (formally the primary and key secondary endpoint for EU).

The primary analysis of change from baseline in mNIS+7 and Norfolk QoL-DN total score at Month 18 was conducted in the mITT population using a mixed-effects model for repeated measures (MMRM) including treatment, visit, genotype, age at symptom onset, categorical baseline NIS (except for NIS-related endpoints) as categorical factors, baseline value as a continuous covariate, and treatment by visit interaction. All efficacy data collected regardless of whether before or after treatment discontinuation were included in the analysis, with the exception of data after initiation of local standard treatment for hATTR amyloidosis or on/after the onset of a serious COVID-19 adverse event.

Efficacy censoring rules for initiation of local standard treatment for hATTR amyloidosis were defined as follows:

- For the APOLLO study, assessments were censored (excluded from analysis) after initiation of any of the following:
  - Orthotopic liver transplant

- Use of TTR stabilizing agents (eg, tafamidis, diflunisal) for >14 days

- For the HELIOS-A study, the APOLLO censoring rules were applied. Additionally, assessments were censored after initiation of any of the following recently approved treatments:
  - Any use of TTR-targeting anti-sense oligonucleotides (eg, inotersen)
  - Any use for patisiran (applicable for the vutrisiran treatment group only)

Missing/censored data were assumed to be missing at random (MAR).

Furthermore, sensitivity and other analyses were conducted:

- Sensitivity analysis 1: Data after initiation of local standard treatment for hATTR amyloidosis or on/after the onset of a COVID-19 SAE were included in the MMRM analysis.
- Sensitivity analysis 2: A propensity score was included as an additional covariate in the MMRM analysis. The propensity score was defined as the probability of being treated with vutrisiran as obtained from a logistic regression model of treatment group [vutrisiran; placebo (APOLLO)]. The logistic regression model included important baseline variables that cover potential differences between the APOLLO and HELIOS-A study populations.
- Sensitivity analysis 3: A pattern-mixture model (PMM) using the ANCOVA/MI method was applied under the assumption that missing at random (MAR) did not apply for patients with missing Month 18 data after stopping study treatment or who died before Month 18 due to reasons unrelated to COVID-19.
- Other analysis 1: An analysis of the binary endpoint, i.e. percentage of patients improving from baseline, was performed using a Cochran-Mantel-Haenszel test with stratification factor of genotype.
- Other analysis 2: The primary analysis was repeated on the Efficacy PP Population.

Analysis of time-averaged trough TTR percent reduction through Month 18

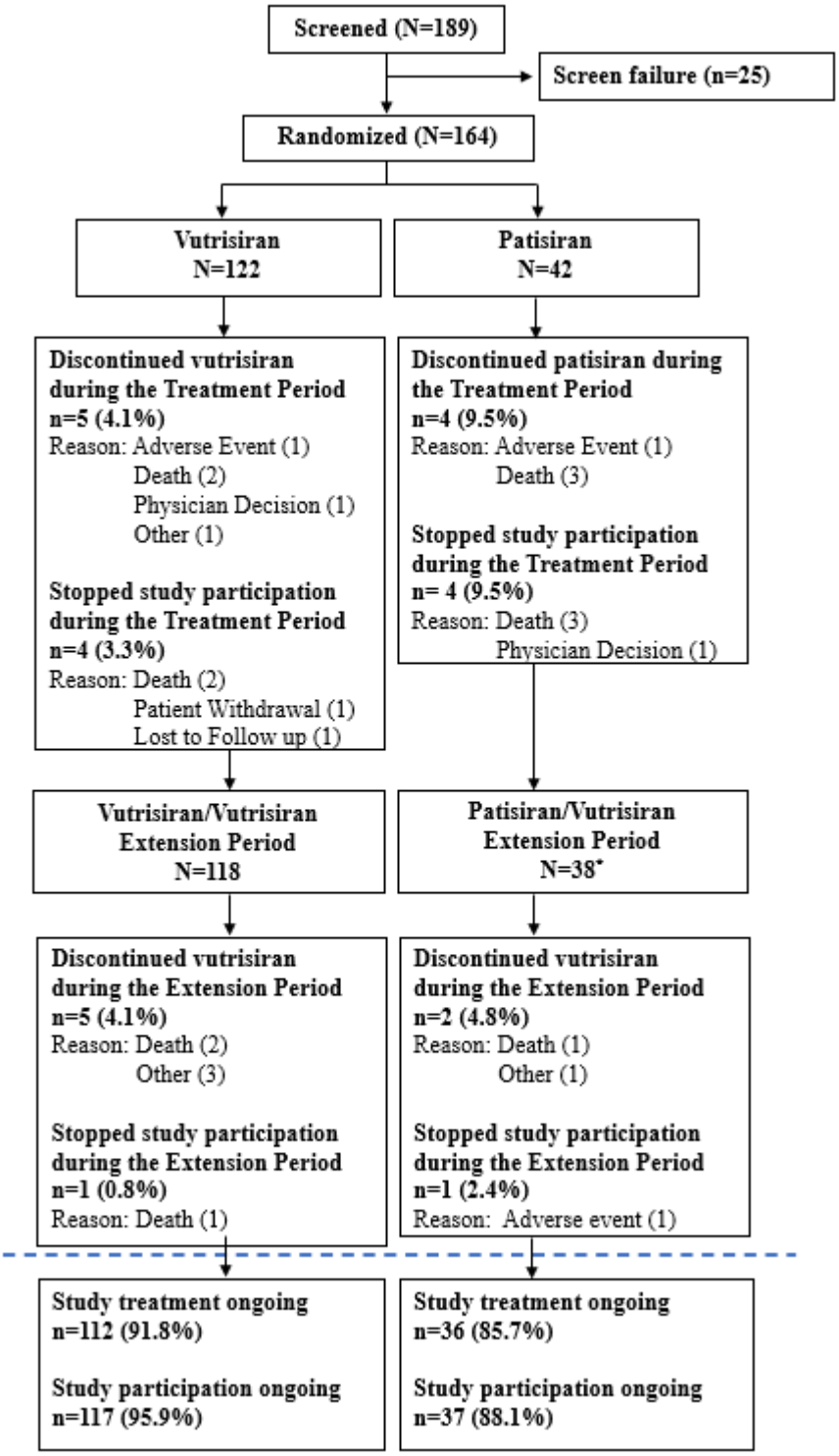
Time-averaged trough TTR percent reduction through Month 18 was defined as the average trough (ie, predose) TTR percent reduction from Month 6 to 18, which is the steady state period for both vutrisiran and patisiran. Only through TTR assessments meeting requirements described in the TTR PP Population definition were included. The Hodges-Lehmann method, stratified by previous TTR stabilizer use (yes vs no), where values within each stratum were first aligned by the within-stratum 1-sample Hodges-Lehmann median, was used to estimate the 95% confidence interval (CI) for the median difference between the vutrisiran and patisiran groups in this study. Non-inferiority of vutrisiran (versus patisiran) was declared if the lower limit of the 95% CI for the median treatment difference in TTR percent reduction (vutrisiran - patisiran) in this study was greater than -10%.

Sensitivity analyses using the same analysis method were conducted to compare the TTR percent reduction through Month 18 between the vutrisiran group from this study and the pooled patisiran group from this study and the APOLLO study.

Results

Participant flow

Figure 5 (from Figure 3 of HELIOS-A CSR2): HELIOS-A Disposition of patients



*As of the data cut-off date, 33 patients in the patisiran group have received treatment in the Treatment Extension Period (including the Legacy Treatment Extension and/or Randomized Treatment Extension Periods), when patients in the patisiran group switch to vutrisiran treatment.

Sources: Table 14.1.1.1.1; Listing 16.2.1.1, Listing 16.2.1.4, and Listing 16.2.5.3

Recruitment

The study was initiated on 14 February 2019 (first patient/first dose). The Month 9 Primary Analysis has been completed with a data cut-off date of 10 November 2020 and the Month 18 a data cut-off date of 26 August 2021. Treatment has continued beyond the Month 18 timepoint and the study is ongoing.

Conduct of the study

A protocol deviation plan was developed by the Sponsor for the assessment and classification of protocol deviations; this plan is stored in the Trial Master File. Major protocol deviations were defined as those that may significantly impact the completeness, accuracy, and/or reliability of the study data; or that may significantly affect a patient's rights, safety and well-being. Deviations not classified as major were assigned as minor. Major protocol deviations were reviewed and approved by the Sponsor prior to the interim database lock for the analyses at Month 18.

Impact of COVID-19 Pandemic on Study Participation

In the vutrisiran group, 89 (73.0%) patients were reported to have had their study visits or dosing impacted by the COVID-19 pandemic (Table 9): 89 (73.0%) patients with a missed, delayed, or partially completed visit; 84 (68.9%) patients with any location change; and 17 (13.9%) patients with a missed or delayed dose. One (0.8%) patient in the vutrisiran group was discontinued from study treatment due to the COVID-19 pandemic; this patient died due to COVID-19-related pneumonia.

In the patisiran group, 21 (50.0%) patients were reported to have had their study visits or dosing impacted by the COVID-19 pandemic (Table 9): 21 (50.0%) patients with a missed, delayed, or partially completed visit; 15 (35.7%) patients with any location change; and 18 (42.9%) patients with a missed or delayed dose. One (2.4%) patient in the patisiran group was discontinued from study treatment due to the COVID-19 pandemic; this patient died due to COVID-19-related pneumonia.

Table 9 (from Table 8 in HELIOS-A CSR2): COVID-19 Study Participation Impact Assessment: Overall Patient Summary (Safety Population)

	HELIOS-A		
	Vutrisiran (N=122)	**Patisiran (N=42)**	**Total (N=164)**
Patients impacted[a], n (%)	89 (73.0)	21 (50.0)	110 (67.1)
Patients with missed, delayed, or partially completed visit[a], n (%)	89 (73.0)	21 (50.0)	110 (67.1)
Patients with any location change[a], n (%)	84 (68.9)	15 (35.7)	99 (60.4)
Patients with missed or delayed doses[a], n (%)	17 (13.9)	18 (42.9)	35 (21.3)

Abbreviations: COVID-19=coronavirus disease 2019.

a Patients whose participation in the study was impacted by the COVID-19 pandemic (e.g., missed, delayed or partially completed visit, missed/delayed study drug dose, or visit location change such as phone visit, etc.).
Source: Table 14.5.1.1

Baseline data

Table 10 (from Table 9 Module 2.7.3 Summary of Clinical efficacy and Table 5 of HELIOS-A CSR2): Baseline Disease Characteristics (Safety Populations)

Characteristic	APOLLO	HELIOS-A
	Placebo (N=77)	**Vutrisiran (N=122)**
Age (years) at hATTR symptom onset, n (%)		
<50	20 (**26.0**)	48 (**39.3**)
≥50	57 (**74.0**)	74 (**60.7**)
Neuropathy impairment score[a,b] , n (%)		
<50	35 (**45.5**)	78 (**63.9**)
≥50 to <100	33 (**42.9**)	39 (**32.0**)
≥100	9 (**11.7**)	5 (**4.1**)
Genotype[a], n (%)		
V30M	40 (51.9)	54 (44.3)
Non-V30M	37 (48.1)	68 (55.7)
Karnofsky Performance Status (KPS), n (%)		
60	22 (28.6)	17 (13.9)
70-80	45 (58.4)	73 (59.8)
90-100	10 (13.0)	32 (26.2)
New York Heart Association (NYHA) classification, n (%)		
No heart failure[c]	-	68 (**55.7**)
I	40 (**51.9**)	11 (**9.0**)
II	36 (**46.8**)	43 (**35.2**)
III	0	0
IV	0	0
Missing	1 (1.3)	0
NT-proBNP, n (%)		
≤3000 ng/L	66 (85.7)	112 (91.8)
>3000 ng/L	9 (11.7)	10 (8.2)
Missing	2 (2.6)	0
Cardiac subpopulation, n (%)[d]		
Yes	36 (46.8)	40 (32.8)

Characteristic	APOLLO Placebo (N=77)	HELIOS-A Vutrisiran (N=122)
No	41 (53.2)	82 (67.2)

Abbreviations: EDC=electronic data capture; hATTR=hereditary transthyretin-mediated (amyloidosis); max=maximum; min=minimum; NT proBNP=N-terminal prohormone B-type natriuretic peptide; NYHA=New York Heart Association; SD=standard deviation; V30M=valine to methionine variant at position 30.
a As recorded in or derived from EDC; included in specified analysis models as categorical factor.
b Mean of nonmissing assessments from scheduled screening visits 2 and 3 after component imputation.
c In the APOLLO study, NYHA class was graded I through IV and "no heart failure" was not an option; thus, in this study, patients classified as NYHA class I included both those without heart failure and those with heart failure who had no symptomatology during ordinary physical activity.
d Patients who had preexisting evidence of cardiac amyloid involvement, defined as patients with baseline left ventricular (LV) wall thickness ≥1.3 cm and no aortic valve disease or hypertension in medical history.
Source: Study 002 CSR1 Table 14.1.3.1

Table 11 (from Table 10 Module 2.7.3 Summary of Clinical efficacy and Table 5 of HELIOS-A CSR2): Baseline Values for Study Endpoint Parameters (mITT Population)

Endpoint Parameter	APOLLO Placebo (N=77)	HELIOS-A Vutrisiran (N=122)
Modified Neuropathy Impairment Score +7 (mNIS+7)		
Mean (SD)	74.61 (**37.04**)	60.55 (**35.99**)
Median (min, max)	71.50 (11.0, 153.5)	63.50 (2.5, 158.0)
Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) total score		
Mean (SD)	55.5 (**24.3**)	47.1 (**26.3**)
Median (min, max)	53.5 (8, 111)	44.0 (-1, 105)
10-meter Walk Test (10-MWT, m/s)		
Mean (SD)	0.790 (0.319)	1.006 (0.393)
Median (min, max)	0.800 (0.00, 1.53)	1.049 (0.08, 1.87)
Modified Body Mass Index (mBMI, kg/m^2)		
Mean (SD)	989.9 (**214.2**)	1057.5 (**234.0**)
Median (min, max)	959.7 (569, 1508)	1047.2 (589, 1723)
Rasch-built Overall Disability Scale (R-ODS)		
Mean (SD)	29.8 (**10.8**)	34.1 (**11.0**)
Median (min, max)	30.5 (3, 48)	35.0 (5, 48)

Endpoint Parameter	APOLLO Placebo (N=77)	HELIOS-A Vutrisiran (N=122)
Polyneuropathy Disability (PND) Score		
I	20 (26.0)	44 (36.1)
II	23 (29.9)	50 (41.0)
IIIA	22 (28.6)	16 (13.1)
IIIB	11 (14.3)	12 (9.8)
IV	1 (1.3)	0
Neuropathy Impairment Score (NIS)		
Mean (SD)	57.02 (32.04)	43.02 (28.63)
Median (min, max)	53.88 (7.0, 125.5)	36.00 (5.0, 127.0)
Serum TTR (mg/L)		
Mean (SD)	198.84 (58.08)	206.11 (61.03)
Median (min, max)	196.43 (58.5, 320.1)	203.25 (58.4, 343.2)

Abbreviations: max=maximum; min=minimum; SD=standard deviation; TTR=transthyretin.

Source: Study 002 CSR1 Table 14.1.6.1

Table 12 (from Table 38 of HELIOS-A CSR1 and Table 33 of HELIOS-A CSR2): FAP Stage, Summary of Change from Baseline at Month 9 and Month 18 (mITT Population)

	Actual/ Comparison	Category	APOLLO		HELIOS-A	
			Placebo (N=77) (n %)	Patisiran-LNP 0.3 mg/kg (N=148) N (%)#	Vutrisiran (N=122) (n %)	Patisiran (N=42) (n %)
Baseline		n	77	148	122	42
		I	37 (48.1)	67 (45.3)	85 (**69.7**)	31 (73.8)
		II	39 (50.6)	81 (54.7)	37 (**30.3**)	11 (26.2)
		III	1 (1.3)	0	0	0
Month 9	Change from baseline comparison	n	67		92	31
		Improved	0		3 (3.3)	2 (6.5)
		No change	54 (80.6)		83 (90.2)	28 (90.3)

		Worsened	13 (19.4)		6 (6.5)	1 (3.2)
Month 18	Change from baseline comparison	Improved	0		5 (4.1)	1 (2.4)
		No change	34 (44.2)		101 (82.8)	36 (85.7)
		Worsened	21 (27.3)		9 (7.4)	1 (2.4)
		Missing	22 (28.6)		7 (5.7)	4 (9.5)

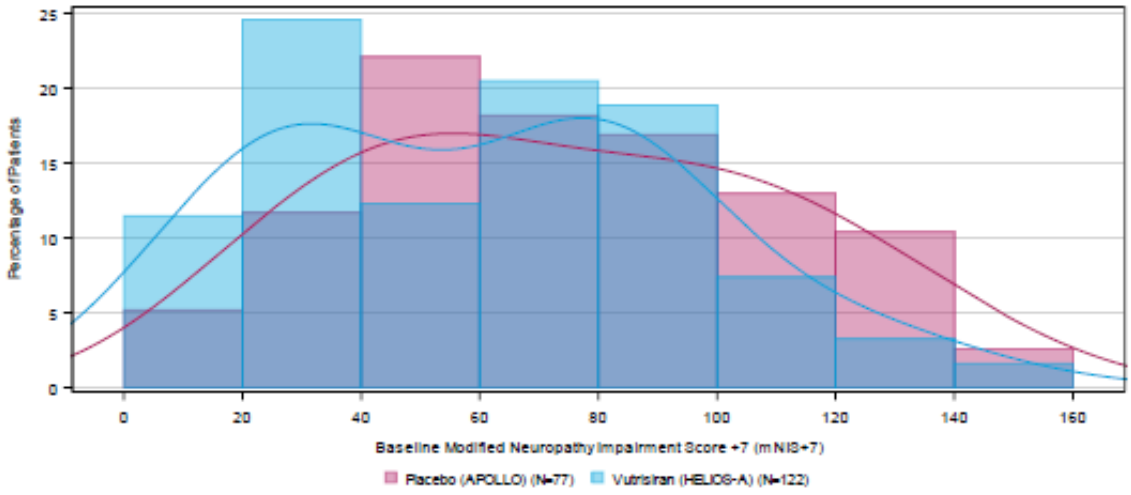
From Table 12: Baseline Disease Characteristics (mITT Population) from the Onpattro EPAR https://www.ema.europa.eu/en/documents/assessment-report/onpattro-epar-public-assessment-report_.pdf

Abbreviations: FAP=familial amyloidotic polyneuropathy; mITT=modified intent-to-treat.

Note: Lower scores indicate greater ambulatory function. For HELIOS-A, the Month 9 assessment of FAP stage was not included in the original protocol and was added in protocol amendment 1; as such, many patients in the HELIOS-A groups have missing data at Month 9.
a For percentage calculations, the denominator is patients with nonmissing values at Month 9.
Source: Table 14.2.4.8

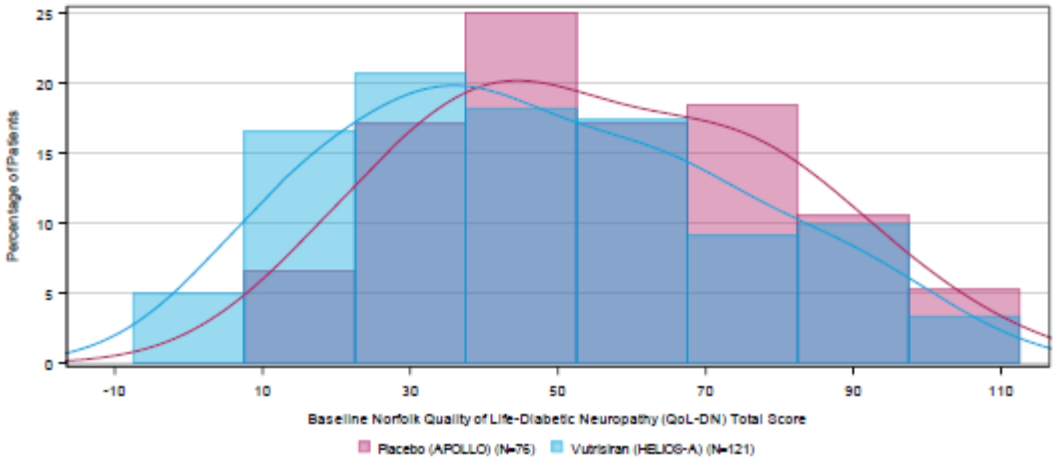
The baseline characteristics and disease severity of APOLLO and HELIOS-A studies are largely overlapping (Figure 6 and Figure 7 from ANNEX A of the Responses to Day 120 LoQ).

Figure 6 (from Figure 6 from ANNEX A of the Responses to Day 120 LoQ): Histogram of Baseline mNIS+7 (mITT Population)



Abbreviations: mITT=modified intent-to-treat; mNIS+7=modified neuropathy impairment score +7.

Figure 7 (from Figure 7 from ANNEX A of the Responses to Day 120 LoQ): Histogram of Baseline Norfolk QoL-DN Total Score (mITT Population)



Abbreviations: mITT=modified intent-to-treat; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy.

Numbers analysed

The numbers analysed are presented below. For the APOLLO study placebo group there were measurements for 77 patients at baseline, for 67 patients at Month 9. For the HELIOS-A vutrisiran group there were measurements for 122 patients at baseline for 114 patients at Month 9 and for 118 at Month 18.

Table 13: Analysis Populations

Analysis Population	APOLLO	HELIOS-A		
	Placebo (N=77)	Vutrisiran (N=122)	Patisiran (N=42)	Total (N=164)
Number of patients				
mITT Population	77	122	42	164
TTR PP Population	0	120	40	160
Month 18 Efficacy PP Population	58	96	-	96
Cardiac Subpopulation[a]	36	40	14	54
Safety Population	77	122	42	164
PK Population	-	122	42	164
All Vutrisiran-treated Population	-	122	33	155

Outcomes and estimation

mNIS+7 at Month 9

The mNIS+7 is a composite measure of neurologic impairment. Higher scores represent a greater severity of disease. The mNIS+7 was assessed at baseline, Month 9, and Month 18. At Month 9, the vutrisiran group showed an improvement in neuropathy compared to baseline (LS mean change from baseline: -2.24 points) while the placebo (APOLLO) group showed a worsening of neuropathy (LS mean change from baseline: +14.76 points). This represents a statistically significant improvement in neuropathy at 9 months for patients in the vutrisiran group compared to the placebo group (LS mean difference between groups: -17.00 points, P=3.542×10^{-12}).

Table 14 (from Table 11 of Module 2.7.3 Summary of Clinical efficacy): mNIS+7, ANCOVA/Multiple Imputation Model, Change from Baseline to Month 9 (mITT Population)

Visit	Actual/ Change	Statistic	APOLLO Placebo (N=77)	HELIOS-A Vutrisiran (N=122)
Baseline	Actual	N	77	122
		Mean (SD)	74.61 (37.04)	60.55 (35.99)
		Median	71.50	63.50
		Min, Max	11.0, 153.5	2.5, 158.0
Month 9	Actual	N	67	114
		Mean (SD)	90.99 (41.31)	57.50 (37.98)
		SE	5.05	3.56
		Median	91.50	57.00
		Min, Max	19.0, 167.5	1.0, 160.1
	Change from baseline [a]	Month 9 LS mean (SE)	14.76 (2.00)	-2.24 (1.43)
		95% CI	(10.84, 18.68)	(-5.04, 0.57)
		LS mean difference (SE) (vutrisiran – placebo)	-	**-17.00 (2.44)**
		95% CI	-	**(-21.78, -12.22)**
		p-value	-	**3.542E-12**

Abbreviations: AE=adverse event; ANCOVA= analysis of variance; CI=confidence interval; COVID-19=coronavirus disease 2019; hATTR=hereditary transthyretin-mediated (amyloidosis); max=maximum; min=minimum; LS=least squares; mITT=modified intent-to-treat; mNIS+7=modified neuropathy impairment score +7; SAP=statistical analysis plan; SD=standard deviation; SE=standard error.
Note: mNIS+7 score = mean of 2 nonmissing assessments planned to be performed ≥24 hours to ≤7 days apart at each scheduled visit, after component imputation; Higher scores represent a greater severity of disease (range = 0 to 304). Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis (considered missing before multiple imputation).

[a]Multiple imputation estimates and p-value derived per combining LS estimates per Rubin's rules based on 100 datasets where missing Month 9 values were imputed using a regression procedure including select baseline variables (see SAP). LS estimates derived from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset) and continuous covariate (baseline value). Source: Study 002 CSR1 Table 14.2.1.1.1

mNIS+7 at Month 18

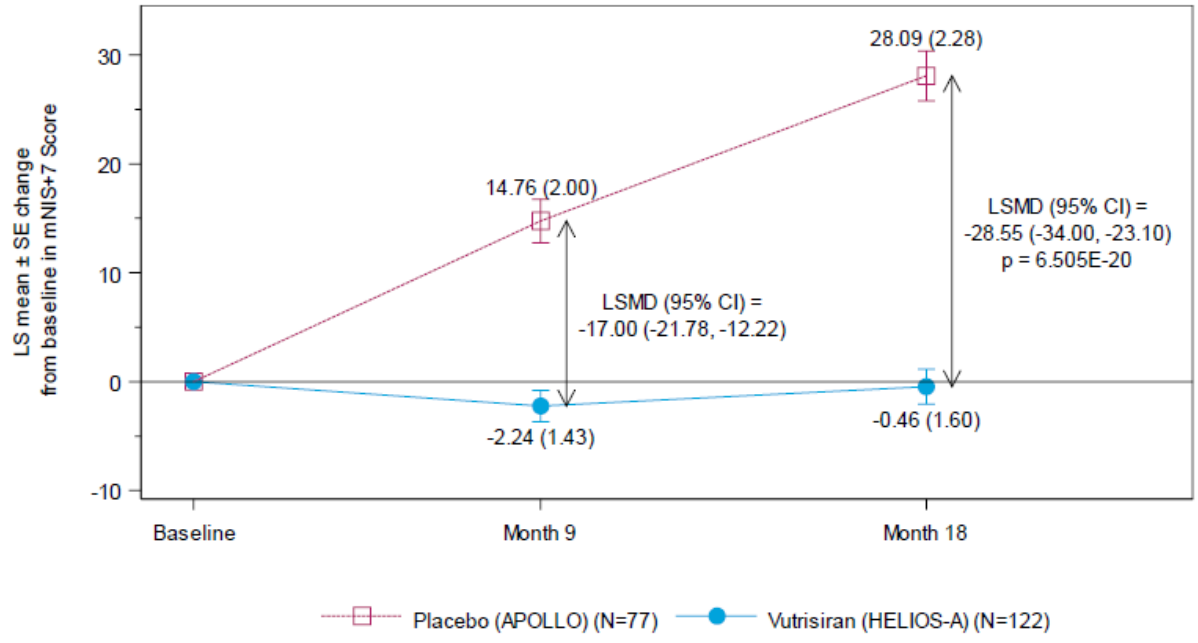
A statistically significant improvement in neuropathy for patients in the vutrisiran group compared to the placebo group was also observed at 18 months (LS mean difference between groups: -28.55 points, P=6.505 × 10^{-20}).

Table 15 (from Table 10 of the HELIOS-A CSR2): mNIS+7, Change From Baseline to Month 18, MMRM Model (mITT Population)

Statistic[a]	**APOLLO**	**HELIOS-A**
	Placebo (N=77)	**Vutrisiran (N=122)**
Month 18 LS mean (SE)	28.09 (2.28)	-0.46 (1.60)
95% CI	(23.58, 32.59)	(-3.61, 2.69)
LS mean difference (SE) (vutrisiran – placebo)	-	**-28.55 (2.76)**
95% CI	-	**(-34.00, -23.10)**
p-value	-	**6.505E-20**

Abbreviations: AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; hATTR=hereditary transthyretin-mediated (amyloidosis); LS=least squares; mITT=modified intent-to-treat; MMRM= mixed-effects model for repeated measures; mNIS+7=modified Neuropathy Impairment Score +7; SE=standard error.
Note: mNIS+7 score = mean of 2 nonmissing assessments planned to be performed ≥24 hours to ≤7 days apart at each scheduled visit, after component imputation; lower scores indicate less neurologic impairment (range = 0 to 304). Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis.
a LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset), continuous covariate (baseline value), and interaction (treatment by visit). Source: Table 14.2.1.1.2

Figure 8 (from Figure 4 of the HELIOS-A CSR2): mNIS+7, Change from Baseline Over Time (mITT Population)



N evaluable			
Placebo	77	67	51
Vutrisiran	122	114	112

Abbreviations: ANCOVA/MI=analysis of covariance model incorporating multiple imputation; CI=confidence interval; LS=least squares; LSMD=least squares mean difference; mITT=modified intent-to-treat; MMRM=mixed effects model for repeated measures; mNIS+7=modified Neuropathy Impairment Score +7; SE=standard error.
Note: The LS mean estimates at Month 9 are from the completed Month 9 primary analysis using ANCOVA/MI while the LS mean estimates at Month 18 are based on MMRM.
Source: Figure 14.2.3.5.2

Of the 122 patients randomized to vutrisiran (HELIOS-A), 118 (96.7%) patients and of 77 placebo patients included in the APOLLO, 67 (87.0%) patients had a baseline assessment for mNIS+7 and at least one post baseline follow-up assessment (either at 9 months, 18 months, or both) were included in the primary Month 18 analysis using a mixed-effects model for repeated measures (MMRM).

Table 16 (from Table 12 of HELIOS-A CSR2): mNIS+7, Summary of Data Available for the Primary Month 18 Analysis (mITT Population)

Parameter Category	APOLLO	HELIOS-A
	Placebo (N=77)	Vutrisiran (N=122)
Included in the primary Month 18 analysis	**67 (87.0)**	**118 (96.7)**
Both Month 9 and Month 18 nonmissing[a]	51 (66.2)	110 (90.2)
Nonmissing Month 9 and missing Month 18	10 (13.0)	4 (3.3)
Nonmissing Month 9 and Month 18 censored	6 (7.8)	2 (1.6)
Missing Month 9 and nonmissing Month 18	0	2 (1.6)

Parameter Category	APOLLO	HELIOS-A
	Placebo (N=77)	Vutrisiran (N=122)
Excluded from the primary Month 18 analysis	**10 (13.0)**	**4 (3.3)**
Missing baseline	0	0
Both Month 9 and Month 18 missing	9 (11.7)	3 (2.5)
Both Month 9 and Month 18 censored	1 (1.3)	1 (0.8)
Month 9 missing and Month 18 censored	0	0

Norfolk QoL-DN at Month 9

The Norfolk QoL-DN is a standardized quality of life questionnaire designed to measure the perception of the effects of polyneuropathy by the patient. Lower scores indicate a higher quality of life. Norfolk QoL-DN was assessed at baseline, Month 9, and Month 18.

At Month 9, the vutrisiran group showed an improvement in quality of life compared to baseline (least squares [LS] mean change from baseline: -3.3 points) while the placebo (APOLLO) group showed a worsening in quality of life (LS mean change from baseline: +12.9 points) (see Table 17). This represents a statistically significant improvement in quality of life at 9 months for patients in the vutrisiran group compared to the placebo group (LS mean difference between groups: -16.2 points, $P=5.426 \times 10^{-9}$).

Table 17 (from Table 14 of Module 2.7.3 Summary of Clinical efficacy): Norfolk QoL-DN, ANCOVA/Multiple Imputation Model, Change from Baseline to Month 9, (mITT Population)

Visit	Actual/ Change	Statistic	APOLLO	HELIOS-A
			Placebo (N=77)	Vutrisiran (N=122)
Baseline	Actual	N	76	121
		Mean (SD)	55.5 (24.3)	47.1 (26.3)
		Median	53.5	44.0
		Min, Max	8, 111	-1, 105
Month 9	Actual	N	66	115
		Mean (SD)	66.2 (27.6)	41.8 (26.6)
		SE	3.4	2.5
		Median	68.0	40.0
		Min, Max	5, 109	-4, 102
	Change from baseline[a]	LS mean (SE)	12.9 (2.2)	-3.3 (1.7)
		95% CI	(8.5, 17.3)	(-6.6, -0.1)

Visit	Actual/ Change	Statistic	APOLLO	HELIOS-A
			Placebo (N=77)	Vutrisiran (N=122)
		LS mean difference (SE) (vutrisiran – placebo)	**-**	**-16.2 (2.8)**
		95% CI	**-**	**(-21.7, -10.8)**
		p-value	**-**	**5.426E-09**

Abbreviations: AE=adverse event; ANCOVA= analysis of variance; CI=confidence interval; COVID-19=coronavirus disease 2019; hATTR=hereditary transthyretin-mediated (amyloidosis); LS=least squares; max=maximum; min=minimum; mITT=modified intent-to-treat; NIS=neuropathy impairment score; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; SAP=statistical analysis plan; SD=standard deviation; SE=standard error.
Notes: Norfolk QoL-DN score = composite of 35 quality of life questions; lower scores indicate higher quality of life (range = -4 to 136). Nonmissing scores for items 27-33 imputed for patients who reported an impact due to COVID-19 pandemic (see SAP). Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis (considered missing before multiple imputation).
[a]Multiple imputation estimates and p-value derived per combining LS estimates per Rubin's rules based on 100 datasets where missing Month 9 values were imputed using a regression procedure including select baseline variables (see SAP). LS estimates derived from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset, baseline NIS) and continuous covariate (baseline value). Source: Study 002 CSR1 Table 14.2.1.2.1

Of the 122 patients randomized to vutrisiran (HELIOS-A), 117 (95.9%) patients had a baseline assessment for Norfolk QoL-DN and at least one post baseline follow-up assessment (either at 9 months, 18 months, or both) and were included in the MMRM model based on change from baseline in Norfolk QoL-DN.

Norfolk QoL-DN at Month 18

A statistically significant improvement in quality of life for patients in the vutrisiran group compared to the placebo group was also observed at Month 18 (LS mean difference between groups: -21.0 points, $P=1.844 \times 10^{-10}$).

Table 18 (from Table 16 of HELIOS-A CSR2 and Table 7 of Responses to Day 120 Q59b): Norfolk QoL-DN, Change From Baseline to Month 18, MMRM Model (mITT Population)

Statistic[a]	APOLLO	HELIOS-A
	Placebo (N=77)	Vutrisiran (N=122)
Month 18 LS mean (SE)	19.8 (2.6)	-1.2 (1.8)
95% CI	(14.7, 24.9)	(-4.8, 2.4)
LS mean difference (SE) (vutrisiran – placebo)	**-**	**-21.0 (3.1)**
95% CI	**-**	**(-27.1, -14.9)**
p-value	**-**	**1.844E-10**

Abbreviations: AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; CSR=Clinical Study Report; hATTR=hereditary transthyretin-mediated (amyloidosis); LS=least squares; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; SAP=Statistical Analysis Plan; SE=standard error.

Notes: Norfolk QoL-DN score = composite of 35 quality of life questions; lower scores indicate higher quality of life (range = -4 to 136). Nonmissing scores for items 27-33 imputed for patients who reported an impact due to COVID-19 pandemic (see SAP). Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis.
a LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariate (baseline value), and interaction (treatment by visit). Source: Study 002 CSR2 Table 14.2.1.2.2

10-MWT, Change from Baseline at Month 9

The 10-MWT is a measure of ambulatory ability and gait speed. An increase in gait speed from baseline represents improvement, and a decrease from baseline represents worsening. 10-MWT was assessed at Baseline, Month 9 and Month 18.

At Month 9, the vutrisiran group showed stable ambulatory function compared to baseline as measured by 10-MWT (LS mean change from baseline: -0.001 m/s) while the placebo (APOLLO) group showed a worsening of 10-MWT (LS mean change from baseline: -0.133 m/s) (see Table 19). This represents a statistically significant improvement in gait speed at 9 months for patients in the vutrisiran group compared to the placebo group (LS mean difference between groups: 0.131 m/s, P=3.103×10^{-5}).

Table 19 (part of Table 17 of Module 2.7.3 Summary of Clinical efficacy): 10-MWT (m/s), ANCOVA/Multiple Imputation Model, Change from Baseline to Month 9 (mITT Population)

Visit	Actual/ Change	Statistic	APOLLO Placebo (N=77)	HELIOS-A Vutrisiran (N=122)
		N	77	122
	Change from baseline [a]	LS mean (SE)	-0.133 (0.025)	-0.001 (0.019)
		95% CI	(-0.182, -0.083)	(-0.038, 0.036)
		LS mean difference (SE) (vutrisiran – placebo)	-	**0.131 (0.031)**
		95% CI	-	**(0.070, 0.193)**
		p-value	-	**3.103E-05**

Abbreviations: 10-MWT=10-meter walk test; AE=adverse event; ANCOVA=analysis of variance; CI=confidence interval; COVID-19=coronavirus disease 2019; LS=least squares; max=maximum; min=minimum; mITT=modified intent-to-treat; NIS=neuropathy impairment score; SAP=statistical analysis plan; SD=standard deviation; SE=standard error.
Notes: 10-meter walk test speed (m/s) =10 meters/mean time (seconds) taken to complete 2 assessments at each visit, imputed as 0 for patients unable to perform the walk; higher speeds indicate greater ambulatory function. Assessments on or after onset of a serious COVID-19 AE excluded from analysis (considered missing before multiple imputation).
[a] Multiple imputation estimates and p-value derived per combining least squares (LS) estimates per Rubin's rules based on 100 datasets where missing Month 9 values were imputed using a regression procedure including select baseline variables (see SAP). LS estimates derived from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset, baseline NIS) and continuous covariate (baseline value).
Source: Study 002 CSR1 Table 14.2.1.3.1

Of the 122 patients randomized to vutrisiran (HELIOS-A), 118 (96.7%) patients had a baseline assessment for 10-MWT and at least one post baseline follow-up assessment (either at 9 months, 18 months, or both) and were included in the MMRM model based on change from baseline in 10-MWT.

10-MWT, Change from Baseline at Month 18

A statistically significant improvement in gait speed for patients in the vutrisiran group compared to the placebo group was also observed at Month 18 (LS mean difference between groups: 0.239 m/s, $P=1.207 \times 10^{-7}$).

Table 20 (from Table 1 of HELIOS-A CSR2): 10-MWT, Change From Baseline to Month 18, MMRM Model (mITT Population)

Statistic[a]	APOLLO	HELIOS-A
	Placebo (N=77)	**Vutrisiran (N=122)**
Month 18 LS mean (SE)	-0.264 (0.036)	-0.024 (0.025)
95% CI	(-0.334, -0.194)	(-0.075, 0.026)
LS mean difference (SE) (vutrisiran – placebo)	-	**0.239 (0.043)**
95% CI	-	**(0.154, 0.325)**
p-value	-	**1.207E-07**

Abbreviations: 10-MWT=10-meter walk test; AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; CSR=Clinical Study Report; LS=least squares; mITT=modified intent-to-treat; MMRM= mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; SE=standard error.
Notes: 10-meter walk test speed (m/s) =10 meters/mean time (seconds) taken to complete 2 assessments at each visit, imputed as 0 for patients unable to perform the walk; higher speeds indicate greater ambulatory function. Assessments on or after onset of a serious COVID-19 AE excluded from analysis.
a LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariate (baseline value), and interaction (treatment by visit). Source: Study 002 CSR2 Table 14.2.1.3.2

Other Secondary Efficacy Endpoints

mBMI

The mBMI is calculated as the product of BMI multiplied by the concentration of serum albumin. mBMI was assessed at Baseline, Day 85, Day 169, Month 9, Day 337, Day 421, Day 505, and Month 18. An increase in mBMI from baseline suggests improvement in the nutritional status, and a decrease from baseline suggests worsening of the nutritional status.

At Month 18, the vutrisiran group in HELIOS-A showed an improvement in mBMI compared to baseline (LS mean change from baseline: 25.0 kg/m2 × albumin g/L) while the placebo group in APOLLO showed a worsening of nutritional status (LS mean change from baseline: -115.7 kg/m2 × albumin g/L), representing an improvement favoring vutrisiran of 140.7 kg/m2 × albumin g/L; P=4.159 × 10-15 (see Table 21).

Table 21 (part of Table 26 of HELIOS-A CSR2 and Table 13 of Responses to Day 120): mBMI, Change from Baseline to Month 18, MMRM Model (mITT Population)

	APOLLO	HELIOS-A

Statistic[a]	Placebo (N=77)	Vutrisiran (N=122)
Month 18 LS mean (SE)	-115.7 (13.4)	25.0 (9.5)
95% CI	(-142.2, -89.1)	(6.3, 43.8)
LS mean difference (SE) (vutrisiran – placebo)	-	**140.7 (16.4)**
95% CI	-	**(108.4, 172.9)**
p-value	-	**4.159E-15**

Abbreviations: AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; CSR=Clinical Study Report; LS=least squares; mBMI=modified body mass index; mITT=modified intent-to- treat; MMRM=mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; SE=standard error.
Notes: mBMI value = albumin (g/L) x weight (kg) / height (m)^2; higher values indicate higher nutritional status. For placebo patients, Month 9 assessment = mean of Day 190 and Day 358 assessments; Month 18 assessment = Day 547 assessment. Assessments on or after onset of a serious COVID-19 AE excluded from analysis.
a LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariate (baseline value), and interaction (treatment by visit).

Of the 122 patients randomized to vutrisiran (HELIOS-A), 120 (98.4%) patients had a baseline assessment for mBMI and at least one post baseline follow-up assessment (at Day 85, 9 months, 18 months, or all assessments) and were included in the MMRM model based on change from baseline in mBMI.

R-ODS

The Rasch-built Overall Disability Scale (R-ODS) is a patient-reported measure of level of disability on a scale of 0-48, with 0 being the worst and 48 the best (no limitations); scores are based on activities of daily living and social participation. R-ODS was assessed at baseline, Month 9, and Month 18.

At Month 18, the LS mean change from baseline in R-ODS was -1.5 points for the vutrisiran group compared to -9.9 points for the placebo group (Table 14 below and Figure 14 in the responses). According to the Applicant, this represents a statistically significant improvement in disability at 18 months for patients in the vutrisiran group compared to the placebo group (LS mean difference between groups: 8.4 points; P=3.541× 10-15). These improvements with vutrisiran treatment were observed as early as Month 9 (Study 002 CSR2 Table 14.2.1.5.1 and Figure 14 in the responses).

Table 22 (from Table 28 of HELIOS-A CSR2 and Table 14 of Response to Day 120): R-ODS, Change From Baseline to Month 18, MMRM Model (mITT Population)

	APOLLO	HELIOS-A
Statistic[a]	**Placebo (N=77)**	**Vutrisiran (N=122)**
Month 18 LS mean (SE)	-9.9 (0.8)	-1.5 (0.6)
95% CI	(-11.5, -8.3)	(-2.6, -0.3)
LS mean difference (SE) (vutrisiran – placebo)	-	**8.4 (1.0)**

95% CI	-	**(6.5, 10.4)**
p-value	-	**3.541E-15**

Abbreviations: AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; CSR=Clinical Study Report; LS=least squares; mITT=modified intent-to-treat; MMRM= mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; R-ODS=Rasch-built Overall Disability Scale; SE=standard error.
Notes: R-ODS score = composite of 24 disability questions; higher scores indicate lower disability (range = 0 to 48). Assessments on or after onset of a serious COVID-19 AE excluded from analysis.
a LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariate (baseline value), and interaction (treatment by visit).
Source: Study 002 CSR2 Table 14.2.1.5.1

Of the 122 patients randomized to vutrisiran (HELIOS-A), 118 (96.7%) patients had a baseline assessment for R-ODS and at least one post baseline follow-up assessment (either at 9 months, 18 months, or both) and were included in the MMRM model based on change from baseline in R-ODS.

Exploratory Endpoint

NT-proBNP

Cardiac amyloid is an important cause of morbidity and mortality, leading to signs and symptoms of worsening congestive heart failure, diminished exertional capacity, and ultimately death from heart failure or arrhythmia.

Serum levels of cardiac biomarkers, including NT-proBNP, troponin T, and troponin I, were used to assess cardiac stress and heart failure severity. These cardiac biomarkers were assessed at baseline, Day 85, Day 169, Month 9, Day 337, Day 421, Day 505, and Month 18. In addition, NT-proBNP, was assessed at Screening.

Table 23 (from Figure 11 of HELIOS-A CSR2): NT-proBNP, Change From Baseline Over Time (mITT Population)

mITT Population		
	APOLLO	**HELIOS-A**
Statistic[a]	**Placebo N=52**	**Vutrisiran N=114**
Baseline, Geometric Mean (SE)	531.291 (86.661)	273.006 (42.240)
Adjusted geometric fold change at Month 18	1.956	0.939
95% CI	(1.628, 2.351)	(0.826, 1.066)
Adjusted geometric fold change ratio (vutrisiran/placebo)		**0.480**
95% CI		**0.383, 0.600)**
p-value		**9.606E-10**
Cardiac Subpopulation		
	APOLLO	**HELIOS-A**
Statistic[a]	**Placebo N=36**	**Vutrisiran N=40***
Baseline, Geometric Mean (SE)	711.100 (151.079)	748.070 (163.184)
Adjusted geometric fold change at Month 18	1.927	0.946
95% CI	(1.443, 2.573)	(0.742, 1.206)
Adjusted geometric fold change ratio (vutrisiran/ placebo)		**0.491**
95% CI		**(0.337, 0.716)**
p-value		**0.0004**

*Cardiac subpopulation has been redefined.
Abbreviations: ANCOVA=analysis of covariance; CI=confidence interval; LS=least squares; mITT=modified intent-to-treat; NIS=neuropathy impairment score; NT-proBNP=*N*-terminal prohormone B-type natriuretic peptide; SAP=statistical analysis plan; SE=standard error.
Note: Lower values indicate less cardiac stress.
[a] Multiple imputation fold-change estimates and p-value derived per combining then exponentiating LS estimates per Rubin's rules based on 100 datasets where missing Month 9 values were imputed using a regression procedure including select baseline variables (see SAP). LS estimates derived from analysis of covariance model of log ratio Month 9 value to baseline, controlling for categorical factor (treatment) and continuous covariate (log-transformed baseline value).
Source: Study 002 CSR2 Table 14.2.4.13.1, Table 14.2.4.15.1

Changes in NT-proBNP levels in response to interventions are predictive of mortality outcomes. Stabilisation of the NT-proBNP levels in vutrisiran-treated patients (0.939-fold change in the mITT or 0.946-fold change in the cardiac subpopulation) as opposed to a clear increase in the placebo group in APOLLO (1.956-fold and 1.927-fold change in the mITT and the cardiac subpopulation, respectively) is suggestive of a beneficial cardiac effect. The adjusted geometric fold change ratio (vutrisiran/placebo)

for the cardiac subpopulation was 0.491 (p = 0.0004). However, it has been noted that the placebo group in APOLLO was in worse disease condition and had a higher degree of cardiac involvement.

Despite the redefinition of the cardiac subpopulation in HELIOS-A and the baseline differences between HELIOS-A and APOLLO, the magnitude of effect of vutrisiran on NT-proBNP is considered similar to that of patisiran obtained in APOLLO. At month 18, geometric mean NT-proBNP decreased to 544.06 ng/L in the patisiran-LNP group and increased to 1116.75 ng/L in the placebo group. At 18 months, the adjusted geometric mean ratio to baseline was 0.89 with Onpattro and 1.97 with placebo (ratio, 0.45; p < 0.001).

Echocardiographic parameters

For the Month 18 analysis, select echocardiogram parameters were re-read due to a staffing change at the central reading site that introduced a potential source of bias impacting the comparison of baseline versus follow-up echocardiogram data. Based on the new measurements for baseline LV wall thickness, the Cardiac Subpopulation was re-derived. As a result, 7 patients receiving vutrisiran were added to the Cardiac Subpopulation and 2 were removed compared to the population defined in the Month 9 analysis.

For prespecified echocardiographic parameters, the difference between vutrisiran (HELIOS-A) and placebo (APOLLO) treatment in the change from baseline at Month 18 was analyzed using an MMRM method in the mITT Population. The echocardiographic parameters analyzed included measures of cardiac structure (mean LV wall thickness, LV mass) and systolic function (global longitudinal strain, cardiac output), and measures related to diastolic function (LV end diastolic volume). Decreases in LV wall thickness, LV mass, and global longitudinal strain and increases in end diastolic volume and cardiac output represent improvements. Overall, the results in the vutrisiran and patisiran groups at Month 18 are comparable (Table 24).

Table 24 (compiled by the assessment teams): Results for Select Echocardiographic Parameters for the Cardiac Subpopulations for vutrisiran and patisiran in Study ALN-TTRSC02-002 (CSR2) and ALN-TTR02-004, respectively

	Placebo (APOLLO) (n=36)	**Vutrisiran (HELIOS-A) (n=40)**	**Patisiran HELIOS-A (n=12)**	**Patisiran APOLLO (n=90)**
Cardiac Biomarker				
NT-proBNP (ng/L)				
Baseline mean	1318.494	1809.284	2283.17	1512.35
Baseline Geometric mean	711.100	748.070	872.034	726.920
Month 18 mean	2942.761	1506.203	2231.828	1321.74
Month 18 Geometric mean	1116.745	614.367	808.716	544.09
Change from Baseline to Month 18 mean	1888.683	275.961	-83.275	55.85
Adjusted Geometric Mean Fold-Change	1.927 (1.97 in APOLLO)	**0.946**		**0.89**
Adjusted Geometric Mean Fold-Change Ratio (Substance vs Placebo)		**0.491 (p=0.0004)**		**0.45 (p=7.736E-08)**
Echocardiographic Parameters				
Mean Left Ventricular (LV) Wall Thickness (cm)				
Baseline mean	1.639	1.649	1.705	1.682
Month 18 mean	1.620	1.602	1.488	1.537
Change from Baseline to Month 18	-0.018	-0.034	-0.142	-0.106

LS Mean (SEM) Difference (Substance - Placebo)		**-0.030 (p=0.5397)**		**-0.093 (p=0.0173)**
Left Ventricular (LV) Mass (g)				
Baseline mean	264.518	269.417	297.740	275.483
Month 18 mean	266.013	255.514	253.337	251.258
Change from Baseline to Month 18	1.575	-8.159	-26.436	-16.143
LS mean difference		**-8.392 (p=0.5527)**		**-15.75 (p=0.150)**
Left Ventricular (LV) End-Diastolic Volume (mL)				
Baseline mean	84.899	84.179	84.322	86.219
Month 18 mean	74.011	79.178	96.618	81.893
Change from Baseline to Month 18	-14.540	-2.419	13.890	-4.884
LS mean difference		**8.780 (p=0.0607)**		**8.34 (p=0.036)**
Cardiac Output (L/min)				
Baseline mean	3.918	3.837	4.122	3.769
Month 18 mean	3.434	3.656	4.486	3.720
Change from Baseline to Month 18	-0.594	-0.115	0.160	-0.152
LS mean difference		**0.407 (p=0.0426)**		**0.38 (p=0.0441)**

Other Exploratory Efficacy Endpoints

The effect of vutrisiran on serum TTR levels and the resultant impact on neuropathy was accompanied by a benefit on other exploratory endpoints evaluating measures impacted by the underlying disease including neuropathy (NIS), and quality of life (EQ-5D-5L, EQ-VAS) (see Table 25). Collectively, these analyses indicated that vutrisiran stabilized disability progression, neuropathy impairment, and quality of life relative to the progression seen in placebo treated patients from the APOLLO study.

Table 25 (from Table 18 of Responses to Day 120): Summary of Exploratory Endpoint Results At Month 18: NIS, EQ-5D-5L, and EQ-VAS (mITT Population)

Exploratory Endpoint	**Statistic[a]**	**APOLLO Placebo (N=77)**	**HELIOS-A Vutrisiran (N=122)**
NIS Score range: 0 to 244 points Higher score=greater severity of disease Decrease/increase from baseline=improvement/worse ning neuropathy	Baseline, Mean (SD)	57.02 (32.04)	43.02 (28.63)
	Month 18, LS mean change (SE)	25.04 (2.05)	2.29 (1.49)
	95% CI	(20.99, 29.08)	(-0.66, 5.23)
	LS mean difference (SE) (vutrisiran – placebo)		**-22.75 (2.53)**
	95% CI		**(-27.74, -17.76)**
	p-value		**3.742E-16**
EQ-5D-5L Score range: 0 to 1 Higher score=better quality of life	Baseline, Mean (SD)	0.6451 (0.1681)	0.7083 (0.1545)
	Month 18, LS mean change (SE)	-0.2104 (0.0202)	-0.0244 (0.0145)
	95% CI	(-0.2502, -0.1706)	(-0.0531, 0.0043)

Increase/decrease from baseline=improvement/ worsening quality of life	**LS mean difference (SE) (vutrisiran – placebo)**		**0.1860 (0.0246)**
	95% CI		**(0.1374, 0.2346)**
	p-value		**2.575E-12**
EQ-VAS Score range: 0 to 100 Higher score=better quality of life Increase/decrease from baseline=improvement/ worsening quality of life	Baseline, Mean (SD)	54.6 (18.0)	64.5 (18.5)
	Month 18, LS mean change (SE)	-11.6 (2.1)	2.1 (1.5)
	95% CI	(-15.7, -7.5)	(-0.9, 5.0)
	LS mean difference (SE) (vutrisiran – placebo)		**13.7 (2.5)**
	95% CI		**(8.7, 18.7)**
	p-value		**2.214E-07**

Abbreviations: CI=confidence interval; CSR=Clinical Study Report; LS=least squares; EQ-5D-5L=EuroQoL5-Dimensions 5-Levels; EQ-VAS=EuroQoL visual analogue scale; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; NIS=neuropathy impairment score; SD=standard deviation; SE=standard error.
a LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariate (baseline value), and interaction (treatment by visit).
Source: Study 002 CSR2 Table 14.2.4.3.2, Table 14.2.4.4.2, Table 14.2.4.5.2

The results from the other exploratory endpoints, especially those evaluating quality of life, can be considered as supporting and complementing the results of the endpoints mNIS+7 and Norfolk QoL-DN at Month 18.

In order to have an overview of the results of the primary, key secondary and secondary endpoints from both of the studies HELIOS-A and APOLLO the following tables are considered useful.

Table 26 (from Table 1 of Responses to Day 120): Clinical Efficacy Results Summary for HELIOS-A at Month 9 and Month 18

Primary and Secondary Endpoints[a]	Baseline, Mean (SD)			Change from Baseline, LS Mean (SEM)		Vutrisiran -Placebo[b] Treatment Difference, LS Mean (95% CI)	*p*-value
	Vutrisiran N=122	Placebo[b] N=77		Vutrisiran	Placebo[b]		
Month 9							
mNIS+7[c]	60.6 (36.0)	74.6 (37.0)		-2.2 (1.4)	14.8 (2.0)	**-17.0** (-21.8, -12.2)	*p*<0.0001
Norfolk QoL-DN[c]	47.1 (26.3)	55.5 (24.3)		-3.3 (1.7)	12.9 (2.2)	**-16.2** (-21.7, -10.8)	*p*<0.0001
10-meter walk test (m/sec)[d]	1.01 (0.39)	0.79 (0.32)		0 (0.02)	-0.13 (0.03)	**0.13** (0.07, 0.19)	*p*<0.0001
Month 18							
mNIS+7[c]	60.57 (35.99)	74.61 (37.04)		-0.46 (1.60)	28.09 (2.28)	**-28.55** (-34.00, -23.10)	*p*<0.0001
Norfolk QoL-DN[c]	47.1 (26.3)	55.5 (24.3)		-1.2 (1.8)	19.8 (2.6)	**-21** (-27.1, -14.9)	*p*<0.0001
10-meter walk test (m/sec)[d]	1.01 (0.39)	0.79 (0.32)		-0.02 (0.03)	-0.26 (0.04)	**0.24** (0.15, 0.33)	*p*<0.0001
mBMI[e]	1057.5	989.9		25	-115.7	**140.7**	*p*<0.0001

Primary and Secondary Endpoints[a]	Baseline, Mean (SD)			Change from Baseline, LS Mean (SEM)		Vutrisiran -Placebo[b] Treatment Difference, LS Mean (95% CI)	*p*-value
	Vutrisiran N=122	Placebo[b] N=77		Vutrisiran	Placebo[b]		
	(233.8)	(214.2)		(9.5)	(13.4)	(108.4, 172.9)	
R-ODS[f]	34.1 (11.0)	29.8 (10.8)		-1.5 (0.6)	-9.9 (0.8)	**8.4** (6.5, 10.4)	*p*<0.0001

Abbreviations: ANCOVA/MI=analysis of covariance with multiple imputation; BMI=body mass index; CI=confidence interval; LS mean=least squares mean; mBMI=modified body mass index; MMRM=mixed-effects model for repeated measures; mNIS=modified Neuropathy Impairment Score; R-ODS=Rasch-built Overall Disability Scale; QoL-DN=Quality of Life - Diabetic Neuropathy; SD=standard deviation; SEM=standard error of the mean.
a All Month 9 endpoints analyzed using the ANCOVA/MI method and all Month 18 analyzed using the MMRM
b External placebo group from APOLLO randomized controlled trial
c A lower number indicates less impairment/fewer symptoms
d A higher number indicates less disability/less impairment
e mBMI: body mass index (BMI; kg/m2) multiplied by serum albumin (g/L); a higher number indicates better nutritional status
f A higher number indicates less disability/less impairment

Table 27 (from Table 2 of Responses to Day 120): Clinical Efficacy Results Summary for the Patisiran Arms of HELIOS-A and APOLLO at Month 9 and Month 18

		Baseline		Change from baseline to Month 9[a]		Change from baseline to Month 18[a]	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
mNIS+7[b]	APOLLO Patisiran (N=148)	148	80.93 (41.507)	141	-0.07 (15.478)	137	-4.19 (18.1.72)
	HELIOS-A Patisiran (N=42)	42	57.68 (33.71)	40	-0.40 (17.21)	36	1.59 (21.50)
Norfolk QoL-DN[b]	APOLLO Patisiran (N=148)	148	59.6 (28.18)	141	-3.9 (18.15)	136	-2.6 (21.28)
	HELIOS-A Patisiran (N=42)	42	47.3 (29.9)	40	-0.6 (18.0)	38	-0.6 (19.3)
10MWT (m/sec)[c]	APOLLO Patisiran (N=148)	147	0.795 (0.4009)	141	0.010 (0.1987)	138	0.040 (0.2649)
	HELIOS-A Patisiran (N=42)	42	1.011 (0.400)	40	-0.037 (0.197)	38	-0.043 (0.276)
mBMI[d,e]	APOLLO Patisiran (N=148)	148	969.7 (210.45)	-	NA	133	-6.5 (111.89)
	HELIOS-A Patisiran (N=42)	42	1058.1 (228.8)	38	-4.2 (103.5)	38	6.9 (91.8)
R-ODS[c]	APOLLO Patisiran (N=148)	148	29.7 (11.51)	141	-0.6 (5.67)	138	-0.8 (6.74)

		Baseline		Change from baseline to Month 9[a]		Change from baseline to Month 18[a]	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
	HELIOS-A Patisiran (N=42)	42	34.0 (10.4)	40	-1.8 (6.3)	38	-1.2 (5.9)

Abbreviations: 10MWT=10-meter walk test; CI=confidence interval; LS=least squares; mBMI=modified body mass index; MI=multiple imputation; MMRM=mixed-effects model for repeated measures; mNIS+7=modified Neuropathy Impairment Score +7; QoL-DN=Quality of Life-Diabetic Neuropathy; R-ODS=Rasch-built Overall Disability Scale; SD=standard deviation; SEM=standard error of the mean.
a Analyzed using MMRM.
b A lower number indicates less impairment/fewer symptoms.
c A higher number indicates less disability/less impairment.
d mBMI value = albumin (g/L) × weight (kg) / height (m2); higher values indicate higher nutritional status.
e mBMI results for the APOLLO patisiran group listed for Month 18 were collected at Day 546.
Sources: Study ALN-TTRSC02-002 CSR2 Tables 14.2.1.1.2, 14.2.1.2.2, 14.2.1.3.2, 14.2.1.4.1, and 14.2.1.5.1; and Study ALN-TTR02-004 CSR1 Tables 14.2.1.1.1, 14.2.1.2.1, 14.2.1.4, 14.2.1.5.1, and 14.2.1.10

It should be noted that the LS mean differences are estimated from statistical models controlling for prespecified covariates and reflect the mean treatment differences for patients with "average" disease severity and baseline scores in the dataset. The HELIOS-A dataset was based on pooled HELIOS-A vutrisiran and APOLLO placebo data, while the APOLLO dataset was based on pooled patisiran and placebo data from APOLLO. The two datasets are not identical, with slightly different data distributions; therefore, the two fitted models, e.g., the relationship between the outcome and covariates, had slight differences, which led to slightly different estimates. Overall, the estimated treatment effects are very similar for vutrisiran and patisiran.

In addition, even though the study was not designed to include formal comparisons of efficacy for these groups, the following Table 28 includes post hoc analysis of key efficacy parameters using mixed-effects model for repeated measures (MMRM) for the within-study comparison of vutrisiran and patisiran groups of the HELIOS-A study, at Month 9 and Month 18. Overall, the HELIOS-A patisiran arm showed similar results as the vutrisiran arm.

Table 28 (from Table 1 of Responses to Day 120): Clinical Efficacy Results Summary for HELIOS-A, Change from Baseline to Month 9 and Month 18, MMRM Model (mITT Population)

		Baseline		Month 9[a]			Month 18[a]		
		n	Mean (SD)	n	LS mean change (SEM)	LS mean difference (95% CI)	n	LS mean change (SEM)	LS mean difference (95% CI)
mNIS+7[b]	Vutrisiran (N=122)	122	60.57 (35.99)	116	-1.37 (1.32)	**-0.96 (-6.14, 4.22)**	112	0.06 (1.48)	**-1.46 (-7.36, 4.43)**
	Patisiran (N=42)	42	57.68 (33.71)	40	-0.42 (2.26)		36	1.53 (2.59)	
Norfolk QoL-DN[b]	Vutrisiran (N=122)	121	47.1 (26.3)	115	-4.0 (1.6)	**-3.6 (-9.8, 2.6)**	111	-2.5 (1.8)	**-1.6 (-8.6, 5.4)**
	Patisiran (N=42)	42	47.3 (29.9)	40	-0.4 (2.7)		38	-0.8 (3.0)	
10MWT (m/sec)[c]	Vutrisiran (N=122)	122	1.006 (0.393)	115	0.002 (0.017)	**0.039 (-0.029, 0.106)**	112	-0.019 (0.025)	**0.034 (-0.064, 0.132)**
	Patisiran (N=42)	42	1.011 (0.400)	40	-0.037 (0.029)		38	-0.053 (0.043)	
mBMI[d]	Vutrisiran (N=122)	122	1057.4 (233.8)	114	4.2 (7.7)	**3.7 (-26.6, 34.1)**	113	21.8 (9.2)	**14.2 (-21.9, 50.3)**
	Patisiran (N=42)	42	1058.1 (228.8)	38	0.5 (13.3)		38	7.6 (15.8)	
R-ODS[c]	Vutrisiran (N=122)	122	34.1 (11.0)	115	-0.4 (0.5)	**1.4 (-0.7, 3.5)**	113	-1.2 (0.5)	**0.1 (-2.0, 2.2)**

		Baseline		Month 9[a]			Month 18[a]		
		n	Mean (SD)	n	LS mean change (SEM)	LS mean difference (95% CI)	n	LS mean change (SEM)	LS mean difference (95% CI)
	Patisiran (N=42)	42	34.0 (10.4)	40	-1.8 (0.9)		38	-1.3 (0.9)	

Abbreviations: 10MWT=10-meter walk test; CI=confidence interval; LS=least squares; mBMI=modified body mass index; MI=multiple imputation; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; mNIS+7=modified Neuropathy Impairment Score +7; QoL-DN=Quality of Life-Diabetic Neuropathy; R-ODS=Rasch-built Overall Disability Score; SD=standard deviation; SEM=standard error of the mean.
a LS estimates derived from MMRM, controlling for categorical factors (treatment, visit), continuous covariate (baseline value), and interaction (treatment by visit).
b A lower number indicates less impairment/fewer symptoms.
c A higher number indicates less disability/less impairment.
d mBMI value = albumin (g/L) × weight (kg) / height (m2); higher values indicate higher nutritional status.
Sources: Table M.5.1, Table M.5.2, Table M.5.3, Table M.5.4, and Table M.5.

Serum TTR (Exploratory Endpoint through Month 9 and Secondary through Month 18)

Reductions in serum TTR observed at Month 9 with vutrisiran treatment were sustained through Month 18 (in the Responses to Day 120 LoQ: Figure 1 in Q59b and Figure 3 in Q59a).

The time-averaged trough TTR percent reduction through Month 18 was 84.7% for vutrisiran and 80.6% for patisiran (Table 29**Table 29** below). The Applicant concluded that vutrisiran demonstrated non-inferiority compared to within-study patisiran as the 95% CI of the median treatment difference in TTR percent reduction (vutrisiran - patisiran) was 1.17, 9.25, in which its lower limit was above -10%.

Mean TTR percent change from baseline over time for the vutrisiran and patisiran groups is presented in the following Table 29 and Figure 9.

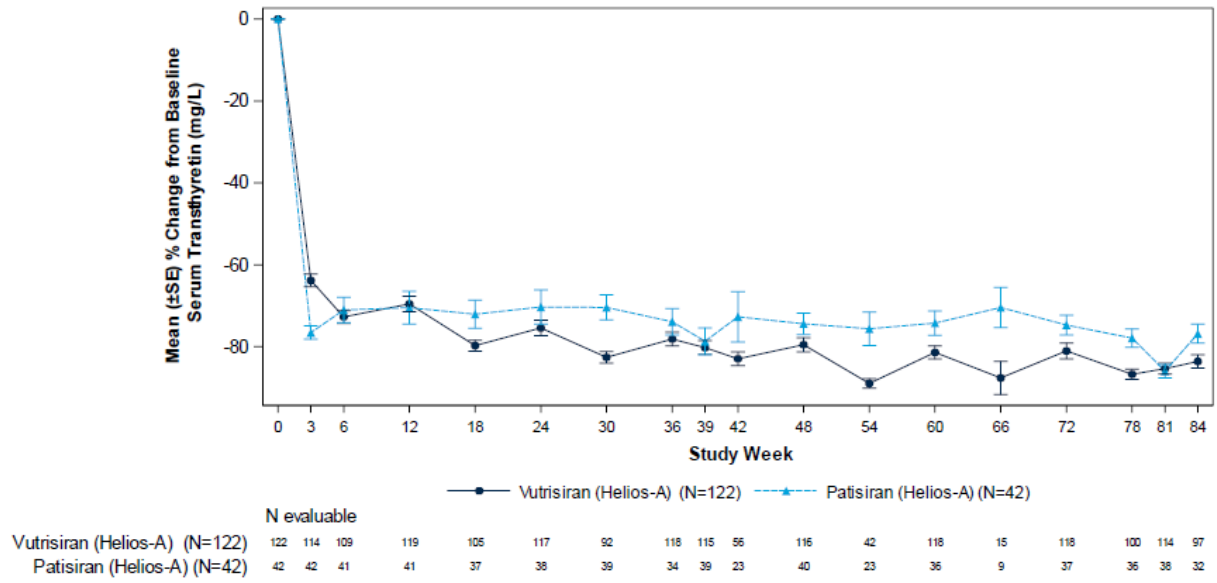
Table 29 (from Table 2 of Response to Day 120): Serum TTR (mg/L): Percent Reduction From Baseline Through Month 18 Hodges-Lehmann Analysis (TTR PP Population)

Visit	Actual/ Change	Statistic	HELIOS-A	
			Vutrisiran (N=120)	Patisiran (N=40)
Baseline	Actual	n	120	40
		Mean (SD)	206.77 (61.23)	209.49 (65.43)
		SE	5.59	10.35
		Median	203.49	207.53
		Min, Max	58.4, 343.2	71.0, 353.2
Month 6-18	Actual	n	120	40
		Mean (SD)	39.37 (41.84)	43.40 (28.42)
		SE	3.82	4.49
		Median	23.62	36.63
		Min, Max	3.0, 224.5	5.2, 132.7
	% Change from baseline	n	120	40
		Mean (SD)	-80.99 (20.96)	-78.56 (13.63)
		SE	1.91	2.16
		Median	-86.19	-81.39
		Min, Max	-98.3, 55.1	-97.2, -27.6
	Percent Reduction Model Estimates	**Pseudomedian (vutrisiran – patisiran)[a]**	**84.67**	**80.60**
		Median Difference[b]	**5.28**	**-**
		95% CI	**(1.17, 9.25)**	**-**
		Noninferiority (95% lower CI > -10%)	**Yes**	**-**

Abbreviations: CI=confidence interval; CSR=Clinical Study Report; max=maximum; min=minimum; PP=per-protocol; SAP=Statistical Analysis Plan; SD=standard deviation; SE=standard error;

TTR=transthyretin.
Note: Patient percent reduction derived from mean trough (predose) TTR assessments between Months 6 through 18; postbaseline TTR assessments included must meet requirements specified in SAP; lower TTR (greater reduction) indicates improvement.
a Hodges-Lehmann 1-sample medians.
b Hodges-Lehmann 2-sample median difference and 95% CI, stratified by previous TTR stabilizer use.
Source: Study 002 CSR2 Table 14.2.1.6

Figure 9 (from Figure 3 of Responses to Day 120): Mean (±SE) TTR Percent Change from Baseline Over Time During the 18-Month Treatment Period (mITT Population)



Abbreviations: CSR=Clinical Study Report; mITT=modified intent-to-treat; SE=standard error; TTR=transthyretin.
Notes: Month 9 and Month 18 non-trough TTR assessments presented at Weeks 39 and 81, respectively. Presented data ⩾5 patients per treatment arm at given study visit.
Source: Study 002 CSR2 Figure 14.2.5.1.2

In the APOLLO study, the patisiran group demonstrated a mean TTR percent reduction from baseline of 82.6% and 84.3% at Month 9 and Month 18, respectively. TTR percent reduction was maintained over the duration of the APOLLO study.

In HELIOS-A at Month 9, the reductions of TTR are similar or even slightly greater with vutrisiran than with patisiran and are in the order of 78-82%. Peak to trough fluctuation was greatly reduced for the vutrisiran group (Δ=1.72%) compared to the patisiran group (Δ=6.80%). However, a formal comparison of the TTR percent reduction between the vutrisiran and patisiran groups at Month 9 was not performed in HELIOS-A.

At Month 18 the reduction in serum TTR levels with vutrisiran (84.67%) was determined to be non-inferior to the within-study patisiran arm (80.60%) based on the pre-specified criteria [Median Difference (95% CI) 5.28 (1.17, 9.25), non-inferiority confirmed if 95% lower CI > -10%], despite the greater fluctuation in the vutrisiran arm. Average TTR reduction at steady state was very similar between the two treatment groups: Mean (SD) 81.37 (18.84) for vutrisiran and 79.68 (11.71) for patisiran and Median (min, max) was 86.88 (-29.0, 98.1) for vutrisiran and 81.51 (37.2, 94.2) for patisiran.

Exploratory endpoints related to staging of polyneuropathy

FAP Stage, PND Score, and KPS Score

According to the Applicant the benefit of treatment with vutrisiran was also seen across the exploratory endpoints of familial amyloid polyneuropathy (FAP) stage, polyneuropathy disability (PND) score, and Karnofsky Performance Status (KPS) score. PND score and FAP stage are frequently used to classify the severity of polyneuropathy in patients with hATTR amyloidosis and are based largely on ambulatory ability. KPS is an assessment of functional status.

FAP Stage

At Month 18, 7 (5.7%) patients in the vutrisiran group and 22 (28.6%) patients in the placebo group had missing FAP stages (Study 002 CSR2 Table 14.2.4.8, please see Q60). Among the patients with FAP stage data available at Month 18, 101 (82.8%) patients in the vutrisiran group and 34 (44.2%) patients in the placebo group had a stable FAP stage compared to baseline (Study 002 CSR2 Table 14.2.4.8). Five (4.1%) patients in the vutrisiran and no patients in the placebo group had an improvement in FAP stage relative to baseline. Nine (7.4%) patients in the vutrisiran group had a worsening in FAP stage relative to baseline compared to 21 (27.3%) patients in the placebo group.

PND Score

At Month 18, 7 (5.7%) patients in the vutrisiran group and 22 (28.6%) patients in the placebo group had missing PND scores (Study 002 CSR2 Table 14.2.4.6). Among the patients with PND score data available at Month 18, 82 (67.2%) patients in the vutrisiran group and 23 (29.9%) patients in the placebo group had stable PND scores compared to baseline (Study 002 CSR2 Table 14.2.4.6). Thirteen (10.7%) patients in the vutrisiran group and no patients in the placebo group had an improvement in PND score relative to baseline. Twenty (16.4%) patients in the vutrisiran group had a worsening in PND score relative to baseline compared to 32 (41.6%) patients in the placebo group.

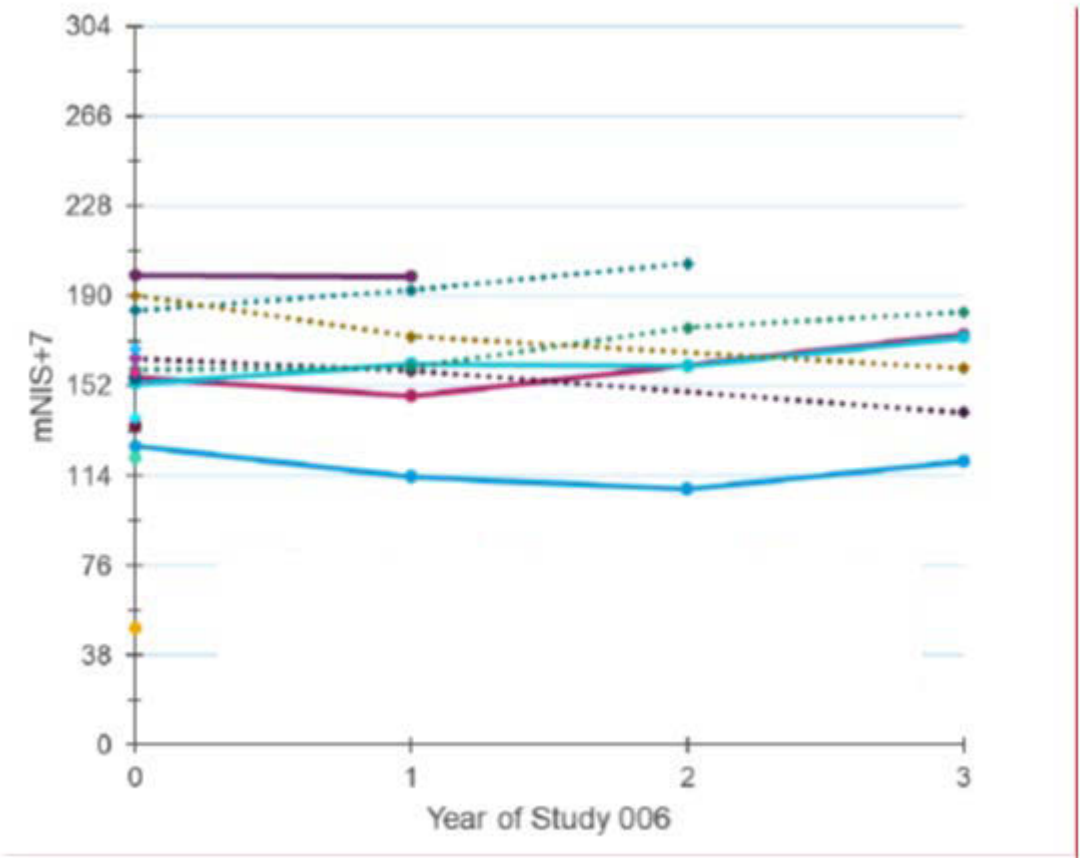
KPS Score

At Month 18, 71 (58.2%) patients in the vutrisiran group had a stable KPS stage compared to baseline (Study 002 CSR2 Table 14.2.4.12). Sixteen (13.1%) patients in the vutrisiran group had an improvement in KPS score relative to baseline. KPS was not collected post-baseline in APOLLO, so the APOLLO placebo group is not featured in this analysis.

Data for FAP stage 3 patients receiving treatment with an RNAi therapeutic is available from patisiran Study 006, which is an ongoing, open-label, extension study designed to evaluate the safety and efficacy of long-term patisiran dosing in patients with hATTR amyloidosis with polyneuropathy who have completed a prior patisiran study. Patients in this study have completed "parent" Study ALN-TTR02-003 (Study 003) or ALN-TTR02-004 (Study 004).

At entry into Study 006, a total of 16 patients had progressed to FAP stage 3, including 8 patients in the 004 Placebo group and 8 patients in the 004 Patisiran group; no patients in the 003 Patisiran group were FAP stage 3 at Study 006 baseline. Among the FAP stage 3 patients who had post-baseline measurements in Study 006, the overall clinical picture suggests relative stability over time in measures of neuropathy impairment (mNIS+7, Figure 10 below).

Figure 10 (from Figure 4 of Responses to Day 120): mNIS+7 Over Time in FAP Stage 3 Patients (Study ALN-TTR02-006)



Abbreviations: FAP=Familial Amyloid Polyneuropathy; mNIS+7=modified Neuropathy Impairment Score +7

Notes: Solid lines represent 004 Patisiran patients; dotted lines represent 004 Placebo patients. 004 Patisiran patients started treatment 18 months before Year 0 in Study 006; 004 Placebo patients started treatment at Year 0 in Study 006. mNIS+7 range is 0 to 304 points; lower scores indicate less severe disease.

With respect to FAP stage 3, only one patient with stage 3 was recruited, and this was in the placebo group in APOLLO. There were no stage 3 patients recruited in HELIOS-A (please see tables below). As the Applicant states, among the patients with FAP stage data available at Month 18, in the vutrisiran group 101 (82.8%) patients had a stable FAP stage, five (4.1%) patients had an improvement and nine (7.4%) patients had a worsening in FAP stage compared to baseline. In the placebo group 34 (44.2%) patients had a stable FAP stage, no patients had an improvement and 21 (27.3%) patients had a worsening in FAP stage relative to baseline.

Table 30 (from Table 14.2.4.8 of HELIOS-A CSR2): Familial Amyloidotic Polyneuropathy (FAP) Stage: Categorical Descriptives by Visit During the Study mITT Population

Table 14.2.4.8
Familial Amyloidotic Polyneuropathy (FAP) Stage: Categorical Descriptives by Visit During the Study
mITT Population

Analysis Visit Category	Placebo (APOLLO) (N=77) n (%)	Vutrisiran (HELIOS-A) (N=122) n (%)	Patisiran (HELIOS-A) (N=42) n (%)
Baseline			
0	0	0	0
I	37 (48.1)	84 (68.9)	31 (73.8)
II	39 (50.6)	38 (31.1)	11 (26.2)
III	1 (1.3)	0	0
Missing	0	0	0
Month 9			
Actual			
0	0	0	0
I	26 (33.8)	62 (50.8)	28 (66.7)
II	34 (44.2)	32 (26.2)	6 (14.3)
III	7 (9.1)	0	0
Missing	10 (13.0)	28 (23.0)	8 (19.0)
Change from baseline			
Improved	0	4 (3.3)	2 (4.8)
No change	54 (70.1)	84 (68.9)	32 (76.2)
Worsened	13 (16.9)	6 (4.9)	0
Missing	10 (13.0)	28 (23.0)	8 (19.0)

Lower scores indicate greater ambulatory function.

Table 14.2.4.8
Familial Amyloidotic Polyneuropathy (FAP) Stage: Categorical Descriptives by Visit During the Study
mITT Population

Analysis Visit Category	Placebo (APOLLO) (N=77) n (%)	Vutrisiran (HELIOS-A) (N=122) n (%)	Patisiran (HELIOS-A) (N=42) n (%)
Month 18			
Actual			
0	0	1 (0.8)	0
I	17 (22.1)	77 (63.1)	30 (71.4)
II	29 (37.7)	35 (28.7)	8 (19.0)
III	9 (11.7)	2 (1.6)	0
Missing	22 (28.6)	7 (5.7)	4 (9.5)
Change from baseline			
Improved	0	5 (4.1)	1 (2.4)
No change	34 (44.2)	101 (82.8)	36 (85.7)
Worsened	21 (27.3)	9 (7.4)	1 (2.4)
Missing	22 (28.6)	7 (5.7)	4 (9.5)

Lower scores indicate greater ambulatory function.

Ancillary analyses

Additional (pre-specified) sensitivity analyses on the primary endpoint (mNIS+7) were performed using the mITT population using the following methods:

- ANCOVA including mNIS+7 without censoring of patients following use of alternative treatment or on/after the onset of a serious COVID-19 AE
- Propensity score approach: propensity score was an analysis covariate in the ANCOVA. The propensity score was defined as the probability of being treated with vutrisiran as obtained from a logistic regression model of treatment group [vutrisiran; placebo (APOLLO)]. The logistic regression model included the following baseline variables: Continuous variables [NT-proBNP (log-transformed), mNIS+7 and Norfolk QoL-DN total score] and Categorical variables {Previous tetramer stabilizer use (tafamidis/diflunisal) [Yes; No], Karnofsky Performance Status (KPS) [60; 70-80; 90-100], Cardiac Subpopulation [Yes; No], PND score [I; II; IIIA; IIIB/IV], Age at hATTR Symptom onset [< 50; ≥ 50], Neuropathy Impairment Score (NIS) [< 50; ≥ 50], Genotype [V30M; non-V30M] and FAP stage [I; II/III]}.

- Pattern-mixture model (PMM) analysis under missing not at random (MNAR) assumptions

Additional (pre-specified) sensitivity analyses were also performed for Norfolk QoL-DN and 10-MWT at Month 9 and at Month 18.

See Module 2.7.3 Summary of Clinical Efficacy for a detailed description of these analyses.

The various sensitivity analyses are briefly outlined below (refer to HELIOS-A, Study 002, CSR2 Table 14.2.2.1.1, Table 14.2.2.2.1, Table 14.2.2.3.1).

Table 31 (from Table 2 of HELIOS-A CSR2): Analysis of Endpoint mNIS+7 and Endpoint Norfolk QoL-DN

Analysis	Description	Population	Analysis Model
Primary Month 18 Analysis	Evaluation where Month 18 data after initiation of local standard treatment OR on or after COVID-19 serious adverse event were censored	mITT	MMRM Continuous covariate: baseline value Categorical factors: treatment, visit, genotype, age at symptom onset, baseline NIS[a] Interaction: treatment by visit
Sensitivity Analysis 1[b]	Sensitivity evaluation where Month 18 data after initiation of local standard treatment OR on or after COVID-19 serious adverse event were included	mITT	MMRM Continuous covariate: baseline value Categorical factors: treatment, visit, genotype, age at symptom onset, baseline NIS[a] Interaction: treatment by visit
Sensitivity Analysis 2	Sensitivity evaluation where a propensity score was included as an analysis covariate, where the propensity score was estimated incorporating important baseline variables that covers potential differences between the APOLLO and HELIOS-A study populations	mITT	MMRM Continuous covariates: baseline value, propensity score Categorical factors: treatment, visit, genotype, age at symptom onset, baseline NIS[a] Interaction: treatment by visit
Sensitivity Analysis 3	Sensitivity evaluation where a pattern-mixture model was applied under the assumption that missing at random did not apply for patients with missing Month 18 data after stopping study treatment or who died before Month 18 due to reasons unrelated to COVID-19	mITT	Pattern-mixture model (modified ANCOVA/MI) Continuous covariate: baseline value Categorical factors: treatment, genotype, age at symptom onset
Other Analysis: Binary endpoint[c]	Evaluation of percentage of patients improving from baseline	mITT	CMH Stratification factor: genotype

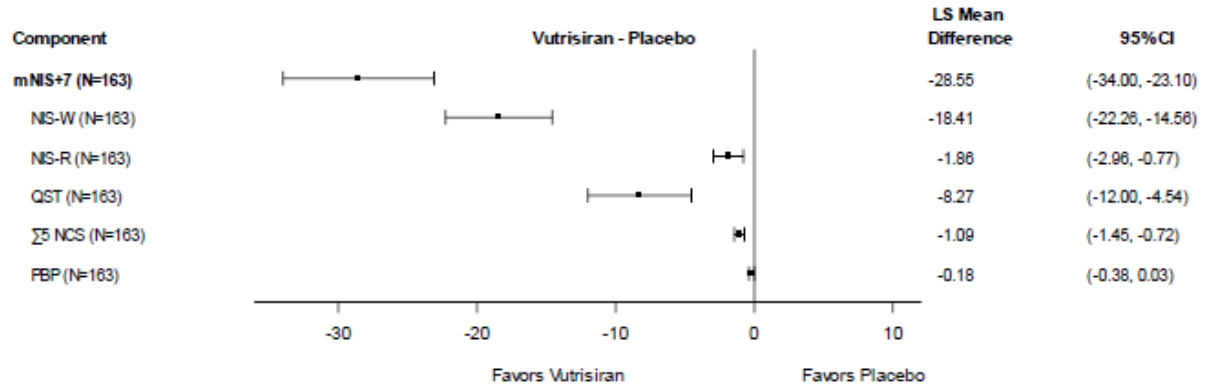
Other Analysis: Efficacy PP	Evaluation representing the hypothetical scenario where the COVID-19 pandemic had not occurred	Month 18 Efficacy PP	MMRM Continuous covariate: baseline value Categorical factors: treatment, visit, genotype, age at symptom onset, baseline NIS[a] Interaction: treatment by visit

Abbreviations: AE=adverse event; ANCOVA/MI=analysis of covariance model incorporating multiple imputation; CMH= Cochran-Mantel-Haenszel; COVID-19=coronavirus disease 2019; hATTR amyloidosis=hereditary transthyretin-mediated amyloidosis; mITT=Modified Intent-to-Treat; mNIS+7= modified Neuropathy Impairment Score +7; MMRM=mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; Norflk QoL-DN= Norfolk Quality of Life–Diabetic Neuropathy; PP=per-protocol.
a For NIS-related endpoints, the categorical baseline NIS score was not included in the model.
b Including data post local standard treatment for hATTR amyloidosis or post serious COVID-19 AE.
c Change from baseline in mNIS+7 of <0 points (decrease or improvement) and ⩾0 points (increase or worsening); change from baseline in Norfolk QoL-DN total score of <0 points (decrease or improvement) and ⩾0 points (increase or worsening).

Component Analysis of mNIS+7

The mNIS+7 components assess motor strength (Neuropathy Impairment Score-weakness [NIS-W]), reflexes (NIS-reflexes [NIS-R]), sensation (quantitative sensory testing [QST]), nerve conduction (nerve conduction studies sum of five attributes [Σ5 NCS]), and sympathetic nerve autonomic function (postural blood pressure [BP]). For each of these components, higher scores represent a greater severity of disease. At Month 18, a consistent improvement compared to placebo (APOLLO) was observed for vutrisiran (HELIOS-A) across all of the mNIS+7 components (see Figure 11).

Figure 11 (from Figure 3 of Responses to Day 120 Q59b): mNIS+7, Component Change From Baseline to Month 18, MMRM Model (mITT Population)



Abbreviations: CI=confidence interval; LS=least squares; mITT=modified intent-to-treat; MMRM= mixed-effects model for repeated measures; mNIS+7=modified Neuropathy Impairment Score+7; NIS-R= Neuropathy Impairment Score-reflexes; NIS-W=Neuropathy Impairment Score-weakness; PBP=postural blood pressure (points); QST=quantitative sensory testing; Σ5 NCS=nerve conduction studies sum of 5 attributes.
Source: Study 002 CSR2 Figure 14.2.3.2.2

<u>mNIS+7, Sensitivity analysis</u>

The result of the sensitivity analysis using a propensity score as a covariate in the ANCOVA is presented in the following Table 32 (from Table 14.2.2.2.1 of HELISO-A CSR) and is similar to the result of the primary analysis. Although a propensity score was included as a covariate in the analysis model, it may not capture all the differences (e.g. unmeasured/unrecognized factors) between the

groups. Furthermore, only patients with non-missing/non-censored data at Month 9 were included in the analysis and missing data were not imputed. The analysis population does not represent the initially randomized population. Therefore, this analysis could only be considered as supportive.

Table 32 (from Table 14.2.2.2.1. HELIOS-A CSR): Modified Neuropathy Impairment Score +7 (mNIS+7): Change from Baseline to Month 9, ANCOVA Model Sensitivity Analysis 2, Propensity Score mITT population

Table 14.2.2.2.1
Modified Neuropathy Impairment Score +7 (mNIS+7): Change from Baseline to Month 9, ANCOVA Model
Sensitivity Analysis 2, Propensity Score
mITT Population

Analysis Visit Statistics	Placebo (APOLLO) (N=77)	Vutrisiran (HELIOS-A) (N=122)	Patisiran (HELIOS-A) (N=42)
Baseline			
n	77	122	42
Mean (SD)	74.61 (37.04)	60.55 (35.99)	57.69 (33.71)
SE	4.22	3.26	5.20
Median	71.50	63.50	53.44
Min, Max	11.0, 153.5	2.5, 158.0	7.0, 137.6

mNIS+7 score = mean of 2 nonmissing assessments planned to be performed ≥24 hours to ≤7 days apart at each scheduled visit, after component imputation; lower scores indicate less neurologic impairment (range = 0 to 304).
Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis.
[1] Estimated from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset) and continuous covariate (baseline value and propensity score).

Table 14.2.2.2.1
Modified Neuropathy Impairment Score +7 (mNIS+7): Change from Baseline to Month 9, ANCOVA Model
Sensitivity Analysis 2, Propensity Score
mITT Population

Analysis Visit Statistics	Placebo (APOLLO) (N=77)	Vutrisiran (HELIOS-A) (N=122)	Patisiran (HELIOS-A) (N=42)
Month 9			
Actual Value			
n	67	114	37
Mean (SD)	90.99 (41.31)	57.50 (37.98)	52.56 (33.25)
SE	5.05	3.56	5.47
Median	91.50	57.00	50.25
Min, Max	19.0, 167.5	1.0, 160.1	6.0, 152.3
Change from baseline			
n	67	114	37
Mean (SD)	15.22 (17.18)	-1.70 (13.22)	-1.41 (17.23)
SE	2.10	1.24	2.83
Median	13.00	-0.44	-2.00
Min, Max	-16.6, 72.0	-35.0, 26.0	-47.0, 62.9
Model estimates [1]			
LS mean (SE)	14.11 (1.93)	-1.88 (1.49)	
95% CI	(10.30, 17.93)	(-4.81, 1.06)	
Vs. Placebo(APOLLO)			
LS mean difference (SE)		-15.99 (2.49)	
95% CI		(-20.90, -11.08)	
P-value		1.175E-09	

mNIS+7 score = mean of 2 nonmissing assessments planned to be performed ≥24 hours to ≤7 days apart at each scheduled visit, after component imputation; lower scores indicate less neurologic impairment (range = 0 to 304).
Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis.
[1] Estimated from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset) and continuous covariate (baseline value and propensity score).

Additional (prespecified) sensitivity analyses on the primary endpoint (mNIS+7) were performed using the Modified Intent-to-Treat (mITT) Population (Table 33). All sensitivity analyses resulted in a consistent estimate of the treatment effect of vutrisiran (HELIOS-A) compared to placebo (APOLLO) on mNIS+7 at Month 18, confirming the robustness of the primary Month 18 analysis.

Table 33 (from Table 4 of Responses to Day 120): mNIS+7, Sensitivity Analyses at Month 18 (mITT Population)

Sensitivity Analysis	Statistic	APOLLO	HELIOS-A
		Placebo (N=77)	**Vutrisiran (N=122)**
Sensitivity Analysis 1[a] No censoring	Month 18 LS mean (SE)	27.24 (2.29)	0.20 (1.65)
	95% CI	(22.72, 31.76)	(-3.05, 3.46)
	LS mean difference (SE) (vutrisiran – placebo)	-	**-27.04 (2.80)**
	95% CI	-	**(-32.57, -21.51)**
	p-value	-	**4.647E-18**
Sensitivity Analysis 2[b] Propensity score approach	Month 18 LS mean (SE)	27.77 (2.30)	-0.06 (1.64)
	95% CI	(23.22, 32.31)	(-3.30, 3.18)
	LS mean difference (SE) (vutrisiran – placebo)	-	**-27.83 (2.85)**
	95% CI	-	**(-33.46, -22.20)**
	p-value	-	**2.105E-18**
Sensitivity Analysis 3[c] PMM analysis	Month 18 LS mean (SE)	29.67 (2.62)	0.38 (1.75)
	95% CI	(24.52, 34.82)	(-3.05, 3.80)
	LS mean difference (SE) (vutrisiran – placebo)		**-29.30 (3.09)**
	95% CI		**(-35.36, -23.23)**
	p-value		**6.925E-21**

Abbreviations: AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; CSR=Clinical Study Report; hATTR=hereditary transthyretin-mediated (amyloidosis); LS=least squares; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; mNIS+7=modified Neuropathy Impairment Score +7; PMM= pattern-mixture model; SAP=Statistical Analysis Plan; SE=standard error.
a All assessments, including those after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE, included in analysis. LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset), continuous covariate (baseline value), and interaction (treatment by visit).
b Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis. LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset), continuous covariates (baseline value, propensity score), and interaction (treatment by visit).
c Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis (considered missing before multiple imputation). Multiple imputation estimates and p-value derived per combining LS estimates per Rubin's rules based on 100 datasets where missing Month 18 values were imputed using a PMM (see SAP). LS estimates derived from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset) and continuous covariate (baseline value).
Source: Study 002 CSR2 Table 14.2.2.1.2, Table 14.2.2.2.2, Table 14.2.2.3.2

Binary analysis

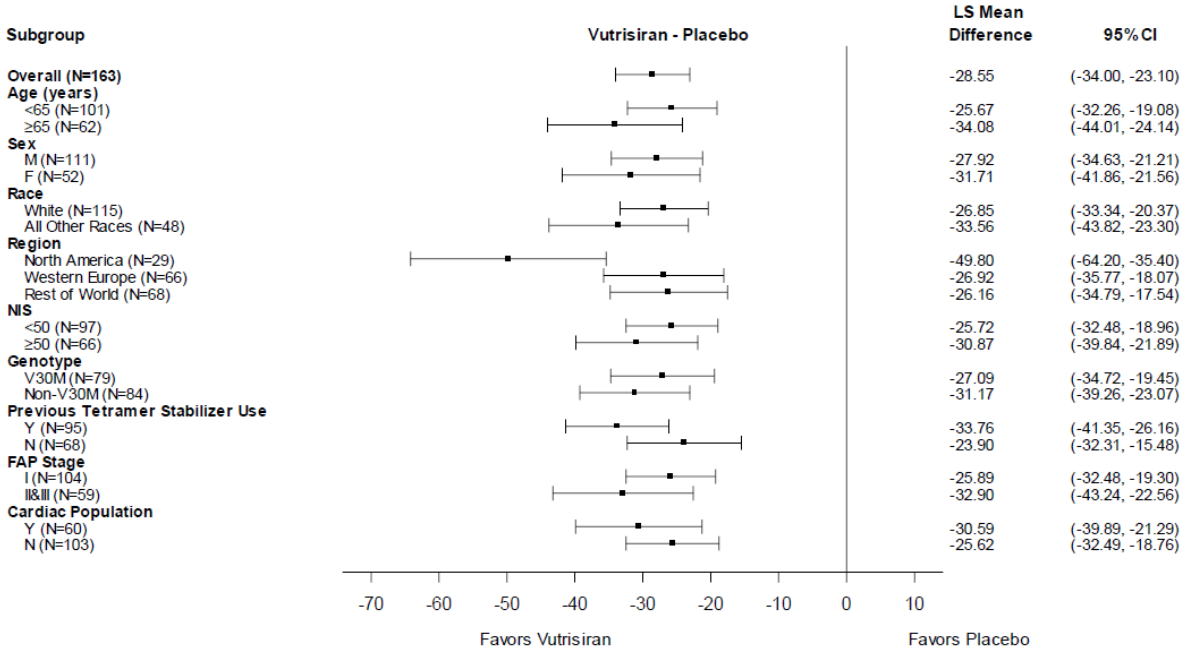
The number and percentage of patients with a change from baseline at Month 18 in mNIS+7 <0 (decrease or improvement) and ≥0 points was compared between the vutrisiran (HELIOS-A) and placebo (APOLLO) groups.

In the vutrisiran group, 48.3% of patients showed an improvement of neuropathy at Month 18 (change from baseline in mNIS+7 <0) as compared to 3.9% of placebo patients (odds ratio of 22.9; 95% CI: 6.8, 76.9) (Table 6 in the Responses to Day 120 LoQ).

Subgroup analyses of mNIS+7

Patients receiving vutrisiran experienced similar improvements relative to placebo (APOLLO) in mNIS+7 at Month 18 across all subgroups including age, sex, race, region, baseline NIS score, genotype, previous tetramer stabilizer use, disease stage, and cardiac subpopulation. Subgroup data are summarized in Study 002 CSR2 Table 14.2.3.1.2. A numerically larger improvement relative to placebo was observed in patients from North America compared to other regions.

Figure 12 (from Figure 4 of Responses to Day 120): mNIS+7, Change from Baseline to Month 18, MMRM Model Subgroup Analysis Forest Plot (mITT Population)



Abbreviations: CI=confidence interval; CSR=Clinical Study Report; FAP=Familial Amyloid Polyneuropathy; LS=least squares; mITT=modified intent-to-treat; mNIS+7=modified Neuropathy Impairment Score +7; MMRM=mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; V30M=valine to ethionine variant at amino acid 30.
Source: Study 002 CSR2 Figure 14.2.3.1.2

Genotype

The effect of vutrisiran was further evaluated across different genotypes.Treatment with vutrisiran resulted in improvements in mNIS+7 at Month 18 regardless of variant type. As several of these

variants are endemic to certain regions, this analysis further supports the consistency of effect across regions shown in Figure 12 above.

Both the component and the subgroup analyses, as well as across different genotypes (despite the small number of some subgroups or genotypes) favoured vutrisiran versus placebo. These results can be supportive of the primary analysis of the primary endpoint.

Sensitivity Analyses of Norfolk QoL-DN

Additional (pre-specified) sensitivity analyses on the secondary endpoint (Norfolk QoL-DN) were performed using the mITT Population (Table 34). All sensitivity analyses resulted in a consistent estimate of the treatment effect of vutrisiran (HELIOS-A) compared to placebo (APOLLO) on Norfolk QoL-DN at Month 18, confirming the robustness of the primary Month 18 analysis.

The sensitivity analysis related to the potential impact of coronavirus disease 2019 (COVID- 19) on quality of life and Norfolk QoL-DN is summarized in Section 2.9 in the responses.

Table 34 (from Table 8 of Responses to Day 120): Norfolk QoL-DN, Sensitivity Analyses at Month 18 (mITT Population)

Sensitivity Analysis	Statistic	APOLLO	HELIOS-A
		Placebo (N=77)	**Vutrisiran (N=122)**
Sensitivity Analysis 1[a] No censoring	Month 18 LS mean (SE)	19.8 (2.5)	-1.1 (1.8)
	95% CI	(14.9, 24.7)	(-4.7, 2.5)
	LS mean difference (SE) (vutrisiran – placebo)	-	**-20.9 (3.0)**
	95% CI	-	**(-26.8, -14.9)**
	p-value	-	**1.064E-10**
Sensitivity Analysis 2[b] Propensity score approach	Month 18 LS mean (SE)	20.3 (2.6)	-1.6 (1.9)
	95% CI	(15.1, 25.5)	(-5.2, 2.1)
	LS mean difference (SE) (vutrisiran – placebo)	-	**-21.9 (3.2)**
	95% CI	-	**(-28.2, -15.5)**
	p-value	-	**1.514E-10**
Sensitivity Analysis 3[c] PMM analysis	Month 18 LS mean (SE)	22.8 (2.9)	-1.3 (2.0)
	95% CI	(17.1, 28.4)	(-5.3, 2.6)
	LS mean difference (SE) (vutrisiran – placebo)		**-24.1 (3.5)**
	95% CI		**(-30.9, -17.3)**
	p-value		**4.928E-12**

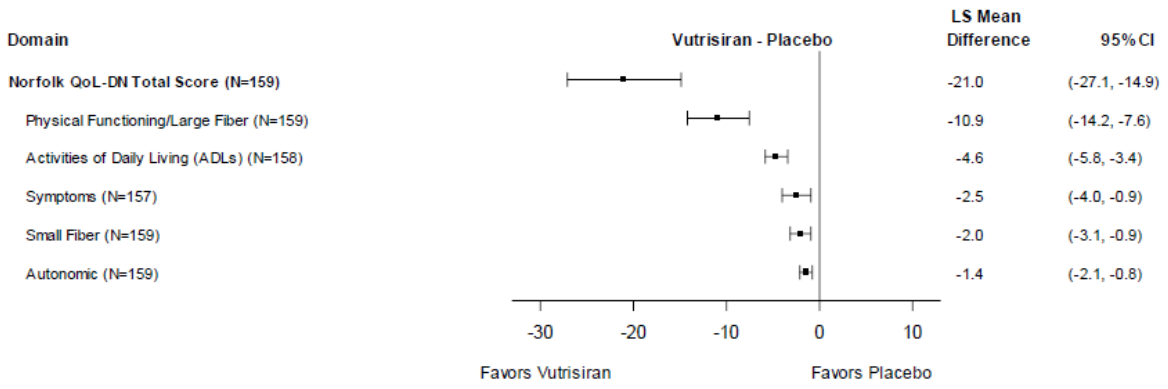
Abbreviations: AE=adverse event; CI=confidence interval; COVID-19=coronavirus disease 2019; CSR=Clinical Study Report; hATTR=hereditary transthyretin-mediated (amyloidosis); LS=least squares; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; PMM= pattern-mixture model; SAP=Statistical Analysis Plan; SE=standard error.
a All assessments, including those after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE, included in analysis. LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariate (baseline value), and interaction (treatment by visit).
b Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis. LS estimates and p-value derived from MMRM, controlling for categorical factors (treatment, visit, genotype, age of disease onset, baseline NIS), continuous covariates (baseline value, propensity score), and interaction (treatment by visit).
c Assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE excluded from analysis (considered missing before multiple imputation). Multiple imputation estimates and p-value derived per combining LS estimates per Rubin's rules based on 100 datasets where missing Month 18 values were imputed using a PMM (see SAP). LS estimates derived from analysis of covariance model, controlling for categorical factors (treatment, genotype, age of disease onset, baseline NIS) and continuous covariate (baseline value).
Source: Study 002 CSR2 Table 14.2.2.4.2, Table 14.2.2.5.2, Table 14.2.2.6.2

Norfolk QoL-DN Domain Analysis

The Norfolk QoL-DN consists of 5 domains (range of possible scores) which include: physical functioning/large fiber (-4 to 56 points), activities of daily living (0 to 20 points), symptoms (0 to 32 points), small fiber (0 to 16 points), and autonomic (0 to 12 points. For each of these domains, a lower score indicates a better quality of life.

At Month 18, a consistent improvement compared to placebo (APOLLO) was observed for vutrisiran (HELIOS-A) across all of the Norfolk QoL-DN domains (see Figure 13).

Figure 13 (from Figure 8 of Responses to Day 120): Norfolk QoL-DN, Change from Baseline to Month 18, MMRM Model Domain Analysis Forest Plot (mITT Population)



Abbreviations: CI=confidence interval; CSR=Clinical Study Report; LS=least squares; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy. Source: Study 002 CSR2 Figure 14.2.3.8.2

Binary analysis

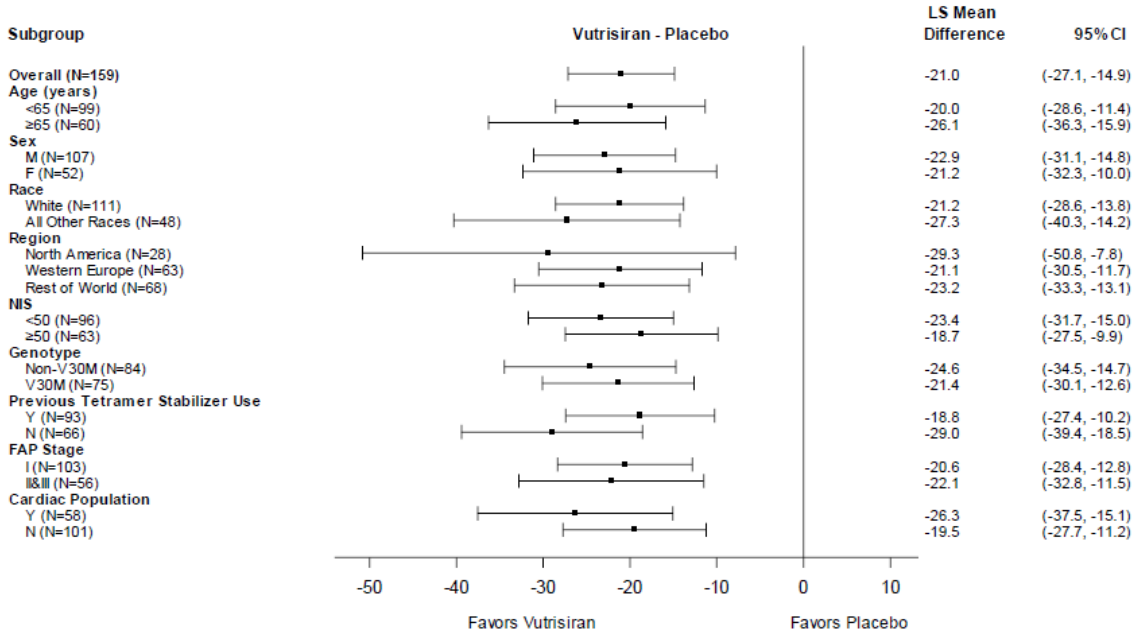
The number and percentage of patients with a change from baseline at Month 18 in Norfolk QoL-DN total score of <0 points (decrease or improvement) and ≥0 points was compared between the vutrisiran (HELIOS-A) and placebo (APOLLO) groups.

In the vutrisiran group, 56.8% of patients showed an improvement in quality of life at Month 18 (change from baseline in Norfolk QoL-DN <0) as compared to 10.4% of placebo patients (odds ratio of 11.3; 95% CI: 5.0, 25.7).

Subgroup analyses of Norfolk QoL-DN

Patients receiving vutrisiran experienced similar improvements relative to placebo (APOLLO) in Norfolk QoL-DN score across all subgroups including age, sex, race, region, baseline NIS score, genotype, previous tetramer stabilizer use, disease stage, and cardiac subpopulation.

Figure 14: (from Figure 9 of Responses to Day 120): Norfolk QoL-DN, Change From Baseline to Month 18, MMRM Model Subgroup Analysis Forest Plot (mITT Population)



Abbreviations: CI=confidence interval; CSR=Clinical Study Report; FAP=Familial Amyloid Polyneuropathy; LS=least squares; mITT=modified intent-to-treat; MMRM=mixed-effects model for repeated measures; NIS=Neuropathy Impairment Score; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; V30M=valine to methionine variant at amino acid 30.

Source: Study 002 CSR2 Figure 14.2.3.7.2

Genotype

The effect of vutrisiran was evaluated across different genotypes and improvements were observed in Norfolk QoL-DN regardless of the individual variant. As several of these mutations are endemic to certain regions, this analysis could further support the consistency of effect across regions.

Other Analysis: Binary Endpoint

The number and percentage of patients with a change from baseline to Month 18 in 10-MWT >0 m/s and ≤0 m/s at Month 18 was compared between the vutrisiran (HELIOS-A) and placebo (APOLLO) groups.

In the vutrisiran group, 49.2% of patients showed an improvement in gait speed at Month 18 (change from baseline in 10-MWT >0 m/s) as compared to 13.0% of placebo patients (odds ratio of 7.2, 95% CI: 3.3, 15.8) (Table 12 in the Day120 LoQ Responses).

Additional analyses of hypothetical estimand and treatment policy estimand based on the "true" mITT population

The Applicant was requested to provide the primary analysis (the primary hypothetical estimand) and the sensitivity analysis (the treatment policy estimand) performed on the "true" mITT population, using appropriate missing data handling strategies for the clinical efficacy endpoints included in the confirmatory testing hierarchy (mNIS+7, Norfolk QoL-DN, 10-MWT, mBMI and R-ODS at Month 18).

The Applicant, as requested, provided two additional PMM analyses which were slightly different from the PMM analysis prespecified in the SAP Version 3.0 Section 4.4.2.3:

- PMM corresponding to the primary hypothetical estimand: assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE were set as missing before multiple imputation.
- PMM corresponding to the treatment policy estimand: assessments after initiation of local standard treatment for hATTR amyloidosis or on or after onset of a serious COVID-19 AE were included in the model without imputation.

The results from MMRM and two PMM methods are summarised in Table 35 from the responses provided over the course of the assessment. The additional analyses were consistent with the prespecified primary MMRM analysis, showing there was little difference between the estimates of treatment effect based upon the two PMM methods or MMRM analyses for the primary, but also for the secondary endpoints.

Table 35 (from Table 1 of the Day 180 responses to LoOI): Comparing the MMRM and PMM Analysis Results for Primary Endpoint and Secondary Endpoints in the mITT Population

	MMRM, LS mean change			**PMM (hypothetical), LS mean change**			**PMM (treatment policy), LS mean change**		
	PBO	**Vutri**	**Vutri-PBO**	**PBO**	**Vutri**	**Vutri-PBO**	**PBO**	**Vutri**	**Vutri-PBO**
mNIS+7 at M9[a]	14.76	-2.24	-17 [-21.78, -12.22]	14.75	-1.89	-16.65 [-21.49, -11.8]	15.12	-1.70	-16.82 [-21.54, -12.09]
Norfolk QoL-DN at M9[a]	12.9	-3.3	-16.2 [-21.7, -108]	13.1	-3.4	-16.4 [-21.8, -11.1]	13.0	-3.4	-16.4 [-21.7, -11.1]
10-MWT at M9[a]	-0.133	-0.001	0.131 [0.070, 0.193]	-0.135	-0.007	0.128 [0.066, 0.190]	-0.135	-0.007	0.128 [0.066, 0.190]
mNIS+7 at M18	28.09	-0.46	**-28.55**	29.67	0.38	-29.30	29.28	1.12	-28.15

	MMRM, LS mean change			PMM (hypothetical), LS mean change			PMM (treatment policy), LS mean change		
	PBO	**Vutri**	**Vutri-PBO**	**PBO**	**Vutri**	**Vutri-PBO**	**PBO**	**Vutri**	**Vutri-PBO**
			[-34.00, -23.10]			[-35.36, -23.23]			[-34.14, -22.17]
Norfolk QoL-DN at M18	19.8	-1.2	**-21.0 [-27.1, -14.9]**	22.8	-1.1	-23.8 [-30.6, -17.1]	23.2	-1	-24.2 [-30.6, -17.9]
10-MWT at M18	-0.264	-0.024	**0.239 [0.154, 0.325]**	-0.304	-0.035	0.269 [0.180, 0.359]	-0.301	-0.036	0.264 [0.172, 0.357]
mBMI at M18	-115.7	25	**140.7 [108.4, 172.9]**	-121.3	18.2	139.5 [103.9, 175.1]	-122.7	20.2	142.9 [107.9, 177.9]
R-ODS at M18	-9.9	-1.5	**8.4 [6.5, 10.4]**	-10.6	-1.8	8.8 [6.8, 10.8]	-10.5	-1.8	8.8 [6.7, 10.8]

a For M9 analysis, the results are based on ANCOVA/MI using Month 9 datasets for CSR1 while the PMM results are based on new analysis using Month 18 datasets for CSR2.

Abbreviations: M9=Month 9; M18=Month 18; MMRM= Mixed-effects model for repeated measures; mNIS+7= Modified Neuropathy Impairment Score +7; Norfolk QoL-DN=Norfolk Quality of Life–Diabetic Neuropathy; 10-MWT=10 meter walk test; mBMI= Modified body mass index; PMM=pattern-mixture model; R- ODS=Rasch-built Overall Disability Scale

- **Summary of main efficacy results**

Table 36 summarises the efficacy results from the main efficacy study supporting this application. These summaries should be read in conjunction with the discussion on clinical efficacy, as well as the benefit risk assessment (see later sections of this assessment report).

Table 36: Summary of efficacy for trial < ALN-TTRSC02-002, HELIOS-A>

Title: HELIOS-A: A Phase 3 Global, Randomized, Open-label Study to Evaluate the Efficacy and Safety of ALN-TTRSC02 in Patients with Hereditary Transthyretin Amyloidosis (hATTR Amyloidosis)	
Study identifier	Study number: ALN-TTRSC02-002 EudraCT number: 2018-002098-23

Design	Global, Phase 3, randomized, open-label study designed to evaluate efficacy, safety, PK, and PD of vutrisiran in adult patients with hATTR amyloidosis with polyneuropathy. Patients were randomized 3:1 to vutrisiran or patisiran, as a reference comparator. The placebo group of the patisiran Phase 3 APOLLO study is used as an external control for the primary and most secondary and exploratory efficacy analyses of vutrisiran.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		18 months Treatment Period (ongoing) not applicable 18 months Treatment Extension Period (ongoing)
Hypothesis	Superiority against external placebo (APOLLO) for clinical efficacy endpoints Non-inferiority against patisiran for serum TTR level reduction		
Treatments groups	HELIOS-A vutrisiran		Treatment: Vutrisiran SC injection 25 mg every 3 months (q3M) Duration: 18 months Treatment Period Number randomized: 122
	HELIOS-A patisiran Reference comparator arm		Treatment: Patisiran IV infusion 0.3 mg/kg once every 3 weeks Duration: 18 months Treatment Period
	APOLLO placebo External control		Treatment: Infusion of saline 0.9% NaCl once every 3 weeks Duration: 18 months Number randomized: 77
Endpoints and definitions	Primary endpoint*	mNIS+7	Change from baseline in the modified neuropathy impairment score +7 (mNIS+7)
	Secondary endpoints:	Norfolk QoL-DN	Change from baseline in Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) total score
		10-MWT	Change from baseline in timed 10-meter walk test (10-MWT)
		R-ODS	Change from baseline in Rasch-built Overall Disability Scale (R-ODS)
		Serum TTR	Percent reduction in serum TTR levels
	Other: select exploratory endpoints	NT-proBNP	Change from baseline in N-terminal prohormone B-type natriuretic peptide (NT-proBNP)
		mBMI	Change from baseline in modified Body Mass Index (mBMI)

		EQ-5D-5L	Change from baseline Increase/decrease from baseline=improvement/ worsening quality of life
		EQ-VAS	Change from baseline Increase/decrease from baseline=improvement/ worsening quality of life
Database lock	Interim database lock: 26 August 2021		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Modified Intent to treat (mITT) population, defined as all randomized patients who received any amount of study drug. For all efficacy endpoints, results are provided for the mITT population, except for NT-proBNP for which results are provided for the mITT population and the cardiac subpopulation. The cardiac subpopulation is defined as all mITT population patients who had pre-existing evidence of cardiac amyloid involvement, defined as patients with baseline left ventricular (LV) wall thickness ≥ 1.3 cm and no aortic valve disease or hypertension in medical history.		

Descriptive statistics and estimate variability	Treatment group	External control: APOLLO placebo	HELIOS-A vutrisiran	HELIOS-A patisiran
	Number of subjects	77	122	42
	mNIS+7 (LS mean change from baseline)	28.09	-0.46	-
	Standard Error	2.28	1.60	-
	Norfolk QoL-DN (LS mean change from baseline)	19.8	-1.2	-
	Standard error	2.6	1.8	-
	10-MWT (LS mean change from baseline)	-0.26	-0.02	-
	Standard error	0.04	0.03	-
	mBMI (LS mean change from baseline)	-115.7	25	-
	Standard Error	13.4	9.5	-

	R-ODS (LS mean change from baseline)	-9.9	-1.5	-
	Standard Error	0.8	0.6	-
	Serum TTR Mean time averaged TTR percent reduction	-	81.37	79.68
	Standard deviation	-	18.84	11.71
	NT-proBNP Adjusted geometric fold change from baseline	1.956	0.939	-
	95% CI	1.628, 2.351	0.826, 1.066	-
	NT-proBNP in cardiac subpopulation Adjusted geometric fold change from baseline	N=36 1.927	N=40 0.946	N=14
	95% CI	1.269,2.005	0.759,1.192	-
	mBMI Month 18, LS Mean Change	N=77 -115.7	N=122 25.0	
	95% CI	(-142.2, -89.1)	(6.3, 43.8)	-
	EQ-5D-5L Month 18, LS Mean Change	-0.2104	-0.0244	
	SE	0.0202	0.0145	-
	EQ-VAS Month 9, LS Mean Change	-11.6	2.1	
	SE	2.1	1.5	-

Effect estimate per comparison	**Primary endpoint** **mNIS+7 at Month 18)** (mean of 2 nonmissing component	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo
		LS mean difference between groups (vutrisiran – placebo)	**-28.55**
		95% Confidence Interval	**(-34.00, -23.10)**

	assessments planned to be performed ≥24 hours to ≤7 days apart at each scheduled visit, after component imputation; higher scores represent a greater severity of disease)	P-value ANCOVA/Multiple Imputation Model	**6.505E-20**
	Secondary endpoint **Norfolk Qol-DN (at Month 18)** (composite of 35 quality of life questions; lower scores indicate higher quality of life (range = -4 to 136)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo
		LS mean difference between groups (vutrisiran – placebo)	**-21.0**
		95% Confidence Interval	**(-27.1, -14.9)**
		P-value ANCOVA/Multiple Imputation Model	**1.844E-10**
	Secondary endpoint **10-MWT (at Month 18)** (10 meters/average time (seconds) taken to complete 2 assessments at each visit, imputed as 0 for patients unable to perform the walk; higher speeds indicate greater ambulatory function)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo
		LS mean difference between groups (vutrisiran – placebo)	**0.239**
		95% Confidence Interval	**(0.154, 0.325)**
		P-value ANCOVA/Multiple Imputation Model	**1.207E-07**
	Secondary endpoint **mBMI** (albumin (g/L) x weight (kg) / height (m)^2; Higher mBMI=better nutritional status)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo
		LS mean difference between groups (vutrisiran – placebo)	**140.7**
		95% Confidence Interval	**108.4, 172.9**
		P-value	**4.159E-15**

		ANCOVA/Multiple Imputation Model		
	Secondary endpoint **R-ODS** (Score range: 0 to 48 points)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo	
		LS mean difference between groups (vutrisiran – placebo)	**8.4**	
		95% Confidence Interval	**6.5, 10.4**	
		P-value ANCOVA/Multiple Imputation Model	**3.541E-15**	
	Secondary endpoint **Serum TTR** (Patient maximum and mean percentage reductions were derived from all nonmissing, postbaseline TTR assessments through the specified timepoint, including nontrough assessments and regardless of missed doses)	Comparison groups	HELIOS-A vutrisiran vs. HELIOS-A patisiran	
		Time averaged TTR % reduction (Represents the mean of assessments between Month 6 through to Month 18) Mean (SD)	Vutrisiran (n=120) -80.99 (20.96) vs. Patisiran (n=40) -78.56 (13.63)	
		Percent Reduction Model Estimates Pseudomedian Stratified Hodges-Lehmann Noninferiority (95% lower CI > -10%)	Vutrisiran (n=120) 84.67 vs. Patisiran (n=40) 80.60 Yes	
	Exploratory **NT-proBNP** (Lower values indicate less cardiac stress)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo	HELIOS-A vutrisiran vs. APOLLO placebo in cardiac subpopulation
		Adjusted geometric fold change ratio (vutrisiran/placebo)	0.480	0.491
		95% Confidence Interval	0.383, 0.600	0.337, 0.716
		P-value ANCOVA/Multiple Imputation Model	9.60E-10	0.0004

	Secondary endpoint **EQ-5D-5L** (Score range: 0 to 1)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo
		LS mean difference between groups (vutrisiran – placebo)	0.1860
		95% Confidence Interval	0.1374, 0.2346
		P-value	2.575E-12
	Secondary endpoint **EQ-VAS** (Score range: 0 to 100)	Comparison groups	HELIOS-A vutrisiran vs. APOLLO placebo
		LS mean difference between groups (vutrisiran – placebo)	13.7
		95% Confidence Interval	8.7, 18.7
		P-value	2.214E-07
Notes	The Clinical Study Report 1 (CSR1) provides an analysis of the Month 9 data with a data cut-off date of 10 November 2020. 0. The confirmatory primary analysis of the HELIOS-A study for EU was performed at Month 18 as in the case of APOLLO study and results were provide with CSR2 with a cut-off date 26 August 2021. The cardiac subpopulation was redefined.		

2.6.5.1. Clinical studies in special populations

There were no clinical studies in special populations. However, the number of older subjects included in the clinical efficacy and safety studies submitted with this application are presented below:

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials APOLLO (placebo group)	24/77 (31.2%)	9/77 (11.7%)	0
Non Controlled trials HELIOS-A (Vutrisiran)	39/122 (32.0%)	6/122 (5.7%)	1/122 (0.8%)
Non Controlled trials HELIOS-A (Patisiran)	9/42 (21.4%)	2/42 (4.8%)	0/42

2.6.5.2. In vitro biomarker test for patient selection for efficacy

Serum levels of the exploratory cardiac biomarker NT-proBNP over time were summarized using descriptive statistics for each visit, since NT-proBNP represents the key assessment informing impact on cardiac manifestations of the disease. However, the following efficacy assessments were performed: NIS, mNIS+7, heart rate variability with deep breathing (HRdb), 10-MWT, FAP and PND, KPS, echocardiogram.

For the Month 9 and Month 18 analyses, the key assessment informing impact on cardiac manifestations of the disease was the cardiac biomarker NT-proBNP, which is used to assess cardiac stress and degree of heart failure. NT-proBNP was assessed at Screening, Baseline, Day 85, Day 169, Month 9 and Month 18.

At Month 9, the geometric mean NT-proBNP levels remained stable in vutrisiran patients (0.99-fold change), while there was a worsening in NT-proBNP in placebo (APOLLO) patients with a 1.59-fold increase.

It is noted that the exploratory cardiac biomarker endpoint, NT-proBNP, was evaluated in both the mITT population and the pre-specified cardiac subpopulation; vutrisiran treatment showed improvement compared to placebo. For the cardiac subpopulation at month 18, the geometric mean NT-proBNP levels for vutrisiran patients had a 0.94-fold change, while there was a worsening in NT-proBNP in placebo (APOLLO) patients with a 1.96-fold increase and a ratio of 0.491 (p=0.0004). In the case of patisiran in APOLLO, similar results were obtained at 18 months, with an adjusted geometric mean ratio to baseline of 0.89 for Onpattro and 1.97 for placebo leading to a ratio of 0.45 ($p < 0.001$). However, this evaluation is not used for patient selection. Instead, the Applicant is conducting a separate clinical development program for patients with ATTR amyloidosis with cardiomyopathy, and a Phase 3 study of (ALN-TTRSC02-003; HELIOS-B), for which enrolment is ongoing, is currently in progress and remains blinded.

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

Pooled analyses or a meta-analysis have not been performed.

It is noted that the main efficacy and safety study, HELIOS-A, included a patisiran reference comparator group to establish similar TTR reduction between vutrisiran and patisiran, as well as to enable a descriptive comparison of clinical efficacy endpoints between the two treatment groups in HELIOS-A. The reference comparator was also used to allow for a descriptive comparison of the patisiran group from HELIOS-A with the efficacy profile of patisiran based on the APOLLO study (ALN-TTR02-004).

2.6.5.4. Supportive study(ies)

Not Applicable

2.6.6. Discussion on clinical efficacy

Vutrisiran is an RNAi therapeutic comprised of a synthetic, chemically modified, double-stranded small interfering RNA (siRNA) that specifically targets variant and wtTTR and silences TTR messenger RNA (mRNA). This is accomplished by incorporation of vutrisiran siRNA into the cellular multiprotein enzyme cleavage complex known as the RNA induced silencing complex (RISC). Reduction of both variant and wild type TTR production in the liver, which are the fundamental pathogenic proteins causing hATTR amyloidosis, will reduce ongoing deposition of amyloid deposits and potentially allow for clearance of existing deposits and consequently, halting or reversing disease progression.

Previous clinical studies with mRNA silencing agents, such as patisiran, and the antisense oligonucleotide (ASO) inotersen, demonstrated that TTR reduction in patients with hATTR amyloidosis with polyneuropathy can have beneficial effects, measured by clinical endpoints evaluating disease manifestations and patient-reported outcomes.

The indication applied for Amvuttra (vutrisiran) has been finally worded as:

Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with stage 1 or stage 2 polyneuropathy.

It is noted that the proposed indication for vutrisiran is the same as that for the approved product Onpattro (patisiran).

Design and conduct of clinical studies

The clinical development program of vutrisiran consists of a safety and clinical pharmacology study assessing PD in healthy subjects (Study 001) and an open-label on-going Phase 3 study in adult patients with hATTR amyloidosis with polyneuropathy (ALN-TTRSC02-002, HELIOS-A), which evaluates the efficacy of vutrisiran, using patisiran as a reference comparator. An external placebo group was used from patisiran study ALN-TTR02-004 (APOLLO), which is a completed, Phase 3, multicenter, multinational, randomized, double-bind, placebo-controlled study in hATTR amyloidosis patients with polyneuropathy. APOLLO was a pivotal trial for the approval of Onpattro (patisiran).

The clinical development program to support the indication and the design of the Phase 3 HELIOS-A study were discussed with multiple regulatory agencies, with scientific advice received from the US FDA (17 July 2018 [FDA PIND 139086]), European Union (EU) European Medicines Agency (EMA; 20 September 2018 [EMA/CHMP/SAWP/615799/2018]), and Japan Pharmaceuticals and Medical Devices Agency (PMDA; 21 February 2019 [P5259, Notification number 0221001]).

Design of the pivotal phase 3 study

HELIOS-A is an ongoing, global, Phase 3, randomized, open-label study designed to evaluate efficacy, safety, PK, and PD of vutrisiran in adult patients with hATTR amyloidosis with polyneuropathy.

The open-label nature of the study would have been of concern in case a reference comparator had not been included in the study, and in case data would have not be available from the reference comparator, which has been developed by the same Applicant. In addition, two independent replicate assessments were performed, approximately 24 hours apart from each other but no greater than 7 days apart.

Patients were randomized 3:1 to vutrisiran 25 mg, administered as a SC injection q3M, or patisiran 0.3 mg/kg, administered as an IV infusion q3w. The primary and most secondary and exploratory endpoints of the study evaluate the efficacy of vutrisiran by comparing to the placebo group of the Phase 3 patisiran APOLLO study. The study has been conducted in 2 parts: an 18-month Treatment Period, with an efficacy analysis at Month 9 and additional efficacy analyses at Month 18, followed by an 18-month Treatment Extension Period, in which all patients are treated with vutrisiran. For regulatory submissions in the US, the primary efficacy analysis was conducted at Month 9. Results for both the endpoint, mNIS+7 change from baseline at Month 9, and endpoint Norfolk QoL-DN total score change from baseline at Month 9, must be statistically significant (i.e., p-value $\leq$0.05) to declare a positive study. However, according to the specifications for EU in the statistical analysis plan (SAP), the analyses performed at month 18 are formally the confirmatory analyses.

The TTR percent reduction through Month 18 was evaluated as the average trough TTR percent reduction from Month 6 to 18 which is the steady state period for both vutrisiran (ALN-TTRSC02) and patisiran. A method was used to estimate the 95% confidence interval (CI) for the median difference between vutrisiran (ALN-TTRSC02) and patisiran groups in this study, and non-inferiority was declared since the lower limit of the 95% CI for the treatment difference was greater than –10%.

Formal non-inferiority testing of vutrisiran compared to patisiran with respect to serum TTR levels as a secondary endpoint was not performed at Month 9 (only descriptive). The Month 18 analyses were confirmatory of the non-inferiority of vutrisiran versus patisiran. Since both molecules were developed by the same Applicant and have the same mechanism of action, i.e. silencing of the TTR RNA, a non-inferiority comparison versus patisiran based on a PD endpoint (measurement of the reduction of TTR protein), and the indirect comparison to the placebo arm of the patisiran APOLLO study, based on a

clinical endpoint, can be considered as a suitable approach. The objectives of the HELIOS-A study are, therefore, considered appropriate.

The study was performed in 57 centers in 22 countries: Argentina, Australia, Belgium, Brazil, Bulgaria, Canada, Cyprus, France, Germany, Greece, Italy, Japan, Korea, Malaysia, Mexico, Netherlands, Portugal, Spain, Sweden, Taiwan, UK, US.

Inclusion and exclusion criteria and baseline characteristics

Patients between 18 to 85 years of age with a diagnosis of hATTR amyloidosis, with documented TTR mutation, Neuropathy Impairment Score (NIS) of 5 to 130 (inclusive), Polyneuropathy Disability (PND) score of ≤3b and Karnofsky Performance Status (KPS) of ≥60% participated to study HELIOS-A. The key inclusion and exclusion criteria are considered appropriate for the investigation of efficacy of vutrisiran in adult patients with hATTR with polyneuropathy. Patients whose neuropathy may be too advanced to permit completion of the study were excluded from the study. This is acknowledged. It should also be noted that the participating patients had Polyneuropathy disability (PND) score of ≤3b and a corresponding FAP stage I or II. It is also noted that in the APOLLO study there was only 1 patient with FAP stage 3 (in the placebo group) and in the HELIOS-A there were no patients in stage 3 recruited. Similarly to HELIOS-A, the study population in the APOLLO study was limited to subjects with Polyneuropathy Disability (PND) Scores of 0-IIIB hATTR/FAP stage 1 and 2. Likewise, as mentioned in section 5.1 of the SmPC for vutrisiran, "*at baseline, 70% of patients had stage 1 disease (unimpaired ambulation; mild sensory, motor, and autonomic neuropathy in the lower limbs), and 30% had stage 2 disease (assistance with ambulation required; moderate impairment of the lower limbs, upper limbs, and trunk)*". As a result, the proposed broad indication could not be considered acceptable.

The unmet medical need for patients with hATTR and stage 3 polyneuropathy can definitely be acknowledged. However, there were no FAP stage 3 patients recruited in HELIOS-A (please see also efficacy data and additional analyses). Therefore, the Applicant restricted the indication, to represent the population included in the study (stage 1 or stage 2 polyneuropathy).

There were no dedicated studies performed in older subjects. The number of older patients included in the clinical efficacy and safety studies with this dossier are very limited. However, this can be attributed to the progress of the disease and the reduced survival of patients with hATTR. The Applicant provided the numbers of older subjects in a Table with information on clinical studies in special populations, especially for the groups 75-84 and above 85 years of age.

Treatments

Vutrisiran 25 mg, either as a vial or as a prefilled syringe with passive needle safety system (PFS-S) for SC injection, is administered q3M (12 weeks ±3 days during the Treatment Period and ±7 days during the Treatment Extension Period). SC administration of vutrisiran four times per year is certainly advantageous for patients when compared to IV administration of patisiran or weekly SC administration of inotersen.

Furthermore, all (100%) of patisiran-treated patients received premedications prior to infusion. The once every 3 months (q3M) subcutaneous (SC) regimen with vutrisiran is infrequent, easy to administer, does not require premedication, and minimizes the need for healthcare encounters. The treatments used in the pivotal study HELIOS-A are either according to the approved posology and method of administration for patisiran, or are considered justified in the case of vutrisiran (please see below). It should be also noted that for vutrisiran four times per year administration is recommended, whilst for another approved treatment for hATTR (Tegsedi-inotersen) subcutaneous administrations are scheduled weekly.

The phrase in section 4.2 of the proposed SmPC "*Therapy should be initiated under the supervision of a physician knowledgeable in the management of amyloidosis*" was not initially considered sufficient. It was thought that more precision and more specific recommendations for the first administration should be included in the SmPC, in addition to the treatment decision by an experienced physician. However, it was later confirmed during the assessment that dedicated human factor (HF) studies for self-administration have not yet been conducted with vutrisiran; therefore, the Notified Body Opinion included in the initial MAA a statement that "*the device was designed for professional use only*".

It has also been confirmed that vutrisiran will be administered by a healthcare professional (HCP) only; thus an appropriate phrase has been included in section 4.2 of the SmPC.

Endpoints

The primary endpoint of the study is formally the difference between vutrisiran (HELIOS-A) and placebo (APOLLO) in the change from baseline to Month 9 for the US, and at Month 18 for the EU, of the composite neuropathy impairment score (mNIS+7), which is considered a disease-specific sensitive and reproducible composite measure of neuropathy progression. The mNIS+7 has been used in multiple clinical studies in hATTR amyloidosis including APOLLO. According to the scientific advice procedure (Procedure No. EMEA/H/SA/3876/1/2018/PA/III and the clarification letter) a preference for analyses of 18 months data was indicated. For EU and other regions, mNIS+7 change from baseline at Month 18 is considered the primary endpoint, and the relevant analyses have been submitted.

An analysis for the change from baseline in Norfolk QoL-DN total score at Month 9 and at Month 18 compared to placebo have also been presented. For the EU, the key secondary endpoint is the change from baseline in Norfolk QoL-DN score at Month 18. Norfolk QoL-DN is a comprehensive, patient-reported, health-related quality of life questionnaire, and a useful tool used in all of the studies in hATTR with polyneuropathy. Norfolk QoL-DN was included to support the clinical relevance of observed changes in mNIS+7.

The 10-meter walk test (10-MWT) was used as indicative of vitality. Rasch-built Overall Disability Scale (R-ODS) was used to investigate improvement in disability at 18 months and mBMI the nutritional status of patients. The impact of neuropathy (NIS) and quality of life (EQ-5D-5L, EQ-VAS) have been also investigated as exploratory endpoints.

Demonstrating non-inferiority of vutrisiran, compared to patisiran in the HELIOS-A study, with regards to TTR reduction through Month 18 or a single clinical endpoint, in a direct comparison supplemented with pooled/indirect comparisons, was required to allow the appropriate use of the external placebo group from patisiran APOLLO data.

All endpoints used in this study are considered appropriate for this patient population to provide useful information on the progression of hATTR or lack of it.

Statistical methods

According to the Applicant's Day 120 Response, the primary estimand would target the treatment effect in a hypothetical scenario, that all patients do not initiate local standard treatment for hATTR amyloidosis and do not have a COVID-19 SAE (using a hypothetical strategy for these two intercurrent events). All assessments after initiation of local standard treatment for hATTR amyloidosis, or the onset of a COVID-19 SAE, were excluded from the primary analysis. In addition, a sensitivity analysis including assessments after initiation of local standard treatment for hATTR amyloidosis, or the onset of a COVID-19 SAE, was aimed to target the treatment effect irrespective of the initiation of local standard treatment for hATTR amyloidosis or the onset of a COVID-19 SAE (using a treatment policy strategy for these two intercurrent events). The Applicant further argued that the number of censored

values is small, with minimum impact on the estimation of treatment effect, which was considered acceptable.

While the proposed primary hypothetical estimand, complemented by the treatment policy estimand, is endorsed, the performed primary and sensitivity analyses at Month 18 using a mixed-effects model for repeated measures (MMRM) could not be fully accepted. Generally, these analyses were not performed on the pre-specified mITT population including all randomized patients who received any amount of study drug. Actually, patients without any post-baseline assessments (both Month 9 and Month 18 assessments missing/censored), both in the vutrisiran and placebo groups, were excluded from the analyses. Missing/censored data should be imputed in alignment with the target estimands. MMRM handles missing data, implicitly based on a missing at random (MAR) assumption, which seems to be implausible in some cases. For example, initiation of alternative treatment may indicate lack of efficacy of study treatment and a poor outcome would be observed, had patients not received alternative treatment. Usually, it is also considered that patients who discontinued study treatment would no longer benefit from it, and would have trajectory similar to placebo patients. Based on a MAR assumption, however, missing data after discontinuation of study treatment, and censored data after initiation of alternative treatment, would be imputed using a mixture of collected data from patients who continued and discontinued study treatment (depending on the follow-up after treatment discontinuation) and did not initiate alternative treatment, which could not be considered appropriate.

Hence, for the primary hypothetical estimand, while missing/censored data due to COVID-19 SAE could be considered MAR, missing/censored data after discontinuation of study treatment and/or initiation of local standard treatment should be imputed using values reflecting lack of efficacy. Considering the frequency and distribution of missing/censored data (more patients in the placebo group had missing/censored data as compared to the vutrisiran group), the placebo multiple imputation is considered appropriate for handling missing/censored data after discontinuation of study treatment, and/or initiation of local standard treatment, or missing data due to other reasons.

For the treatment policy estimand, assessments after initiation of local standard treatment for hATTR amyloidosis or the onset of a COVID-19 SAE are included in the analysis, and missing data can generally be handled using the placebo multiple imputation.

In summary, the primary analysis (the primary hypothetical estimand) and the sensitivity analysis (the treatment policy estimand) performed on the "true" mITT population, using appropriate missing data handling strategies, as discussed above, have been requested, and provided by the Applicant for the clinical efficacy endpoints included in the confirmatory testing hierarchy (mNIS+7, Norfolk QoL-DN, 10-MWT, mBMI and R-ODS at Month 18).

Efficacy data and additional analyses

With respect to the dosing regimen, the recommended 25 mg q3M regimen of vutrisiran achieves similar or slightly higher TTR reduction than patisiran. This magnitude of TTR reduction has been shown to result in favorable clinical outcomes across clinical studies of hATTR amyloidosis patients with polyneuropathy, including the APOLLO study, in the patisiran program.

Collectively, the results of Study 001 and Study 002 (HELIOS-A), as well as pooled population PK, population PK/PD modeling, and disease progression analyses support the recommended vutrisiran dosing regimen of 25 mg administered q3M across all subgroups.

It appears that this dosing regimen provides clinical benefit with an acceptable safety profile for treatment of all patients with hATTR amyloidosis with polyneuropathy. No dose adjustment is necessary for any of the subpopulations.

In HELIOS-A, 164 adult patients with hATTR amyloidosis with polyneuropathy were randomized 3:1 to vutrisiran or patisiran (122 vutrisiran and 42 patisiran). Randomization was stratified by TTR genotype (V30M vs. non-V30M) and baseline NIS score (<50 vs. ≥50).

The number of patients is considered sufficient to allow collection of useful information for both vutrisiran and patisiran group in the HELIOS-A study, and is very similar to the numbers of studies for other medicinal products recently approved for hATTR. The numbers from the APOLLO study have already been assessed and discussed during the approval procedure of Onpattro (patisiran) (https://www.ema.europa.eu/en/medicines/human/EPAR/onpattro).

Efficacy data from HELIOS-A for the Month 9 early analysis timepoint (data cut-off date of 10 November 2020), as well as for the Month 18 (data cut-off date of 26 August 2021), have been submitted.

During the period of COVID-19 pandemic, a small number of protocol deviations were reported that are not considered to have an impact on patient safety or the overall interpretation of study results.

It can be agreed that the deviations are not considered to have seriously affected patient safety or the overall interpretation of study results, taking into consideration the unique and difficult period of COVID-19 pandemic.

Delayed assessments due to COVID-19 were included in the efficacy analyses of HELIOS-A, based on the Month 18 Efficacy PP Population using an MMRM model. Only a few number of patients had a missed or delayed dose of vutrisiran [17 and only 1 (0.8%) patient had a missed dose], or had their Month 18 efficacy visit impacted by the COVID-19 pandemic (13). It can be agreed that the mitigation efforts promptly implemented by the Applicant to address potential trial risk due to the COVID-19 pandemic successfully ensured continued administration of study drug and collection of study data, thus minimizing impact of the pandemic on study conduct. A GCP inspection is not considered necessary. The results of additional analyses for mNIS+7 and Norfolk QoL-DN were consistent with the primary Month 18 analysis, supporting that the impact on the conclusions of HELIOS-A was negligible from the COVID-19 pandemic.

Furthermore, the Applicant´s argument that the adverse impact on patients from the COVID-19 pandemic could potentially result in an underestimate of treatment effects of HELIOS-A vutrisiran, as compared to the placebo arm from the APOLLO study, which was conducted in the pre-COVID-19 era, is quite valid.

It is clearly noted that the disease characteristics of the patients are worse in the placebo group of the APOLLO study compared to HELIOS-A. The percentage of patients with NIS ≥50 was 54.6% in the APOLLO compared to 34.6% in HELIOS-A. The percentage of patients with low Karnofsky Performance Status (KPS) percentage score of 60 (indicative of lower ambulatory function) was 28.6% in APOLLO versus 13.9% in HELIOS-A. Furthermore, the patients in the placebo group in APOLLO had clearly a higher cardiac involvement of the disease. In HELIOS-A study 55.7%, 9.0% and 35.2% of patients had no heart failure, NYHA I and NYHA II, respectively, whilst in APOLLO 51.9% had NYHA I and 46.8% had NYHA II. It is noted that in the APOLLO study, NYHA class was graded I through IV and "no heart failure" was not an option; thus, in this study, patients classified as NYHA class I included both those without heart failure and those with asymptomatic heart failure.

APOLLO population was older, with more advanced disease as per NIS, Norfolk QoL, or 10MWT and virtually all patients had heart failure and a lower mBMI: either leaner or deproteinised. The Applicant discussed, within the Responses to Day 120 LoQ, the impact of the observed baseline differences (APOLLO placebo and HELIOS-A vutrisiran) on study results and the validity of the evaluation of the effect of vutrisiran versus external placebo.

It can be agreed that the clinical course of the placebo group in hATTR amyloidosis clinical studies and the natural history study at different study periods (1997-2017) is similar, with a change from baseline in mNIS+7 ranging from 11.20 to 17.80 (with study durations 8 months to 1 year), and a change in NIS at 1 year of 12.50. Similar values were also obtained in the change from baseline for Norfolk QoL-DN in APOLLO (7.50) and Inotersen Phase 3 study (6.95) at 9 and 8 months, respectively.

It can also be agreed that the baseline differences in between HELIOS-A vutrisiran and APOLLO placebo patients are largely overlapping. It is noted that the Applicant has access to individual patient data in APOLLO, which allows for the impact of baseline differences to be understood and well accounted for, and also enables adjustment for baseline values and key prognostic factors in the treatment comparison.

The above differences are also a reason why more weight is put on the formal non-inferiority testing of the vutrisiran group versus the within-study control group of patisiran regarding TTR reduction or a direct comparison of one of the clinical parameters (preferably mNIS+7) at Month 18. Such a non-inferiority comparison of the vutrisiran group to the within-study control group of patisiran was informative, as anticipated. The relevant analyses have been provided by the Applicant.

From the results at month 9, a large improvement in neuropathy was observed for patients in the vutrisiran group compared to the placebo group, with a LS mean difference of the mNIS+7 change from baseline between groups of -17.00 points. In addition, a noticeable difference in improvement in quality of life at 9 months for patients in the vutrisiran group compared to the placebo group with a LS mean difference between groups in Norfolk QoL-DN change from baseline of -16.2 points occurred.

For mNIS+7, 50.4% of patients in the vutrisiran group showed an improvement of neuropathy (change from baseline mNIS+7 <0 points) at Month 9 compared to 18.2% of placebo (APOLLO) patients. For Norfolk QoL-DN, 53.4% of patients in the vutrisiran group had an improvement (<0-point LS mean change) at Month 9 compared to 23.4% of placebo patients.

The efficacy was maintained at Month 18. For the primary endpoint of modified Neuropathy Impairment Score +7 (mNIS+7) a statistically significant, large and clinically meaningful difference in the change from baseline at Month 18 was observed between vutrisiran in HELIOS-A and placebo in APOLLO with a treatment difference, LS Mean (95% CI) of -28.55 (-34.00, -23.10). Pre-specified sensitivity analyses with no censoring, propensity score and pattern-mixture model showed consistent and supportive results with the primary analysis, with treatment differences, LS Mean (95% CI) of -27.04 (-32.57, -21.51), -27.83 (-33.46, -22.20) and -29.30 (-35.36, -23.23), respectively.

Component (all components), binary (48.3% of patients in vutrisiran compared to 3.9% of placebo patients) and subgroup (all subgroups including age, sex, race, region, baseline NIS score, genotype, previous tetramer stabilizer use, disease stage, and Cardiac Subpopulation) analyses for mNIS+7 showed improvement of neuropathy at Month 18 in support of the primary analysis.

Similarly, for the key secondary endpoint Norfolk QoL-DN, a statistically significant, large and clinically meaningful difference in the change from baseline at Month 18 was observed between vutrisiran in HELIOS-A and placebo in APOLLO with a treatment difference, LS Mean (95% CI) of -21.0 (-27.1, -14.9). Pre-specified sensitivity analyses with no censoring, propensity score and pattern-mixture model showed consistent and supportive results with the primary analysis, with treatment differences, LS Mean (95% CI) of -20.9 (-26.8, -14.9), -21.9 (-28.2, -15.5) and -24.1 (-30.9, -17.3), respectively.

As in the case of the primary endpoint, mNIS+7, also for the key secondary endpoint Norfolk QoL-DN domain (all domains), binary (56.8% of patients in vutrisiran compared to 10.4% of placebo patients) and subgroup (all subgroups including age, sex, race, region, baseline NIS score, genotype, previous tetramer stabilizer use, disease stage, and Cardiac Subpopulation) analyses showed improvement of neuropathy at Month 18 in support of the primary analysis.

Supportive efficacy results were obtained at Month 9 with 10-meter walk test (10-MWT) (LS mean difference of 0.131), which is a measure of ambulatory ability and gait speed. However, the Applicant was requested to provide the respective value for the improvement with patisiran in APOLLO, and to discuss the clinical meaningfulness of the small difference between vutrisiran (HELIOS-A) and placebo group (APOLLO). Since a mean increase of 0.10 m/s can represent a clinically meaningful change in gait speed, and since 0.10 m/s decrements of baseline gait speed were associated with incremental decreases in survival in adults aged 65 years or older, an LS mean difference of 0.131 m/s (95% CI: 0.071, 0.191) improvement in gait speed at Month 9 for patients between the vutrisiran group in HELIOS-A, compared to the APOLLO placebo group, can be considered clinically relevant, albeit small.

For the 10-MWT, there was also a statistically significant improvement in gait speed at 18 months for patients in the vutrisiran group compared to the placebo group with a LS mean difference between groups (95% CI): 0.239 m/s; P=1.207 × 10-7 (0.154, 0.325). In the vutrisiran group, 49.2% of patients showed an improvement in gait speed at Month 18 (change from baseline in 10-MWT >0 m/s) as compared to 13.0% of placebo patients (odds ratio of 7.2, 95% CI: 3.3, 15.8) (HELIOS-A Clinical Study Report ALN-TTRSC02-002 CSR2).

At Month 9, the vutrisiran group demonstrated a 7.6 unit LS mean increase (improvement) in mBMI, while a -60.2 unit LS mean decline (worsening) was seen in the placebo (APOLLO) group, indicating an improvement in nutritional status for vutrisiran-treated patients compared to placebo, and suggesting that treatment with vutrisiran effectively halted the expected decline in mBMI. A statistically significant improvement in mBMI was maintained at 18 months for patients in the vutrisiran group compared to the placebo group with a LS mean difference between groups (95% CI): 140.7 kg/m2 × albumin g/L; P=4.159 × 10-15 (108.4, 172.9), demonstrating a higher nutritional status. This is considered a relevant aspect in hATTR, since it expresses the improvement on dysautonomia, which can impact the patient more than the loss of sensation and partially the motor function.

A statistically significant improvement in disability at 18 months for patients in the vutrisiran group compared to the placebo group with a LS mean difference between groups (95% CI): 8.4 points; P=3.541× 10-15, (6.5, 10.4) was recorded for R-ODS.

For the other exploratory endpoints: neuropathy (NIS), and quality of life (EQ-5D-5L, EQ-VAS) statistically significant differences were observed at Month 18 favoring vutrisiran compared to placebo.

Several sensitivity and subgroup analyses have been performed, which are supportive of the beneficial effects of vutrisiran. Effects observed across all mNIS+7 components favour vutrisiran (HELIOS-A) compared to placebo (APOLLO) and across all patient subgroups, with an effect seen across the full range of baseline neuropathy severity and across different genetic variants. Consistent improvement favouring vutrisiran in HELIOS-A relative to placebo (APOLLO) across all Norfolk QoL-DN domains and across all patient subgroups were recorded.

Of the 122 patients randomized to vutrisiran (HELIOS-A), 118 (96.7%) of these patients and 77 placebo patients included in the APOLLO, 67 (87.0%) patients had a baseline assessment for mNIS+7, and at least one post baseline follow-up assessment (either at 9 months, 18 months, or both) were included in the primary Month 18 analysis using a mixed-effects model for repeated measures (MMRM).

Thus, the performed primary and sensitivity analyses at Month 18 using a MMRM was initially questioned. Generally, these analyses were not performed on the pre-specified mITT population including all randomized patients who received any amount of study drug. Actually, patients without any post-baseline assessments (both Month 9 and Month 18 assessments missing/censored), both in the vutrisiran and placebo groups, were excluded from the analyses. Furthermore, the MMRM handles missing data implicitly based on a missing at random (MAR) assumption, which was not fully in

alignment to the defined estimands. Therefore, analyses performed on the "true" mITT population and in alignment to the defined estimands were requested and provided.

Two additional PMM analyses were implemented: PMM 1, corresponding to the primary hypothetical estimand, and PMM 2, corresponding to the treatment policy estimand.

For the primary endpoint mNIS+7 at Month 18 the differences in LS mean change between vutrisiran from HELIOS-A study and placebo from APOLLO study were -28.55, -29.30 and -28.15 using the MMRM, PMM 1 (hypothetical) and PMM 2 (treatment policy), respectively.

For the secondary endpoint Norfolk QoL-DN at Month 18, the differences in LS mean change between vutrisiran from HELIOS-A study and placebo from APOLLO study were -21.0, -23.8 and -24.2 using the MMRM, PMM 1 (hypothetical) and PMM 2 (treatment policy), respectively.

It can be agreed that there was little difference between the estimates of treatment effect based upon the two PMM methods or MMRM analyses for the primary (PMM being slightly larger than MMRM) endpoint, but also for the secondary endpoints.

Caveats for the positive observations could have been the fact that the population was possibly less diseased in HELIOS-A, and patients in that study knew they were all on treatment, thus increasing the expectations from treatment and a placebo-like effect. For population comparison, it is not enough to demonstrate that all quartiles behaved similar to the entire placebo or active treatment component. If patients on one of the trials were all similarly shifted towards one of the ends, all quartiles may be similarly compared, yet results will be shifted. Interestingly, patisiran had shown similar results in the APOLLO study by Month 9, as vutrisiran in HELIOS-A.

The different results for patisiran in studies HELIOS-A and APOLLO were discussed for the better understanding and assessment of the comparative effects. In HELIOS-A, patisiran has been used as a reference comparator to contextualise the results from both studies and to evaluate the size and relevance of the differences observed between vutrisiran and placebo.

A thorough comparison of within HELIOS-A study vutrisiran vs. patisiran at Month 9 and Month 18 with the important endpoints have been provided by the Applicant. Informal post hoc analyses of key efficacy parameters using MMRM for the within-study comparison of vutrisiran and patisiran groups of the HELIOS-A study at Month 18 showed very small mean differences (95% CI) between these treatment groups -1.46 (-7.36, 4.43), -1.6 (-8.6, 5.4), 0.034 (-0.064, 0.132) and 0.1 (-2.0, 2.2) in the change from baseline for mNIS+7, Norfolk QoL-DN, 10MWT and R-ODS, respectively. A relatively larger LS mean difference 14.2 (-21.9, 50.3) was observed for the change in mBMI, but this was in favour of vutrisiran. These results are supportive of the non-inferiority comparison of vutrisiran versus patisiran with respect to reduction in serum TTR levels, and it can be agreed that comparable results in clinical endpoints have been observed for vutrisiran and patisiran in HELIOS-A.

A comparison of the results of patisiran arms in HELIOS-A vs. APOLLO have also been submitted. A descriptive summary of key efficacy parameters (change from baseline) for the patisiran arms of the HELIOS-A study and APOLLO study at Month 9 and Month 18 showed also similar results and consistency, despite that the sample size of patisiran in HELIOS-A was small, with greater variability as a consequence, and it was not designed for comparisons between the patisiran groups in these studies. It can be agreed that the two datasets are not identical, with slightly different data distributions and, as a result, the two fitted models, e.g., the relationship between the outcome and covariates, had slight differences, which led to slightly different estimates. It can also be agreed that the numerical differences between the HELIOS-A patisiran and APOLLO patisiran groups are minor for all important efficacy endpoints and unlikely to have an impact on the conclusions of the HELIOS-A study.

Changes in NT-proBNP levels in response to interventions are predictive of mortality outcomes. Stabilisation of the NT-proBNP levels were observed in patients on vutrisiran vs. an increase in patients on placebo. Although the obvious limitations including higher mean NT-proBNP baseline levels in the APOLLO study and cross-study comparison, the similar fold-change ratios (substance vs. placebo) suggest similar effects of vutrisiran and patisiran.

It should be also noted that for the Month 18 analysis, select echocardiogram parameters were re-read due to a staffing change at the central reading site that introduced a potential source of bias impacting the comparison of baseline versus follow-up echocardiogram data. Based on the new measurements for baseline LV wall thickness, the Cardiac Subpopulation was re-derived. As a result, seven patients receiving vutrisiran were added to the Cardiac Subpopulation and two were removed compared to the population defined in the Month 9 analysis.

Despite the above, it appears that changes in select echocardiographic parameters were of a similar magnitude with vutrisiran and patisiran when compared to the placebo group in APOLLO study.

TTR reduction at Month 9 was similar or even slightly greater with vutrisiran than with patisiran, and in the order of 78-82%, which is considered a very high TTR reduction. Such a large PD effect on the TTR could be indicative of the efficacy of vutrisiran. The % reduction of patisiran in APOLLO study was not presented, but it is known from phase 2 patisiran development study 002 that reductions of up to 96% and an average reduction of 92.5% were observed by two years. It is interesting to note that TTR reduction was nearly as similar with vutrisiran in HELIOS-A, as patisiran in APOLLO, in spite of the differences with the population. Nevertheless, as in the case of endpoints mNIS+7 and Norfolk QoL-DN at Month 9, and based on the same mechanism of action, and the very similar pharmacodynamic effects, the objective of this study was met at Month 18, as anticipated, and a demonstration of the non-inferiority of vutrisiran compared to within-study patisiran group, with respect to serum TTR levels through Month 18, was provided. The pre-specified criteria for declaring non-inferiority of vutrisiran (versus patisiran) was if the lower limit of the 95% CI for the median treatment difference in TTR percent reduction (vutrisiran - patisiran) was greater than -10%. The time-averaged trough TTR percent reduction through Month 18 was 84.7% for vutrisiran and 80.6% for patisiran (Table 35 in HELIOS-A CRS2 or Table 2 in Q59b). It can be concluded that vutrisiran demonstrated non-inferiority compared to within study patisiran, because the 95% CI limits of the median treatment difference in TTR percent reduction (vutrisiran - patisiran) was 1.17 and 9.25, in which the lower limit was above -10% [H0: Vutrisiran is inferior to patisiran: difference in median TTR reduction (vutrisiran - patisiran) $\leq$ -10%].

Last, the results from different analysis models MMRM, PMM 1 (hypothetical estimand) and PMM 2 (treatment policy estimand) appear consistent, and the slight differences do not have any impact on the clinical interpretation of the results. Therefore, the use of the MMRM results in the SmPC is considered acceptable, in order to keep all public information consistent.

2.6.7. Conclusions on the clinical efficacy

Hereditary transthyretin amyloidosis (hATTR) is a rare, progressive and fatal disease which manifests as destabilization of the tetrameric structure of the TTR protein. Vutrisiran is a siRNA molecule that uses RNA interference mechanisms to target and silence the expression of wild type and variant TTR mRNA and inhibit the synthesis of the TTR protein.

Large reductions in TTR and large improvements in neuropathy have been observed from baseline to Month 9 and to Month 18 with vutrisiran (in HELIOS-A), compared to an external placebo group from the pivotal study of patisiran (APOLLO). The results for the primary and secondary endpoints at Month 18, as pre-specified in the statistical analysis plan, together with the demonstration of non-inferiority

of vutrisiran to patisiran on the TTR percent reduction, confirmed the efficacy of vutrisiran in patients with hATTR with FAP stage 1 and 2 polyneuropathy. Finally, the indication for vutrisiran, as it is agreed, reflects the population studied and is the same as that for the approved product Onpattro (patisiran).

2.6.8. Clinical safety

2.6.8.1. Patient exposure

Safety data of vutrisiran in patients with hATTR amyloidosis with polyneuropathy is derived from the active controlled, open-label HELIOS-A study with a completed 18-months Treatment and ongoing Extension Period. With the response to the D120 LoQ, the Applicant has provided updated safety data as of the cut-off date of 26th August 2021. As of this date, overall, 155 patients with hATTR amyloidosis with polyneuropathy were exposed to vutrisiran in the clinical development program, with a cumulative exposure of 233.0 patient years; 118 patients have overall been exposed for ≥ 18 months and five subjects have been exposed for ≥ 27 months.

Posology:

During the Treatment Period, vutriran posology was 25 mg s.c. every three months (q3M); during the ongoing Extension Period as (per protocol Amendment 4.0) subjects who had already entered, or are entering the Extension Period, were randomised to either 25 mg vutrisiran s.c. q3M or 50 mg vutrisiran s.c. q6M. The all vutrisiran population comprises all subjects who received at least one dose of vutrisiran during HELIOS-A Treatment Period or Extension Period (irrespective of posology).

According to the Applicant, the patisiran group (N=42) in the HELIOS-A study was not intended to inform the safety profile of vutrisiran, but mainly the PK and a descriptive efficacy comparison to patisiran; as such, the patisiran data were not included in the Clinical Overview (CO) or the body of the Summary of Clinical Safety (SCS) and of the SUR#1, respectively of the original submission. The main safety comparison provided by the Applicant was made to the external placebo group of the APOLLO study (N=77). HELIOS-A and APOLLO enrolled patients with the same disease and nearly identical inclusion and exclusion criteria, aiming to provide a fair representation of the type of disease-related events commonly reported in this patient population. Differences between the studies included double-blind vs open-label design, exposure duration and frequency and mode of study drug administration, respectively. In addition, patients in the APOLLO placebo group but not in the HELIOS-A vutrisiran group received pre-medications (including corticosteroids, antihistamines and paracetamol) prior to the infusion. With regard to baseline differences, see section 3.3.4.2 of this report.

At the time of the current submission, Helios-B study, performed in patients with hATTR amyloidosis with cardiomyopathy, was ongoing. 348 subjects had been included in this study, randomised 1:1 to vutrisiran and placebo; however, as this study was still blinded at the time of writing this report, no detailed safety data had been provided.

2.6.8.2. Adverse events

An overview of AEs as of the CRS2 data cut-off date (26-AUG-2021) reported during the HELIOS-A Treatment Period for the vutrisiran and patisiran groups, as well as for the APOLLO placebo group, is presented in the following Table 37.

Table 37: Overview of Adverse Events HELIOS-A Treatment Period and APOLLO placebo group (CSR2-cut-off date 26-AUG-2021, Safety Population)

Category	No. of Patients (%)/No. of Events		
	APOLLO Placebo (N=77; PY=96.1)	HELIOS-A Vutrisiran (N=122; PY=191.3)	HELIOS-A Patisiran (N=42; PY=63.3)
At least 1 AE	75 (97.4)/1231	119 (97.5)/1057	41 (97.6)/433
At least 1 AE related to study drug	30 (39.0)/190	29 (23.8)/64	15 (35.7)/74
At least 1 SAE	31 (40.3)/99	32 (26.2)/63	18 (42.9)/42
At least 1 SAE related to study drug	0	2 (1.6)/2	5 (11.9)/7
At least 1 severe AE	28 (36.4)/88	19 (15.6)/40	16 (38.1)/25
At least 1 severe AE related to study drug	2 (2.6)/2	3 (2.5)/9	2 (4.8)/2
At least 1 AE leading to treatment discontinuation	11 (14.3)/15	3 (2.5)/3	3[b,c] (7.1)/3
At least 1 AE leading to treatment discontinuation related to study drug	0	0	0
At least 1 AE leading to stopping study participation	9 (11.7)/11	3 (2.5)/3	2[b,c] (4.8)/2
At least 1 AE leading to stopping study participation related to study drug	0	0	0
Death[a]	6 (7.8)	2 (1.6)	3[b] (7.1)

Abbreviations: AE=adverse event; CSR=Clinical Study Report; PY=patient years; SAE=serious adverse event.
[a] All deaths (including non-treatment emergent) are included in this table.

Common adverse events

During the Treatment Period, AEs reported in ≥10% of patients in the vutrisiran group were fall, pain in extremity, diarrhoea, oedema peripheral, urinary tract infection, arthralgia and dizziness. In the patisiran group, AEs reported in ≥10% of patients were infusion related reaction, urinary tract infection, diarrhoea, fall, constipation and headache.

Adverse events reported in ≥5% of patients in the vutrisiran group are summarized in the following Table 38.

Table 38: Adverse Events in ⩾5% of Patients in the Vutrisiran Group during the HELIOS-A Treatment Period by Preferred Term (As of CSR2 data-cut 26-AUG-2021, Safety population)

Preferred Term	No. of Patients (%)/No. of Events		
	APOLLO Placebo (N=77; PY=96.1)	HELIOS-A Vutrisiran (N=122; PY=191.3)	HELIOS-A Patisiran (N=42; PY=63.3)
At least 1 AE	**75 (97.4)/1231**	**119 (97.5)/1057**	**41 (97.6)/433**
Fall	22 (28.6)/43	22 (18.0)/39	6 (14.3)/8
Pain in extremity	8 (10.4)/12	18 (14.8)/23	3 (7.1)/3
Diarrhoea	29 (37.7)/95	17 (13.9)/31	7 (16.7)/18
Oedema peripheral	17 (22.1)/35	16 (13.1)/18	4 (9.5)/5
Urinary tract infection	14 (18.2)/23	16 (13.1)/25	8 (19.0)/13
Arthralgia	0	13 (10.7)/16	4 (9.5)/5
Dizziness	11 (14.3)/37	13 (10.7)/13	0
Nausea	16 (20.8)/22	12 (9.8)/18	4 (9.5)/10
Syncope	8 (10.4)/9	12 (9.8)/13	1 (2.4)/1
Abdominal pain	1 (1.3)/1	11 (9.0)/13	1 (2.4)/1
Headache	9 (11.7)/10	11 (9.0)/15	5 (11.9)/24
Cough	9 (11.7)/11	9 (7.4)/9	1 (2.4)/1
Nasopharyngitis	6 (7.8)/11	9 (7.4)/11	1 (2.4)/2
Upper respiratory tract infection	5 (6.5)/6	9 (7.4)/10	4 (9.5)/11
Vomiting	8 (10.4)/30	9 (7.4)/14	4 (9.5)/6
Atrial fibrillation	5 (6.5)/7	8 (6.6)/12	1 (2.4)/1
Thermal burn	4 (5.2)/4	8 (6.6)/11	0
Vitamin A decreased[a]	0	8 (6.6)/8	2 (4.8)/2
Gait disturbance	3 (3.9)/4	7 (5.7)/7	0
Neuralgia	5 (6.5)/13	7 (5.7)/8	3 (7.1)/3
Orthostatic hypotension	7 (9.1)/8	7 (5.7)/7	2 (4.8)/2
Rash	3 (3.9)/5	7 (5.7)/8	1 (2.4)/1

Abbreviations: AE=adverse event; CSR=Clinical Study Report; PY=patient years.
Preferred terms are sorted by decreasing frequency in the vutrisiran column.
[a] In the APOLLO study, the AE "vitamin A decreased" was not reported because vitamin A levels were blinded.
Source: Study 002 CSR2 Table 14.3.1.2.1

Relationship of adverse events to study drug/Related adverse events/Adverse events by causality

According to the CO, the Applicant defined Adverse drug reactions (ADRs) as follows:

ADRs are AEs for which there is a basis to believe there may be a causal relationship to the medicinal product. For vutrisiran, ADRs are defined as the most frequently reported AEs in vutrisiran-treated subjects in the HELIOS-A study, while accounting for commonly reported AEs in the background disease population using the data from the external reference APOLLO placebo group. Additional considerations included temporal relationship and biologic plausibility. AEs were analysed by individual preferred term (PT) and also grouping by medical concept. Based upon a frequency criterion of AEs (individual PT and/or grouped medical concept) reported in >5% of vutrisiran-treated subjects in HELIOS-A (Treatment Period) and >3% higher frequency compared to the APOLLO placebo group, the following events were observed:

- Arthralgia was reported in 10.7% of vutrisiran-treated subjects, compared with none in the APOLLO placebo group. AEs of arthralgia were mostly mild or moderate in severity, with one patient having a severe AE; none of the events were serious. No AEs of arthralgia led to treatment discontinuation. AEs of arthralgia did not increase over time.

- The medical concept of dyspnoea, which includes the PTs dyspnoea, dyspnoea exertional, and dyspnoea paroxysmal nocturnal, was reported in 6.6% of vutrisiran treated subjects compared with no subjects in the APOLLO placebo group. These AEs of dyspnoea were mild or moderate in severity; none of the events were severe or serious. None of the AEs led to treatment discontinuation. AEs of dyspnoea did not increase over time.

- As a result of the updated safety data of the HELIOS-A Treatment Period (as of 26-AUG-2021), the Applicant further proposes to include "pain in extremity" in SmPC section 4.8 as a common ADR, which occurred in 14.8% vutrisiran-treated subjects compared with 10.4 % APOLLO placebo subjects. These events were generally mild or moderate, and did not lead to treatment discontinuation in any subject. There was neither a clear pattern in time to onset of pain in extremity since start of vutrisiran or since last dose, respectively nor a pattern regarding associated TEAEs.

- In addition, based on the subcutaneous administration mode of vutrisiran, injection site reactions (ISRs) are considered an ADR. ISRs were reported in 4.1% of vutrisiran-treated subjects during the Treatment Period. ISRs were all mild, non-serious, transient, and did not lead to treatment discontinuation. Symptoms included bruising, erythema, pain, pruritus, and warmth. One further subject experienced a single ISR during the HELIOS-A study Extension Period, with the PT of traumatic haematoma, apparently reported as moderate.

- Vitamin A decreased was excluded as an ADR as it is an expected PD effect of any TTR-lowering therapy.

As of the CSR2, during the HELIOS-A Treatment Period, AEs considered related to study drug by the investigator and reported in ≥ two vutrisiran-treated subjects were vitamin A decreased (in 8 (6.6%) HELIOS-A vutrisiran, two (4.8%) HELIOS-A patisiran and no APOLLO placebo subjects), Injection site reaction in four (3.3%) vutrisiran and no patisiran and APOLLO placebo subjects, respectively, in whom study drug was infused intravenously), dry eye in three (2.5%) vutrisiran, no patisiran and one (1.3%) APOLLO placebo subjects), dyspepsia in two (1.6%) vutrisiran and no patisiran or APOLLO placebo subjects), fatigue in two (1.6%) vutrisirian, no patisiran and four (5.2%) APOLLO placebo subjects, oedema peripheral in two (1.6%) vutrisirian, no patisiran and five (6.5%) APOLLO placebo subjects and scleral discolouration in two (1.6%) vutrisiran and no patisiran or APOLLO placebo subjects), respectively.

Vitamin A results collected on HELIOS-A were not blinded, and some investigators reported this known PD effect as an AE, with reporting varying by investigator.

Adverse Events over time

An analysis of AEs over time by 3-month intervals in the vutrisiran group during the Treatment Period was performed, showing that the proportion of patients with AEs was generally stable over time (CSR2 Table 14.3.1.2.3).

Adverse events by severity

As of the CSR2, one or more severe AEs were reported for 15.6% vutrisiran and 38.1% patisiran subjects during the HELIOS-A Treatment Period and for 36.4% APOLLO placebo subjects, respectively. Severe AEs by preferred term (PT) that were reported in more than a single vutrisiran subject were sepsis, atrial fibrillation, hypokalaemia, and syncope (reported in two subjects each and not related each). During the Treatment Period, three vutrisiran-treated patients had severe AEs that were

considered related to study drug by the investigator (all were already reported in the SCS of the originally submitted documentation): Patient, experiencing Escherichia urinary tract infection, which was also an SAE (see Section 3.3.7.3, below); patient, experiencing vitamin A decreased and oedema peripheral; patient experienced six severe, transient events, i.e. dizziness, dry mouth, hyperhidrosis, hyperthermia, scleral discolouration, and dyspepsia, on Day 1 after the first dose of study drug. For all three patients with severe, treatment-related events, no action was taken with the study drug and all three patients continued in the study. One further treatment related severe AE was reported in the all vutrisiran population as of 26-AUG-2021, i.e. an SAE of transaminases increased (see AECI, Hepatic events and SAEs, respectively, below).

Adverse events of clinical interest (AECI)/other adverse events of interest

Injection site reactions (ISRs) of clinical interest concerned severe or serious injection site reactions (ISRs), ISRs that are associated with a recall phenomenon (reaction at the site of a prior injection with subsequent injections), recurrent ISRs that are increasing in severity, or ISRs that lead to temporary dose interruption or permanent discontinuation of vutrisiran. A systemic reaction that includes the injection site (e.g. generalized urticaria) was not considered an ISR. The frequency of ISRs was evaluated by analysis of AEs mapping to the MedDRA high level term (HLT) of Injection Site Reactions. As of the CSR2 data cut-off (26-AUG-2021), during HELIOS-A Treatment Period, ISRs were reported in five (4.1%) vutrisiran-treated patients, and in 0.6% of the 836 total vutrisiran doses administered. In HELIOS-A, vutrisiran was initially administered using injections prepared from vials; some patients were transitioned to prefilled syringe (PFS) injections, as that presentation became available. By presentation, ISRs occurred in 0.8% vutrisiran doses administered as vials and in 0.3% vutrisiran doses administered as prefilled syringes. ISR signs and symptoms included bruising, erythema, pain, pruritus, and warmth in one subjects each. No patient had more than one event of ISR. All ISRs were non-serious, transient, and considered mild in severity; no ISR led to treatment discontinuation. One further subject experienced one single ISR during the Extension Period (as of 26-AUG-2021); apparently, the signs and symptoms in this subject were reported as traumatic haematoma and this ISR was reported as moderate (according to Table 14.3.1.2.5 of CSR2).

As of CSR2 data cut-off (26-AUG-2021), during HELIOS-A Treatment Period, in the patisiran group, infusion related reactions (IRRs) were reported for 10 (23.8%) patients, who had a total of 50 IRRs; the maximum severity of the events was mild for five patients, moderate for four patients, and severe for one patient. Three patients had a total of four serious IRRs, all treatment related. For two (4.8%) patients, the SAEs of IRR resulted in an interruption of the patisiran infusion. No patient discontinued study treatment due to an IRR.

During the completed Helios-A Treatment period, the incidence of TEAEs mapping to the Hypersensitivity Standardised MedDRA Query (SMQ; based on broad and narrow scope terms) in the Helios-A vutrisiran group (21.3%) was lower as compared to the incidences in the APOLLO placebo (29.9%), and the Helios-A or pooled patisiran groups, respectively (33.3% and 41.1%). Even, when infusion related reactions, which could not occur with vutrisiran due to the administration mode, were excluded from the analysis, the incidence of TEAEs in the Hypersensitivity SMQ was very similar or lower compared to the other treatment groups.

Hepatic events

During the HELIOS-A Treatment period as of the CSR2, AEs mapping to the drug-related Hepatic Disorders SMQ were reported for 6 (4.9%) patients in the vutrisiran group. These concerned hepatobiliary disorders in two (1.6%) subjects, i.e. hepatic cyst and hepatic function abnormal in one subject each, and Investigations in four (3.3%) subjects, respectively. In the APOLLO placebo group, AEs in the drug-related Hepatic Disorders SMQ were reported for six (7.8%) patients, hepatobiliary

disorders concerned one (1.3%) subject and investigations two (2.6%) subjects, respectively. In the HELIOS-A patisiran group, hepatic AEs mapping to the SMQ were reported in six (14.3%) subjects.

In the vutrisiran group, all hepatic AEs were mild or moderate in severity, none was serious, and none led to discontinuation of study drug. Three patients had hepatic AEs that were considered related to treatment by the investigator, all concerning investigations. However, in all cases no action was taken with study drug. In one of these subjects increased alkaline phosphatase was not reported as resolved; however, alkaline phosphatase declined to near baseline values despite continued treatment, and all other related hepatic AEs resolved despite continued treatment. Confounding factors were present in two of these cases.

In the **all vutrisiran group** (HELIOS-A Treatment+ Extension Period), AEs mapping to the drug-related Hepatic Disorders SMQ were reported for 7.7% of subjects (the majority being within the Investigations SOC). Three severe events occurred in two subjects; all these severe events occurred during the Extension Period, concerned increased liver function test (LFT) parameters, and are described in the respective section, directly below.

Overall, four related hepatic AEs occurred, three during the Treatment Period (described above) and no hepatic AE led to discontinuation of study drug.

Liver function test (LFT) parameters

In subjects treated with vutrisiran during the HELIOS-A Treatment Period, mean absolute and mean change from baseline in bilirubin, remained generally stable over the course of the study, however, a minor increase from baseline in mean transaminases (<4.37IU/L) was found, peaking at Day 505, which trended back towards baseline thereafter. Further, when disregarding measurements at time-points at which values are available for only few subjects, there appeared to be a rather steady increase in alkaline phosphatase (ALP) over time in both, the vutrisiran as well as the patisiran group (see CSR2, Table 14.3.5.3.1). Of note, no corresponding increase in ALP was found in the patisiran group of the Apollo patisiran study.

As of the CSR2, regarding worst post-baseline LFT results derived from routine monitoring in HELIOS-A, increased alanine aminotransferase (ALT) was found in 35.2% vutrisiran and 47.6% patisiran subjects, increased aspartate aminotransferase (AST) in 24.6% vutrisiran and 31.0% patisiran subjects and total bilirubin increased was found in 8.2% vutrisiran and 9.5% patisiran subjects, respectively. Central and local routine safety laboratory monitoring indicated that values of ALT or AST >3×ULN were reported for 1 patient, who had an AST elevation of 5.4×ULN and an ALT elevation of 3.9×ULN (considered not related to study treatment, but attributable to eating a potentially hepatotoxic wild herb). No action was taken with regard to study drug, the event resolved.

In the all vutrisiran-treated population (derived from HELIOS-A Treatment and Extension Period), three subjects overall had values of ALT or AST >3×ULN: one subject during the Treatment Period, described above, two subjects during the Extension Period:

- Subject had a severe SAE of transaminases increased, considered related. ALT 6.36xULN, AST 5.22xULN, AP 1.42xULN, and GGT 4.06xULN were reported in a patisiran-vutrisiran subject, 42 Days after the first dose of 50 mg vutrisiran during randomised Extension (RTE) period. Medical history included amongst others heart failure, hypertension and a BMI of 31.0 mg/m². CT scan showed diffuse hepatic steatosis, hepatic cysts and gallstones without complication. Information provided after the data cut-off date included variable descriptions of symptoms from jaundice and right upper quadrant pain to isolated ALT/AST elevations without symptoms. However, bilirubin was normal at the time of the event, no value of total bilirubin >2×ULN was reported throughout the study. Updated information provided by the applicant over the course of the assessment showed, that a milder transaminase increase occurred in this subject, peaking approx. four weeks after the second vutrisiran injection at

ALT of >2.4 ULN and AST of >2.3. In both instances transaminase levels returned to near normal or normal, respectively, no specific treatment or hospitalisation was required during either event and the subject is ongoing in the study (as of 08 April 2022). Of note, the subject received a different posology than that currently recommended, i.e. twice the recommended single dose administered with a longer treatment interval (of six months).

Beyond this case, no further related SAE of transaminases increased occurred as of 26-AUG-2022.

- In subject non-serious AEs of moderate transaminases increased and severe drug-induced liver injury occurred during the Helios-A Treatment Extension Period and were attributed to the subject's vitamin A supplement. Transaminase increase started after the 6th vutrisiran administration in this subject and was reported as AE after the 8th dose. Transaminase levels peaked to 2.81xULN AST and 3.96xULN ALT and improved to <2xULN AST and <3xULN ALT (at the last available measurement) after the vitamin A supplement was withdrawn, while vutrisiran administration was continued unchanged. The exact vitamin A dose taken by the subject is not known. All values for bilirubin were normal during the study. The patient is continuing to receive vutrisiran in the study.

No values of total bilirubin >2×ULN were reported and no patients met biochemical Hy's law criteria.

Alkaline phosphatase (ALP) increases (> 1.5xULN) during the HELIOS-A Treatment Period were reported in 11 (9.0%) vutrisiran vs. 1 (2.4%) patisiran subject (as of the CSR2) and in 1 (1.3%) APOLLO placebo subject. Of the 11 vutrisiran subjects with ALP increase, four had confounding factors or no clear increase in ALP from baseline. For the remaining seven subjects, any concurrent elevations in LFTs were typically transient and mild with all ALT and AST values < 3xULN and all bilirubin levels <2xULN. There was no pattern in time-to onset of ALP elevation. Overall, only a single ALP elevation was reported as TEAE in the vutrisiran group, which returned to near baseline valued despite continued vutrisiran.

Cardiac events

At baseline in the HELIOS-A vutrisiran group, 68.0% of patients had a medical history of at least one term within the Cardiac disorders SOC, including 31.1% with terms in the Heart failure NEC HLT and 13.1% with terms in the Cardiac conduction disorders HLT. In addition, 9.8% of patients had a medical history of at least 1 term within the Cardiac device therapeutic procedures HLT (eg, pacemaker and/or defibrillator) and two patients (1.6%) had heart transplant. At baseline in the patisiran group, 73.8% of patients had a medical history of at least one term within the Cardiac disorders SOC, including 35.7% with terms in the Heart failure NEC HLT and 16.7% with terms in the Cardiac conduction disorders HLT. In addition, 21.4% of patients had a medical history of at least one term within the Cardiac device therapeutic procedures HLT and one patient (2.4%) had a heart transplant.

A summary of cardiac safety found in the HELIOS-A study and in the APOLLO placebo group is presented in the following Table 39.

Table 39: Summary of Cardiac Safety during the HELIOS-A Treatment Period in the Vutrisiran Group compared to HELIOS-A patisiran and APOLLO placebo group, respectively (CSR2 data cut-off, 26-AUG-2022, Safety population)

Category	No. of Patients (%)/No. of Events		
	APOLLO Placebo (N=77; PY=96.1)	HELIOS-A Vutrisiran (N=122; PY=191.3)	HELIOS-A Patisiran (N=42; PY=63.3)
AEs in the Cardiac disorders SOC	28 (36.4)/45	37 (30.3)/65	10 (23.8)/15
SAEs in the Cardiac disorders SOC	10 (13.0)/15	11 (9.0)/17	6 (14.3)/6
Cardiac arrhythmia HLGT AEs	22 (28.6)/29	30 (24.6)/47	3 (7.1)/4
Supraventricular arrhythmias HLT	13 (16.9)/16	10 (8.2)/15	1 (2.4)/2
Rate and rhythm disorders NEC HLT	0	8 (6.6)/8	2 (4.8)/2
Cardiac conduction disorders HLT	7 (9.1)/7	10 (8.2)/18	0
Ventricular arrhythmias and cardiac arrest HLT	6 (7.8)/6	6 (4.9)/6	0
Torsade de Pointes/QT Prolongation SMQ AEs	14 (18.2)/15	14 (11.5)/16	2 (4.8)/3
Cardiac Failure SMQ (narrow) AEs	8 (10.4)/12	7 (5.7)/9	8 (19.0)/8

Vutrisiran group in HELIOS-A Treatment Period as of CSR2 data cut-off (26-AUG-2021):

30.3% vutrisiran subjects had AEs in the Cardiac disorders SOC, 24.6% had AEs that mapped within the Cardiac arrhythmia HLGT, the majority (22 of 30) of these latter patients had cardiac amyloidosis at baseline. The most frequent AEs within the Cardiac arrhythmia HLGT were atrial fibrillation (6.6%), bundle branch block left (4.1%), atrioventricular block first degree (3.3%), ventricular extrasystoles (2.5%), ventricular tachycardia (2.5%), and tachycardia (2.5%). In the vutrisiran group, cardiac SAEs were reported for 11 (9%) patients (one patient each with acute myocardial infarction, atrial fibrillation, atrioventricular block, atrioventricular block complete, atrioventricular block second degree, cardiac failure, cardiac failure acute, cardiac failure chronic, cardiac failure congestive, conduction disorder, myocardial ischaemia, and pericarditis). To evaluate whether there were any events that may be associated with QTc prolongation, an analysis of AEs within the Torsade de pointes/QT prolongation SMQ (broad terms) was performed. In the vutrisiran group, 11.5% of patients had AEs that mapped within the Torsade de pointes/QT prolongation SMQ; no confirmed events of Torsade de pointes were reported. No patient had a cardiac AE that was considered related to the study drug, one patient had an AE in the Cardiac disorders SOC (cardiac failure acute, considered not related) that resulted in discontinuation from the study drug and from the study. One patient death was adjudicated as a cardiovascular death (PT, iliac artery occlusion, which occurred after an SAE of cardiac failure), considered not related to study drug.

All Vutrisiran-Treated Population – HELIOS-A Treatment Period + Extension Period (as of 26-AUG-2021)

In the all vutrisiran-treated population, 24.5% of patients had AEs within the Cardiac disorders SOC. Cardiac SAEs were reported for 9.0% patients. Most events occurred in the Treatment Period (as described above). Cardiac SAEs that occurred in the Treatment Extension Period were bradycardia (one patient), sinus node dysfunction (one patient), and atrioventricular block second degree (one patient); none of these SAEs was considered related to the study drug and none resulted in discontinuation from the study drug. One patient died due to an SAE of sudden cardiac death, which was adjudicated as a CV death.

No patient had a cardiac AE (including SAEs) that was considered related to the study drug.

Cardiac events in the cardiac subpopulation, HELIOS-A Treatment Period (provided in CSR2, cut-off date of 26-AUG-2021):

In the HELIOS-A study, 40 of the 122 patients (32.8%) in the vutrisiran group and 14 of the 42 patients (33.3%) in the patisiran group had pre-existing evidence of cardiac amyloid involvement, defined as baseline LV wall thickness ≥1.3 cm and no aortic valve disease or hypertension in medical history. In the cardiac subpopulation, 15 (37.5%) of subjects in the vutrisiran group and four (28.5%) in the patisiran group had AEs in the Cardiac disorders SOC. Cardiac AEs reported in ≥5% of the vutrisiran-treated cardiac subpopulation were atrial fibrillation (15.0%), atrioventricular block (5.0%), cardiac failure (5.0%), and cardiac failure congestive (5.0%). Cardiac disorder SAEs were reported for six subjects (15.0%) in the vutrisiran cardiac subgroup, with the respective PTs occurring in one subject each and for four (28.6%) subjects in the patisiran cardiac subgroup.

In the cardiac subpopulation, during the HELIOS-A Treatment Period, AEs that mapped within the HLGT of Cardiac arrhythmia occurred in 32.5% HELIOS-A vutrisiran subjects (with the most common being atrial fibrillation with an incidence of 15.0%), in 7.1% HELIOS-A patisiran subjects and in 30.6% APOLLO placebo subjects. Torsade de pointes/QT prolongation SMQ AEs occurred in 17.5% vutrisiran and 7.1% patisiran subjects. No confirmed torsade de pointes were reported with vutrisiran. The incidences of events within the Torsade de pointes/QT prolongation SMQ in the cardiac subpopulation were mainly driven by the PTs of syncope, which occurred in 6 (15.0%) HELIOS-A vutrisiran vs. one (7.1%) HELIOS-A patisiran and three (8.3%) APOLLO placebo subjects. None of the syncope events in the HELIOS-A vutrisiran cardiac subpopulation was considered related by the Investigator, all concerned isolated events with no apparent pattern in temporal relationship to study drug administration or the total number of doses applied until the event, respectively.

Renal disorders

In HELIOS-A, at baseline 23.8% vutrisiran and 28.6% patisiran subjects had a medical history of terms within the Renal and urinary disorders SOC. In the vutrisiran group, the most common terms mapped to the HLT of bladder and urethral symptoms (8.2%) and the HLT of renal failure and impairment (5.7%). As of the CSR2 (26-AUG-2021), in the HELIOS-A Treatment Period, five (4.1%) vutrisiran subjects had an AE within the Acute renal failure SMQ, none of which was considered related to study drug or led to study drug discontinuation. In the HELIOS-A patisiran group two (4.8%) subjects and in the APOLLO placebo group nine (11.7%) subjects had an AE within the Acute renal failure SMQ.

Renal function parameters/Urinalysis

As of the CSR2 (26-AUG-2021), in the vutrisiran group, mean absolute values and mean change from baseline in eGFR and creatinine were relatively stable throughout the HELIOS-A Treatment Period. The majority of patients in the vutrisiran group as well as in the patisiran group did not exhibit shifts in eGFR. During the Treatment Period, two subjects in the vutrisiran group and three subjects in the

patisiran group met the criterion of Grade 3 chronic kidney disease (eGFR <30 ml/min/1.73m2) during the Treatment Period. No additional shifts to eGFR <30 ml/min/1.73m² occurred during the Extension Period.

No patient in the HELIOS-A study (vutrisiran or patisiran group) met the criteria of laboratory abnormalities corresponding to CTCAE v5.0 Grade 3 creatinine increased (creatinine >3.0×baseline or >3.0-6.0×ULN) or Grade 3 acute kidney injury (creatinine >3.0×ULN or >4.0 mg/dL).

As of the CSR2 (26-AUG-2021), in the HELIOS-A study the majority of patients in the vutrisiran group had no abnormalities in urinalysis parameters during the Treatment (and Extension Period). For patients with urinalysis abnormalities, there did not appear to be any pattern to the abnormalities.

Malignancies

As of the CSR2 (26-AUG-2021), in the HELIOS-A Treatment Period, five (4.1%) vutrisiran subjects and no patisiran subjects experienced a malignancy. These included a subject with a recent history of gynecological pain and bleeding, who had an SAE of endometrial neoplasm on Day 87, with prior metrorrhagia on Day 79 and a subject, with no relevant medical history, and a non-serious AE of basal cell carcinoma on Day 50, a subject with no relevant medical history with an SAE of adenocarcinoma of the cervix, serious on Day 413, a subject with no relevant medical history with an SAE of high grade transitional cell carcinoma on Day 451, and a subject with no relevant medical history with an AE of basal cell carcinoma on Day 335. None of the cases was considered related to study drug and no action was taken with study drug. In the All Vutrisiran Treated Population – Treatment Period + Extension Period no additional malignancy was reported.

In the APOLLO placebo group, four (5.2%) subjects had malignancies, two patients had malignant skin neoplasms (basal cell carcinoma and malignant melanoma in situ), and three patients had solid organ malignancies (two colon cancers and one prostate cancer). One of the patients with metastatic colorectal cancer had a prior history of Stage 3 colon cancer.

Ocular events

As of the CSR2 cut-off (26-AUG-2021), during the HELIOS-A Treatment Period, 28.7% vutrisiran vs. 23.8% patisiran subjects had an AE within the Eye disorders SOC. In the vutrisiran group, the AEs mapping within the Eye disorder SOC reported in ≥2% of subjects were vision blurred (4.1%), dry eye (4.1%), conjunctival haemorrhage (4.1%), visual impairment (2.5%), and eye pain (2.5%). One event (scleral discolouration) was severe, no AE in the Eye disorder SOC was serious.

Treatment-related AEs in the Eye disorders SOC occurred in no patisiran and in 5.7% vutrisiran subjects, the PTs were dry eye in 3 (2.5%) subjects, scleral discolouration in two (1.6%) subjects, and night blindness and vision blurred, respectively in one (0.8%) subject each. The one patient with the AE of night blindness had a medical history of optic ischemic neuropathy that was ongoing at the start of the study and a history of regular eye exams. The ophthalmologic examination conducted after the AE of night blindness did not report findings suggestive of vitamin A deficiency, e.g., xerophthalmia, the patient's visual acuity was assessed as requiring glasses. No action was taken with the study drug and the patient continued in the study. Overall three events of scleral discolouration occurred in two subjects, and all were considered related. One event was considered severe and occurred over study Day 1-2, with several concurrent severe non-ocular AEs, which were all transient. This subject had another event of (mild) scleral discolouration on Study day 168. In one further subject scleral discolouration was reported, which was mild and occurred from study Day 3-6.

No additional treatment-related ocular events occurred during the Extension Period.

In the APOLLO placebo group, 26.0% subjects had an AE within the Eye disorders SOC. Treatment related AEs in the Eye disorders SOC occurred in 6.5% subjects, with PTs of dry eye, eyelid ptosis, night blindness, retinal disorder, and vision blurred in one subject each.

Depression and suicidality

As of the CSR2, in the HELIOS-A Treatment Period, AEs within the Depression and suicide/self-injury SMQ were reported for no patisiran and for eight (6.6%) vutrisiran patients, the PTs were depression in four (3.3%) subjects and adjustment disorder with depressed mood, major depression, memory impairment, and terminal insomnia, respectively in one subject (0.8%) each. All events were mild or moderate in severity, none was serious, and none was considered related to study drug.

In the APOLLO placebo group, AEs within the Depression and suicide/self-injury SMQ were reported for 10.4% subjects, the respective PTs were depression in 7.8% subjects and poor-quality sleep as well as psychomotor hyperactivity in 1.3% subjects each.

C-SSRS (HELIOS-A Treatment Period, results presented as of 26-AUG-2021): In the vutrisiran group, at baseline, 85.2% of patients had no suicidal ideation or behaviour, 13.1% had suicidal ideation, and 1.6% of patients had suicidal behaviour. At patients' worst post-baseline assessment, 86.9% of patients had no suicidal ideation or behaviour, 8.2% had suicidal ideation, 2.5% had suicidal behaviour, and responses were missing for 2.5% of patients. In the patisiran group, at baseline, 97.6% of patients had no suicidal ideation or behaviour, 2.4% had suicidal ideation, no patient had suicidal behaviour, and no responses were missing. At patients' worst post-baseline assessment, 92.9% of patients had no suicidal ideation or behaviour, 2.4% had suicidal ideation, no patient had suicidal behaviour, and responses were missing for 4.8% of patients.

2.6.8.3. Serious adverse event/deaths/other significant events

Deaths

As of the updated data cut-off (26-AUG-2021), a total of 5 patients died during the Treatment Period of the HELIOS-A study, including 2 (1.6%) patients in the vutrisiran group and 3 (7.1%) patients in the patisiran group. None of the deaths was considered related to study drug by the Investigators, all were considered related to the underlying disease or other factors. Two of the deaths, 1 in each treatment group, were due to COVID-19 infection.

Overall, in the vutrisiran clinical development program there have been five deaths in vutrisiran-treated patients as of 19 October 2021. All deaths occurred in the HELIOS-A study, two cases during the Treatment Period, three during the Extension Period (one of the latter occurred before data cut-off date and two of the latter occurred after data cut-off date but before database lock, i.e. before 19 Oct 2021). Two cases were cardiovascular, one was non-cardiovascular; the two cases that occurred after cut-off date were not adjudicated.

The 1st case concerned a death due to pneumonia secondary to Covid-19 infection in a subject with risk factors for an unfavourable Covid-19 outcome (age > 80 years, male sex, pre-existing cardiac condition). In the 2nd case, death due to occlusion of the iliac artery during hospitalisation with cardiac failure and pneumonia occurred in a subject with pre-existing cardiac conditions, i.e. cardiac amyloidosis and a history of atrial fibrillation, cardiac failure and circulatory disorders of the brain. The 3rd case concerned the event of sudden cardiac death in a subject with a history of cardiac amyloidosis and congestive heart failure. The two death cases that occurred after database lock concerned a sudden cardiac death in two subjects, each with a history of cardiac amyloidosis with multiple pre-existing cardiac conditions. None of the five fatal events in vutrisiran treated subjects was considered related to study drug.

Serious adverse events (SAEs)

As of 26-AUG-2021 (CSR2), in the HELIOS-A Treatment Period, one or more SAEs were reported for 26.2% of patients in the vutrisiran and 42.9% subjects in the patisiran group. In the APOLLO placebo group, SAEs were reported in 40.3% subjects. The SOCs in which ≥5% vutrisiran subjects reported SAEs were Cardiac disorders (9.0% of patients) and Infections and infestations (7.4% of patients). Apart from Covid-19 related events, the type of SAEs observed with vutrisiran was generally consistent with those reported in the patient population. Serious events reported in at least 2 (≥1%) vutrisiran subjects were pneumonia, acute kidney injury, ventricular tachycardia, COVID-19 pneumonia, pyelonephritis, sepsis, fall, hypokalaemia, and syncope. In addition to the data derived from HELIOS-A Treatment Period, further SAEs that occurred in at least 2 subjects in the all vutrisiran-treated population (Treatment + Extension Period) were orthostatic hypotension, atrioventricular block second degree, and ankle fracture (in two subjects each).

An SAE of pyelonephritis occurred in 2 (1.6%) vutrisiran subjects vs. no APOLLO placebo subjects; in one of these subjects, ultrasound scan revealed a markedly enlarged prostate and significant residual volume post micturition at the time of the event. Both events were considered not related to treatment, did not lead to change of study drug and according to the provided narratives were moderate in intensity and resolved. Also, an SAE of syncope occurred in 2 (1.6%) vutrisiran vs. no APOLLO placebo or HELIOS-A-patisiran subjects, respectively (see AECI, Cardiac events, above). Beyond this, there are no imbalances in SAEs by PT in HELIOS-A vutrisiran vs. APOLLO placebo subjects, which would per se raise concerns.

As of 26-AUG-2021, during the HELIOS-A Treatment Period, two SAEs in the vutrisiran group were considered related to treatment by the Investigator. These concerned dyslipidaemia and Escherichia urinary tract infection (UTI), respectively and concerned one subject each. Neither related SAE led to change in study drug dose, both events resolved. Causality of the SAE of Escherichia UTI is questionable based on the medical history of UTI as well as the reported surgery for prostatic hypertrophy shortly before the onset of the event. A contributory role of vutrisiran with the case of moderate dyslipidaemia cannot be fully excluded based on the temporal relationship, however, the dyslipidaemia resolved with atorvastatin treatment despite continued vutrisiran. According to SUR#1 Table 14.3.1.2, within the Metabolism and nutrition disorders SOC, no further AEs with the PT of dyslipidaemia, hypercholersterolaemia, or hypertriglyceridaemia, respectively were reported with vutrisiran in the HELIOS-A Treatment Period.

As of 26-AUG-2021, a third SAE, i.e. transaminases increased was considered related in the all vutrisiran population (see AECI, LFT parameters, above).

2.6.8.4. Laboratory findings

Haematology evaluations (as of 26-Apr-2021):

For the HELIOS-A vutrisiran group mean values for hematology parameters, including red blood cell parameters (hemoglobin and hematocrit), white blood cell parameters (leukocytes, lymphocytes, and neutrophils), and platelet parameters (including platelet counts and mean platelet volume) were generally stable over the course of the study. Potentially clinically significant abnormalities in haematology parameters were infrequent in the vutrisiran group and respective decreases in haematology parameters occurred somewhat less frequently in vutrisiran compared to patisiran subjects. Increase in neutrophils (≥12x10^9/L) and in leukocytes (≥16x10^9/L) occurred in one vutrisiran subject each vs. in no patisiran subject. Potentially clinically significant abnormalities regarding platelets were not reported in any subject. In the vutrisiran group, two subjects had Grade 3 haematology abnormalities; both subjects were receiving chemotherapy.

Blood chemistry evaluations (as of 26-Apr-2021):

In the HELIOS-A vutrisiran group, the mean absolute values and mean percent changes from baseline for the blood chemistry parameters, including sodium, potassium, chloride, bicarbonate, calcium, glucose, phosphate, protein, urate, and albumin, remained generally stable over time.

During the HELIOS-A Treatment Period, potentially clinically significant abnormality in blood chemistry reported in >3 vutrisiran subjects was low sodium (<130 mmol/L) which was reported in 3.3% vutrisiran and 4.8% patisiran subjects, respectively. Four patients in the vutrisiran group had Grade 3 abnormalities in chemistry parameters, i.e. low sodium and low potassium levels in two subjects each, none of which was considered related with study drug.

Coagulation parameters (as of 26-Apr-2021):

In the vutrisiran group, mean values for coagulation parameters, including activated partial thromboplastin time, prothrombin time, and prothrombin international normalized ratio (INR), were stable over time during the Treatment Period. Three subjects in the all vutrisiran group had Grade 3 abnormalities in coagulation parameters, all of whom were on anticoagulants and two of whom had Grade 3 elevated prothrombin INR also at baseline.

Vital signs, physical findings, and other observations related to safety

The incidence of potentially clinically significant abnormalities in vital sign assessments during the HELIOS-A Treatment Period in the vutrisiran group compared to the patisiran group did not show any consistent pattern which would raise new safety concerns for vutrisiran. While the incidence of weight increment ≥ 7% during the HELIOS-A Treatment Period (as of CSR2) was very similar in the vutrisiran and patisiran treatment groups (22.1% vs. 21.4%), the incidence of weight decrement ≥ 7% was lower in vutrisiran compared to patisiran subjects (17.2% vs. 28.6%).

Electrocardiograms (ECGs):

As of the CSR2, in the vutrisiran group, mean ECG parameters, including QTc corrected by Fridericia's formula (QTcF) interval and all other ECG parameters, remained stable throughout the Treatment Period in both, the safety population and the cardiac subpopulation.

As of the CSR2, during the 18-Month Treatment Period, the number of relevant shifts of QTcF was low and consistent with the underlying disease. No clear imbalance between vutrisiran and patisiran was seen with regard to the number of relevant shifts of QTcF, neither in the safety population nor in the cardiac subpopulation.

Clinically significant change from baseline was infrequent, nevertheless was only reported in the vutrisiran but not in the patisiran group i.e. in two (1.7%) subjects at day 85, four (3.5%) subjects at months 9, four (3.6%) subjects at day 337, and four (3.5%) subjects at Month 18, respectively.

2.6.8.5. In vitro biomarker test for patient selection for safety

N/A

2.6.8.6. Safety in special populations

During the Helios-A Treatment Period (as of the cut-off date of 26-AUG-2021), the safety profile, including the type and nature of AEs, including SAEs, in the vutrisiran-treated subjects was generally consistent across demographic and baseline disease characteristics, i.e. the **Intrinsic factors** age (<65 vs ≥65 years), sex (male vs female), race (White vs all other races), weight (<65 vs ≥65 kg),

genotype (V30M vs non-V30M), and familial amyloid polyneuropathy (FAP) stage (I vs II/III) as well as the **extrinsic factor** geographic region (North America; Western Europe; Rest of world). From the provided AE table by age category (below) a higher incidence of e.g. SAEs, and TEAEs within the accidents and injuries SMQ, cardiac disorders SOC and vascular disorders SOC, respectively with increasing age was found within the vutrisiran group, as would be expected. However, in the subgroup aged ≥ 65 years, the safety profile of vutrisiran derived from the Helios-A treatment group, compared to the respective subgroup of the Apollo placebo group, does not raise safety concerns with regard to age.

The safety profile of vutrisiran in the cardiac subpopulation was described by the Applicant as overall similar to that observed in the safety population, with similar proportions of patients experiencing AEs and SAEs. As of the CSR2 (16-AUG-2021), TEAEs in the cardiac subpopulation occurred in 97.5% vutrisiran and 92.9% patisiran subjects during the HELIOS-A Treatment Period, and in 97.2% of APOLLO placebo subjects, respectively. SAEs in the cardiac subpopulation occurred in 32.5% vutrisiran and 35.7% patisiran subjects during the HELIOS-A Treatment Period, and in 50.0% of the APOLLO placebo subjects, respectively.

Through 18 Nov 2021, there have been no reported pregnancies in the vutrisiran clinical development program.

No instances of accidental or intentional overdose of vutrisiran have been reported during the clinical development program (as of the SUR#1 cut-off date). Vutrisiran is not expected to have a withdrawal or rebound effect as the onset of the PD effects occurs within days and recovery occurs over months.

Table 40: Age distribution for TEAEs (All Vutrisiran-treated Population)

Category	No. of Patients (%)			
Age category (years)	**<65 (N=102)**	**≥65 to <75 (N=45)**	**≥75 to <85 (N=7)**	**≥85 (N=1)**
At least 1 AE	85 (83.3)	41 (91.1)	7 (100.0)	1 (100.0)
At least 1 SAE	19 (18.6)	18 (40.0)	3 (42.9)	1 (100.0)
Death	1 (1.0)	1 (2.2)	0	1 (100.0)
Inpatient hospitalization or prolongation of existing hospitalization	15 (14.7)	17 (37.8)	3 (42.9)	1 (100.0)
Life threatening event	5 (4.9)	2 (4.4)	0	1 (100.0)
Persistent or significant disability/incapacity	0	1 (2.2)	0	1 (100.0)
Important medical event	7 (6.9)	8 (17.8)	0	1 (100.0)
AEs leading to stopping study participation	1 (1.0)	2 (4.4)	0	1 (100.0)
Psychiatric disorders SOC	15 (14.7)	6 (13.3)	0	0
Nervous system disorders SOC	38 (37.3)	16 (35.6)	3 (42.9)	0
Accidents and injuries SMQ	38 (37.3)	18 (40.0)	4 (57.1)	1 (100.0)
Cardiac disorders SOC	23 (22.5)	12 (26.7)	3 (42.9)	0
Vascular disorders SOC	12 (11.8)	6 (13.3)	2 (28.6)	0
Cerebrovascular and spinal vascular disorders HLT	0	0	0	0
Infections and infestations SOC	46 (45.1)	19 (42.2)	6 (85.7)	1 (100.0)
Anticholinergic syndrome PT	0	0	0	0
Quality of life decreased PT	0	0	0	0
Sum of postural hypotension PT, fall PT, black outs (loss of consciousness PT), syncope PT, dizziness PT, ataxia PT, and fractures (bone and joint injuries HLGT)	30 (29.4)	16 (35.6)	5 (71.4)	1 (100.0)
AE appearing more frequently in older patients (ie, ≥10% greater incidence for patients ≥65 years of age vs <65 years of age)				
Fall	13 (12.7)	10 (22.2)	3 (42.9)	0
Oedema peripheral	7 (6.9)	8 (17.8)	2 (28.6)	0
Contusion	0	3 (6.7)	2 (28.6)	0

AE=adverse event; HLGT=high-level group term; HLT=high-level term; PT=preferred term; SMQ=standardized MedDRA queries; SAE=serious adverse event; SOC=system organ class.
Source: Study 002 CSR2 Tables 14.3.1.20, M.1.5.1, M.1.5.2, M.1.5.3, M.1.5.4, and M.1.5.5.

2.6.8.7. Immunological events

Antidrug antibody (ADA) assessments

As of the CSR2 (26-AUG-2021), during the HELIOS-A Treatment Period, the incidence of treatment-emergent ADA in the vutrisiran group was 3.3% (4 of 121 patients). Treatment-emergent ADA were low-titer (50) and generally transient. One subject had tested positive for ADA at baseline, remained positive at each post-baseline assessment through data cut-off with ADA titres between 50 to 100. PK concentrations for vutrisiran and TTR levels for the 4 (3.3%) vutrisiran-treated patients who tested positive for ADA were comparable at any time during the study with those of subjects with negative ADA status. One additional patient had a treatment-emergent ADA in the Extension Period that was low titer (50) and the subject tested negative at the next visit. Apparently, no patients who switched from patisiran during the Treatment Period to vutrisiran in the Extension Period reported a treatment-emergent ADA.

There was no pattern of AEs in patients with positive ADA status to suggest an impact of ADA on the safety profile of vutrisiran. There was no evidence of an association of ADA and anaphylactic reactions, or severe hypersensitivity.

In the phase I (single-dose) study, transient low ADA occurred in 1 out of 60 (1.9%) vutrisiran subjects; there was no impact of ADAs on the subject's PD response or PK profile.

2.6.8.8. Safety related to drug-drug interactions and other interactions

It is anticipated that drug-drug interactions are unlikely to occur with vutrisiran. Therefore, no formal studies have been conducted by the Applicant, and such studies are considered unnecessary.

2.6.8.9. Discontinuation due to adverse events

As of 26 August 2021, according to CSR2, three (2.5%) vutrisiran and three (7.1%) patisiran subjects had AEs leading to treatment discontinuation during the HELIOS-A Treatment Period compared to 12 (15.6%) placebo subject in the APOLLO study.

AEs leading to study drug discontinuation occurred in four (3%) subjects in the all vutrisiran population (Treatment + Extension Period) and concerned the three death cases during vutrisiran treatment as well as one case of acute cardiac failure (non-fatal) which occurred during the HELIOS-A Treatment Period (between original and SUR#1 data cut-off); none of these cases was considered treatment related.

In the all vutrisiran population, two (1.3%) subjects had an AE that led to interruption of study drug. These concerned the related SAE of transaminases increased and one fatal COVID-19 pneumonia.

2.6.8.10. Post marketing experience

N/A

2.6.9. Discussion on clinical safety

Overall, 155 patients with hATTR amyloidosis with polyneuropathy were exposed with vutrisiran in the clinical development program (i.e. in the single pivotal, open-label, active controlled HELIOS-A study) with a cumulative exposure of 233.0 person years as of the latest safety cut-off (26-AUG-2021). Of these, 118 patients have been exposed for ⩾ 18 months and 5 subjects for ≥ 27 months. Taking into

consideration that hATTR amyloidosis is an orphan disease and that vutrisiran is a second-generation siRNA-GalNAc conjugate, this could be acceptable for an orphan drug; however, long-term data above two years are still limited. In line with the proposed posology, 25 mg vutrisiran was injected s.c. every three months during the Helios-A Treatment Period. With protocol amendment 4.0 of the Helios-A study, subjects, who had already entered or who were entering the Extension Period, were randomised to receive either 25 mg vutrisiran s.c. q3M or 50 mg vutrisiran s.c. q6M. The safety analyses for the Extension Period include both dose groups without an evaluation on safety by dose. However, as only the 25 mg dose is proposed for approval, this is not further pursued.

HELIOS-A included a small (N=42) within-study patisiran reference comparator group in order to establish similar TTR reduction with vutrisiran and patisiran, as well as to enable a descriptive efficacy comparison with patisiran, however, the main safety comparison of the HELIOS-A vutrisiran group was made to the external APOLLO placebo group. This latter safety comparison is considered informative in order to distinguish disease related from drug related adverse events. However, although both studies were sponsored by Alnylam Pharmaceuticals and the HELIOS-A study was designed in order to be highly similar to the APOLLO study, there are nevertheless some baseline differences regarding the APOLLO placebo compared to the HELIOS-A vutrisiran population which limit comparability of results. These differences pertain mainly to the APOLLO placebo population appearing rather consistently somewhat more severely affected by the hATTR amyloidosis including the degree of cardiac involvement. Further differences across both studies relate to blinded treatment in APOLLO vs. open label treatment in HELIOS-A and to the premedication also received by APOLLO placebo subjects but not by vutrisiran subjects in HELIOS-A. Further, duration of drug exposure differed between studies, nevertheless, it was longer in HELIOS-A compared to APOLLO placebo as of the latest safety data cut-off provided. Due to these limitations, the within-study comparison of vutrisiran safety with patisiran is also considered relevant. As the HELIOS-A patisiran group was rather small, the Applicant has further provided a comprehensive analysis of the safety profile of the HELIOS-A vutrisiran group compared to that of the pooled (HELIOS-A and APOLLO) patisiran group as requested (using the completed 18-Months Helios-A Treatment Period).

As only interim data of the pivotal study had been presented with the original submission (with approx. 14 months of HELIOS-A Treatment Period completed as of the Safety Update Report SUR#1, with a cut-off date of 09-APR-2021) the Applicant has presented updated safety data over the course of the assessment with the completed 18-Months Helios-A Treatment Period and the ongoing Extension Period as of the current data-cut-off date of 26-AUG-2021 (in the Clinical Study Report 2, CSR2).

As of CSR2, the vast majority (>97%) of subjects across all treatment groups (HELIOS-A vutrisiran/patisirian as well as APOLLO placebo) had reported at least one TEAE. The incidence of SAEs considered related was higher in the vutrisiran compared to APOLLO placebo group (1.6% vs. 0%), nevertheless the incidence was low and also lower than in the HELIOS-A patisiran group (11.9%). Apart from this, the overview of safety (including the incidence of ADRs, severe AEs, SAEs, AEs leading to treatment discontinuation and to stopping study participation, respectively as well as the incidence of death cases) in the HELIOS-A vutrisiran group compared relatively favourably to both, the APOLLO placebo as well as the HELIOS-A patisiran group. None of the five death cases that occurred in vutrisiran treated subjects in the clinical development program, as of 19 October 2021, was considered treatment related.

The majority of TEAEs reported in the HELIOS-A study was consistent with common symptoms of hATTR amyloidosis with polyneuropathy. A decrease in serum levels of vitamin A is an expected secondary pharmacodynamic effect of reducing serum TTR protein and occurred in both HELIOS-A treatment groups. A warning regarding decreased vitamin A levels is proposed for section 4.4 of the SmPC, but no respective adverse drug reaction (ADR) is given in SmPC section 4.8, in line with the respective PI of patisiran and inotersen. This is endorsed, provided no clear vitamin A related ADR will

be identified. Subjects participating in the APOLLO study were generally supplemented with 2500 IU vitamin A, which is recommended accordingly in the patisiran SmPC. In contrast, daily substitution with 3000 IU vitamin A is recommended in the inotersen SmPC. According to the Applicant's clarification, subjects in the HELIOS-A study were supplemented with a dose of 2500 IU to 3000 IU of vitamin A. In line with this, the recommended daily vitamin A dose has been amended (from 2500 IU) to 2500 IU – 3000 IU in sections 4.2 and 4.4 of the vutrisiran SmPC.

Ocular involvement of hATTR amyloidosis is frequent particular with longer disease duration, the most frequent ocular disorders being dry eye syndrome (nearly 70%), amyloid deposition on the iris (38%) or on the anterior capsule of the lens (33%), pupillary disorders (as scalloped iris in about 28%), glaucoma (20%) and vitreous opacity [Luigetti M et al., 2020]. On the other hand, vitamin A deficiency is potentially related to ocular symptoms such as reduced night vision/night blindness, persistent dry eyes, eye inflammation and corneal inflammation/ulceration/thickening/perforation, respectively. However, in nonclinical studies, the reduction of serum vitamin A after chronic administration of vutrisiran had no effects on electroretinograms, ophthalmology examinations, and histopathology of the eye. In the inotersen and patisiran clinical study program, no negative findings, such as ocular toxicity, related to lower serum vitamin A levels have been identified. As further the reduction of TTR levels as well as vitamin A levels were generally comparable with vutrisiran compared to patisiran ocular toxicity would therefore not be anticipated for vutrisiran.

As of the CSR2 cut-off date, the incidence of TEAEs in the Eye disorders SOC in the HELIOS-A vutrisiran group was higher compared to the HELIOS-A patisiran group (28.7% vs. 23.8%) but similar compared to the incidence in the APOLLO placebo group (26.0%). The majority of ocular AEs by PT in the vutrisiran group was consistent with the underlying disease and age of subjects, respectively. However, as there is overlap in hATTR amyloidosis and vitamin A deficiency related ocular symptoms, interpretation of the available data is difficult. AEs in the Eye disorders SOC were considered treatment related in 5.7% vutrisiran but no patisiran subjects. The latter may be owed to the open-label study design and the fact that ocular toxicity has not been identified with patisiran. It is considered reassuring, that the incidence of treatment related AEs in the Eye disorders SOC in the HELIOS-A vutrisiran group was similar to that of the APOLLO placebo group (6.5%). Regarding related ocular AEs in the vutrisiran group (i.e. dry eye in three (2.5%) subjects, scleral discolouration in two (1.6%) subjects, and night blindness and vision blurred, respectively in one (0.8%) subject each), the ophthalmological evaluation of the only subject with an AE of night blindness did not report findings suggestive of vitamin A deficiency. Transient AEs of scleral discolouration occurred already within the first days of treatment in both vutrisiran subjects concerned, which also makes a causal relationship with vitamin A deficiency implausible. Dry eye considered related by investigator occurred in 2.5% subjects in the vutrisiran and with a similar incidence in the APOLLO placebo group (1.3%); of note, TEAEs of dry eye independent of causality occurred with a similar incidence in the HELIOS-A vutrisiran (4.1%) and patisiran group (4.8%) and dry eye syndrome is described in literature as the most frequent ocular disorder in hATTR amyloidosis. Apparently none of the related AEs in the Eye disorders SOC led to study drug discontinuation, none was serious. Although a numerical imbalance with regard to (related as well as overall) TEAEs in the Eye disorders SOC was found within the HELIOS-A study in the vutrisiran compared to the patisiran group, taking the totality of data into consideration, including pre-clinical and pharmacological findings for patisiran and vutrisiran as well as the respective comparison with the APOLLO placebo group and the detailed analysis of the related ocular AEs, no clear safety signal with regard to ocular toxicity due to vitamin A deficiency is identified.

ADR of vutrisiran were mainly identified based on the most frequently reported TEAEs in vutrisiran treated patients in HELIOS-A accounting for commonly reported AEs in the background disease population, i.e. compared to APOLLO placebo subjects. Additionally temporal relationship and biologic plausibility were considered and AEs were analysed by PT and grouping by medical concept,

respectively. Using a frequency criterion of an incidence >5% of vutrisiran-treated patients in HELIOS-A and >3% higher frequency compared to APOLLO placebo, the following ADRs were identified by the Applicant: arthralgia (reported in 10.7% of vutrisiran vs. 0 placebo subjects), dyspnoea (including the PTs dyspnoea, dyspnoea exertional, and dyspnoea paroxysmal nocturnal; reported in 6.6% vutrisiran vs. no placebo subjects), and pain in extremity (reported in 14.8% vutrisiran-treated subjects vs. 10.4 % placebo subjects). As of the CSR2, one subject had a severe AE of arthralgia, apart from this, AEs of arthralgia and dyspnoea, respectively were mild or moderate, no event was serious, no AE of arthralgia or dyspnoea led to treatment discontinuation, or increased over time. Arthralgia and dyspnoea are also commonly associated with patisiran. Similarly, events of pain in extremity were mild or moderate, no event was serious or led to treatment discontinuation, and no clear pattern in time to onset of pain in extremity was recognizable with regard to start of vutrisiran or since the last vutrisiran dose.

Based on the administration mode of vutrisiran, injection site reactions (ISRs) were also regarded ADRs and were reported in 4.1% vutrisiran subjects during the Helios-A Treatment Period and no APOLLO placebo subjects. ISRs included the symptoms bruising, erythema, pain, pruritus, and warmth and the respective symptoms are included in the description of ISRs in section 4.8 of the SmPC. One further subject experienced a single ISR during the HELIOS-A study Extension Period, with the PT of traumatic haematoma, apparently reported as moderate. In the all vutrisiran population, five mild and one moderate ISRs occurred, no event led to treatment discontinuation and no subject had more than one event. There was no increase in ISRs over time and the incidence of ISRs was low, irrespective of presentation (i.e. vials or pre-filled syringes). As of the CSR2, the incidence of antidrug antibody (ADA) formation in vutrisiran subjects was low, i.e. 4.1% (5/121 subjects) in the all-vutrisiran population of the HELIOS-A Treatment + Extension Period, and 1.9% (1/60) in healthy volunteers in study 001. Titres were low and generally transient. One subject had tested positive for ADA at baseline, remained positive at each post-baseline assessment (through data cut-off) with ADA titres between 50 to 100. No impact of ADA on PD activity of vutrisiran or on the safety profile of vutrisiran was identified. It is noted, that a systemic reaction that includes the injection site was not considered an ISR. The comparative incidences of TEAEs mapping to the broad Hypersensitivity SMQ, as well as the presented analyses by relatedness and severity, respectively, do not raise serious concerns. In two cases with the PT of "drug hypersensitivity" other medicinal products were suspected. The severe related AEs reported on Day 1 in one subject, which included dizziness, dyspepsia, hyperhidrosis and hyperthermia, do not correspond to anaphylaxis; however it could not be excluded, that these events were caused by other non-IGE-mediated or non-immunologic drug hypersensitivity reactions. Apart from this case, no clear safety signal arose with regard to hypersensitivity. Therefore, it can be agreed with the Applicant, that at present no specific conditions for vutrisiran home administration are required. It should be noted, however, that according the SmPC, Amvuttra should be administered by a healthcare professional in any case. Nevertheless, taking into consideration, that the overall vutrisiran exposure at the time of the safety data cut-off is limited to 155 subjects, drug related hypersensitivity should be further evaluated.

The mechanism of action, available pre-clinical data, ECG data derived from the Phase I vutrisiran study performed in healthy volunteers, and the known safety profile of approved siRNAs, respectively are not indicative of a cardiac safety concern with regard to vutrisiran. However, vutrisiran has the same sequence but different modifications than revusiran, the development of which in hATTR amyloidosis with cardiac predominant phenotype was discontinued due to an increase of mortality in the revusiran arm compared to placebo, though not compared to the range expected from natural history. A revusiran-related effect on mortality could not be excluded. Therefore, and as patients with hATTR amyloidosis often have cardiac involvement, cardiac safety of vutrisiran was thoroughly evaluated in the overall safety population as well as the cardiac subpopulation. In the HELIOS-A study, in general, the type of cardiac AEs in vutrisiran-treated patients was consistent with those previously reported in patients with hATTR amyloidosis, based on findings in the APOLLO placebo group. Also, the

comparison of incidences and event rates, respectively of the evaluated cardiac events in HELIOS-A vutrisiran compared to APOLLO placebo does not raise concerns (as these appeared consistently numerically lower in the HELIOS-A vutrisiran group). No cardiac AE in the all vutrisiran HELIOS-A study population was considered related to study drug, and only one cardiac AE, although not considered related, led to study drug withdrawal.

During the 18-Months HELIOS-A Treatment Period, the incidence of TEAEs in the Cardiac disorders SOC was 30.3% in the vutrisiran and 23.8% in the patisiran group. In particular, the incidence of cardiac arrhythmia HLGT AEs was approx. three times higher in HELIOS-A vutrisiran compared to HELIOS-A patisiran subjects (26.6% vs. 7.1%). However, no cardiac safety concerns derived from ECG evaluations. In the overall safety population the number of relevant shifts of QTcF was low and consistent with the underlying disease and in both, the overall safety population and the cardiac subpopulation, no imbalance between the treatment groups to the disadvantage of vutrisiran was seen in the number of relevant shifts of QTcF during the Helios-A Treatment Period. Patisiran is not associated with a cardiac safety concern, the observed shifts in QTcF appear therefore consistent with the underlying disease, i.e. hATTR amyloidosis with cardiac involvement. The overall rate of Helios-A vutrisiran subjects with clinically significant ECG changes from baseline was low for a hATTR amyloidosis population (i.e. $\leqslant$ 3.5% at different time points throughout Month 18 as of the CSR2), and the finding of no respective changes in the Helios-A patisiran group could be a chance finding due to the low sample size. In contrast to the imbalance regarding cardiac arrhythmias, TEAEs in the cardiac failure SMQ (narrow) occurred with a considerably lower frequency in the Helios-A vutrisiran (5.7%) compared to the Helios-A patisiran group (19.0%). It is further reassuring, that SAEs in the cardiac disorders SOC were reported with a lower incidence in the Helios-A vutrisiran vs. Apollo placebo as well as the Helios-A patisiran group (9.0% vs. 13.0% and 14.3%, respectively).

In the cardiac subpopulation but not in the overall safety population, a somewhat higher incidence of syncope was found in HELIOS-A vutrisiran (15.0%) compared to both, HELIOS-A patisiran (7.1%) and APOLLO placebo (8.3%) subjects. The Applicant has evaluated all six cases of syncope of the Helios-A vutrisiran cardiac subpopulation: no event was considered related by the Investigator, all concerned isolated events with no apparent pattern in temporal relationship to study drug administration or the total number of doses applied until the event, respectively. In four of the six subjects concerned, a medical history of (orthostatic) hypotension or loss of consciousness was reported. Up to now no subject experienced a further TEAE of syncope during subsequent injections. Based on the provided data regarding syncope (including the fact, that syncope occurred generally with a similar incidence in the overall safety population of the Apollo placebo vs. Helios-A patisiran group) a causal relationship with vutrisiran appears rather unlikely. Apart from syncope, the evaluation of cardiac events in the cardiac subpopulation (while limited particularly due to the low number of subjects in the HELIOS-A patisiran cardiac subgroup) appears to be generally in line with that of the overall safety population. It is therefore generally agreed with the Applicant, that both findings, the imbalance in TEAEs in the Cardiac arrhythmia HLGT within the Helios-A study (but not compared to the Apollo placebo group) as well as the higher incidence of syncope in the cardiac subpopulation in the Helios-A vutrisiran vs. both, the Helios-A patisiran and the Apollo placebo group, could be chance findings, due to the low subject numbers of the Helios-A patisiran and the cardiac subpopulation, respectively.

Taken altogether, the currently available cardiac findings with vutrisiran do not raise serious cardiac safety concerns.

Compared to the Apollo placebo group and the Helios-A patisiran group, the incidences of TEAEs in the drug-related hepatic disorders SMQ during the Helios-A Treatment given for the vutrisiran group do not raise concerns. Four subjects in the all vutrisiran group had hepatic AEs that were considered treatment related. In three of these subjects, no event was serious or severe, no action was taken with study drug and all related hepatic AEs resolved despite continued treatment or increased LFT

parameters returned to near baseline, respectively. Confounding factors were present in two of these cases. However, in the fourth case a related severe hepatic SAE of transaminases increased (ALT >6xULN, AST >5xULN) was reported 42 Days after the first dose of 50 mg vutrisiran s.c.. After the second vutrisiran injection, a milder transaminase increase occurred, peaking approx. four weeks afterwards at ALT of 2.4xULN and AST of 2.3xULN. In both instances transaminase levels returned to near normal or normal, respectively, no specific treatment or hospitalisation was required during either event, and the subject is ongoing in the study. Concomitant medication that was reportedly ongoing at the time of SAE onset is associated with liver function test disorders, and a non-alcoholic fatty liver disease was reported within two weeks of the SAE. Nevertheless, the considerable increase of transaminases from mostly normal elevated levels after the first dose of vutrisiran as well as a positive re-challenge point towards a causal relationship with vutrisiran. Of note, the subject received a different posology than that currently recommended, i.e. twice the recommended single dose administered with a longer treatment interval (of 6 months). The Sponsor validated a signal of transaminase elevations which is currently ongoing and has stated to continue careful monitoring the reports of transaminase elevations. Further evaluation of events of transaminases increased is indicated; however, no labelling changes is considered necessary at present. In subject (moderate) transaminases increased and severe drug-induced liver injury were not considered related to vutrisiran but attributed to the subject's vitamin A supplement. Transaminase increase started after the sixth vutrisiran administration and was reported as AE after the eight dose. Transaminase levels improved to <2 ULN AST and <3 ULN ALT (at the last available measurement) after vitamin A supplement taken at an unknown dose was withdrawn, while vutrisiran administration was continued unchanged. The dose of vitamin A supplementation of 2500-3000 U recommended in the SmPC of vutrisiran is considered established and well below toxic levels. In order to ensure, that patients do not take too high doses of vitamin A, e.g. in the attempt to counteract vitamin A lowering, the PL was be amended in order to emphasize, that the daily Vitamin A dose should be taken as recommended by the treating physician.

Alkaline phosphatase (ALP) increases (> 1.5 ULN) during the HELIOS-A Treatment Period were reported in 11 (9.0%) vutrisiran vs. 1 (2.4%) patisiran subjects (as of the CSR2) and in one (1.3%) Apollo placebo subject. The requested evaluation showed a low incidence of ALP increase >1.5 ULN without alternative explanations, with only one event being considered a TEAE by investigator. Further, ALP increase was not associated with clinically significant transaminase or bilirubin elevations. The observations of ALP increase >1.5 ULN are thus not of clear clinical relevance. Nevertheless, in order to inform the treating physicians and patients, respectively, ALP increased has been added to the PI as an ADR within the Investigations SOC based on the higher incidence in the Helios-A vutrisiran compared to the patisiran but also the Apollo placebo group. There appears to be a rather steady increase in alkaline phosphatase (ALP) over time, which by the way appears to develop generally similarly in the Helios-A patisiran group, with a mean absolute change (SD) from baseline of 22.19 (35.38) U/L in the vutrisiran and of 23.82 (18.17) U/L in the patisiran group at day 673, constituting a mean relative change of 36.71% for vutrisiran and 32.24% for patisiran, respectively. Yet, the mean ALP values measured still remained within the normal reference values. Of note, no corresponding increase in ALP was found in the patisiran group of the Apollo study. As discussed in the non-clinical part of this Report, ALP elevations might be possibly explained by a slower ALP clearance from the circulation because of its competition with vutrisiran in binding to hepatic ASGPR. The clinical relevance of this seemingly continuous ALP increase is currently unclear and should be further evaluated.

Arthralgia and pain in extremity (with very common frequency each) as well as dyspnoea, blood alkaline phosphatase increased and ISR (with common frequency each) are the only ADR identified and listed in SmPC section 4.8. Further discussion regarding noticeable findings from the evaluation of common TEAEs/ADRs/severe AEs led to the following conclusions (incidences are updated as of the CSR2):

Although the TEAE of abdominal pain was reported with a higher incidence in the HELIOS-A vutrisiran group (9.0%) vs. the APOLLO placebo group (1.3%) and the HELIOS-A patisiran group (2.4%), and dyspepsia considered related by the investigator was reported in 1.6% subjects in the HELIOS-A vutrisiran vs. no subjects in the APOLLO placebo or HELIOS-A patisiran group, respectively, the provided justification for not including abdominal pain and dyspepsia, respectively in section 4.8 of the SmPC can be accepted. The frequency of TEAEs within the abdominal pain medical concept was very similar in the Helios-A vutrisiran vs. the Apollo placebo group, no event was considered treatment related, and the severity of respective events was low. The frequency of dyspepsia TEAEs (irrespective of causality) was numerically lower in the vutrisiran vs. the Apollo placebo as well as the pooled patisiran group. Both vutrisiran subjects with dyspepsia considered related to study drug by the investigator continued vutrisiran without recurrence of dyspepsia.

As influenza like illness and rash could potentially have also been symptoms of a systemic hypersensitivity reaction, or, in the case of rash, also of an injection site reaction, further evaluation was requested. During the 18 Months Treatment Period, influenza like illness occurred at a similar frequency, the respective incidences have been clarified by the Applicant to be 4.1% in the Helios-A vutrisiran group vs. 2.6% in the APOLLO placebo group. Although a causal relationship of influenza like illness with vutrisiran e.g. in the context of drug hypersensitivity cannot be fully excluded for all subjects, the incidence was low and the currently available data do not justify inclusion of influenza like illness in the SmPC as an ADR. Overall, in the Helios-A vutrisiran group (Treatment Period) compared to the APOLLO placebo group the incidence of TEAEs of rash by PT (5.7% vs. 3.9%) or TEAEs mapped to the rash custom query (7.4% vs. 9.1%) was similar. Within the rash medical concept grouping, none of the events in the vutrisiran group was considered an injection site reaction, two events were considered treatment related by the investigator (one of which had the PT of rash) as were three events (in three subjects) in the APOLLO placebo group. In three of the nine vutrisiran subjects with TEAEs in the rash medical concept grouping, the events occurred within a week of the previous dose. However, these latter events were all non-serious, vutrisiran was continued and no additional events of rash were reported in these subjects. It is therefore agreed, that labelling of rash as an ADR is currently not warranted. With regard to muscle spasm and sinusitis, respectively, which are both labelled for patisiran, it can be agreed that the presented data do not provide evidence to conclude a class effect for TTR-lowering RNAis. In particular, none of the events of sinusitis or within the medical concept of muscle spasm was considered treatment related and incidences of respective TEAEs were quite low.

Based on the information regarding subject provided with the Response to the CHMP Day 120 LoQ (including a medical history of cardiac amyloidosis, cardiac failure and peripheral oedema, respectively as well as the time course of onset and increase of peripheral oedema), the event of severe peripheral oedema, which was considered related by the investigator, is not indicative of a hypersensitivity reaction and causality with vutrisiran is uncertain. Comprehensive data provided as of the CSR2 data cut-off (26-AUG-2021) showed, that the safety profile of vutrisiran was generally consistent across the analysed subgroups by age, sex, race, weight, genotype (V30M or non-V30M mutation), FAP stage and geographic region, respectively.

Based on the mechanism of action and non-clinical studies, in which there was no evidence of genotoxic potential, the risk of vutrisiran for carcinogenicity in humans is considered low. While no malignancies were reported in the HELIOS-A patisiran group, the incidence of malignancies in the HELIOS-A vutrisiran group (4.1%) did not exceed that of the APOLLO placebo group (5.2%). It is noted, that the mean age was approx. 5 years younger in the HELIOS-A vutrisiran group compared to the APOLLO placebo group (57.8 vs. 62.2 years). However, the malignancies reported with vutrisiran do not demonstrate a striking pattern and the type and incidence appear consistent with that reported in the general population in this age group.

Overall, vutrisiran is therefore not considered to be associated with an increased cancer risk.

No safety signal derived from evaluation of renal adverse events, renal function parameters or urinalysis, respectively. No clear safety signal is raised from the evaluation of AEs within the Depression and suicide/self-injury SMQ and analyses of Columbia-Suicide Severity Rating Scale, respectively.

2.6.10. Conclusions on the clinical safety

Overall, 155 patients with hATTR amyloidosis with polyneuropathy were exposed to vutrisiran in the clinical development program with a cumulative exposure of 233.0 person years as of the latest safety cut-off (26-AUG-2021). Of these, 118 patients have been exposed for ⩾ 18 months. Taking into consideration that hATTR amyloidosis is an orphan disease and that vutrisiran is a second-generation siRNA-GalNAc conjugate, this could be acceptable for an orphan drug, however, long-term data above 2 years are still limited.

After completion of the Helios-A Treatment period and with the Extension Period ongoing (as of the 26-AUG-2021) the only ADRs identified are arthralgia and pain in extremity with very common frequency each, as well as dyspnoea, blood alkaline phosphatase increased and injection site reactions (ISRs), with common frequency each. IRSs occurred in 5 (4.1%) vutrisiran subjects during the Helios-A Treatment Period and in one further subject during the Extension Period and none of the overall six subjects concerned experienced more than one event. Five ISRs were mild one event was moderate, no ISR led to treatment discontinuation, despite no administration of premedication. Vitamin A level reduction with vutrisiran in the proposed posology is comparable to patisiran, and as with patisiran no clear ocular toxicity due to vitamin A deficiency toxicity was identified. The identified ADRs have been appropriately implemented in the Summary of Product Characteristics.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 41: Summary table of safety concerns

Summary table of safety concerns	
Important identified risks	• None
Important potential risk	• Clinical consequences of vitamin A deficiency, including delayed symptoms • Hypersensitivity reactions
Missing information	• Longer-term safety (>2 years) • Use in patients with moderate or severe hepatic impairment • Use in pregnant women and effects on pregnancy outcomes

2.7.2. Pharmacovigilance plan

Table 42: Summary table of Ongoing and Planned Additional Pharmacovigilance Activities in the PV plan

Study Number **Short Name** **Status**	**Summary of Objectives**	**Safety Concerns Addressed**	**Milestones**	**Due Dates**
Category 3 - Required additional pharmacovigilance activities				
HELIOS-A Randomised Treatment Extension The HELIOS-A-RTE[a] study is a Phase 3 global, open-label study to evaluate the safety and efficacy of ALN-TTRSC02 in patients with hATTR Amyloidosis. (Ongoing)	The aim of the study is to collect further longer-term safety and efficacy data on vutrisiran in patients with hATTR amyloidosis with polyneuropathy.	• Longer-term safety (>2 years)[a]	Final CSR (planned)	2025
ALN-TTR02-013 ConTTRibute Study: A global prospective observational multicenter long-term study of patients with hATTR amyloidosis (Ongoing; protocol to be amended to include vutrisiran)	The primary objective of this study is to document the natural history, clinical characteristics, and management of ATTR amyloidosis as part of routine clinical care. This study is enrolling hATTR and wtATTR amyloidosis patients and was initiated in November 2020. Long-term safety data for vutrisiran will be collected as part of ConTTRibute.	• Clinical consequences of vitamin A deficiency, including delayed symptoms • Longer term safety (>2 years) • Use in patients with moderate or severe hepatic impairment • Use in pregnancy and pregnancy and infant outcomes	Protocol Concept	Protocol Concept Sheet provided in RMP Annex 3 Submission of amended protocol to include vutrisiran: within 3 months of EC Decision.
			Final Protocol	Final protocol submission date: within 3 months of EC Decision *(e.g. December 2022)*
			Start of data collection	November 2020
			Start of vutrisiran data collection	As soon as amended protocol is agreed by regulatory authorities
			Interim updates	Interim analyses will be provided annually via PAM procedure.
			Final Report	Final study report: 2034

Abbreviations: CSR=clinical study report; EC=European Commission; hATTR amyloidosis=hereditary transthyretin-mediated amyloidosis; PAM=post-authorisation measure; RTE=Randomised Treatment Extension; TBD=to be determined.

[a] HELIOS-A Protocol Amendment 3, in place at the time of database lock for this document, included an 18-month extension period (for a total study duration of 36 months); as of 19 February 2021, Protocol Amendment 4 lengthened the extension period to a maximum of 36 months (for a total study duration of up to 54 months).

Of note, a targeted follow-up questionnaire will be implemented for reports of vitamin A deficiency/ocular toxicity in order to collect additional information on these events; these data will be analysed and presented in the Periodic Safety Update Reports (PSURs) to further characterise this risk.

2.7.3. Risk minimisation measures

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Important Potential Risk:		
Clinical consequences of vitamin A deficiency, including delayed symptoms	Routine risk minimisation measures: • The secondary pharmacologic effect on serum vitamin A levels is described in SmPC sections 4.4, 4.5, 5.1, and 5.3, and PIL Section 2. • Legal status: Prescription-only Additional risk minimisation measures: • None	Routine PV activities beyond adverse reactions reporting and signal detection: • Specific targeted follow-up of vitamin A deficiency/ocular toxicity Additional PV activities: • Evaluation of data from the HELIOS-A Randomised Treatment Extension (HELIOS-A RTE) • Evaluation of data from the ConTTRibute Study
Hypersensitivity Reactions	Routine risk minimisation measures: • SmPC Section 4.3 and PIL Section 2 • Legal status: Prescription-only Additional risk minimisation measures: None	Routine PV activities beyond adverse reactions reporting and signal detection: • None Additional PV activities: • Evaluation of data from the HELIOS-A Randomised Treatment Extension (HELIOS-A RTE) • Evaluation of data from the ConTTRibute Study
Missing Information:		
Longer-term safety (>2 years)	Routine risk minimisation measures: SmPC Section 4.8- Additional risk minimisation measures: • None	Routine PV activities beyond adverse reactions reporting and signal detection: • None Additional PV activities: • Evaluation of data from the HELIOS-A Randomised Treatment Extension (HELIOS-A RTE) • Evaluation of data from the ConTTRibute Study
Use in patients with moderate or severe hepatic impairment	Routine risk minimisation measures: • SmPC sections 4.2 and 5.2 Additional risk minimisation measures: • None	Routine PV activities beyond adverse reactions reporting and signal detection: • None Additional PV activities: • Evaluation of data from the ConTTRibute Study
Use in pregnant women and effects on pregnancy outcomes	Routine risk minimisation measures:	Routine PV activities beyond adverse reactions reporting and signal detection:

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
	• SmPC sections 4.4, 4.6, and 5.3, and PIL Section 2- Additional risk minimisation measures: • None	• None Additional PV activities: • Evaluation of data from the ConTTRibute Study

Abbreviations: PIL=Patient Information Leaflet; PV=Pharmacovigilance; RTE=Randomised Treatment Extension; SmPC=Summary of Product Characteristics.

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. 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.

2.8.2. 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 13.06.2022. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Non-Conformity of paediatric studies

Not applicable

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, Amvuttra (vutrisiran) 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

Hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis), also known as variant transthyretin-mediated amyloidosis, is a rare, autosomal dominant, rapidly progressive, multi systemic disease caused by variants in the transthyretin (TTR) gene that results in debilitating morbidity and high mortality. Amyloid deposits accumulate in multiple organs, particularly the peripheral nervous system, gastrointestinal tract, kidney, and heart, which manifests in progressive polyneuropathy including sensorimotor neuropathy and autonomic neuropathy. Cardiomyopathy, nephropathy, and gastrointestinal dysfunction frequently develop simultaneously. The phenotypic presentation of the disease is dependent on the pattern of affected organs. The most common manifestations of hATTR amyloidosis are polyneuropathy and cardiomyopathy.

The worldwide prevalence of hATTR-PN has been estimated at approximately 10,000 patients. In Europe, the incidence is estimated from 0.003 to 0.10 cases per 10,000 per year (between 5000 to 6000 patients or 0.3 new cases per year per 1 million inhabitants), with the majority of cases in Portugal, France, Italy, and the United Kingdom. In Europe, the prevalence is highest in northern Portugal and northern Sweden (as high as 50 per 100,000 inhabitants).

There are over 120 reported TTR genetic variants associated with hATTR amyloidosis with heterogeneity in disease presentation from predominantly neuropathic, predominantly cardiac or mixed phenotypes. Worldwide, the most common disease-causing variant results in a valine to methionine mutation at position 30 in the TTR molecule, V30M (p. TTRV50M). V30M is predominantly associated with polyneuropathy and is found primarily in families with heritage from Portugal, Sweden, Japan, and Brazil. In the US, the isoleucine substitution for valine at position 122 in TTR, V122I (pV142I), is the most prevalent TTR associated variant with a prevalence of approximately 4% in West Africans and African Americans. V122I is associated with predominantly cardiac manifestations but also can be associated with concurrent polyneuropathy.

3.1.2. Available therapies and unmet medical need

Before the approval of inotersen and patisiran, therapeutic strategies to treat hATTR included orthotopic liver transplant (OLT) or pharmacotherapy with tafamidis or off-label use of diflunisal, both of which are TTR stabilizers that work by preventing dissociation of the tetramer into amyloid-forming monomers.

Tafamidis ("Vyndaqel"; EMEA/H/C/2294) was approved across the EU for the treatment of ATTR in adult subjects with Stage 1 symptomatic polyneuropathy to delay peripheral neurological impairment and has also been licensed in Japan and several other countries. Diflunisal is a non-steroidal anti-inflammatory drug (NSAID) that is presently used off-label in subjects with Stage 1 and Stage 2 disease; however, the cardiovascular and renal side effects associated with the NSAID class limit the use of this drug in older patients with hATTR-PN or patients with hATTR CM.

There are currently three European Commission (EC) approved therapies available in the European Union (EU) for the treatment of hATTR amyloidosis in adults with polyneuropathy: ONPATTRO (patisiran), TEGSEDI (inotersen) and VYNDAQEL (tafamidis). Patisiran and inotersen act by targeting the production of TTR synthesis in the liver by acting on messenger RNA (mRNA): patisiran through

ribonucleic acid interference (RNAi); and inotersen through RNAse H-mediated cleavage. Tafamidis acts by binding to the thyroxine-binding site on TTR to reduce its dissociation into misfolded amyloidogenic monomers. Both ONPATTRO (patisiran) and TEGSEDI (inotersen) are also approved in the United States (US) and Japan. VYNDAQEL (tafamidis), is also approved in Japan for the treatment of polyneuropathy in adults with transthyretin amyloidosis, however it is not approved in the US for this indication.

Other treatment approaches currently used in clinical practice for hATTR amyloidosis include: orthotopic liver transplantation (OLT), which eliminates variant TTR from the circulation but does not negate the hepatic production of wtTTR, and another TTR tetramer stabilizer (diflunisal).

There are treatment options available for patients with hATTR amyloidosis with polyneuropathy. In spite of the several treatments available, there are patients who do not tolerate or have shown adverse reactions to some of the above mentioned products; therefore, a new efficacious and safety agent will be most welcome. There is still a need for improved products that address the underlying physiological basis of the disease (not stabilisers), are highly effective in improving neuropathy and delay or stop disease progression, have convenient dosing, minimize the need for health care encounters and have acceptable safety profiles without the need for intensive laboratory or clinical monitoring.

3.1.3. Main clinical studies

The pivotal trial for this application is HELIOS-A (ALN-TRSC02-002), which is an ongoing, global, Phase 3, randomized, open-label study designed to evaluate efficacy, safety, PK, and PD of vutrisiran in adult patients with hATTR amyloidosis with polyneuropathy. The primary and most secondary and exploratory endpoints of the study evaluate the efficacy of vutrisiran by comparing to the placebo group of the Phase 3 patisiran APOLLO study. The study is being conducted in 2 parts: an 18month Treatment Period, with the early efficacy analyses at Month 9 (primary for US) and additional efficacy analyses at Month 18 (primary for EU), followed by an 18-month Treatment Extension Period, in which all patients are treated with vutrisiran. The study was performed in 57 centers in 22 countries.

Patients with 18 to 85 years of age with a diagnosis of hATTR amyloidosis with documented TTR mutation, Neuropathy Impairment Score (NIS) of 5 to 130 (inclusive), Polyneuropathy Disability (PND) score of ≤3b and Karnofsky Performance Status (KPS) of ≥60% participated in HELIOS-A.

164 adult patients with hATTR amyloidosis with polyneuropathy were randomized 3:1 to receive vutrisiran 25 mg administered as a SC injection q3M or patisiran (active comparator) 0.3 mg/kg administered as an IV infusion q3w (122 vutrisiran and 42 patisiran). Randomization was stratified by TTR genotype (V30M vs. non-V30M) and baseline NIS score (<50 vs. ≥50). A small number of patients were excluded from the analysis for justified reasons: 8 in the group of vutrisiran and 5 in the group of patisiran.

Efficacy data from HELIOS-A are available at the Month 9 analysis timepoint (data cut-off date of 10 November 2020).

A direct comparison between vutrisiran and patisiran was performed only on a pharmacodynamic endpoint: the TTR % reduction from baseline.

3.2. Favourable effects

At month 9, a statistically significant improvement in neuropathy was observed for patients in the vutrisiran group compared to the placebo group with a LS mean difference of the mNIS+7 change from baseline between groups of -17.00 points. For the EU formal primary analysis of the primary endpoint

of mNIS+7 a statistically significant, large and clinically meaningful difference in the change from baseline was also observed at Month 18 between vutrisiran in HELIOS-A and placebo in APOLLO with a treatment difference, LS Mean (95% CI) of -28.55 (-34.00, -23.10).

In addition, a noticeable difference in improvement in quality of life at 9 months for patients in the vutrisiran group compared to the placebo group with a LS mean difference between groups in Norfolk QoL-DN change from baseline of -16.2 points occurred. For the key secondary endpoint Norfolk QoL-DN a statistically significant, large and clinically meaningful difference in the change from baseline at Month 18 was demonstrated between vutrisiran in HELIOS-A and placebo in APOLLO with a treatment difference, LS Mean (95% CI) of -21.0 (-27.1, -14.9).

For mNIS+7 48.3% of patients in the vutrisiran group showed an improvement of neuropathy (change from baseline mNIS+7 <0 points) at Month 18 compared to 3.9% of placebo (APOLLO) patients. For Norfolk QoL-DN, 56.8% of patients in the vutrisiran group had an improvement (<0-point LS mean change) at Month 18 compared to 10.4% of placebo patients.

Several pre-specified sensitivity analyses with no censoring, propensity score and pattern-mixture model as well as subgroup analyses have been performed, which are supportive of the beneficial effects of vutrisiran observed in the primary analyses of the primary and key secondary endpoints. Effects observed across all mNIS+7 components and all domains of Norfolk QoL-DN are in favour of vutrisiran (HELIOS-A) compared to placebo (APOLLO) and across all patient subgroups, with an effect seen across the full range of baseline neuropathy severity and across different genetic variants.

Supportive statistically significant and clinically relevant results were also obtained with 10-meter walk test (10-MWT) (with a LS mean difference between groups (95% CI): 0.239 m/s), which is a measure of ambulatory ability and gait speed.

At Month 18, a statistically significant improvement in mBMI for patients in the vutrisiran group compared to the placebo group with a LS mean difference between groups of 140.7 kg/m2 × albumin g/L was recorded, indicating an improvement in nutritional status for vutrisiran-treated patients compared to placebo. This is considered a relevant aspect in hATTR, since it expresses the improvement on dysautonomia, which can impact the patient more than the loss of sensation and partially the motor function.

Informal post hoc analysis of key efficacy parameters using MMRM for the within-study comparison of vutrisiran and patisiran groups of the HELIOS-A study at Month 18 showed very small mean differences (95% CI) between these treatment groups -1.46 (-7.36, 4.43), -1.6 (-8.6, 5.4), 0.034 (-0.064, 0.132) and 0.1 (-2.0, 2.2) in the change from baseline for mNIS+7, Norfolk QoL-DN, 10MWT and R-ODS, respectively. At Month 18 the estimated difference of vutrisiran versus patisiran in the change from baseline for mNIS+7 is -1.46 (-7.36, 4.43) leading to a very similar upper bound of 4.43 compared to Month 9 results (-0.96 [95% CI: -6.14, 4.22]). An "informal non-inferiority" margin of 4 points is considered acceptable. A relatively larger LS mean difference 14.2 (-21.9, 50.3) was observed for the change in mBMI, but this was in favour of vutrisiran.

TTR reduction at Month 9 was similar or even slightly greater with vutrisiran than with patisiran and in the order of 78-82%, which is considered a very high TTR reduction. At Month 18 the reduction in serum TTR levels with vutrisiran (84.67%) was determined to be formally non-inferior to the within-study patisiran arm (80.60%) based on the pre-specified criteria [Median Difference (95% CI) 5.28 (1.17, 9.25), non-inferiority confirmed if 95% lower CI > -10%].

Overall, the confirmatory data at month 18 are considered very relevant considering the rapidly progressive nature of the disease and show maintenance of the effect.

3.3. Uncertainties and limitations about favourable effects

The study population in HELIOS-A was limited to stage 1 and 2 patients with hATTR with polyneuropathy and patients with FAP stage 3 were not included. Extrapolation of efficacy data to stage 3 patients is not considered appropriate. Very limited data with no patients in vutrisiran who progressed to stage 3 at Month 9 and only 2 patients at Month 18 exist. These numbers are very low to justify an extrapolation of the indication to include FAP stage 3 patients with hATTR with polyneuropathy. The initially proposed indication for Amvuttra (vutrisiran) was modified to accurately reflect the population studied and has been agreed as the same as that for the approved product Onpattro (patisiran).

Analyses of the "true" mITT population, implementing two slightly different PMM methods PMM 1 (hypothetical estimand) and PMM 2 (treatment policy estimand) were provided upon request. There was little difference between the estimates of treatment effect based upon the two PMM methods or MMRM analyses for the primary, but also for the secondary endpoints. The treatment effect sizes from PMM being slightly larger than MMRM did not have any impact on the clinical interpretation of the results. Since MMRM was the prespecified analysis, the use of the MMRM results in the SmPC is considered acceptable, in order to keep all public information consistent.

The sustained effect of treatments by month 18 in the important clinical endpoints was shown with comparisons between vutrisiran and patisiran in HELIOS-A and patisiran vs. patisiran between HELIOS-A and APOLLO studies. The estimated difference of vutrisiran versus patisiran at Month 18 (-1.46 [CI: -7.36, 4.43]) was very similar to the results at Month 9 (-0.96 [95% CI: -6.14, 4.22]) in the change from baseline for mNIS+7, showing an "informal non-inferiority" margin of 4 points, which is considered acceptable. However, this should be included in the EPAR for future reference.

Regarding cardiomyopathy, vutrisiran treatment led to favourable trends for NT-proBNP and exploratory pre-specified echocardiographic parameters (such as LV wall thickness, LV mass, and increases in end diastolic volume and cardiac output) when compared to placebo (from APOLLO). Despite methodological limitations, including cross-study comparison and higher baseline NT-proBNP values in APOLLO compared to HELIOS-A, the magnitude of effect on these biomarkers/PD parameters appears similar for vutrisiran and patisiran. However, a clinically relevant cardiac benefit still needs to be confirmed.

3.4. Unfavourable effects

Based on the SC administration mode of vutrisiran, injection site reactions (ISRs) were regarded ARDs. As of the completed 18-Months HELIOS-A Treatment Period, ISRs were reported in 5 out of 122 (4.1%) vutrisiran subjects and in 5 (0.6%) of the total 836 doses of vutrisiran administered.

ISRs included the symptoms bruising, erythema, pain, pruritus, and warmth and the respective symptoms are included in the description of ISRs in section 4.8 of the SmPC. ISRs in vutrisiran treated subjects were all mild, did not lead to treatment discontinuation and no subject had more than one event. There was no increase in ISRs over time. One further subject experienced one single ISR during the Extension Period; apparently, the signs and symptoms in this subject were reported as traumatic haematoma and this ISR was reported as moderate.

Using a frequency criterion for TEAES of an incidence >5% of vutrisiran-treated patients in HELIOS-A and >3% higher frequency compared to APOLLO placebo, the following ADRs were also identified and reflected in the labelling (based on incidences reported in the completed 18-Months Treatment Period):

- Arthralgia was reported in 13 (10.7%) of HELIOS-A vutrisiran vs. no APOLLO placebo subjects. One subject had a severe AE of arthralgia, apart from this, AEs of arthralgia were mild or moderate, no

event was serious, no AE of arthralgia led to treatment discontinuation, or increased over time. Arthralgia is also commonly associated with patisiran and was reported in 4 (9.5%) HELIOS-A patisiran subjects.

- Dyspnoea; the medical concept of dyspnoea, which includes the PTs dyspnoea, dyspnoea, exertional, and dyspnoea paroxysmal nocturnal, respectively was reported in 8 (6.6%) of HELIOS-A vutrisiran vs. no APOLLO placebo subjects. AEs within the dyspnoea medical concept were mild or moderate in severity, none of the events was severe or serious, or led to treatment discontinuation, respectively. AEs of dyspnoea did not increase over time. Dyspnoea is also known to be commonly associated with patisiran.
- Pain in extremity occurred in 18 (14.8%) vutrisiran-treated subjects compared to 8 (10.4 %) APOLLO placebo subjects. These events were generally mild or moderate, no event was severe or led to treatment discontinuation. No clear pattern with regard to onset of these events in relation of vutrisiran administration was apparent.

Alkaline phosphatase (ALP) increases (> 1.5 ULN) during the HELIOS-A Treatment Period were reported in 11 (9.0%) vutrisiran vs. 1 (2.4%) patisiran subjects (as of the CSR2) and in 1 (1.3%) Apollo placebo subject. In the vutrisiran group, one event was considered a TEAE, related, by investigator. ALP increase was not associated with clinically significant transaminase or bilirubin elevations. In order to inform the treating physicians and patients, respectively, ALP increased has been added to the PI as a common ADR within the Investigations SOC based on the higher incidence in the Helios-A vutrisiran compared to the patisiran but also the Apollo placebo group.

The TEAE of abdominal pain was reported with a higher incidence in the HELIOS-A vutrisiran group (9.0%) vs. the APOLLO placebo group (1.3%) and the HELIOS-A patisiran group (2.4%), however, the frequency of TEAEs within the abdominal pain medical concept was very similar in the Helios-A vutrisiran vs. the Apollo placebo group, no event was considered treatment related, and the severity of respective events was low. Dyspepsia considered related by the investigator was reported in 1.6% subjects in the HELIOS-A vutrisiran vs. no subjects in the APOLLO placebo or HELIOS-A patisiran group, respectively, however, the frequency of dyspepsia TEAEs (irrespective of causality) was numerically lower in the vutrisiran vs. the Apollo placebo as well as the pooled patisiran group. In both vutrisiran subjects with dyspepsia considered related to study drug by the investigator, the subjects continued vutrisiran without recurrence of dyspepsia.

A decrease in serum levels of vitamin A is an expected secondary pharmacodynamic effect of reducing serum TTR protein and occurred in both HELIOS-A treatment groups: Over the course of the study, median percent reduction in vitamin A levels through Month 18 was 63.3% in the vutrisiran group and 63.4% in the patisiran group (as of the CSR2, 26_AUG-2021). In Helios-A vitamin A was generally supplemented at a dose of 2500 to 3000 IU and the SmPC recommendations have been amended accordingly. In subject, non-serious AEs of moderate transaminases increased and severe drug-induced liver injury were attributed to the subject's vitamin A supplement. In order to ensure that patients do not take too high doses of vitamin A, information has therefore been added to the PL that the daily vitamin A dose should be taken as recommended by the treating physician. In addition, the SmPC warnings and precautions regarding vitamin A deficiency/supplementation during pregnancy have been amended as requested over the assessment.

In the HELIOS-A study, in general, the type of cardiac AEs in vutrisiran-treated patients was consistent with those previously reported in patients with hATTR amyloidosis, based on the APOLLO placebo group, and the incidences appeared consistently numerically lower in the HELIOS-A vutrisiran group. No cardiac AE in the all vutrisiran HELIOS-A study population was considered related to study drug, and only one cardiac AE, although not considered related, led to study drug withdrawal. During the 18-Months HELIOS-A Treatment Period, the incidence of TEAEs in the Cardiac disorders SOC was 30.3% in

the vutrisiran and 23.8% in the patisiran group. In particular, the incidence of cardiac arrhythmia HLGT AEs was approx. three times higher in HELIOS-A vutrisiran compared to HELIOS-A patisiran subjects (26.6% vs. 7.1%). However, no cardiac safety concerns derived from ECG evaluations. In contrast to the imbalance regarding cardiac arrhythmias, TEAEs in the cardiac failure SMQ (narrow) occurred with a considerably lower frequency in the Helios-A vutrisiran (5.7%) compared to the Helios-A patisiran group (19.0%). It is further reassuring, that SAEs in the cardiac disorders SOC were reported with a lower incidence in the Helios-A vutrisiran vs. Apollo placebo as well as Helios-A patisiran group (9.0% vs. 13.0% and 14.3%, respectively). In the cardiac subpopulation but not in the overall safety population, a somewhat higher incidence of syncope was found in HELIOS-A vutrisiran (15.0%) compared to both, the HELIOS-A patisiran (7.1%) and APOLLO placebo (8.3%) subjects. Of the six cases of syncope in the Helios-A vutrisiran cardiac subpopulation, no event was considered related by the Investigator, all concerned isolated events with no apparent pattern in temporal relationship to study drug administration or the total number of doses applied until the event, and without recurrenc at subsequent injections, respectively. In four of the six subjects concerned, a medical history of (orthostatic) hypotension or loss of consciousness was reported. A causal relationship with vutrisiran therefore is considered unlikely. Apart from syncope, the evaluation of cardiac events in the cardiac subpopulation is considered to be generally in line with that of the overall safety population. Taken altogether, the currently available cardiac findings with vutrisiran do not raise serious cardiac safety concerns.

The safety profile of vutrisiran was generally consistent across the analysed subgroups (by age, sex, race, weight, genotype, FAP stage and geographic region, respectively).

Justification has been satisfactorily provided over the course of the assessment , labelling inclusion of the following AEs in the PI is currently not warranted:

- rash: In the Helios-A vutrisiran group (Treatment Period) compared to the APOLLO placebo group the incidence of TEAEs of rash by PT (5.7% vs. 3.9%) or TEAEs mapped to the rash custom query (7.4% vs. 9.1%) was similar. Within the rash medical concept grouping, none of the events in the vutrisiran group is considered an injection site reaction.
- muscle spasm (with an incidence in the HELIOS-A vutrisiran vs. APOLLO placebo and HELIOS-A patisiran group of 4.1% vs. 1.3% and 4.8%) and sinusitis (with an incidence of 2.5% vs. no subject in both control groups) are both labelled for patisiran. However, the presented data do not provide evidence to conclude a class effect for TTR-lowering RNAis. In particular, none of the events of sinusitis or within the medical concept of muscle spasm was considered treatment related.
- peripheral oedema; based on the information provided regarding a severe related event of peripheral oedema , including a medical history of cardiac amyloidosis, cardiac failure and peripheral oedema, respectively as well as the time course of onset and increase of peripheral oedema, the event is not indicative of a hypersensitivity reaction and causality with vutrisiran is uncertain.

The Applicant has presented the requested updated safety data covering the completed 18 Months Treatment Period and the ongoing Extension Period of the Helios-A study as of 26-AUG-2021 as well as a comparison of the safety profile of vutrisiran to the pooled (HELIOS-A and APOLLO) patisiran population.

3.5. Uncertainties and limitations about unfavourable effects

A numerical imbalance with regard to (related as well as overall) TEAEs in the Eye disorders SOC was found within the HELIOS-A study in the vutrisiran compared to the patisiran group (reported in 5.7% vutrisiran vs. 0 patisiran and 28.7% vutrisiran vs. 23.8% patisiran subjects, respectively as of the

CSR2). However, the respective comparison with the APOLLO placebo group as well as the detailed analysis of the reported related AEs in the HELIOS-A group does not clearly identify a safety signal with regard ocular toxicity due to vitamin A deficiency. Based on a generally comparable reduction of TTR levels as well as vitamin A levels with vutrisiran compared to patisiran, ocular toxicity would not be anticipated for vutrisiran in line with the available knowledge from patisiran. Therefore, the recommendation of vitamin A supplementation as well as a warning regarding ocular signs of vitamin A deficiency and recommendation for ophthalmological assessment if such symptoms occur, in line with the respective PI of patisiran and inotersen, respectively could generally be endorsed for vutrisiran.

In Helios-A (in Table 14.3.5.3.1 of the CSR2), a steady increase in alkaline phosphatase (ALP) over time was seen, with a mean absolute change (SD) from baseline of 22.19 (35.38) U/L in the vutrisiran and of 23.82 (18.17) U/L in the patisiran group at day 673, constituting a mean relative change of 36.71% for vutrisiran and 32.24% for patisiran, respectively. The mean ALP values still remained within the normal reference values. No corresponding increase in ALP was found in the patisiran group of the Apollo study. In pre-clinical studies, alkaline phosphatase levels were moderately increased upon long-term quarterly dosing of vutrisiran ≥10 mg/kg in rats and in all repeat-dose toxicity studies with monthly injections ≥100 mg/kg in monkeys. ALP elevations might be possibly explained by a slower ALP clearance from the circulation because of its competition with vutrisiran in binding to hepatic ASGPR. The clinical relevance of this seemingly continuous ALP increase is currently unclear and should be further evaluated. In the context of this initial MAA, the reflection of this ADR in the SmPC was considered sufficient.

The incidences of TEAEs in the drug-related hepatic disorders SMQ during the Helios-A Treatment have been clarified by the Applicant and do not raise concerns compared to the indicences in the Apollo placebo group. However, in a case of a related, severe hepatic SAE of transaminases increased reported during the Helios-Extension Period (in a subject, who had received patisiran during study the Treatment Period), the considerable increase of transaminases from mostly normal and at most mildly elevated levels to > 5xULN AST and > 6xULN ALT after the first dose of vutrisiran as well as a positive (milder) re-challenge point towards a causal relationship with vutrisiran. In both instances transaminase levels returned to near normal or normal without specific treatment. It should be noted, that the subject received a different posology than that currently recommended, i.e. twice the recommended single dose administered with a longer treatment interval (of 6 months). The Sponsor validated a signal of transaminase elevations which is currently ongoing and has stated to continue careful monitoring the reports of transaminase elevations. Further evaluation of events of transaminases increased is indicated, however, no labelling changes is considered necessary at present.

The severe related AEs reported on Day 1 in a subject, which included dizziness, dyspepsia, hyperhidrosis and hyperthermia, do not correspond to anaphylaxia, however it could not be excluded, that these events were caused by other non-IGE-mediated or non-immunologic drug hypersensitivity reactions. Apart from this case, no clear safety signal arose with regard to hypersensitivity. Therefore, it can be agreed with the Applicant, that at present no specific conditions for vutrisiran home administration are required. It should be noted, however, that according the SmPC, Amvuttra should be administered by a healthcare professional in any case. Nevertheless, taking into consideration, that the overall vutrisiran exposure at the time of the safety data cut-off is limited to 155 subjects, drug related hypersensitivity should be further evaluated.

No data is available in patients with moderate or severe hepatic impairment, in patients with severe renal impairment and in pregnant or lactating women.

3.6. Effects Table

Table 43: Effects Table for Amvuttra for the treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with polyneuropathy (data cut-off: 26-AUG-2021)*

Effect	Short Description	Unit	Treatment	Control	Control	Uncertainties/ Strength of evidence	References
Favourable Effects							
mNIS+7	Comprehensive composite neuropathy impairment score developed for hATTR amyloidosis. Score encompasses the totality of the motor, sensory, and autonomic neurologic impairment in hATTR amyloidosis.	Points Score range: 0 to 304 Higher score = greater severity of disease Decrease from baseline = improvement in neuropathy	**Vutrisiran (HELIOS-A)** LS Mean (SE) Change at Month 18: -0.46 (1.60)	**PLACEBO (APOLLO)** LS Mean (SE) Change at Month 18: 28.09 (2.28)	**Patisiran (HELIOS-A)** Mean Change from Baseline to Month 18 Mean (SD): 1.59 (21.50)	**SoE:** LS Mean Difference (vutrisiran vs placebo) (SE): **-28.55 (2.76)** 95% CI: -34.00, -23.10 (p=6.505E-20) Large and clinically relevant treatment effect favouring vutrisiran	HELIOS-A Module 2.5 Sect. 4.3.1.1 And Responses to Day 120 LoQ

Effect	Short Description	Unit	Treatment	Control	Control	Uncertainties/ Strength of evidence	References
Norfolk QOL-DN	Standardized quality of life questionnaire designed to measure the perception of the effects of polyneuropathy by the patient. Initially developed for diabetic neuropathy, but modified and shown to be reliable and valid in assessing QoL in hATTR amyloidosis patients: correlated / associated with disease stage (e.g., FAP stage) and severity (e.g., NIS).	Points Score range: -4 to 136 Lower score = higher QoL Decrease from baseline = improvement in QoL	**Vutrisiran (HELIOS-A)** LS Mean (SE) Change at Month 18: -1.2 (1.8)	**PLACEBO (APOLLO)** LS Mean (SE) Change at Month 18: 19.8 (2.6)	**Patisiran (HELIOS-A)** Mean Change from Baseline to Month 18 Mean (SD): -0.6 (19.3)	**SoE:** LS Mean Difference (vutrisiran vs placebo) (SE): **-21.0 (3.1)** 95% CI: -27.1, -14.9 (p=1.844E-10) Large and clinically relevant treatment effect in favour of vutrisiran	HELIOS-A Module 2.5 Sect. 4.3.1.1 And Responses to Day 120 LoQ
10-MWT	Measure of ambulation that assesses how fast a patient can walk a distance of 10 meters, reflecting overall ambulatory ability. Gait speed improvement has been shown to correlate with improved quality of life.		**Vutrisiran (HELIOS-A)** LS Mean (SE) Change at Month 18: -0.024 (0.025)	**PLACEBO (APOLLO)** LS Mean (SE) Change at Month 18: -0.264 (0.036)	**Patisiran (HELIOS-A)** Mean Change from Baseline at Month 18 Mean (SD): -0.043 (0.276)	**SoE:** LS Mean Difference: 0.239 (0.043) 95% CI: 0.154, 0.325 (p=1.207E-07) Statistically significant and clinically relevant improvement on gait speed.	HELIOS-A Module 2.5 Sect. 4.3.1.1

Effect	Short Description	Unit	Treatment	Control	Control	Uncertainties/ Strength of evidence	References
Time averaged TTR % reduction Mean (SD) Median (min, max)	Transthyretin percent reduction. Represents the mean of assessments between Month 6 and Month 18	%	**Vutrisiran (HELIOS-A):** 80.99 (20.96) -86.19 (-98.3, 55.1)	**Patisiran (HELIOS-A):** 78.56 (13.63) -81.39 (-97.2, -27.6)	**Patisiran (APOLLO)** mean TTR percent reduction from baseline of 82.6% at Month 9	**SoE:** Pseudomedian vutrisiran 84.67 and patisiran 80.60 Median Difference 5.28 - 95% CI (1.17, 9.25) non-inferiority of vutrisiran compared to within study patisiran was demonstrated: the 95% CI of the median treatment difference in TTR percent reduction (vutrisiran – patisiran) was 1.17, 9.25, in which its lower limit was above -10%	HELIOS-A Module 2.5
Unfavourable Effects							
Injection site Reactions (ISRs)	Incidence of ISRs (TEAE)	n (%)	Vutrisiran 5 (4.1)	APOLLO Placebo NA	HELIOS-A patisiran (not relevant, as different administration mode)	Based on the SC mode of administration, ISRs are regarded ADR; reported in 5 (0.6%) of the total 836 doses of vutrisiran administered. ISRs were all mild, nonserious, transient, did not lead to treatment discontinuation. Symptoms included bruising, erythema, pain, pruritus, warmth. One further, single event during Extension Period, moderate, PT: traumatic haematoma	(1) HELIOS-A Treatment Period. (2) HELIOS-A Extension Period

Effect	Short Description	Unit	Treatment	Control	Control	Uncertainties/ Strength of evidence	References
Arthralgia	Incidence of arthralgia (PT, TEAE)	n (%)	Vutrisiran 13 (10.7)	APOLLO Placebo 0	HELIOS-A patisiran 4 (9.5)	Imbalance compared to APOLLO placebo group; also common ADR of patisiran; as of 26-AUG-2021, AEs of arthralgia were mostly mild or moderate in severity, with 1 patient having a severe AE; none of the events were serious. No AEs of arthralgia led to treatment discontinuation; AEs of arthralgia did not increase over time.	(1)
Dyspnoea	Evaluation of the medical concept of dyspnoea, which includes the PTs dyspnoea, dyspnoea exertional, and dyspnoea paroxysmal nocturnal	n (%)	Vutrisiran 8 (6.6)	APOLLO Placebo 0	HELIOS-A patisiran Not given	Imbalance compared to APOLLO placebo group; also common ADR of patisiran; as of 26-AU-2021, none of the AEs of dyspnoea were severe or serious, none led to treatment discontinuation; AEs of dyspnoea did not increase over time.	(1)
Pain in extremity	Incidence of arthralgia (PT, TEAE)	n (%)	Vutrisiran 18 (14.8)	APOLLO Placebo 8 (10.4)	HELIOS-A patisiran 3 (7.1)	Mild to moderate; no apparent pattern in onset regarding vutrisiran administration; symptom of underlying disease, but imbalance vs. comparator groups	(1)

Effect	Short Description	Unit	Treatment	Control	Control	Uncertainties/ Strength of evidence	References
Alkaline phosphatase (ALP) increased	Incidence of increase >1.5 ULN	n (%)	Vutrisiran 11 (9.0%)	APOLLO Placebo 1 (1.3)	HELIOS-A patisiran 1 (2.4)	Not associated with clinically significant transaminase/bilirubin increase	(1)
	Steady increase in mean ALP	Mean relative change from baseline (%), Day 673	36.71	Not given	32.24	Mean values still within normal range; may be explained by slower clearance due to competition with vutrisiran in binding to hepatic receptors; clinical relevance in long-term vutrisiran treatment is unclear and listed as investigations	(1), Table 14.3.5.3.1

Abbreviations:
Notes: (1) HELIOS-A study Treatment Period compared to external placebo group from APOLLO study as of Clinical study report 2 (cut-off date: 26-AUG-2021); (2) HELIOS-A Extension period as of Clinical study report 2 (ongoing, cut off-date: 26-AUG-2021)
* Patients without any post-baseline assessments (both Month 9 and Month 18 assessments missing/censored) both in the vutrisiran and placebo groups were excluded from the analyses and this cannot be accepted. Therefore, analyses performed on the "true" mITT population as pre-specified should be provided and these should be used inform the appropriate sections of the SmPC for the mNIS+7, Norfolk QoL-DN, 10-MWT, mBMI and R-ODS results.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

An open-label study (HELIOS-A) was performed with vutrisiran and patisiran, as a reference comparator, in patients with hATRR with polyneuropathy. In order to show superiority to placebo, the vutrisiran results from HELIOS-A study were compared to an external placebo group from a double-blind, placebo-controlled study performed with patisiran (APOLLO). Clinically relevant improvements were observed with vutrisiran in appropriate and relevant endpoints evaluating the severity of neuropathy (mNIS+7) as well as the quality of life (Norfolk QoL-DN). TTR reduction at Month 18 with vutrisiran demonstrated non-inferiority with patisiran in HELIOS-A. A large PD effect on the TTR is indicative of the efficacy of vutrisiran, which was demonstrated also with clinical endpoints at Month 18.

Vutrisiran is a very similar agent as patisiran with the same MoA and very similar pharmacodynamic effect (%TTR reduction, with misfolded TTR being the main pathological aetiology for hATTR), developed by the same company. By demonstrating non-inferior TTR reduction from Month 6 to 18

between vutrisiran and patisiran in HELIOS-A and because vutrisiran and patisiran share the same mechanism of action, a comparison to the external placebo group of the APOLLO study is considered possible. Despite some differences in study setting and baseline characteristics of the patient populations of HELIOS-A and APOLLO, as well as potentially different patient expectations (all patients received active treatment in HELIOS-A, while half of the patients received placebo in APOLLO), efficacy of vutrisiran was shown across the clinical endpoints. Also similarity of results on clinical endpoints between vutrisiran and patisiran in HELIOS-A and between the patisiran arms in HELIOS-A and APOLLO was observed. Analyses of the "true" mITT population, implementing two slightly different PMM methods PMM 1 (hypothetical estimand) and PMM 2 (treatment policy estimand) were provided upon request and showed little differences between the estimates of treatment effect. The treatment effect sizes from PMM being slightly larger than MMRM did not have any impact on the clinical interpretation of the results. Since MMRM was the prespecified analysis, the use of the MMRM results in the SmPC is considered acceptable, in order to keep all public information consistent.

The initially proposed broad indication including also stage 3 patients has been modified to reflect the population studied and it is worded the same as that for the approved product Onpattro (patisiran) based on similar findings regarding biomarker/PD parameters and clinical endpoints.

Taking into consideration that hATTR amyloidosis is an orphan disease and that vutrisiran is a second-generation siRNA-GalNAc conjugate, the provided safety data could be considered comprehensive, however, long-term data > 2 years are still limited. The safety profile of vutrisiran as derived from the updated data as of 26-AUG-2021 is considered acceptable and manageable with appropriate labelling in the product information.

As of now the only ADRs identified are arthralgia and pain in extremity, with very common frequency each, as well as dyspnoea and injection site reactions (ISRs), occurring commonly each. In addition, ALP increased has been added to the PI as a common ADR within the Investigations SOC based on the higher incidence in the Helios-A vutrisiran compared to the patisiran but also the Apollo placebo group. Vutrisiran is administered subcutaneously once every three months and IRSs occurred in 5 (4.1%) vutrisiran subjects during the Helios-A Treatment Period and in 6 vutrisiran subjects overall. Five ISRs were mild, one event was moderate, no ISR led to treatment discontinuation, despite no administration of premedication and none of the 6 subjects concerned had more than one event. The frequency of ISRs was low irrespective of vutrisiran presentation (as a vial or as a prefilled syringe). In contrast to vutrisiran, patisiran, which is also indicated for the treatment of hATTR amyloidosis with polyneuropathy, is administered intravenously every three weeks and requires premedication including corticosteroids in order to reduce the risk of infusion related reactions (IRRs). Nevertheless, in the HELIOS-A patisiran group, IRRs were reported for 10 (23.8%) patients and included 4 SAEs. Dyspnoea and arthralgia are also commonly associated with patisiran. In vutrisiran treated subjects, AEs within the dyspnoea medical concept were mild or moderate in severity; none of the events were severe or serious.

In one subject other than IGE-mediated or non-immunologic drug hypersensitivity reactions could not be excluded, while the case is not compatible with anaphylaxia. Apart from this case, no clear safety signal arose with regard to hypersensitivity. Taking the limited safety data-base into consideration, drug related hypersensitivity should be further evaluated.

Vitamin A level reduction with vutrisiran with the proposed posology is comparable to patisiran, and as with patisiran no clear ocular toxicity due to vitamin A deficiency toxicity was identified. Nevertheless, ocular changes can occur in patients with hATTR amyloidosis and it is important to assess if these changes could potentially be related to vitamin A deficiency. Therefore a warning about the ocular signs and symptoms of vitamin A deficiency and recommendation for ophthalmological assessment if such symptoms occur, as well as recommendation for vitamin A supplementation are included in the

proposed SmPC. In line with the dose used in the Helios-A study, the recommended daily vitamin A supplementation has been amended to 2500 IU – 3000 IU and detailed instructions regarding planned and unplanned pregnancies have been implemented. The proposed addition that vitamin A supplementation should not be adjusted based on serum vitamin A levels has been deleted from the PI as requested as it had rather complicated the decision-making of the physician, as were the suggested explanations on vitamin A testing in section 4.5 which did not coincide with the warnings in section 4.4. Considering also the potential risk of hypervitaminosis A due to inadequate vitamin A supplementation, information was added to the SmPC, that the daily vitamin A dose should not exceed 2500 -3000 IU, and to the PL, that the daily vitamin A dose should be taken as recommended by the treating physician.

The incidence of TEAEs in the drug-related hepatic disorders SMQ in the Helios-A vutrisiran group compared to the Apollo placebo group does not raise concerns. However, further evaluation of events of transaminases increased is indicated based on a case of a related, severe, reversible SAE of transaminases increased with a positive, milder re-challenge. These events occurred in a subject receiving twice the single vutrisiran dose recommended in the SmPC with a longer treatment interval (i.e. 50 mg s.c. q6M) though.

In addition to the AEs of ALP increased, a continuous relative increase in mean ALP by approx. 37% was found (but within normal limits of the reference range up to day 673 of the Helios-A study) the clinical relevance of which with regard to long-term vutrisiran administration is currently unclear and which should be further evaluated.

Regarding safety, all the outstanding issues have been resolved and the PI has been amended as requested.

3.7.2. Balance of benefits and risks

Hereditary transthyretin amyloidosis (hATTR) is a rare, progressive and eventually fatal disease which manifests as destabilization of the tetrameric structure of the TTR protein. Vutrisiran is a siRNA molecules that uses RNA interference mechanisms to target and silence the expression of wild type and variant TTR mRNA and inhibit the synthesis of the TTR protein.

A large reduction in TTR and large, statistically significant and clinically meaningful differences in the change from baseline at Month 18 using clinically appropriate tools evaluating the improvement in neuropathy have been observed with vutrisiran in an open-label study (HELIOS-A) compared to an external placebo group from the clinical program of patisiran (APOLLO). In addition, reduction in TTR levels was found to be non-inferior to patisiran used at the approved dosing regimen.

These results are considered confirmatory for the efficacy of vutrisiran in patients with hATTR with stage 1 or stage 2 polyneuropathy. The safety profile of vutrisiran as derived from the presented safety data is considered acceptable and manageable with appropriate labelling in the product information.

Therefore, the benefits of vutrisiran in the proposed target population are considered to outweigh the risks.

3.8. Conclusions

The overall benefit/risk balance of Amvuttra is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Amvuttra (Vutrisiran) is not similar to Onpattro (patisiran), Tegsedi (inotersen) and Vyndaqel (tafamidis) within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity.

Outcome

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

Treatment of hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with stage 1 or stage 2 polyneuropathy

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 marketing authorisation holder (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.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Post-authorisation measure(s)	Motivation
Proposed post-authorisation measure with proposed classification:	Motivation/Background information on measure, including due date:
Submission of the reports from carcinogenicity studies in rats and mice	Carcinogenicity studies were not submitted and are presently ongoing in rats and mice. The schedule proposed by the Applicant for submission of the reports of both carcinogenicity studies has been agreed Q1 2024
The active substance specification limits for duplex purity, purity and impurities including specified impurities by denaturing AX-HPLC UV as well as IPRP-UPLC UV, melting temperature, sodium content, assay, pH and water content should be re-assessed when there are available data from an additional 10 batches manufactured with the commercial process.	The specification limits have been partly revised during the procedure. The active substance specification limits should be re-assessed once additional data from commercial batches become available. Q4 2027
The finished product specification limits for purity and impurities should be re-assessed when there are available data from an additional 10 batches manufactured with the commercial process.	The specification limits have been partly revised during the procedure. The finished product specification limits should be re-assessed once additional data from commercial batches become available. Q4 2027

Post-authorisation measure(s)	Motivation
Proposed post-authorisation measure with proposed classification:	Motivation/Background information on measure, including due date:
PASS Category 3 (classification as PASS)	ALN-TTRSC02-002 HELIOS-A-RTE study is a Phase 3 global, randomised, open-label study to evaluate the safety and efficacy of ALN-TTRSC02 in patients with Hereditary Transthyretin Amyloidosis (hATTR Amyloidosis). The aim of the study is to collect further longer-term safety and efficacy data on vutrisiran in patients with hATTR amyloidosis with polyneuropathy Due date to be agreed on based on updated study synopsis.

Post-authorisation measure(s)	Motivation
PASS Category 3	ALN-TTR02-013 ConTTRibute Study: A global prospective observational multicenter long-term study of patients with hATTR amyloidosis The primary objective of this study is to document the natural history, clinical characteristics, and management of ATTR amyloidosis as part of routine clinical care. This study is enrolling hATTR and wtATTR amyloidosis patients and was initiated in November 2020. Long- term safety data for vutrisiran will be collected as part of ConTTRibute. Due date to be agreed on based on updated study synopsis.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

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

Refer to Appendix on new active substance (NAS).

Paediatric Data

Not applicable

5. Appendices

5.1. CHMP AR on similarity dated 21 July 2022

5.2. CHMP AR on New Active Substance (NAS) dated 21 July 2022