26 July 2018
EMA/554262/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Onpattro

International non-proprietary name: patisiran

Procedure No. EMEA/H/C/004699/0000

Note

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

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List of abbreviations

Abbreviation	Definition
10-MWT	10-meter walk test
ADA	Antidrug antibodies
AE	Adverse event
AL	Immunoglobulin light chain amyloid
ALN-18328	Patisiran drug substance
ALN-TTR02	Patisiran drug product; also known as patisiran-LNP
ALT	Alanine transaminase
ANC	Absolute neutrophil count
ANSM	French National Agency for Medicine and Health Products Safety
ASO	Antisense oligonucleotide
AST	Aspartate transaminase
ATTR	Amyloid transthyretin
AX-HPLC	Anion-exchange high performance liquid chromatography
BUN	Blood urea nitrogen
CAS	Central assessment sites
CFU	Colony Forming Units
CHMP	Committee for Medicinal Products for Human use
CI	Confidence interval
CIPN-R-ODS	Chemotherapy-induced Peripheral Neuropathy Rasch-built Overall Disability Scale
CMAP	Compound muscle action potential
C_{max}	Maximum observed plasma concentration
C_{max2}	Maximum observed plasma concentration for the second phase
C_{trough}	Observed lowest concentration before next dose
C_{max}_ss	C_{max} at steady state
C_{trough}_ss	C_{trough} at stead state
COMPASS 31	Composite Autonomic Symptom Score
CNS	Central nervous system
CPP	Critical process parameter
CQA	Critical Quality Attribute
CRF	Case report forms
CSR	Clinical study report
CYP	Cytochrome P450
DDI	Drug-drug interaction
DLS	Dynamic light scattering

Abbreviation	Definition
DLin-MC3-DMA	Patisiran-LNP lipid excipient; (6Z, 9Z, 28Z, 31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate
DMC	Data monitoring committee
DoE	Design of experiments
DSPC	Patisiran-LNP lipid excipient; 1,2-Distearoyl-sn-Glycero-3-Phosphocholine
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EOI	End of infusion
EQ-5D-5L	EuroQoL 5 dimensions 5 levels
EQ-VAS	EuroQoL visual analogue scale
EU	European Union
FAC	Familial amyloidotic cardiomyopathy
FAAS	Flame Atomic Absorption Spectroscopy
FAP	Familial amyloidotic polyneuropathy
FAP-R-ODS	hATTR Amyloidosis with Polyneuropathy Rasch-built Overall Disability Scale
FDA	United States Food and Drug Administration
FTIR	Fourrier Transform Infrared Spectroscopy
GCP	Good Clinical Practice
hATTR	Hereditary transthyretin-mediated amyloidosis
HDPE	High Density Polyethylene
HGLT	High level group term
HLT	High level term
HPLC	High performance liquid chromatography
HPLC-CAD	High performance liquid chromatography charged aerosol detector
HPLC-ELSD	High performance liquid chromatography evaporative light scattering detection
HRdb	Heart rate response to deep breathing
ICH	International Conference on Harmonization
IENFD	Intraepidermal nerve fiber density
INFARMED	National Authority of Medicines and Health Products; Portugal
ICP-MS	Inductively coupled plasma mass spectrometry
IPC	In-process control
IPRP-HPLC MS	Ion pairing reversed phase high performance liquid chromatography mass spectrometry
IRR	Infusion-related reaction
ITT	Intent-to-treat

Abbreviation	Definition
IV	Intravenous
KF	Karl Fischer titration
LC-MS	Liquid chromatography mass spectrometry
LDPE	Low density polyethylene
LFT	Liver function test
LNP	Lipid nanoparticle
LS	Least squares
LV	Left ventricular
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
MAD	Multiple-ascending dose
MALDI-TOF-MS	Matrix assisted laser desorption-time of flight-mass spectrometry
mBMI	Modified body mass index
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicine and Healthcare Products Regulatory Agency; United Kingdom
MI/ANCOVA	Multiply-imputed analysis of covariance
mITT	Modified intent-to-treat
MMRM	Mixed-effect model repeated measures
MMN-R-ODS	Multifocal Motor Neuropathy Rasch-built Overall Disability Scale
mNIS+7	Modified Neurologic Impairment Score +7
MPA	Medicinal Products Agency; Sweden
MS	Mass Spectrometry
mRNA	Messenger RNA
NCS	Nerve conduction study
NIS	Neurologic Impairment Score
NIS-LL	Neurologic Impairment Score Lower Limb
NIS-R	Neurologic Impairment Score Reflex
NIS-W	Neurologic Impairment Score Weakness
NMR	Nuclear Magnetic Resonance
NMT	Not more than
NNTTR	Non-native transthyretin
NOR	Normal Operating Range
Norfolk QoL-DN	Norfolk Quality of Life-Diabetic Neuropathy
NSAID	Nonsteroidal anti-inflammatory drug
NT-proBNP	N terminal pro b-type natriuretic peptide
NYHA	New York Heart Association

Abbreviation	Definition
OFAT	One factor at a time
OLT	Orthotopic liver transplantation
PAR	Proven Acceptable Range
patisiran-LNP	Patisiran drug product; also known as ALN-TTR02
PBS	Phosphate buffered saline
PCS	Patient Care Site
PD	Pharmacodynamics
PDCO	Paediatric Committee
PE	polyethylene
PEG	polyethylene glycol
PEG_{2000}-C-DMG	Patisiran-LNP lipid excipient; (R)-methoxy-PEG_{2000}-carbamoyl-di-O-myristyl-sn-glyceride
PES	polyethersulfone
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetics
PMDA	Japanese Pharmaceuticals and Medical Devices Agency
PMM	Pattern-mixture model
PND	Polyneuropathy disability
PP	Polypropylene
PPQ	Process performance qualification
PT	Preferred term
q3w	Once every 3 weeks
q4w	Once every 4 weeks
QbD	Quality by design
QoL	Quality of life
QST	Quantitative sensory testing
QST-BSA_{HP}	Heat pain measured by QST – small fiber
QST-BSA_{TP}	Touch pressure measured by QST – large fiber
QTc	Corrected QT interval
QTcB	QT obtained using Bazett's formula
QTcF	QT obtained using Fridericia's formula
R_{AC}	Accumulation ratio
RBP	Retinol binding protein
RHD	Recommended human dose
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid

Abbreviation	Definition
RNAi	RNA interference
R-ODS	Rasch-built Overall Disability Scale
RP-HPLC	Reverse phase high performance liquid chromatography
RP-HPLC-ELSD	Reverse phase high performance liquid chromatography evaporative light scattering detection
SAD	Single-ascending dose
SAP	Statistical Analysis Plan
SAE	Serious adverse event
SE-HPLC	Size exclusion high performance liquid chromatography
SEM	Standard error of the mean
SGNFD	Sweat gland nerve fiber density
siRNA	Small interfering ribonucleic acid
SmPC	Summary of Product Characteristics
SMQ	Standardized Medical Dictionary for Regulatory Activities (MedDRA) query
SNAP	Sensory nerve action potential
SOC	System organ class
T3	Triiodothyronine
T4	Thyroxine
TAMC	Total Aerobic Microbial Count
TI	Tolerance interval
TLC	Thin layer chromatography
TSE	Transmissible Spongiform Encephalopathy
TSH	Thyroid stimulating hormone
TTC	Threshold of toxicological concern
TTR	Transthyretin
TYMC	Total Combined Yeasts/Moulds Count
UF	Ultrafiltration
ULN	Upper limit of normal
US	United States
UV	Ultraviolet
V30M	Valine to methionine mutation at position 30
VDT	Vibration detection threshold
wt	Wild type

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Alnylam UK Limited submitted on 18 December 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Onpattro, 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 10 November 2016.

During the procedure (Day91), the Marketing Authorisation applicant was transferred to Alnylam Netherlands B.V.

Onpattro, was designated as an orphan medicinal product EU/3/11/857 on 15 April 2011 in the following condition: Treatment of transthyretin-mediated amyloidosis.

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).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Onpattro 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: ema.europa.eu/Find medicine/Human medicines/European public assessment reports. (http://www.ema.europa.eu/ema/index.jsp?curl=/pages/medicines/human/medicines/004699/human_med_002307.jsp)

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0304/2013 on the granting of a product-specific waiver.

Information relating to orphan market exclusivity

Similarity

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

New active Substance status

The applicant requested the active substance patisiran 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.

Protocol assistance

The applicant received Protocol assistance from the CHMP on 25 July 2013 (EMEA/H/SA/2585/1/2013/PA/III). The Protocol assistance pertained to quality, non-clinical and clinical aspects of the dossier.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Kristina Dunder Co-Rapporteur: Alexandre Moreau

The application was received by the EMA on	18 December 2017
Accelerated Assessment procedure was agreed-upon by CHMP on	09 November 2017
The procedure started on	25 January 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	28 March 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	28 March 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	3 April 2018
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	12 April 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	24 April 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	25 May 2018
The following GMP and GCP inspections were requested by the CHMP and their outcome taken into consideration as part of the	

Quality/Safety/Efficacy assessment of the product	
– A routine GCP inspection (GCP/2018/001) at 1 investigator site in Spain and at the Sponsor between 12/03 – 06/04/2018 and a triggered inspection (GCP/2018/009) at 1 investigator site in Mexico and at the CRO. The outcome of the inspection carried out was issued on 04/06/2018.	4 June 2018
– A GMP inspection at one site responsible for manufacture of the finished product in the USA between 15 - 17 May 2018. The outcome of the inspection carried out was issued on 22 May 2018.	22 May 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	15 June 2018
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	26 June 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	03 July 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	12 July 2018
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	23 July 2018
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 Onpattro on	26 July 2018
The CHMP adopted a report on similarity of Onpattro with Vyndaqel and Tegsedi on (Appendix 1)	26 July 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) is a rare, life-threatening, autosomal dominant multi-systemic disease caused by mutations in the TTR gene that results in rapidly progressive, debilitating morbidity and high mortality. It has a male predominance (approximately 3:1 male to female ratio) with diagnosis typically occurring in the seventh decade. The cardinal manifestations of hATTR amyloidosis are polyneuropathy and cardiomyopathy.

2.1.2. Epidemiology

The estimated European prevalence of hATTR amyloidosis is 0.10 per 10,000 (between 5000 to 6000 patients), with the majority of cases in Portugal, France, Italy, and the United Kingdom It has a male predominance (approximately 3:1 male to female ratio) with a median age at diagnosis of 63 years (Swiecicki, Zhen et al. 2015). In Europe, the prevalence is highest in northern Portugal and northern Sweden. In northern Sweden is as high as 50 per 100,000 inhabitants. The age of onset of disease varies between countries and regions due to differences in the most common mutation types, which is also related to founder effects. For example, in Portugal the median age of onset is 30-40 years, whereas it is 50 years in Sweden and the applicant refers to a median age of onset of 63 years. The progression rate appears somewhat faster in early onset disease.

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). Indeed, Swiecicky et al. (2015) show in their single-centre study at the Mayo Clinic that 50% of the patients survived 56.8 months. In comparison, the Swedish National Board of Health and Welfare (Socialstyrelsen) states that the survival is 9-13 years from onset.

2.1.3. Aetiology and pathogenesis

TTR, also known as prealbumin, is a tetrameric protein produced by hepatocytes, the choroid plexus and retina. More than 95% of TTR in the circulation is derived from the liver. The primary physiological role of TTR is to serve as a carrier of retinol (also known as vitamin A), which involves TTR binding to the retinol binding protein (RBP): vitamin A complex. Importantly, TTR knockout mice have little to no circulating vitamin A or RBP; they have normal RBP levels and vitamin A stores in the liver and exhibit no signs or symptoms of vitamin A deficiency provided they have access to dietary vitamin A. TTR also serves as a minor carrier for thyroxine (T4); however, while TTR knockout mice show a 3-fold reduction of T4, triiodothyronine (T3) is only modestly affected and thyroid stimulating hormone (TSH) remains normal. In humans, thyroid binding globulin (TBG) rather than TTR is the main carrier of T4.

There are more than 120 reported TTR genetic mutations associated with hATTR amyloidosis, and almost all patients are heterozygous for the mutated TTR allele. The most common genotype is the valine to methionine mutation at position 30 (V30M), accounting for approximately 50% of cases worldwide, and

occurring primarily in families with heritage from Portugal, Sweden, Japan, and Brazil.(9) Mutations in the TTR gene lead to destabilization of the tetrameric protein and disassociation of the TTR subunits into dimers and individual mutant and wt monomers, which subsequently misfold. These misfolded TTR monomers can then self-assemble into oligomers and form amyloid fibrils and plaques in the extracellular space of various tissues, including the peripheral nervous system, heart, gastrointestinal tract, kidney, central nervous system (CNS) and eye leading to cellular injury and organ dysfunction with corresponding clinical manifestations.

2.1.4. Clinical presentation, diagnosis and stage / prognosis

Historically, due to incomplete understanding of etiology and pathogenesis, 2 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. However, while patients with hATTR amyloidosis may present with predominantly polyneuropathy or cardiomyopathy, most patients with hATTR amyloidosis manifest signs and symptoms of both polyneuropathy and cardiomyopathy over the course of their disease. Therefore, clinicians caring for these patients have evolved to refer to 1 hereditary disease with a spectrum of clinical manifestations rather than attempt to classify the disease into 2 distinct syndromes.

The diagnosis of hATTR amyloidosis is based on the establishment of characteristic signs and symptoms of polyneuropathy and/or cardiomyopathy in conjunction with confirmation of a mutant TTR genotype and absence of other known causes of peripheral neuropathy or cardiomyopathy (eg, chronic inflammatory demyelinating polyneuropathy [CIDP] or immunoglobulin light chain [AL] amyloidosis). Further support for the diagnosis can come from a tissue biopsy (including abdominal fat pad, salivary gland, or rectal biopsy) demonstrating amyloid deposits.

The clinical manifestations of the length-dependent, symmetrical polyneuropathy are the result of amyloid-mediated injury to large and small peripheral nerve fibers. Sensory abnormalities include painful dysesthesias in the feet and hands, as well as loss of sensation leading to thermal burns involving the feet and hands and to joint injury in the lower limbs. Progressive muscle atrophy and motor weakness in both lower and upper limbs leads to impaired ambulation and inability to perform other activities of daily living, such as being able to hold eating utensils or a drinking glass, or button a shirt or zip up a coat. Autonomic dysfunction results in debilitating orthostatic hypotension, severe gastrointestinal symptoms (including early satiety, chronic nausea/vomiting, and both diarrhea and constipation), 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. 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. In these patients, neuropathy progression is relatively rapid, with data from natural history and a placebo-controlled interventional study showing a 10- to 14-point increase (worsening) per year in neurologic impairment as measured by the Neurologic Impairment Score (NIS). This is in contrast to diabetic polyneuropathy, where NIS progression is typically less than 1 point per year.

Cardiac infiltration with amyloid leads to heart wall thickening and cardiomyopathy characterized by heart failure due to diastolic and systolic dysfunction, as well as conduction disturbances and arrhythmias. Patients with symptomatic heart failure experience rapid progression of their amyloid cardiomyopathy, 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.

This constellation of progressive morbidity from amyloid infiltration results in severe disability, and 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 median survival is 4.7 years following diagnosis with a reduced survival (3.4 years) for patients presenting with cardiomyopathy.

2.1.5. Management

The treatment of hATTR amyloidosis requires a multidisciplinary approach primarily involving neurology, gastroenterology, and cardiology specialties. Limited treatment options, namely orthotopic liver transplant (OLT) and TTR tetramer stabilizers, exist for a small subset of patients. However, availability is limited and most patients continuing to experience significant morbidity and mortality associated with disease progression. Palliative/symptomatic therapies directed at specific symptoms such as pain, nausea, vomiting, and diarrhea have been the mainstay of treatment despite their limited effectiveness.

OLT essentially eliminates mutant TTR from the circulation but does not affect the hepatic production of wt TTR, which continues to be made by the transplanted liver. OLT is only effective in slowing the progression of disease in patients with an early age of onset (<50 years of age), especially for those with the V30M mutation and short disease duration before transplant. Consequently, almost two-thirds of patients with hATTR amyloidosis are not transplant-eligible. Even when OLT is possible, morbidity and mortality are substantial; patients require life-long immunosuppressive medications, with their attendant risks of infection and renal injury. One-year mortality rates of up to 10% have been reported.

TTR tetramer stabilizers (including tafamidis and diflunisal) act by binding to the T4-binding site on TTR to reduce its dissociation into misfolded amyloidogenic monomers. Tafamidis is not approved in the United States. It received approval from the European Medicines Agency (EMA) under exceptional circumstances in November 2011 ‘for the treatment of transthyretin amyloidosis in adult patients with stage I symptomatic polyneuropathy to delay peripheral neurologic impairment’. Tafamidis has been reported to be generally well tolerated. Long-term follow-up of the early stage V30M patients who continued tafamidis treatment showed continued neuropathy progression over time. Diflunisal, a generic, oral nonsteroidal anti-inflammatory drug (NSAID) that has been demonstrated to bind to and stabilize the TTR tetramer in a manner similar to tafamidis, was shown to reduce neuropathy progression compared with placebo in V30M and non-V30M patients with hATTR amyloidosis with both early and late stage neuropathy in a Phase 3 United States (US) National Institutes of Health-sponsored study. While used off-label in some countries where available, diflunisal is not an approved treatment for hATTR amyloidosis in any country. In the United States, diflunisal carries boxed warnings for cardiovascular thrombotic events and gastrointestinal risk. Overall, the data to date with TTR tetramer stabilizers indicate some slowing of neuropathy progression, with effects limited in the case of tafamidis to early stage V30M patients, and no effect on the cardiac manifestations of the disease.

2.2. About the product

Patisiran-LNP ranges in size from approximately 60 to 100 nm and contains 4 lipid excipients: DLin-MC3-DMA; PEG2000-C-DMG; DSPC; and cholesterol. These lipid excipients protect ALN-18328 from degradation

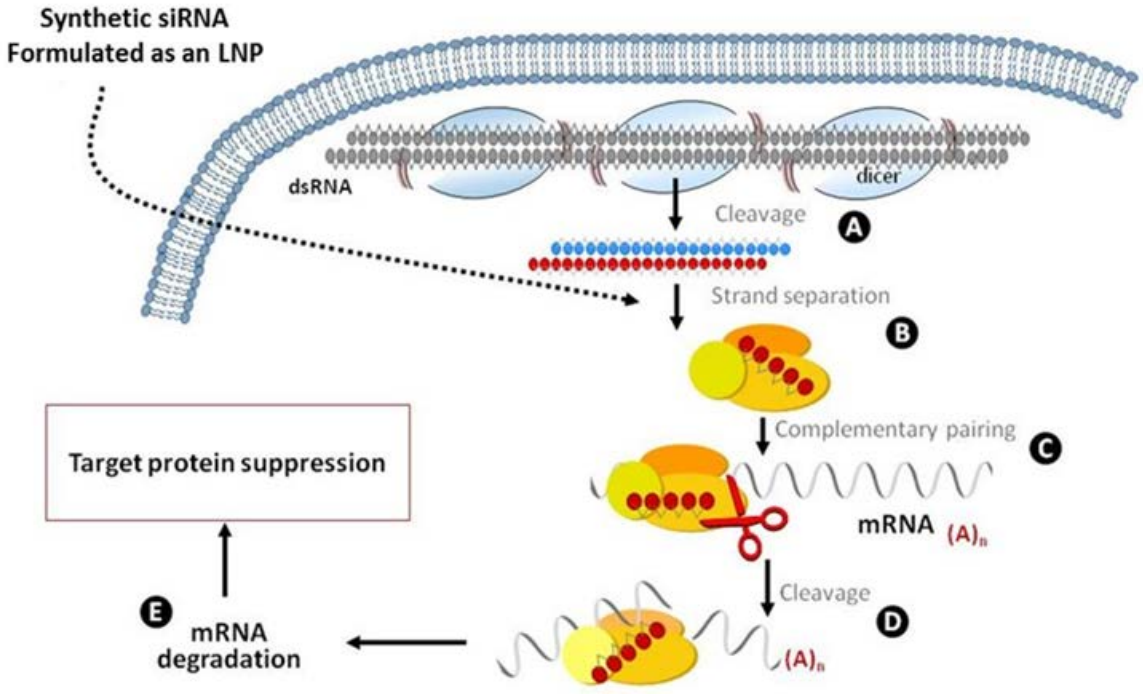
by endo- and exo-nucleases in the circulatory system and facilitate delivery to the target site of action, the liver. DLin-MC3-DMA and PEG2000-C-DMG are novel lipid excipients, that are not constituents of any approved drugs. DLin-MC3-DMA is important for particle formation, fusogenicity, cellular uptake, and endosomal release of the siRNA. PEG2000-C-DMG aids in patisiran-LNP stability in the circulation and provides optimum circulation time enabling uptake of patisiran-LNP into the liver. Cholesterol is multicompendial (United States Pharmacopeia/National Formulary, European Pharmacopoeia, and Japanese Pharmacopoeia). DSPC is a synthetic phosphatidylcholine that is very similar to naturally occurring phosphatidylcholines, is included in the inactive ingredient (excipient) list by the United States (US) Food and Drug Administration (FDA) for approved products, and is a constituent of 2 drugs that are currently approved in the US and the European Union (DaunoXome®[liposomal daunorubicin] and ONIVYDE®[irinotecan liposome injection]); these excipients provide physicochemical stability to the LNP.

ALN-18328, the active ingredient in patisiran-LNP, is a first-in-class siRNA that utilizes the naturally occurring mechanism of RNAi to reduce the expression of mutant and wt TTR mRNA and its corresponding protein. ALN-18328 is formulated as lipid nanoparticles (patisiran-LNP) to target delivery to hepatocytes in the liver, the primary source of TTR protein in the circulation.

Following intravenous infusion, coating of the LNP (60-100nm in diameter) by apolipoprotein E facilitates binding to the low density lipoprotein receptor on hepatocytes and subsequent endocytosis. Fusion of the ionizable lipid component of the LNP with the endosomal membrane then leads to release of the siRNA into the cytoplasm where it can bind to and activate the RISC. The ALN-18328 RNA duplex then unwinds and the antisense strand specifically binds to a genetically conserved sequence in the 3' untranslated region of TTR mRNA, and hence can bind both wt and mutant mRNA regardless of the specific pathogenic mutation (Figure 1). The Argonaute-2 endonuclease within the RISC/siRNA enzyme complex catalytically degrades wt and mutant TTR mRNA, resulting in a reduction of both wt and mutant TTR protein synthesis.

Figure 1

Mechanism of RNA Interference and Therapeutic Concept



Abbreviations: dsRNA=double stranded RNA; LNP-lipid nanoparticle; mRNA=messenger RNA; RISC=RNAi induced silencing complex; RNAi=RNA interference; siRNA=small interfering ribonucleic acid; TTR=transthyretin.
Note: In the naturally occurring process, intracellular double stranded RNA (dsRNA) is processed by the "Dicer" complex pathway (A) to produce siRNAs which become integrated into a multi-subunit protein complex, the RNAi induced silencing complex (RISC) (B), which guides the siRNAs to the target mRNA sequence (C). The siRNA duplex unwinds, and the antisense strand remains bound to RISC and directs degradation of the complementary mRNA sequence (D), resulting in target protein suppression (E). As a therapeutic using this RNAi pathway, patisiran delivers a synthetic siRNA specific for TTR mRNA into the cytoplasm of hepatocytes. Once in the cytoplasm, the siRNA bypasses the Dicer complex pathway (A) and interacts with RISC downstream of the Dicer complex pathway (B, C, D) to mediate the specific degradation of TTR mRNA (E), thereby reducing the amount of TTR protein.

Patisiran-LNP mechanism of action is distinct from that of OLT and TTR tetramer stabilizers, and is unique in its ability to reduce the levels of both mutant and wt TTR using the catalytic process of RNAi. The siRNA-mediated RNAi mechanism of action of patisiran-LNP is also distinct from that of the TTR-targeting ASO inotersen, a single-stranded RNA/DNA hybrid that acts on its mRNA target in the nucleus via the RNase H endonuclease.

The therapeutic hypothesis is that the reduction of liver-derived circulating mutant and wt amyloidogenic TTR protein by patisiran-LNP will reduce the deposition and promote the stabilization or clearance of TTR amyloid deposits, thereby stabilizing (or maybe even improving) the disease manifestations including polyneuropathy and cardiomyopathy.

2.3. Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on

- hATTR is a rare life-threatening genetic disorder, which leads to death within 5-13 years from diagnosis.
- There remains a high unmet medical need for a safe and effective therapy that can be used by the broader hATTR amyloidosis population.
- Patisiran represents a therapeutic innovation with a completely new mechanism of action to treat ATTR amyloidosis with RNAi resulting in a suppression of the production of both wild-type and mutated TTR protein from the liver.
- From the single pivotal placebo-controlled study, it appears that patisiran was able to stabilise disease severity over 18 months. Patisiran had significant effects on neuropathy, nutritional, autonomic, and QoL-related endpoints and an acceptable safety profile.

Alnylam currently holds orphan drug designations for patisiran-LNP in the United States (Number 12 3711) and the European Union (EU/3/11/857) for the condition ATTR amyloidosis. Additionally, in the United States, patisiran-LNP was granted Fast Track designation for the "treatment of TTR-Familial Amyloid Polyneuropathy" (FDA Ref ID 3398118) and Breakthrough Therapy designations for the treatment of adults with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis)" (FDA Ref ID 4182831).

As a disease that occurs mainly in adults, a pediatric investigational plan waiver was granted by the EMA Paediatric Committee (PDCO) for patisiran-LNP as a treatment of FAP (P/0304/2013). The EMA has confirmed that the "treatment of hereditary transthyretin-mediated amyloidosis" also remains within scope of the Decision. Similarly, patisiran-LNP is exempt from the requirements under the US Paediatric Research Equity Act.

During the development of patisiran-LNP, Alnylam sought scientific advice from the following health authorities: United States Food and Drug Administration (FDA); European Medicines Agency (EMA); French National Agency for Medicine and Health Products Safety (ANSM); Medicine and Healthcare products Regulatory Agency; United Kingdom (MHRA); National Authority of Medicines and Health Products; Portugal (INFARMED); Medicinal Products Agency; Sweden (MPA); and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA). These interactions covered aspects of manufacturing, nonclinical, and clinical development, including the pivotal Phase 3 study (Study 004).

Taking into consideration the rarity of the condition, it was discussed and agreed upon with FDA, EMA, and European national health authorities that a single randomized controlled study (Study 004) could serve as the basis for the marketing authorization application for patisiran-LNP provided that internal and external validity of the study could be demonstrated. A double blind, randomized, placebo-controlled study was therefore considered appropriate. An active comparator was not used in Study 004, as this was a global trial and there is no standard of care worldwide for treatment of the full spectrum of disease severity occurring in hATTR amyloidosis. A double-blind, randomized, placebo-controlled study was therefore considered appropriate.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as concentrate for solution for infusion containing 2.0 mg/mL of patisiran (present as 2.1 mg/mL patisiran sodium) as active substance.

Other ingredients are:

DLin-MC3-DMA ((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate)
PEG_{2000}-C-DMG (α-(3'-{[1,2-di(myristyloxy)proponoxyy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene)
DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine)
Cholesterol
Sodium phosphate, dibasic, heptahydrate
Potassium phosphate, monobasic, anhydrous
Sodium chloride
Water for injections

The product is available as 5 mL concentrate in a Type I glass vial with a Flurotec®-coated chlorobutyl stopper and an aluminium flip-off cap as described in section 6.5 of the SmPC.

2.4.2. Active Substance

General information

The active substance, patisiran, is a chemically-synthesized, double-stranded oligonucleotide. The sense strand and the antisense strand each contain 21 nucleotides. Nineteen nucleotides of the sense strand

hybridize with the complementary 19 nucleotides of the antisense strand, thus forming 19 nucleotide base pairs and leaving the two 3'-terminal nucleotides on each strand as un-hybridized overhangs.

The chemical names of patisiran sense and antisense strands are:

- Sense strand:

  Guanylyl-(3'-5')-(2'-O-methyl)uridylyl-(3'-5')-adenylyl-(3'-5')-adenylyl-(3'-5')-(2'-Omethyl) cytidylyl-(3'-5')-(2'-O-methyl)cytidylyl-(3'-5')-adenylyl-(3'-5')-adenylyl-(3'-5')-guanylyl-(3'-5')-adenylyl-(3'-5')-guanylyl-(3'-5')-(2'-O-methyl)uridylyl-(3'-5')-adenylyl-(3'-5')-(2'-O-methyl)uridylyl-(3'-5')-(2'-O-methyl)uridylyl-(3'-5')-(2'-O-methyl)cytidylyl-(3'-5')-(2'-O-methyl)cytidylyl-(3'-5')-adenylyl-(3'-5')-(2'-O-methyl)uridylyl-(3'-5')-thymidylyl-(3'-5')-thymidine, 20 sodium salt.

- Antisense strand:

  Adenylyl-(3'-5')-uridylyl-(3'-5')-guanylyl-(3'-5')-guanylyl-(3'-5')-adenylyl-(3'-5')-adenylyl-(3'-5')-(2'-O-methyl)uridylyl-(3'-5')-adenylyl-(3'-5')-cytidylyl-(3'-5')-uridylyl-(3'-5')-cytidylyl-(3'-5')-uridylyl-(3'-5')-uridylyl-(3'-5')-guanylyl-(3'-5')-guanylyl-(3'-5')-uridylyl-(3'- 5')-(2'-O-methyl)uridylyl-(3'-5')-adenylyl-(3'-5')-cytidylyl-(3'-5')-thymidylyl-(3'-5')-thymidine, 20 sodium salt.

The molecular formula and relative molecular mass of the patisiran active substance, the sense and antisense strands, as free acid and sodium salt, are summarised in Table 1.

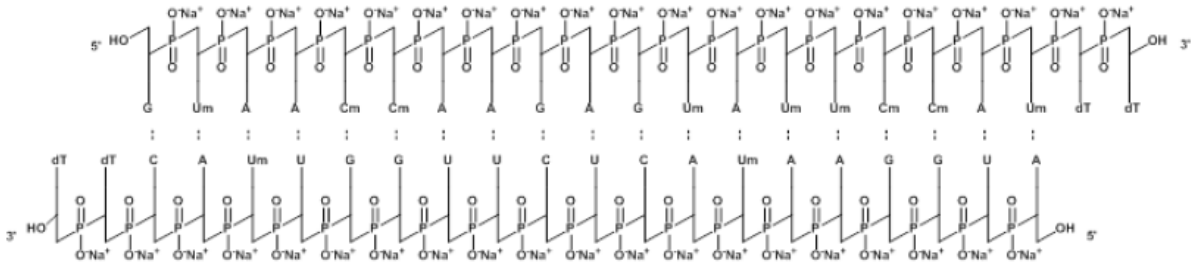
Table 1 Molecular formula and relative molecular mass of patisiran

	Drug Substance	**Sense Strand**	**Antisense Strand**
Molecular formula of the free acid	C_{412} H_{520} N_{148} O_{290} P_{40}	C_{210} H_{268} N_{76} O_{143} P_{20}	C_{202} H_{252} N_{72} O_{147} P_{20}
Molecular formula of the sodium salt	C_{412} H_{480} N_{148} Na_{40} O_{290} P_{40}	C_{210} H_{248} N_{76} Na_{20} O_{143} P_{20}	C_{202} H_{232} N_{72} Na_{20} O_{147} P_{20}
Molecular weight of the free acid	13,424 Da	6764 Da	6660 Da
Molecular weight of the sodium salt	14,304 Da	7204 Da	7100 Da
Monoisotopic molecular weight of free acid	13,418 Da	6761 Da	6657 Da

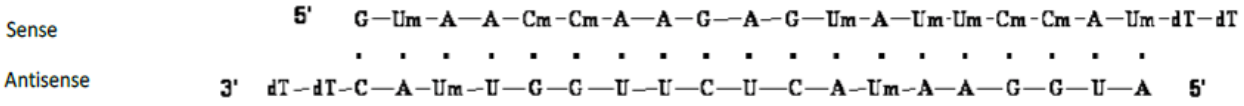
The chemical structure and nucleotide sequence of patisiran active substance is presented in Figure 2, where A, C, G, and U represent adenosine, cytidine, guanosine, and uridine ribonucleotide residues, respectively; Cm and Um represent 2'-O-methylcytidine and 2'-O-methyluridine residues, respectively; dT represents thymidine deoxyribonucleotide residues.

Figure 2: active substance structure

Sense Strand



Antisense Strand



The chemical structures and physical properties of patisiran and the single strand intermediates were elucidated and confirmed by a combination of LC-MS, MS-MS sequence confirmation, thermal dependent UV absorbance, SE-HPLC UV, FAAS, UV absorption, UV spectroscopy, ^{1}H-NMR, Imino-^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, FTIR, circular dichroism, differential scanning calorimetry and thermogravimetric analysis.

The active substance is a white to off-white powder, hygroscopic, soluble in water and phosphate buffered saline.

With respect to stereochemistry, all the pentose moieties of the nucleotides in the patisiran active substance are in the naturally occurring D-ribose form. The ribo phosphoramidite starting materials are diastereoisomeric in nature caused by the chiral centre at the phosphorus atom. The diastereoisomeric purity of these starting materials is confirmed using two orthogonal methods. The chirality of the D-ribose is maintained during the synthesis of the oligonucleotide sequences. Since the ribose moieties in an RNA sequence are restricted to C-3' endo puckering, patisiran molecules adopt the classic A-form. A number of isomeric impurities have been observed under stressed degradation and stability studies.

Polymorphism has not been observed for patisiran.

Manufacture, characterisation and process controls

Patisiran active substance is synthesised in seven main steps, from well-defined starting materials with acceptable specifications.

Step 1 Conventional solid-phase synthesis
Step 2 Cleavage and deprotection
Step 3 Crude ultrafiltration
Step 4 Purification
Step 5 Ultrafiltration
Step 6 Duplex formation (annealing)
Step 7 Lyophilisation

The planned commercial process scale for patisiran active substance has been adequately defined. The specifications and control methods for starting materials and reagents have been presented. Protected phosphoramidites are considered suitable starting materials for synthetic oligonucleotides. Detailed information on the impurity profiles of the phosphoramidite starting materials and classification of impurities as critical or non-critical has been provided. Critical impurities are those which can be incorporated into the product during various steps of synthesis cycle. Non-critical impurities are those species that cannot be incorporated into oligonucleotide during synthesis and thus do not impact the purity of the final product. Unspecified phosphoramidite impurities are by default considered critical. The limits of critical impurities have been justified and are accepted The manufacturing process has been developed using a combination of conventional univariate studies and elements of QbD such as risk assessment, design of experiment (DoE) studies and models. Using small scale models, a process characterization combining previous manufacturing knowledge, multifactorial design of experiment (DoE) and one factor at a time studies (OFAT) were conducted to identify potential critical process parameters, parameter set points, and the normal operating ranges (NORs) and proven acceptable ranges (PARs). A final risk assessment using scientific rationale, process characterization data and manufacturing knowledge was used to assess all process parameters and establish a final set of CPPs.

Based on these studies, proven acceptable ranges have been defined within each of the steps and sub-steps of the manufacturing process of the active substance. The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs. Adequate in-process controls are applied during the synthesis. The in-process controls, analytical methods and their relationship to the active substance CQAs are defined.

The specifications and control methods for intermediate products have been presented. The quality of double-stranded oligonucleotides is pre-determined by the quality of the single strand intermediates, therefore the control of these intermediates by adequate specifications is critical. For example, some of the impurities can only be controlled at the level of the single strand intermediates. Identity by molecular weight and by sequence confirmation is performed for the sense and antisense strand. Consequently, the sequence is of the resulting duplex is also proven. The description for the sequencing method is acceptable and the method has been validated with respects to specificity which is acceptable for an identity test. Batches of sense strand and the antisense strand have been included in the justification of the specifications. The applicant has committed to re-evaluate the specifications for the single strand intermediates specification on the data basis of additional commercial batches. Stability studies for the single strands have also been performed. No degradation has been observed at storage for 6 months at 2-8°C and only minimal degradation has been observed at 25°C/65% RH for three months.

Holding times are proposed for each step of the active substance synthesis. The applicant provided data demonstrating acceptable batch results in relation to these proposed holding times.

The characterisation of the active substance 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.

Impurities referred to as addition sequences are oligonucleotides that contain at least one additional nucleotide. These are referred to as N plus x species (N+x), where N refers to the expected number of nucleotides and x refers to the name of additional nucleotides present. The most common impurity in this category is the N+1 species. These impurities are primarily formed through detritylation of the incoming phosphoramidite during coupling with the acidic activator resulting mainly in a double coupling.

Impurities referred to as deletion sequences are oligonucleotides which are missing one or more nucleotides. The internal deletions are referred to as N minus x species (N-x), where N refers to the expected number of nucleotides in the full-length oligonucleotide and x refers to the deleted nucleotide. These impurities occur due to multiple events, which include incomplete detritylation, incomplete oxidation, and incomplete coupling followed by incomplete capping. These impurities contribute a relatively small amount to the overall impurity profile. The most common impurity in this group is the N-1 species.

Impurities referred to as truncated failure sequences, or terminal deletion sequences which are lacking at least one terminal nucleotide from either end, are another class of oligonucleotide related impurities. Similar to internal deletion sequences, failure sequences are often referred to as N minus x species (N-x), where N represents the number of nucleotides in the desired product and x represents the number of nucleotides missing from that sequence. These impurities are mainly generated during synthesis when the incoming amidite fails to couple and the 5´ hydroxyls of the support bound oligonucleotide intermediate is capped, resulting in capped failure sequences.

The adequacy of the impurity control strategy and suitability of the analytical methods and specifications are discussed further under Specifications. Degradation impurities are discussed under Stability.

Potential genotoxic impurities have been adequately addressed. Some compounds were identified as putative mutagenic/carcinogenic impurities. They are depleted during the manufacturing process and it is agreed that no further controls are necessary for these impurities. Residual solvents are consistently removed in the drug substance manufacturing process and therefore it is acceptable that only the solvent used in the purification process is tested at release Elemental impurities are tested in patisiran by a highly sensitive and selective ICP-MS method. All data have been confirmed to meet the acceptance criterion

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Changes to manufacturers, scale of production and manufacturing process made over the course of patisiran active substance development are described. An analytical comparability evaluation on chemical, physical and bioanalytical attributes of patisiran active substance was performed to demonstrate that patisiran active substance manufactured by the intended commercial manufacturing process and scale is analytically comparable to material manufactured by the previous processes and scales used in clinical development. The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The active substance is packaged in a container which complies with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The active substance specification includes tests for visual appearance, identification by duplex retention time (SE-HPLC UV), identification by molecular mass (IPRP-HPLC MS), identification by Tm(UV Spectrophotometry (Thermal)), sodium content (Flame AAS), impurities (SE-HPLC UV and AX-HPLC UV), assay (AX-HPLC UV), pH (Ph. Eur.), water content (KF), elemental impurities (ICP-MS), residual solvents(GC), bacterial endotoxins (Ph. Eur.) and bioburden (Ph. Eur.).

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines.

There was extensive discussion during the procedure on the adequacy of the impurity test methods to detect and quantify individual impurities and the need for orthogonal methods. The stability indicating non-

denaturing SE-HPLC and denaturing AX-HPLC methods were developed and optimized to detect synthesis impurities or those that result during chain elongation, as well as potential degradants that may occur following exposure to harsh conditions during manufacturing or storage of the active substance. The SE-HPLC method utilizes a size based separation mechanism to ensure that the active substance exists predominantly in the duplex state. The AX-HPLC method separates the target full length sense and antisense strands from synthesis impurities and potential degradants using a charge based separation, primarily determined by length of the phosphodiester backbone.

Impurities present at higher than the qualification threshold were qualified by toxicological and clinical studies and appropriate specifications have been set. The applicant initially proposed to establish the specification limits for active substance based on statistical analysis of release and stability data using a sliding tolerance interval (TI) approach. TIs account for uncertainty in estimation to establish bounds that include a specific proportion of the data with a specific confidence. They have the undesired property of resulting in intervals that are wider the higher the uncertainty is (i.e. the smaller the sample size is). This approach was not accepted by the assessors, as due to the limited amount of batch data available, the resulting specification limits were in certain instances considerably wider than the observed min-max range. In response, the applicant re-evaluated the specifications for duplex purity, sodium content and pH based on ±3SD ranges instead of TI approach. The re-evaluated specifications resulted in tightening of the specifications for each of the attributes. As more information is gathered using the validated process, the applicant is committed to reassessment of the specifications and adjusting, as appropriate based on process capability.

Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. Batch analysis data at commercial scale of the sense and antisense strands and the final active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from four commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 30-48 months under long term conditions (-20°C±5°C) and for up to 6 months under accelerated conditions (25ºC / 60% RH) according to the ICH guidelines were provided. Data from one supportive development batch stored up to 60 months under long term conditions (-20°C±5°C) was also provided.

The following parameters were tested*:* appearance, impurities (SE-HPLC UV and AX-HPLC UV), assay (AX-HPLC UV) and water content (KF). The analytical methods used were the same as for release and were stability indicating.

All tested parameters were within the specification limits at long term storage conditions. Degradation products increased under accelerated storage conditions; impurity peaks that coincide with expected thermal degradants (2',5'-isomers, shortened sequences) were generated.

Photostability testing following the ICH guideline Q1B was performed on two batches. Following exposure, samples were tested for physical appearance, duplex purity by non-denaturing SE-HPLC, assay by denaturing AX-HPLC and purity by denaturing AX-HPLC. The direct exposure sample and its respective dark control met all acceptance criteria following exposure to both cool white fluorescent and near UV light demonstrating that major photolytic impurities were not formed.

Results for forced degradation studies at stress conditions were also provided. Patisiran active substance was exposed to extremes of thermal, acidic, basic, oxidative, and photolytic stress. The level of degradation was

calculated by comparison of the peak area response of the challenge sample to that of a non-stressed control sample. In the denaturing AX-HPLC method, the duplex structure denatures into roughly equimolar mixture of the sense and antisense strands; the peak area response was calculated as the ratio of the stress sample peaks to that of the non-stressed control. The major degradation products of the patisiran substance observed in the forced degradation study include products of depurination, depyrimidation, base cleavage (nucleobases missing from the 3' or 5' end), thymine dimers (only upon extreme photolytic stress), isomers (primarily 2'5'- isomers) or strand cleavage. The major thermal degradants obtained during the forced thermal degradation study are consistent with those obtained following accelerated storage of the active substance. There is little or no occurrence of these degradants at the long-term storage conditions (-20°C±5°C).

The stability results indicate that the active substance manufactured by the proposed supplier(s) is sufficiently stable. The stability results justify the proposed retest period of 36 months stored at -20°C±5°C in the proposed container.

2.4.3. Finished Medicinal Product

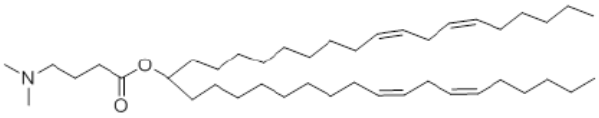
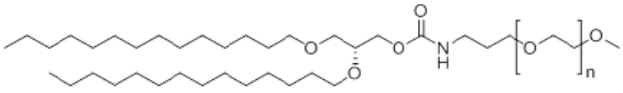
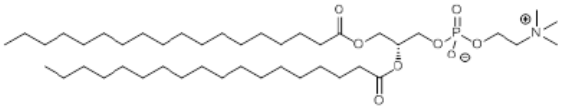
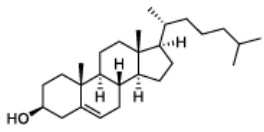
Description of the product and Pharmaceutical development

The finished product is presented as concentrate for solution for infusion containing 2.0 mg/mL of patisiran (present as 2.1 mg/mL patisiran sodium) as active substance. The active substance is incorporated into lipid nanoparticles in a phosphate buffer. The nanoparticles are formed by a mixture of four lipid excipients.

The full list of excipients is included in section 6.1 of the SmPC and in section 2.4.1 of this report. Cholesterol, dibasic sodium phosphate heptahydrate, monobasic anhydrous potassium phosphate, sodium chloride and water for injections are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) is a known pharmaceutical ingredient and its quality controlled to an appropriate in-house specification. DLin-MC3-DMA and PEG_{2000}-C-DMG are novel excipients.

The lipid excipients associate with the siRNA, protect it from immediate degradation in the circulatory system, and aid in its delivery to the target site in the liver. The components of the LNP and the quantitative proportion of each have been determined based on physicochemical and biological properties, as well as stability of the formulation.

Table 2 Lipid excipients in Patisiran finished product

Lipid	Molecular Weight (g/mol)	Chemical Name and Structure
DLin-MC3-DMA	642.09	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate $C_{43}H_{79}NO_2$ 
PEG_{2000}-C-DMG	2650[a]	(R)-2,3-bis(tetradecyloxy)propyl 1-(methoxypoly(ethylene glycol)2000)propyl carbamate or α-(3'-{[1,2-di(myristyloxy)propanoxyy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene $(C_2H_4O)_nC_{36}H_{73}NO_5$ 
DSPC	790.16	1,2-distearoyl-*sn*-glycero-3-phosphocholine $C_{44}H_{88}NO_8P$ 
Cholesterol	386.65	5-cholesten-3β-ol; 3β-hydroxy-5-cholestene $C_{27}H_{46}O$ 

[a]Average molecular weight

DLin-MC3-DMA

The ionisable aminolipid DLin-MC3-DMA confers distinct physicochemical properties that regulate particle formation, encapsulation of siRNA, cellular uptake, fusogenicity (ability to fuse with the endosomal membrane), and endosomal release of the siRNA. This particular lipid was chosen after extensive studies of related cationic lipids. It has been demonstrated that neutral nanoparticles display longer circulation lifetimes resulting potentially in broader biodistribution profiles as compared to more highly charged particles that are more rapidly cleared from the blood.

PEG_{2000}-C-DMG

PEG-lipids stabilize the particle by forming a protective hydrophilic layer that shields the hydrophobic lipid particle. Additionally, by shielding the particles' surface charge, the PEGylated lipid prevents the association with serum proteins and resulting rapid uptake by the reticuloendothelial system when the particles are administered intravenously. It has been found that modulating the alkyl chain length of the PEG-lipid anchor controls the rate of PEG-lipid dissociation from the particle, thereby impacting the pharmacokinetics and pharmacodynamics of the encapsulated siRNA. PEG_{2000}-C-DMG was chosen to allow for dissociation of the PEG-lipid from the circulating particle over time, revealing a particle without PEG that is taken up by hepatic cells, leading to siRNA release.

DSPC

DSPC is a structural lipid for the LNPs, the incorporation of which optimizes stability and encapsulation. DSPC is a naturally occurring phospholipid. The lipid has a phosphotidylcholine head group which is zwitterionic at neutral pH. Diastearoylphosphatidylcholine has been used in approved drug products and is listed in the current FDA Inactive Ingredient Guide.

Cholesterol

Cholesterol is also a structural lipid for the LNPs. It is largely hydrophobic with a polar head group. From a functional perspective, its structure is planar and conformational inflexible. Cholesterol has the ability to affect permeability of lipid systems into which it is incorporated by filling in the gaps created by imperfect packing of other lipid species. It is used in several approved drug products.

Phosphate Buffered Saline (PBS)

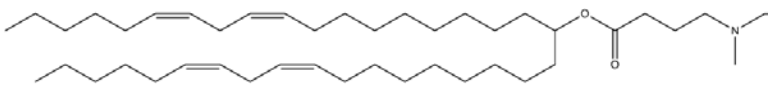
Phosphate buffered saline (PBS) pH 7.4 consists of 10 mM sodium/potassium phosphate and 150 mM sodium chloride and provides an isotonic, neutral medium for parenteral administration.

Novel Excipients

DLin-MC3-DMA

DLin-MC3-DMA is a cationic lipid. with two long unsaturated (linoleyl, C18:2) chains in the structure, each containing four double bonds in the Cis (Z) configuration at the 6 and 9 position. The chemical name of DLin-MC3-DMA is (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate. It has a molecular formula of $C_{43}H_{79}NO_2$ corresponding to a relative molecular mass of 642.09 and a structure as outlined in **Figure 3**. It is not a chiral molecule.

Figure 3 Structure of DLin-MC3-DMA



It is a colourless to pale yellow liquid, very soluble in organic solvents, sparingly soluble in methanol and practically insoluble in water. The molecular structure of DLin-MC3-DMA has been elucidated and confirmed by a combination of UV, IR, MS, ^{13}C-NMR, ^{1}H-NMR and elemental analysis testing.

DLin-MC3-DMA is a chemically-synthesized lipid compound manufactured from well-defined starting materials with acceptable specifications;

The process does not involve any use of animal or human derived materials. Sufficiently detailed information on the route of synthesis is provided in the dossier. Critical quality attributes and critical process parameters have been defined and the in-process controls applied are adequate for this type of manufacturing process.

The information provided on potential and actual impurities in DLin-MC3-DMA comprise related substances, residuals solvents, peroxides, elemental impurities and genotoxic impurities. Characterization of related substances in DLin-MC3-DMA batches has been performed using HPLC-ELSD and samples exposed to stress conditions (70°C, 21 days and oxidation by 0.3% H_2O_2, 3 days) have been used for impurity characterization by LC-MS. The susceptibility of DLin-MC3-DMA to oxidative degradation was confirmed during stress experiments. Lipid oxidation and isomerization impurity structures are assigned based on known literature

precedent and are expected to be mixtures of isomers. Additionally, DLin-MC3-DMA was shown to be susceptible to photolytic degradation. To control the formation of oxidative or photolytic impurities, the excipient is stored at sub-ambient temperature, under an inert cover gas, and under protection from light. Residual solvents and inorganic impurities (including elemental impurities) are adequately characterised and controlled.

Evaluation of potential genotoxic impurities in the synthesis of DLin-MC3-DMA was performed in accordance with the principles stipulated in the ICH M7 guideline. An assessment concluded that the likelihood of the presence of the potential impurities containing structural alerts in DLin-MC3-DMA is low because there are multiple purification steps downstream from formation or use of these compounds. The control strategy proposed has been justified.

The excipient specification includes tests for visual appearance, identification (HPLC-UC, IR, ^{1}H-NMR,^{13}C-NMR) related substances (HPLC-ELSD, HPLC-CAD), assay (HPLC-UV), solubility, water content (KF), peroxide (FOX assay), elemental impurities (ICP-MS), residual solvents (GC), residue on ignition (USP), bacterial endotoxins (Ph. Eur.) and microbiological purity (Ph. Eur.).

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. The applicant has committed to validate the accuracy of the HPLC-ELSD test procedure in accordance guideline ICHQ2 for the known impurity. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Specification limits have been based on pharmacopoeia requirements and statistical evaluation of the available batch results. The presented test results for assay, known impurities, unknown impurities, total impurities, water content, and peroxides from clinical and PPQ batches are consistently lower than the proposed specification limits. The proposed specification limits are accepted however the applicant has committed to reassessment of the specifications once data has been collected on lots of excipient post process qualification. All specifications will be assessed and any modifications will be based on the assessment of process capability, assay performance, and stability.

The container closure system adequately described.

Stability data from production scale batches of excipient from the proposed manufacturer stored in packaging representative for marketing for up to 12-60 months under long term conditions (-20°C±5°C) and for up to 6 months under accelerated conditions (5°C±3°C) according to the ICH guidelines were provided.

During development, the stability of DLin-MC3-DMA has been assessed using analytical methods that evaluate quality, purity, and content. These analytical methods have been modified over time, as product development progressed. Prior to testing of the latest three PPQ batches, the stability protocol was updated to include a more comprehensive summary of specified impurities and their corresponding limits. In order to ensure that the first three stability batches were of comparable quality with respect to key quality attributes, an analytical evaluation was performed by re-testing the first three batches using the updated analytical methodology at 78, 78, and 64 months.

Photostability testing was performed on the excipient in accordance with ICH Guideline Q1B. Significant degradation occurred. Both known and unknown impurities increased . Storage of the excipient at a sub-ambient temperature, under an inert cover gas, and protection from light is expected to limit oxidative and photolytic degradation of this excipient.

Results for forced degradation studies at stress conditions were also provided. The excipient was exposed to thermal, humidity, acidic, alkaline and oxidative stress. At all conditions except for oxidative conditions little

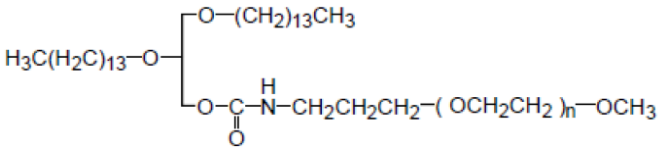
or small increase in impurities occurred As expected at oxidative conditions an increase in some impurities occurred. The assay result decreased correspondingly.

All tested parameters were within the specification limits at long term storage conditions. The stability results indicate that the excipient manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 48 months stored at -20°C±5°C in the proposed container.

PEG_{2000}-C-DMG

The chemical name of PEG_{2000}-C-DMG is (α-(3'-{[1,2-di(myristyloxy)proponoxyy]carbonylamino}propyl)-ω-methoxy, polyoxyethylene). It has a molecular formula of $(C_2H_4O)_nC_{36}H_{73}NO_5$ (n: about 47) corresponding to a relative molecular mass of 2650 ± 300 Da and a structure as outlined in Figure 4.

Figure 4 Structure of PEG_{2000}-C-DMG



It is a white to off white powder, very soluble in water, chloroform and acetonitrile. The molecular structure of PEG_{2000}-C-DMG has been elucidated and confirmed by a combination of orthogonal methods.

PEG_{2000}-C-DMG is a chemically-synthesized lipid compound manufactured from well-defined starting materials with acceptable specifications. The process does not involve any use of animal or human derived materials. Sufficiently detailed information on the route of synthesis is provided in the dossier. Critical quality attributes and critical process parameters have been defined and the in-process controls applied are adequate for this type of manufacturing process.
The information provided on potential and actual impurities in PEG_{2000}-C-DMG comprise process- and product-derived organic impurities, residuals solvents, inorganic impurities (including elemental impurities) and genotoxic impurities is sufficiently well detailed. The origin and fate of impurities has been adequately characterised and an appropriate control strategy has been developed. Residual solvents and inorganic impurities (including elemental impurities) are adequately characterised and controlled.

Evaluation of potential genotoxic impurities in the synthesis of PEG_{2000}-C-DMG was performed in accordance with the principles stipulated in the ICH M7 guideline. The absence of a test for the impurities has been justified in line with Option 4 in ICH M7.

The excipient specification includes tests for visual appearance, identification (^{1}H-NMR), purity and related substances (TLC), average molecular weight (MALDI-TOF-MS), free NHS content (^{1}H-NMR), organic volatile impurities (GC), water content (KF), bacterial endotoxins (Ph. Eur.) and microbiological purity (Ph. Eur.). 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. The specification limits have been justified based on pharmacopoeia requirements and available batch results.

The container closure system including the material of construction was specifically developed for PEG_{2000}-C-DMG and its quality is controlled in line with relevant guidelines.

Stability data from production scale batches of excipient from the proposed manufacturer stored in packaging representative for marketing for up to 36-60 months under long term conditions (-20°C±5°C) and for up to 6 months under accelerated conditions (25°C 60%RH) according to the ICH guidelines were provided.During

development, the stability of PEG_{2000}-C-DMG has been assessed using stability indicating analytical methods. Photostability studies have not been performed on PEG_{2000}-C-DMG. The material upon manufacture is always stored away from light and is explicitly stated on the certificate of analysis "protect from light". Therefore, absence of a specific photostability study was deemed acceptable. All tested parameters were within the specification limits at long term storage conditions and no trends were seen. The stability results indicate that the excipient manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period in the proposed container.

Formulation Development

The therapeutic hypothesis of patisiran finished product is that a small interfering RNA (siRNA; patisiran active substance) targeting transthyretin (TTR) formulated in lipid nanoparticles (LNPs) and administered via intravenous (IV) infusion will enable delivery of the siRNA to the liver, result in RNA interference-mediated reduction in TTR protein and thereby prevent the formation of amyloid fibrils that form the basis of the pathophysiology of hereditary TTR-mediate amyloidosis (hATTR amyloidosis). In order to achieve its therapeutic effect, synthetic siRNAs must be delivered into the cells of the target tissue.

A comprehensive description of the pharmaceutical development of the finished product formulation has been provided. The focus of the development was to enable delivery of the RNA containing lipid nanoparticles (LNPs) to the liver cells, allowing the RNA interference-mediated reduction in TTR protein.

The manufacturing process has been developed using a combination of conventional univariate studies and elements of QbD such as risk assessment, design of experiment (DoE) studies and models. Using small scale models, a process characterization combining previous manufacturing knowledge, multifactorial design of experiment (DoE) and one factor at a time studies (OFAT) were conducted to identify potential critical process parameters, parameter set points, and the normal operating ranges (NORs) and proven acceptable ranges (PARs). A final risk assessment using scientific rationale, process characterization data and manufacturing knowledge was used to assess all process parameters and establish a final set of CPPs.

The LNP is formed by mixing between an aqueous solution containing the siRNA (patisiran active substance) and an ethanol solution containing the 4 lipids. A systematic evaluation of variations of these lipid components was used to develop the LNP formulation used for the finished product. Upon optimisation of the final formulation a model siRNA able to silence the expression of a particular protein expressed in hepatocytes was used. An iterative approach was used that simultaneously examined the effects of using different ionisable lipids and the relative molar ratios of the four lipids. The efficacy of the formulation with patisiran active substance was verified in animal models prior to initiation of Phase 1 clinical studies. Although not a liposome, the physicochemical attributes described in the draft FDA Guidance for Industry: Liposome Drug Products (October 2015) was referenced when characterizing the physicochemical attributes of the LNP in the patisiran finished product. The finished product has the following physicochemical properties.

Morphology

Cryo-transmission electron microscopy (cryo-TEM) images of the product confirm that the product consists of particles. In addition, the images show that the patisiran DP particles do not have the typical bilayer structure of standard liposomes but are spherical nanoparticles with a high electron density core.

Net charge

Electrokinetic potential experiments have shown the range of zeta potential values that patisiran DP has at pH 7.4. A neutral charged nanoparticle is important as a charged particle will have safety issues when

infused. The neutral nature at biologically relevant pH (~7.4) enables adequate distribution within the circulatory system without significant degradation. Once out of biologically relevant pH and in the endosomes of the liver where the pH is acidic (~5), the LNP is broken down and the active substance is released following disruption of endosomal membrane.

Particle size

The finished product particle size impacts the pharmacokinetic parameters. Therefore, it is controlled during manufacture through the LNP formation process. Based on experience with previous LNP formulations, the finished product particle size was chosen to enable delivery primarily to the liver.

The formulation of the LNPs in phosphate-buffered saline and the primary container-closure system remained unchanged throughout all non-clinical and clinical studies of patisiran finished product, and is the same for the intended commercial finished product. The finished product commercial manufacturing process is a scale up of the manufacturing process used for the initial clinical studies. Key changes are increase of the scale and changes to the buffer used to dissolve the active substance. The CPP, non-CPPs, in-process controls are justified in relation to CQAs and risk assessment. Detailed information on the development on the nanoparticle formation step is provided.

The choice of sterile filtration and aseptic processing and filling for patisiran finished product has been justified by the applicant.

An *in vitro* TTR mRNA suppression assay was developed for characterization of finished product activity using the Hep3B human hepatoma cell line. The results from finished product batches demonstrated suppression of TTR mRNA expression by >95%. Cells treated with control articles exhibited no suppression of TTR mRNA levels.

Comparability data to demonstrate similar delivery and release of the siRNA to the hepatocytes for the different size batches tested includes an *in vitro* functional bioassay, *in vitro* release assay, and TTR reduction in hATTR amyloidosis patients in the Phase 3 studies. The limited data submitted with regards to *in vitro* release of the active substance and bioassay does not indicate any substantial differences in quality between the batches. The similarity of product manufactured with the development and commercial scale processes cannot be fully evaluated on quality data alone, due to lack on data from the smaller batch size. The similarity of these batches is concluded based on the combined pharmaceutical, pre-clinical, clinical and pharmacokinetic data. This is discussed further in the clinical part of the assessment report.

Compatibility of the finished product mixed with 0.9 % NaCl solution in two concentration levels using four different application configurations with different materials of construction (DEHP free PVC, or non-PVC infusion components) has been demonstrated for up to 19 hours in the infusion bag, and during an approximate 5 hour infusion time period at 30°C. No data generated at the preferred storage temperature of 5°C has been provided however, based on the storage conditions for the unopened vials it can be concluded that also the diluted product can be stored at 2-8°C.

The primary packaging is a Type I glass vial with a Flurotec®-coated chlorobutyl stopper and an aluminium flip-off cap as described in section 6.5 of the SmPC. The material complies with Ph.Eur. and EC requirements. Extractables and leachables have been studied in sufficient detail. Details of the sterilisation processes for primary packaging components are described in sufficient detail. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

During development the appearance of a white coating on the inner surface of the glass vial at or near the liquid-headspace interface was observed. A root-cause analysis indicated that formation of aggregates

following shipping is an inherent property of the LNP as a reaction to mechanical stress. In the SmPC the user is instructed to withdraw the product with a filter needle to eliminate any aggregates, and to not shake or vortex the finished product vials but to gently invert the infusion bag after adding the solution to avoid detrimental mechanical stress for the product. The filtration step prior to administration effectively removes any aggregates so the presence of the coating does not impact the quality of the product delivered to the patient. During the procedure, additional data has been provided by the applicant with regards to the morphology and internal structure of the lipid nanoparticles and uniformity of composition across the particle size range. The proposed particle size distribution and free siRNA specification limits have been justified from a pharmacokinetic perspective. It has been shown that there is no change in these other quality attributes in the presence of the white coating or following the remedial pre-administration preparation steps proposed in the SmPC.

The impact of the white coating is under confirmatory evaluation using finished product vials subjected to extensive shipping via a transportation study to induce the formation of the white coating under realistic conditions of use, and stored as per the stability protocol at long term (2-8°C) and accelerated (25°C/60% RH) conditions. To date 6 months of stability data on product in which the white coating is present in varying intensities have been provided. The results obtained to date, both at the recommended and accelerated conditions, are consistent with samples that do not display a white coating as well as with a prior study in which 15 months of data were collected. The applicant has committed to continue the stability study to test the impact of the white coating on the finished product quality with commercial scale batch to 36 months of storage at 2-8°C. Out of specification results and obvious trends not in accordance with drug product without the white coating should be immediately communicated to the agency.

Manufacture of the product and process controls

During the procedure a major objection was raised relating to missing confirmation of GMP compliance for one of the manufacturing sites. A GMP inspection of the site was completed by Dutch (NL) inspectorate on 17 May 2018 with no major or critical deficiencies cited. Confirmation of GMP compliance was confirmed and the major objection was resolved.

The finished product manufacturing process consists of five main steps as outlined below:

1. Preparation of active substance and lipid solutions
2. Mixing of solutions to form lipid nanoparticles (LNP)
3. Ultrafiltration, exchange of buffer and initial concentration
4. Dilution to final concentration and bioburden filtration
5. Sterile filtration and filling into vials

Proven acceptable ranges have been defined within each of the steps and sub-steps of the manufacturing process of the finished product. The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs. The in-process controls applied are adequate for this type of manufacturing process and pharmaceutical form. The in-process controls, analytical methods and their relationship to the finished product CQAs are defined.

Holding times are proposed for each step of the finished product manufacture. The applicant provided data demonstrating acceptable batch data and media fill data in relation to these proposed holding times. During the procedure a major objection was raised on the absence of bioburden testing directly before the final

filtration and filling operation The issue was resolved by the applicant agreeing to apply a bioburden test immediately prior to the final filtration and filling operation

The major steps of the manufacturing process have been validated by a number of studies (4 full scale process performance qualification (PPQ) batches of bulk drug product and 3 full scale PPQ batches of final patisiran finished product filled in vials). It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

Product specification

The finished product release and shelf-life specifications include appropriate tests for this kind of dosage form; visual appearance, identification by molecular mass (IPRP-HPLC MS), identification by single strand retention time (AX-HPLC UV), purity and impurities (IPRP-HPLC UV and AX-HPLC UV), assay (AX-HPLC UV), lipid identity (RP-HPLC ELSD), lipid content (RP-HPLC ELSD), duplex (siRNA) encapsulation (fluorometric assay), pH (Ph. Eur.), osmolality (Ph. Eur.), particle size (DLS), elemental impurities (ICP-MS), residual ethanol (GC), residual EDTA (IPRP-HPLC UV), particulate matter (Ph. Eur.) bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), content uniformity (Ph. Eur.), volume in container (Ph. Eur.) and container closure integrity (dye ingress) and siRNA *in vitro* release (kinetic fluorometric assay). The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines.

As for the active substance, there was extensive discussion during the procedure on the adequacy of the impurity test methods to detect and quantify individual impurities in the finished product and the need for orthogonal methods. The specification limits were set following the same rationale as the active substance. The use of the tolerance interval (TI) approach to setting specifications was not accepted by the CHMP, as due to the limited amount of batch data available, the resulting specification limits were in certain instances considerably wider than the observed min-max range. The applicant was asked to propose specifications for the active substance based on ±3SD ranges or smaller. In response, the applicant re-evaluated the specifications for lipid excipient content, particle size, pH, encapsulation, purity and impurities were evaluated against historical process performance and against a shelf life of 24-months based on ±3SD ranges and max-min values instead of TI approach. The re-evaluated specifications resulted in tightening of the specifications for some of the attributes. Based on the results of the evaluation, it may be appropriate to tighten the lipid release specifications beyond their current ± 3 SD ranges following the review however, at present, a sufficient number of data points for the validated larger scale process is not available that would justify tightening of the specifications beyond the ± 3 SD ranges. The historical process performance during the production of clinical lots supports the adequacy of the originally proposed specifications.

A test for *in vitro* release of patisiran from the nanoparticles (by kinetic fluorometric assay) has been included in the finished product specification. The test and release limits are considered acceptable as the limits are based on results from clinical batches. In general, the validation of the current analytical method is accepted, however the acceptance criteria for precision and accuracy, which were related to the specification limits, need to be justified. The applicant has committed to validate a scaled-up method for the siRNA in-vitro release (kinetic fluorometric assay). The validated method along with the adequate dataset, will be submitted in a variation to the EMA, with a target completion by March 2019.

Satisfactory information regarding the reference standards used for assay and impurities testing has been presented. Batch analysis results are provided for several batches manufactured at different scales confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The applicant has committed to continual assessment of manufacturing process capability and the appropriateness of the specifications with respect to process performance. The reassessment is planned once 30 lots of the finished product have been produced using the validated process (approximate timeline from process validation = 2 years).

Stability of the product

Stability data from three batches of finished product stored for up to 36 months under long term conditions (2-8ºC) and for up to 6 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing, were packed in the primary packaging proposed for marketing, and stored in upright and inverted orientations.

Samples were tested for appearance, purity of active substance (duplex and single strands), assay, lipid content, siRNA encapsulation, pH, osmolality, particle size, particulate matter, bacterial endotoxins, sterility and container integrity (dye ingress). All results meet the proposed commercial specifications throughout the 36 months of data collection.

Photostability testing has been performed on two batches of finished product in accordance with ICH Guideline Q1B. The direct exposure sample and its respective dark control met all acceptance criteria following exposure to the light at 1 x ICH Q1B exposure equivalent. Accordingly, no special protection from light is required during storage of the patisiran finished product. Exposure of the patisiran finished product to a 4 x ICH Q1B exposure equivalent resulted in a decrease in the content of the unsaturated lipid DLin- MC3-DMA and a concomitant increase in its likely oxidation products. Photostability studies performed on DLin-MC3-DMA independently of the patisiran finished product indicated sensitivity of the unsaturated lipid to photolytic degradation. However, the rate and extent of degradation is substantially less in the formulated patisiran finished product than in the neat lipid.

Results for forced degradation studies at stress conditions were also provided. The finished product was exposed to heat), acid stress basic stress and oxidative stress. There were varying levels of degradation of active substance and lipid components under all stress conditions. Under all stressed conditions on the encapsulated finished product, reduction in duplex purity was observed). In contrast, the duplex purity for non-encapsulated patisiran active substance was shown to undergo significant reduction under less severe conditions.

An in-use stability study of the chemical and physical stability of the admixture has been assessed under simulated conditions of use, and utilizing clinically relevant concentrations of the patisiran finished product representing two-fold differences in patient weight (50 and 104 kg) at 30°C±2°C/75%±5% RH and ambient fluorescent lighting and following a simulated infusion using clinically relevant equipment, filters, administration sets, and supplies. The results of the in-use stability assessment confirm the physical and chemical stability of the admixture at clinically relevant concentrations following a 16-hour hold-time in the infusion bag (0.9% NaCl) at a customary room temperature (up to ICH climatic Zone IVb), and followed by a simulated infusion under ambient lighting.

Based on available stability data, the proposed shelf-life of 24 months when stored at 2-8°C as stated in the SmPC (section 6.3) are acceptable.

Adventitious agents

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

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.

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. Recommendation(s) for future quality development

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

S.4

1. The applicant is committed to reassessment of the active substance specifications and adjusting as appropriate based on process capability when more batch data is available.

P.2

2. The applicant is committed to continue the new stability study to test the impact of the white coating on the drug product quality with the ongoing commercial scale drug product batch to 36 months of storage at 2°C-8°C. Out of specification results and obvious trends not in accordance with drug product without the white coating should be immediately communicated to the agency.

P.3

3. The applicant has committed to re-evaluate the specifications for the single strand intermediates specification on the data basis of additional commercial batches.

P.4, Control of Novel excipient DLin-MC3-DMA

4. The applicant is committed to perform supplemental validation covering the accuracy of method for DLin-MC3-DMA, including accuracy of the qualified reference impurity, in accordance to the ICHQ2(R1).

5. The applicant is committed to reassessment of the specifications of the excipient DLin-MC3-DMA once data has been collected on additional lots of excipient post process qualification. All specifications will be part of the assessment, and their limits modified, as applicable, based on the assessment of process capability, assay performance, and stability.

P.4, Characterisation of Novel excipient PEG_{2000}-C-DMG

6. For the excipient PEG2000-C-DMG the applicant commits to developing and validating the specific optical rotation method, and setting specification after additional batches of PEG2000-C-DMG have been tested.

P.5

7. The applicant commits to validate a scaled-up method for the siRNA in-vitro release in accordance with regulatory guidelines. The validated method along with the adequate dataset, will be submitted in a variation to the EMA, with a target completion by March 2019.

8. The applicant has committed to continual assessment of manufacturing process capability and the appropriateness of the finished product specifications with respect to process performance. The reassessment is planned once additional lots of the finished product have been produced using the validated process (approximate timeline from process validation = 2 years).

2.5. Non-clinical aspects

2.5.1. Introduction

The drug product (Onpattro) has the form of a lipid nanoparticle (LNP) that contains the double-stranded siRNA (ds-siRNA) patisiran sodium as active pharmaceutical ingredient (patisiran for short, experimental name ALN-18328, CAS no: 1386913-72-9; the whole particle is also described as patisiran-LNP). Patisiran consists of two RNA single strands (A-32345, the sense strand, and A-32346, the antisense strand) and has the chemical formula $C_{412}H_{520}N_{148}O_{290}P_{40}$ (free acid) alternatively $C_{412}H_{480}N_{148}Na_{40}O_{290}P_{40}$ (sodium salt) and a molecular weight of 13424Da (free acid) alternatively 14304Da (sodium salt). The purpose of the ds-siRNA is to inhibit the mRNA expression of human mutant and wild-type transthyretin mRNA (mostly produced in the liver) via the RNA interference pathway. Besides the ds-siRNA, there are four excipients (constituting the LNP) whereof two are novel ones (see below).

2.5.2. Pharmacology

Primary pharmacodynamic studies

The ds-siRNA was designed against a target region in the human TTR gene that seems to be 100% conserved across different human populations (i.e. no polymorphisms). The ds-siRNA was also effective in-vitro against common human transthyretin alleles (including Val30Met, the most common mutation linked to the disease). Potential off-target mRNAs similar to TTR mRNA were identified through bioinformatics analysis, but no clear in-vitro suppression of their expression was measured when tested. The *in-vivo* studies show that the ds-siRNA reduces liver tissue TTR mRNA and serum TTR protein in mice and cynomolgus monkeys. After a single patisiran-LNP dose in naïve cynomolgus monkeys, liver TTR mRNA is reduced between 43% (0.03mg/kg) and94% (0.3mg/kg). Serum TTR protein levels are reduced between 46% (0.03mg/kg) and 75% (0.3mg/kg). For a single dose in cynomolgus monkeys, it takes 4-8d after administration before the greatest TTR reduction is seen and at least 24-28d before TTR levels start to approach pre-exposure levels. The extent of TTR protein suppression in cynomolgus monkeys (65-91%, 0.15-0.5mg/kg) after repeat dosing seems roughly similar between an every 3 week alternatively every 4 week exposure schedule (q3w or q4w) in a repeat-dose study spanning 24w +12w recovery. With repeated dosing in cynomolgus monkeys, it takes 9-

12w (q3w or q4w) before lowest steady state TTR suppression is reached/observed. After the end of repeated dosing (q3w or q4w) over 24w it takes around 12w recovery for TTR protein normalization. In a mouse disease model (the V30M/Hsf-1 KO mouse model), the tissue TTR protein suppression is 97-100% after 6 doses of ALN-TTR01 (LNP with same siRNA as patisiran-LNP) at 3mg/kg (bi-weekly; only one dose was tested).

Secondary pharmacodynamic studies

Patisiran-LNP treatment (0.3-3mg/kg) in cynomolgus monkeys reduced serum TTR protein levels in a manner that was correlated with a reduction in TTR protein associated molecule levels (i.e. Retinol binding protein (RBP), Vitamin A and T_4 – but not T_3). After repeated dosing between 0.3mg/kg and 3mg/kg for 4 doses bi-weekly and 14 doses every three weeks, a max T_4 hormone reduction of 46-50% was measured. Vit A was reduced starting after 48-168h in cynomolgus monkeys at all tested doses (0.3 to 3 mg/kg) with 42-70% at the lowest dose and between 50-79% at the highest dose (increasing reduction with additional doses). Overall, the treatment effects on TTR interaction targets tend to occur rapidly (within 2-3w of the first dosing) but the recovery to pre-exposure levels is much slower after repeated dosing at higher doses (i.e. ≤60d for lower doses but >60d at the max dose of 3mg/kg). Treatment impact on cytokine levels have also been studied (see toxicology section).

Safety pharmacology programme

In-vitro hERG testing was not conducted for the ds-siRNA but only for the possible impact from the LNP (with patisiran exchanged for an insect luciferase siRNA with no target in the human genome). There was no indication that the LNP in itself can inhibit the hERG channel (see Discussion on non-clinical aspects). Increased body temperature and heart rate were seen in cynomolgus monkeys after a single IV 60min infusion dose at ≥3mg/kg (max dose at 6mg/kg), giving a NOEL and NOAEL for patisiran-LNP at 1mg/kg and a LOAEL of 3mg/kg (see Discussion on non-clinical aspects). For one animal at 3mg/kg and two animals at 6mg/kg, the effects remained 39-47h after infusion. No neurological effects were detected.

Excipients: The LNP component of patisiran-LNP has the overall purpose of supporting the targeting of and uptake in liver cells and contains four lipid excipients relevant for its structure and functionality. Among those, DLin-MC3-DMA and PEG_{2000}-C-DMG are novel excipients. The DLin-MC3-DMA is a 0.642kDa lipid and the PEG_{2000}-C-DMG is a 2.5kDa PEGylated lipid. The stated role of the DLin-MC3-DMA lipids is to support particle formation, cellular uptake via endocytosis, and endosomal release of the ds-siRNA into the cytosol. DLin-MC3-DMA on its own is highly insoluble in aqueous solutions, the lowest detected soluble concentration being 3uM (4.3% of 1x Cmax from Clinical Phase I studies). PEG_{2000}-C-DMG is exposed on the surface of patisiran-LNP and has the purpose to stabilize the LNP particle by forming a protective hydrophilic layer that shields the hydrophobic lipid particle and inhibits association with plasma proteins and cell surfaces.

Pharmacodynamic drug interactions

No studies have been conducted to evaluate pharmacodynamic drug interactions. The potential for pharmacodynamic drug interactions with patisiran-LNP in humans is expected to be low since there are no other TTR suppressive agents or other agents that affect TTR production. This has been considered acceptable by the CHMP.

2.5.3. Pharmacokinetics

The non-clinical pharmacokinetics studies for patisiran-LNP characterized the ADME aspects of the ds-siRNA and the two novel excipients in the LNP (DLin-MC3-DMA and PEG_{2000}-C-DMG) but not the other two more established LNP excipients (cholesterol and DSPC). The siRNA measurements in plasma used LC-MS/MS methods while measurements in other tissues (e.g. liver, spleen) used binding-based methods (primarily non-validated PNA-probe HPLC methodology). Excipients were measured using LC-MS/MS.

Plasma kinetics: In Sprague Dawley rat exposed to a single IV bolus dose (0.03, 0.30 or 1.0mg/kg) of patisiran-LNP, the systemic plasma levels (Cmax: low dose 0.587-0.595ug/mL to max dose 25.1-26.65ug/mL; AUC_{0-t}: low dose 0.212-0.294ug x h/mL to max dose 12.7-13.8ug x h/mL) of the ds-siRNA were more than dose-proportional primarily between the low and middle dose (12-13x vs external 10x dose ratio) and the low and max dose (42x-60x vs external 33x dose ratio). The rat clearance was between 72.3 and 101mL/kg/h and the $t_{1/2}$ 0.23-0.44h. In cynomolgus monkeys exposed to a single dose (same doses as rat) of patisiran-LNP via 1h infusion, the systemic plasma levels of the siRNA (Cmax: low dose 0.203-0.258ug/mL to max dose 16.5-18.1ug/mL; AUC_{0-t}: low dose 0.292-0.336ug x h/mL to max dose 19.9-28.8ug x h/mL) were also more than dose-proportional between the low and middle dose (12-19x vs external 10x dose ratio) and the low and max dose (64x-89x vs external 33x dose ratio). The monkey clearance reduced with increasing dose (135-164 to 29.8-49.5mL/kg/h) while the $t_{1/2}$ increased with dose (males 3.52 to 14.7h, females 10.2 to 19.1h). This dose-related increase in $t_{1/2}$ was also observed in toxicological repeated dose studies in monkey.

DLin-MC3-DMA: In Sprague Dawley rat, DLin-MC3-DMA is roughly dose-proportional in rat plasma levels compared to the external doses (0.03 to 1.00mg/kg single bolus dose of patisiran-LNP). The differences for Cmax (low dose 3.55-3.72ug/mL to max dose 141-151ug/mL) and AUC_{0-t} (low dose 5.97-6.23ug x h/mL to max dose 218-227ug x h/mL) between the low and max dose is slightly more than dose-proportional. No dose-relation for the clearance is observed but the half-life increases with dose (from low dose 82.3-177h to high dose 246-288h). In cynomolgus monkey plasma, DLin-MC3-DMA is more than dose proportional for Cmax (low dose 1.82-2.02ug/mL to max dose 122-136ug/mL) and somewhat less than dose-proportional for AUC_{0-t} (low dose 50.7-63ug x h/mL to max dose 1551-1850ug x h/mL). There is no change in clearance and no consistent dose-dependent increase in $t_{1/2}$ (ranging between 428 and 780h).

PEG_{2000}-C-DMG: Only the max dose (1.0mg/kg) of patisiran-LNP was evaluated for PEG_{2000}-C-DMG plasma levels in the single dose rat and cynomolgus monkeys studies. The Cmax was 15.1-16.5ug/mL for rat (bolus dose) and 16-8-18.2ug/mL for monkey (1h infusion). For the AUC_{0-t}, rat has 20.6-23.5ug x h/mL whereas monkey has 220-225ug x h/mL. Clearance is 36.5mL/kg/h for rat and 3.48-3.49 mL/kg/h for monkey. The half-life is much longer for PEG_{2000}-C-DMG in monkey (160-162h) than in rat (21.3h). Based on the publication of Mui & Tam (2013; DOI: 10.1038/mtna.2013.66), PEG_{2000}-C-DMG desorbs from the LNP in mouse plasma following IV infusion with an estimated desorption rate of 45% per hour. Within 2 hours after IV administration, 80% of PEG_{2000}-C-DMG has desorbed from the LNP.

Anti-drug antibodies (ADA) against PEG_{2000}-C-DMG were reported in rats and monkey studies, indicating a potential decrease of exposure in the 26-week study in rats (measured in toxicology studies). There were some issue with some preclinical studies showing pre-dose ADA (in the 6-week rat and 6-week monkey toxicology studies) but these measurements are considered unlikely to be relevant. The applicant specified that this measure is a false positive due to a nonspecific binding in serum when compared to diluent. This is supported by the very limited number of animals having confirmed ADA in the long term GLP studies.

Tissue distribution: Patisiran-LNP had a low binding to rat serum albumin (0.89%), human serum albumin (0.46%), and human α1-acid glycoprotein (2.07%). No data were given for the monkeys despite being a pharmacologically-relevant species but the applicant has justified this with a pharmacodynamics rational (i.e. monkey and human having a similar profile of decrease in serum TTR for the same dose level).

Based on male adult rat autoradiography (QWBA) studies with 0.3mg/kg single dose IV patisiran-LNP with radiolabelled ^{14}C-DLin-MC3-DMA, most of the radioactivity (>90%) is rapidly distributed to the liver (Cmax 69.5ug equiv/g tissue > ULOQ and tmax 1-3.5h in Sprague Dawley rat; 73.9ug equiv/g tissue > ULOQ and tmax 1-6h in Long Evans rat) which is also the main target for organ toxicity (see the toxicology section and Discussion on non-clinical aspects). While the clear majority of the ALN-TTR02 dose was found in the liver, the highest signal was found in the posterior lymph node (Cmax 176.6-275.7ug equiv/g tissue (> ULOQ) with tmax 48-168h). Other weak to moderate uptake organs ranked on estimated uptake of total dose were spleen (1.61-1.67% of total dose), kidney (0.14-0.42% of total dose), lungs (0.21-0.57% of total dose), heart (0.07-0.24% of total dose) and adrenals (0.03-0.14% of total dose). Additional organs/tissues with a weak to moderate uptake are the small intestine, the bone marrow and the prostate (0.90-0.292ug equiv/g) (see also Discussion on non-clinical aspects). There was very little uptake in the central nervous system tissues (≤0.070ug equiv/g, tmax 0.25-24h) in both rat stocks, corresponding to less than 0.02% of the total dose. That being said, there was some initial uptake in the meninges of rats (1.16-4.2ug equiv/g, tmax 0.25-1h) directly after infusion. No pigmentation-dependent differences were observed.

Overall, mainly the lipid part of the LNP is taken up and broken down in liver cells, endothelial cells and phagocytic cells. Organs/tissues with cells rich in LDL-like and related scavenger receptors and/or where a fenestrated endothelium exists are also capable of taking up the LNP (e.g., lymph nodes, spleen, bone marrow vessels, and adrenal gland). The rat quantitative QWBA study and repeat-dose toxicity findings are consistent with uptake of the LNP in these organs (i.e., spleen, lymph nodes, bone marrow, and adrenal gland).

ds-siRNA liver distribution: While much of the patisiran-LNP is rapidly shifted to the liver (tmax ~1h), the initial uptake of patisiran-LNP into hepatocytes is slower as most of the radioactivity signal is found in the liver sinusoid lumens. For cynomolgus monkeys, the liver tmax for the ds-siRNA is estimated to 2h. In rat, after 24h-72h, the radioactive signal starts to increase in the hepatocyte cell cytoplasm and in cells of the hepatic vasculature, becoming roughly equal to vascular lumen signals (unclear if also uptake in Kupffer cells) (see also Discussion on non-clinical aspects).

DLin-MC3-DMA liver distribution: The DLin-MC3-DMA excipient (measured with LC-MS/MS) had a rat liver specific tmax between 4 and 24h and a cynomolgus monkeys liver tmax of 5-15h (no clear dose-dependency). Both liver Cmax and AUC in monkeys increase with increasing dose with a profile indicating that the uptake is more than dose proportional (11.1-16x vs external 10x dose ratio) between the low dose (0.03mg/kg) and middle dose (0.30mg/kg) and proportional between the middle dose and high dose (1.0mg/kg). In rat, it is estimated that 77.7% of the DLin-MC3-DMA in the total patisiran-LNP dose is located in the liver (50% left after 7d, 25% left after 14d and 3.14% left after 60d). In monkeys, the liver has >97% of the DLin-MC3-DMA of the total patisiran-LNP dose for the first 7d after the dose, 43.2% after 14d and 25% after 28d.

Regarding the impact of DLin-MC3-DMA on the liver, the applicant estimates DLin-MC3-DMA to have unbound plasma Cmax of ~3.1uM and a liver Cmax of ~6.7uM. Based on that in-vitro tests used up to 30uM and did not detect CYP induction/inhibition effects, the applicant argues that it is unlikely that clinical DLin-MC3-DMA concentrations would result in significant clinical inhibition or induction of liver CYP enzymes. Also, for tests using up 3uM (e.g. efflux proteins), there is no indication of a trend that would indicate a molecular effect.

PEG$_{2000}$-C-DMG liver distribution: The PEG$_{2000}$-C-DMG excipient (measured with LC-MS/MS, and only based on one dose: 1mg/kg) had a rat liver specific tmax of ~1h (single bolus dose) and a cynomolgus monkey liver tmax of 2h (1h infusion). After 1h, there is 48.9% of the PEG$_{2000}$-C-DMG from the total patisiran-LNP dose in the liver and 10.4% after 24h and 0.81% after 7d. In cynomolgus monkeys liver, 10.5% is left after 24h and 0.86% after 7d. The PEG$_{2000}$-C-DMG liver Cmax is 7.27-8.71ug/mL in rat and 1.92-3.4ug/mL in cynomolgus monkeys. The AUC$_{0-t}$ is 196-207ug x h/mL in rat and 5.52-6.19ug x h/mL in cynomolgus monkeys. The liver $t_{1/2}$ is calculated to 207h in male rat (not reported in female rat or cynomolgus monkeys).

Metabolism: Based on biochemical testing with different species serum samples, the proportion of intact siRNA in a patisiran-LNP formulation after 24h was 62-71% in rodents (CD-1 mouse and Sprague-Dawley rat), 42-44% in cynomolgus monkeys and ~100% in human. In cynomolgus monkeys liver microsomes from patisiran-LNP treated animals (1mg/kg/day 1h IV, biweekly for 4 doses), there were no significant changes in the protein levels/activities of CYP1A1/2, CYP2C76, CYP2C43, CYP2D6, CYP3A4 or UGT1A1. For liver cytosol, there were some indications (although but not fully replicated) that ALN-18328 was also more stable over time in human samples compared to cynomolgus monkey and rat. In contrast, for free siRNA, and supporting the functionality of the LNP, there is <1% left intact after 24h in serum or liver+S9 samples independent of species. The degradation of the siRNA occurs via exonuclease activity. When measured in rat urine, no intact ds-siRNA can be detected within 29d after a single bolus dose.

Table 3 A cross species comparison of metabolite profiles in vitro and in vivo

		ALN-18328	DLin-MC3-DMA	PEG$_{2000}$-C-DMG
Rat	In vitro	Fragments of oligo [a]	Single and double oxidation metabolites [d]	Stable with low level of species independent O-detetradecylation products [g]
	In vivo	Plasma: Predominantly unchanged ALN-18328 [b] Urine: Major metabolites: S(N-3)3', S(N-4)3', and AS(N-3)3' [b]	Plasma: Predominantly unchanged parent with <10% each of single, double oxidation metabolites, and DMBA [b, e] Urine: DMBA as a major metabolite [b, e]	Urine: no metabolites associated with PEG$_{2000}$-C-DMG [b]
Monkey	In vitro	Fragments of oligo [a]	Single and double oxidation metabolites [d]	Stable with low level of species independent O-detetradecylation products [g]
	In vivo	Plasma: Predominantly unchanged ALN-18328 [c]	Plasma: Predominantly unchanged parent with <5% each of single and double oxidation metabolites [c] Urine: DMBA as a major metabolite [c]	N/A
Human	In vitro	Fragments of oligo [a]	Single and double oxidation metabolites [d]	Stable with low level of species independent O-detetradecylation products [g]
	In vivo	N/A	Urine: DMBA as a major metabolite [f]	N/A

Abbreviations: DMBA=4-(dimethylamino)butyric acid; N/A=not applicable; PEG$_{2000}$-C-DMG=(R)-methoxy-PEG$_{2000}$-carbamoyl-di-O-myristyl-sn-glyceride.
[a] Source: TTR-ST10-008, BA16027
[b] Source: TTR02-DSM17-002
[c] Source: TTR02-DSM16-013
[d] Source: 319N-1002
[e] Source: 319N-1201
[f] Source: Clinical Study ALN-TTR02-001 and ALN-TTR02-002
[g] Source: 319N-1305

DLin-MC3-DMA: The in-vitro metabolite profiles of DLin-MC3-DMA were similar based on mouse, rat, monkey, and human liver microsomes or S9 incubations. In rat, 6 DLin-MC3-DMA metabolites (mostly various mono and di-oxidation metabolites) were identified whereof one, the hydrolysis metabolite M6 (4-Dimethylaminobutyric acid or DMBA; also detected in humans) was found in all tissues studied (only at <3% in plasma). In cynomolgus monkeys 65-68% of the DLin-MC3-DMA radioactivity was based on the parent compound. Three metabolites were detected in cynomolgus monkeys;one corresponded to DBMA and was found in both plasma (<1% of the total dose) and excretions (mostly in urine but also in faeces). A second plasma metabolite, Dioxy-DLin-MC3-DMA, was found in monkey plasma at 2-5%. Overall, in rat and monkey plasma, no metabolite was >10% of the total dose of DLin-MC3-DMA.

PEG_{2000}-C-DMG: Based on mouse, rat, monkey, and human liver microsome or S9 samples, a total of 6 PEG_{2000}-C-DMG O-detetradecylation metabolites were detected in cynomolgus monkeys and human (but not in rodents). None of the PEG_{2000}-C-DMG or its metabolites was detected in rat urine within 29d after a single bolus dose.

Elimination: In both rats and monkeys, patisiran-LNP radioactivity was primarily recovered in the urine (~49% in rats and ~50% in cynomolgus monkeys) with some excreted in the faeces (~24% in rats and ~10% in monkeys). In rats, most of the excreted dose is found in the urine from the first post-dose week (~34.5% of the total dose). No intact ds-siRNA was excreted in urine or faeces in rats or monkeys. Intact rats showed a urinary DLin-MC3-DMA elimination of ~33% of the total dose. No intact DLin-MC3-DMA was found in monkey urine.

PEG_{2000}-C-DMG: In cynomolgus monkeys, there was approximately 44% of PEG_{2000}-C-DMG excreted in the faeces. PEG_{2000}-C-DMG was not detected in urine.

Drug interactions:

In a 6-week monkey toxicity study, following 4 doses of patisiran-LNP by IV infusion (1 mg/kg, q2w x 4), there were approximately 97% reductions in liver TTR mRNA concentrations (relative to control), 90% reductions in TTR serum protein concentrations (relative to baseline), and no inhibition or induction of hepatic CYP1A1/2, CYP2C76, CYP2C43, CYP2D6, CYP3A4 (both midazolam and testosterone as substrate), and UGT1A1 was observed. This data suggests that patisiran-mediated reductions in hepatic TTR mRNA and serum TTR protein did not impact the activity of several CYP isozymes.

ALN-18328, PEG2000-C-DMG, or DMBA, the hydrolysis metabolite of DLin-MC3-DMA do not appear to be inhibitors or substrates of the transporters OATP1B1 (CHO), OATP1B3 (CHO), OAT1 (CHO), OAT3 (HEK293), OCT1 (CHO), OCT2 (CHO), MATE1 (MDCKII), and MATE2-K (MDCKII), at concentrations up to 50X the observed Cmax in humans at 0.3 mg/kg (7.15 μg/mL [ALN-18328], 40.2 μg/mL [DLin-MC3-DMA], 4.22 μg/mL [PEG2000-C-DMG]).

Taken together, the in vivo and ex vivo data on enzyme inhibition/induction, protein binding and transporter interactions on the components of patisiran-LNP (ALN-18328, DLin-MC3-DMA, and PEG2000-C-DMG) suggest a low propensity to cause drug interactions with co-medications likely to be administered with patisiran-LNP.

2.5.4. Toxicology

A comprehensive program of toxicology studies has been performed with patisiran-LNP, which includes single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, and reproductive toxicity.

Supplementary studies have been performed to evaluate excipients and immunostimulation. TK monitoring has been included in the pivotal studies and some additional studies. All pivotal studies are considered GLP compliant. However, it is noted that site in the USA that performed for the phases of analysis of cytokine and complement in the pivotal toxicity study on monkeys (TR02-NCD12-001) has never been inspected by US FDA to verify its claim of GLP compliance. However, cytokine and complement were not changed when measured after single –dose monkey study with ALN-18328 (study TTR-NCD09-004). During the repeated studies in monkeys, it was observed that administration of patisiran-LNP or AF-011-1955 resulted in transient complement activation (C3a and Bb) shortly after dosing that was not accompanied by any adverse clinical signs. Moreover complement activation in monkeys is a known effect following siRNA administration (Mustonen et al. 2017). Thus, the analysis performed by the laboratory report expected effects.

Patisiran is a siRNA directed against human TTR mRNA and has cross-reactivity in the cynomolgus monkeys, but not in rat mouse or rabbit. As a consequence, general toxicology studies have been performed in cynomolgus monkeys (pharmacologically active) and rats (off-target). To determine whether any observed toxicities were related to the specific siRNA contained in the formulation, nonspecific siRNA effects, or to the LNP formulation itself an LNP that contained siRNA directed against insect luciferase mRNA (AD-1955) was included in general toxicology studies. To evaluate on-target reproductive and developmental toxicities related to siRNA- mediated reduction in serum TTR concentrations (TTR reduction and associated reductions in vitamin A and thyroxine) the rodent reproductive and developmental toxicity studies included a rodent surrogate siRNA (AD-18534). IV infusion was used in the repeat-dose toxicology studies with in general a once every two- or three-week dosing interval to support the clinical route and timing of dose administration.

Separate toxicology studies were not conducted with the 2 novel lipids, DLin-MC3-DMA, and PEG_{2000}-CDMG. However, the TKs of the lipids were evaluated in almost all general toxicity, carcinogenicity, and reproductive and developmental toxicity studies, why it can be argued that the lipids have been evaluated in the studies.

Due to a change in manufacturing scale, a single-dose intravenous infusion bridging comparability study in monkey was performed. Similar PD, PK, and safety were observed following a single IV infusion of patisiran-LNP produced by either Alnylam or NLI. However, it should also be pointed out that an in vivo study with the above described design is not sensitive enough to pick up (safety) issues related to the relatively small changes made in the composition.

The liver is the primary site of patisiran-LNP uptake and accumulation, and hepatotoxicity determined the NOAELS in all non-clinical species. Further target organs of toxicity include spleen, bone marrow, lymph nodes and adrenal gland, which is also consistent with the uptake of patisiran LNP into sinusoidal organs with fenestrated endothelium. Overall, the toxicities seen are not primarily related to the pharmacology of the TTR-targeting siRNA but are more likely derived from the LNP. This is supported by the similarities in toxicological profile in the general toxicology studies regardless of siRNA target in the LNP (insect luciferase or human TTR).

Single dose toxicity

A single-dose IV study with the unformulated siRNA in monkeys at 100 mg/kg showed no toxicities even at the highest dose. ALN-18328 is not heavily chemically modified for stability, and when unformulated, it is rapidly metabolized and cleared from the circulation.

Repeat dose toxicity

Primary target organs of toxicity for patisiran-LNP were the liver and spleen. Secondary target organs included spleen, adrenal glands, testis, lymph nodes and the infusion sites.

Liver**:** Hepatocyte single-cell necrosis was a common finding across all general toxicology studies and species and ranging from minimal to marked severity. It was noted in rats at ≥0.1 mg/kg/q4w for 4 weeks and 0.15 mg/kg/q2w for 6 weeks. In monkey the finding was evident at 3 mg/kg/q2w for 6 weeks and at ≥1 mg/kg/q3w for 39 weeks. The necrosis was partially or fully reversible across studies. In addition, hepatocellular vacuolation (ranging from minimal to marked severity) was present in rats at ≥0.15 mg/kg/q2w for 6 weeks and at 0.3 mg/kg/q2w for 26 weeks, and monkeys at 3 mg/kg/q2w for 6 weeks and at ≥1 mg/kg/q3w for 39 weeks. In all of the species, reversible increased mixed cell inflammation/infiltration (of minimal tomarked severity) was observed that was sometimes accompanied by inflammation-related changes in hematology. Overall, the liver findings were correlated with changes in liver enzymes and were the primary drivers of NOAELs in the studies.

It is noted that no single cell necrosis or adverse findings are found in the 26-week rat study. However, as noted in the toxicokinetics section, systemic exposure to ALN-18328 was not detected at the end of the dosing period in any dose group and around 50% of the animals had detectable ADA directed against PEG_{2000}-C-DMG. Thus, this study is not considered to fully reflect the toxicity of patisiran-LNP.

Infusion site findings**:** Infusion site findings were noted in all repeated dose toxicology studies, and were associated with vascular/perivascular inflammation at the injection site. Overall the findings were correlated with increases in inflammatory markers (monocytes, lymphocytes and NEU counts). Similar findings were noted in the 6-week monkey study and 39-week monkey study (similar incidence and severity in controls and treated animals).

Spleen and Lymph nodes**:** The white pulp and marginal zone of the spleen were affected across rodent studies with lymphoid depletion/atrophy/necrosis observed in mice and rats along with histiocytosis in rats. No concomitant decreases in circulating lymphocytes or other signs of immunotoxicity were noted.

Increased extramedullary haematopoiesis was observed in spleen of rats and mice following patisiran-LNP administration. In monkeys, red pulp hypocellularity was observed following patisiran-LNP administration. All of the splenic changes in rodents and monkeys were reversible.

In rats, reversible lymphoid and stromal cell hyperplasia, histiocytosis, and inflammation were observed in lymph nodes in the 6-week (q2w×4) toxicity study but not in subsequent studies. There were no lymph node findings in mice or monkeys.

Bone marrow**:** Bone marrow findings were present but of minor toxicological concern across species. They were limited to a minimal to moderate increases in myeloid cellularity. In rats, minimal to slight increases in hematopoietic hypercellularity in the bone marrow were reported at ≥0.15 mg/kg/q2w for 6 weeks (lowest dose tested) and at 1 mg/kg/q4w for 4 weeks. In the 26-week study in rats, increased M:E ratios were observed at ≥0.l mg/kg/q2w. In the 6-week (q2w×4) monkey study, increased M:E ratios were observed at≥ 1 mg/kg/q2w. No findings were evident in the 39-week study in cynomolgus monkeys. However, as noted above, clearing antibodies significantly reduced exposures.

Testis**:** In the 6-week general toxicity study (q2w×4), testicular/epididymal toxicity was noted in 2 males ≥1.8 mg/kg. Findings included severe degeneration/atrophy of the seminiferous epithelium and marked oligo/aspermia. It is notable that the findings in the testis were still present in the LD and HD even after the

lengthy 60-day recovery period. However, the findings were not observed in longer duration rat or monkey studies, and no effects were evident on male rat fertility parameters or on sperm parameters in the 39 week monkey studies. Thus, while recognized, the finding is not further pursued.

Adrenal gland**:** Minimal to slight cortical hypertrophy and increased absolute and relative adrenal weights (cortical hypertrophy) were noted in the 6-week rat study at ≥0.8 mg/kg. There were no findings after recovery and no adrenal gland findings in the 26-week study. Decreases in cortical vacuolation zona fasciculata) at 3 mg/kg/q2w in the monkey 6-week study was not replicated in the longer monkey study.

Genotoxicity

Patisiran-LNP (10 mg/mL) was neither mutagenic in the bacterial reverse mutation assay, nor clastogenic or aneugenic in the mammalian chromosome aberration assay in human blood peripheral lymphocytes. While precipitation was a major problem in the in vitro studies, the validity of the studies has not been compromised. Single IV doses of patisiran-LNP at ≤ 30 mg/kg (MTD) did not induce micronucleus formation in the bone marrow of male and female CD-1 mice.It can be concluded that patisiran-LNP was not genotoxic in either the in vitro or in vivo assays.

Carcinogenicity

A 2-year carcinogenicity study of patisiran-LNP in rats was not conducted because of the technical challenges of long term IV administration of patisiran-LNP, and the immunogenicity (development of ADA) that was observed in the 26-week chronic rat study. However, a 26-week carcinogenicity was performed in TgRasH2 mice.

Survival rate was high (88-100%) in both controls and patisiran-LNP-treated groups, whereas in the positive control group there were significant decreases in male and female survival rates (40% and 16% respectively). Lung neoplastic findings (bronchioalveolar carcinoma and adenoma) were observed in treated male rats (0/25, 2/25, 4/25, and 3/25 males at 0, 0.5, 2, and 6 mg/kg/q2w patisiran-LNP, respectively). It is striking that no lung neoplastic findings is noted in the control group, whereas a total of 9 cases (in 75 animals) were found in the treated groups. However, these tumors are common spontaneous tumors in TgRasH2 mice, and the findings in the study are not further considered.

Reproduction Toxicity

Since ALN-18328 is not pharmacologically active in rats or rabbits, the rat-specific surrogate formulation AF-011-18534 was used in the reproductive and developmental toxicity studies in rats to assess potential on-target pharmacological effects of patisiran-LNP on reproduction and development.

Fertility and embryo fetal development**:** No effects were noted on male or female reproductive parameters in a combined fertility and embryo fetal development study. Despite reductions in serum TTR(-90%), vitamin A (-79%) and thyroxine (-68%) which demonstrated the lack of a pharmacological effect of the patisiran-LNP surrogate on fertility or reproduction in males. At 1.5mg/kg AF-011-18534 there were increased foetal and litter incidences of foetuses with fused, wavy and thickened ribs. In addition, a statistically significant increase in the litter incidence of bifid thoracic centra was noted at 0.15mg/kg patisiran, and also at lower incidence 1.5mg/kg patisiran and AF-011-18534. However, these findings are considered developmental delays.

One foetus in the AF-011-18534 group had severe dilation of the lateral ventricles of the brain and a domed head. While a serious malformation, a single foetus without similar findings in other foetuses is considered a chance finding.

In the rabbit embryo foetal development study, an increased incidence of adverse clinical signs, reduced maternal body weight gain, and reduced food consumption were observed at 0.6mg/kg. Thus, maternal NOAEL for patisiran-LNP is considered to be 0.3 mg/kg. Foetal visceral malformations, skeletal malformations and skeletal variations are present in single foetuses scattered at across dose groups without apparent systematicity. However, at 0.3mg/kg, one embryo presented dilated aortic arch and narrow pulmonary trunk and another embryo from a different litter presented persistent truncus arteriosus and ventricular septum defect. While seemingly disparate, they are all aspects of heart malformations sometimes seen in syndromes. Thus, the applicant was asked to further discuss these malformations and pay particular attention to a possible mode of action and transfer over the placenta of the different parts of the product. In the response, it was agreed that the mode of action behind the slight increase in foetal heart malformations at the mid-dose (0.3 mg/kg) in the pivotal embryo-foetal development study in rabbits is unknown. The Applicant further described with data that in the dose range-finding embryo-foetal development study in pregnant rabbits, the foetal concentrations of foetal ALN-18328 and PEG_{2000}-C-DMG concentrations were below detection limits at 0.3 mg/kg. In addition, the mean foetal DLin-MC3-DMA concentration was 0.18% of its corresponding maternal plasma Cmax value. It is thus considered unlikely that the malformations are attributable to the patisiran-LNP components.

Prenatal and postnatal development, including maternal function**:** The NOAEL for maternal toxicity and for viability and growth in the offspring for patisiran-LNP and AF-011-18534 were 1.5 mg/kg, the highest dose tested for patisiran-LNP and a dose of AF-011-18534 that essentially inhibited liver TTR production during organogenesis and lactation.

Two hours post-dose on LD 12, no ALN-18328 or AD-18534 was detected in milk. Also no AF-011-18534-related effects on serum TTR, vitamin A, or thyroid hormones in the F1 generation. Milk DLin-MC3-DMA and PEG_{2000}-C-DMG concentrations in the 1.5 mg/kg patisiran-LNP were around 5% of their respective maternal plasma concentrations at LD12.

Immunogenicity and immunostimulation

Immunotoxicity has been evaluated in the repeat-dose toxicity studies in rat and monkey. The spleen is a target organ of toxicity in both species but there is no evidence of immunosuppression. Immunogenicity to biotherapeutic drugs in nonclinical species (as seen in the 26-week repeat-dose toxicity study in rats) is not generally predictive of the potential immunogenicity in humans. For this reason, the ADA responses in animals may be of limited value in predicting these responses in humans following patisiran-LNP administration.

Serum complement and cytokines were evaluated in the single-dose and repeated dose (6-week and after the third dose in the 39-week) studies in cynomolgus monkey. Data from the 6-week study indicate that administration of patisiran-LNP or AF-011-1955 resulted in transient complement activation after 15 minutes, 6h and 24 h after dosing. No adverse clinical signs were noted. The 39-week monkey study suggests that these transient increases do not lead to a long-term complement depletion. Also, the similarity in response for both patisiran-LNP and AF-011-1955 indicates that the response is independent of the specific siRNA.

The immunostimulatory potential of the siRNA alone or when formulated in the LNP has also been investigated in vitro and in CD-1 mice. No signals indicative of a strong immunostimulatory potential were noted.

Metabolites and impurities

Metabolite toxicity studies were not conducted. The in vitro metabolic profiles and stability of ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG in serum and liver S9 were similar among nonclinical species and humans. Overall, impurities have been qualified or adequately controlled. However, no bibliographic reference was given about the LOAEL which allowed to determine the PDE value of acetamide. The Applicant was asked to clarify this point and in the response specified the reference of the value used for the PDE calculation. This value was issued from the known Carcinogenic Potency Database (Gold database), from a chronic exposure carcinogenicity study performed on mice. At the low dose level, malignant lymphoma were reported. As no NOAEL was established, a specific safety factor of 10 was added.

For linoleyl methane sulfonate, the PDE calculation proposed by the applicant was based on bibliographic data. Otherwise, the applicant refers to another acceptance limit of 60ppm described in module 3, but it was missing in the toxicological part of the dossier. In the response, the applicant specified that acceptance limit of 60ppm was a preliminary value. Given that actual concentration of linoleyl methanesulfonate is below the limit of detection of 0.6ppm, and considering the qualification of this impurity, this is acceptable. Considering that treatment with Onpattro is potentially life-long, the choice of employing less than lifetime (LTL) calculations in the estimations of total intake of potential genotoxic impurities was not adequately discussed by the applicant. However, according to ICH M7, it is possible to establish less-than-lifetime acceptable intakes for mutagenic impurities in pharmaceuticals. In the Application, the total number of dosing days for patisiran-LNP was determined by considering the once every 3-week dosing frequency and the total duration of treatment; the latter was determined considering the onset of hereditary transthyretin-mediated amyloidosis (typically between 30-50 years of age) and the life expectancy of a 30-year old in the US/Europe population (approximately an additional 50 years).

Phototoxicity

Patisiran-LNP has a peak absorbance at 260 nm and a molar extinction coefficient of approximately 42,500 L mol-1 cm-1 at 290 nm. It can thus be concluded that no dedicated phototoxicity studies are considered necessary.

Hemolysis / Hemocompatibility

No clinically relevant haemolysis or flocculation was observed in vitro in human whole blood treated with patisiran-LNP at final concentrations ≤400 µg/mL. These concentrations exceed the ALN-18328 plasma Cmax (7.15 µg/mL) at the RHD.

2.5.5. Ecotoxicity / environmental risk assessment

An ERA for Patisiran-LNP was submitted in accordance with the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA/CHMP/SWP/4447/00 corr 2*), 2006.

Introduction

Patisiran-LNP, a novel synthetic ribonucleic acid interference (RNAi) therapeutic, is being developed for the treatment of adult patients with hereditary transthyretin (TTR)-mediated amyloidosis (hATTR amyloidosis), with a dose regimen of 0.3 mg/kg by intravenous (IV) infusion, once every 3 weeks for chronic administration (i.e. lifetime dosing).

The drug product, patisiran-LNP, is a sterile, preservative-free formulation of the siRNA, containing patisiran drug substance (ALN-18328) with lipid excipients in isotonic buffer, formulated as a concentrate for solution for infusion. The lipid excipients associate with the siRNA, protect it from immediate degradation in the circulatory system, and aid in delivery to the liver. The drug product is a homogenous solution of nucleic acid/lipid nanoparticles with an average size of approximately 60-100 nm.

Phase I: Estimation of Exposure

Screening for Persistence, Bioaccumulation and Toxicity

Thelog10Pow for patisiran drug substance (siRNA) was determined in buffer solutions of varying pH (4, 7, 9) using the OECD 107 octanol-water shake-flask method (Table 4). The study was GLP compliant.

The octanol/water partition coefficient (Pow) of patisiran drug substance was found to be less than 7.7 in pH 4, 7 and 9 solutions at 20°C. The log10Pow value of < -1.2 is clearly below log10Kow (log10Pow) of >4.5 indicated by OSPAR convention and the CHMP guidance to be the threshold for further screening of persistence, bioaccumulation and toxicity (PBT).

Table 4 Octanol / Water Partition Coefficient Results for Patisiran-LNP

Buffer Solution	P_{ow}	$Log_{10}P_{ow}$
4	$< 6.3 \times 10^{-2}$	< -1.2
7	$< 7.7 \times 10^{-2}$	< -1.1
9	$< 6.7 \times 10^{-2}$	< -1.2

Calculation of the Predicted Environmental Concentration (PEC)

For orphan drug submissions, **Fpen** can be refined based on the prevalence on which the medicinal orphan drug designation, as adopted by the Committee for Orphan Medicinal Product (COMP), was based. In the approved orphan drug designation for patisiran, the prevalence of ATTR-mediated amyloidosis was considered to be not more than 0.2 in 10,000 people in the European Union (EU/3/11/857). Accordingly, the prevalence of 0.2 in 10,000 people (**0.00002**) is used in the calculation for PECsw.

The proposed dose of patisiran is 0.3 mg/kg once every 3 weeks. This is equivalent to 17.4 doses per year (365 days/21 [21 days = 1 treatment cycle]) giving rise to a total dose of 5.22 mg/kg/year per patient, or 313 mg/patient/year based on 60 kg body weight or 0.86 mg/patient/day based on 60 kg body weight.

The PEC_{SW} was calculated as follows:

$$PEC_{sw} = \frac{DOSEai * Fpen}{WASTEWinhab * DILUTION}$$

Where:

Parameter	Symbol	Value	Unit
Input			
Maximum daily administered dose per inhabitant	DOSEai	0.86	$mg.inh^{-1}.d^{-1}$
Market penetration	Fpen	0.00002	-
Amount of wastewater per inhabitant per day	WASTEWinhab	200 (default)	$L.\ inh^{-1}.d^{-1}$
Dilution factor	DILUTION	10 (default)	-
Output			
Local surface water concentration	PEC_{sw}	-	mg/L

Based on these data, the PEC_{sw} was calculated to 0.0000086 µg/L. This value is below the action limit of 0.01 therefore a Phase II environmental fate and effects analysis was not considered required.

Table 5 Summary of main study results

Substance (INN / Invented Name): Patisiran-LNP			
CAS-number (if available): siRNA (patisiran) CAS #1386913-72-9			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	≤-1.2	Potential PBT (N)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	≤-1.2	not B
	BCF	NA	not B
Persistence	DT50 or ready biodegradability	NA	not P
Toxicity	NOEC or CMR	NA	not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			

Calculation	Value	Unit	Conclusion
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.0000086	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)	N	N	(N)

2.5.6. Discussion on non-clinical aspects

The drug product of Onpattro contains the active substance patisiran (a ds-siRNA) and in a lipid nanoparticle composed of four excipients whereof two (the lipid DLin-MC3-DMA and the PEGylated lipid PEG_{2000}-C-DMG) are novel (and therefore requiring additional non-clinical assessment besides patisiran). The excipients are part of the LNP that has the purpose of both stabilizing and targeting the ds-siRNA towards the liver. The novel excipient DLin-MC3-DMA supports LNP particle formation, cellular uptake of the LNP via endocytosis, and endosomal release of the ds-siRNA into the cytosol. PEG_{2000}-C-DMG is exposed on the surface of patisiran-LNP and has the purpose to stabilize the LNP.

Pharmacology: The target of the active substance, patisiran, is the TTR mRNA expression in the human liver (and consequently an overall reduction of serum TTR protein levels). It is a primate and human gene sequence specific siRNA that is able to reduce TTR expression (mRNA and protein) in various test systems (this has led to the use of a rodent specific surrogate ds-siRNA in some of the non-clinical studies). While it is not clear if the patisiran inhibition effect is the same for all possible TTR polymorphisms, the in-vitro inhibition range for the five more common human transthyretin disease-linked polymorphisms (V30M, T60A, S77Y, S77F, and V122I) is relatively similar at an IC50 between ~16pM and 36pM. The LNP formulation does not seem to influence the extent of the inhibition in-vitro. The cynomolgus monkeys serum TTR level reduction ranges between 46% and 75% (0.03-0.3mg/kg) after a single dose and between 65% and 91% after repeated dosing (q3w or q4w; 0.15-0.5mg/kg). The TTR suppression works slowly in-vivo, taking 4-8d to reach a maximum effect in cynomolgus monkeys after a single dose and 9-12w with repeated dosing (~3 doses). Increasing the number of doses increases the recovery period before the TTR protein levels return to pre-exposure levels (with 24w administration requiring 9-12w recovery in both rodents and cynomolgus monkeys). Secondary effects to patisiran-LNP treatment are ~40-80% reductions in blood levels of RBP, T4 (but not T3) and Vitamin A (indicating that care has to be taken in the context of planned pregnancies) within 2-3w of the first dosing. The recovery period to pre-exposure levels of these downstream biomolecules increases with longer administration periods and with higher doses (to >60d at the max dose of 3mg/kg in cynomolgus monkeys). The safety pharmacological assessment did not find that the LNP with a siRNA directed against insect luciferase has a hERG effect. Cynomolgus monkeys demonstrated a transient (up to ~50h) increased body temperature and heart rate after single patisiran=LNP infusion at 3 to 6mg/kg but the mechanisms underlying this effect remains unclear.

Pharmacokinetics: The PK studies used animal exposure to patisiran-LNP and then subsequent specific measurement of the siRNA and the two excipients, making it difficult to assess the exact kinetic properties of the whole of patisiran-LNP. Overall, systemic plasma exposure was higher and half-lives longer in cynomolgus monkeys compared to rats for all the three patisiran-LNP components (DLin-MC3-DMA remaining the longest in the body) with no indication of a sex-specific profile. The patisiran-LNP had low reported

protein-binding ability (~0.5-2.1%) but the exact values remain unclear due to the semi-quantitative nature of the quantification method (SDS-PAGE/densitometry). The levels of the ds-siRNA were more than dose proportional in rats and in primates (based on comparing the low-to-middle and low-to-high doses). The cynomolgus monkey clearance reduced with increasing dose and increased t1/2 (up to 14.7-19.1h). This dose-related increase in ds-siRNA t1/2 was also observed in toxicological repeated dose studies in monkey. Regarding the novel excipients, the t1/2 of DLin-MC3-DMA increased with dose in rat but not cynomolgus monkeys. That being said, overall, the t1/2 was longer in cynomolgus monkeys (up to 428-780h) compared to rat (up to 246-288h). The t1/2 for PEG_{2000}-C-DMG was also longer for cynomolgus monkeys (160-162h after 1h infusion) than for rat (21.3h after single bolus dose). PEG_{2000}-C-DMG is believed to desorb from the LNP with an estimated desorption rate of 45% per hour. Anti-drug antibodies (ADA) against PEG_{2000}-C-DMG were found in both rats and cynomolgus monkeys.

The liver was the main uptake organ for patisiran-LNP followed by weak to moderate uptake in spleen (1.61-1.67% of total dose), kidney (0.14-0.42% of total dose), lungs (0.21-0.57% of total dose), heart (0.07-0.24% of total dose) and adrenals (0.03-0.14% of total dose) – indicating a preference for sinusoidal organs. Based on a rat QWBA study (and based on a radiolabelled DLin-MC3-DMA), the LNP-based liver targeting approach of Onpattro is effective as the liver seems to receive most of the patisiran-LNP dose (>90%) with a tissue tmax between 1h and 6h. A siRNA specific method (the PNA-probe HPLC method) indicates that much of the ds-siRNA is rapidly removed as the rat liver contains 25.6% of all siRNA at 1h but only 0.56% after 24h. For DLin-MC3-DMA, the uptake into the liver was 77.7% of the total dose in rat (with 25% left after 2w) and >97% of the total dose in cynomolgus monkeys (with 43% left after 2w). The levels of DLin-MC3-DMA in the cynomolgus monkeys liver indicate that the uptake is more than dose proportional between the low dose (0.03mg/kg) and middle dose (0.30mg/kg) and proportional between the middle dose and high dose (1.0mg/kg) and that there may be a possibility for DLin-MC3-DMA to accumulate in the liver with a q3w administration scenario. For PEG_{2000}-C-DMG, ~49% of the total dose was taken up in the rat liver within 1h (with a rapid elimination to 0.81% after 7d). The 1h levels in cynomolgus monkeys liver are unclear but, similar to rat, 0.86% remains after 7d.

In liver microsomes from patisiran-LNP treated animals (1mg/kg/day 1h IV, biweekly for 4 doses), there were no significant changes in the protein levels/activities of CYP1A1/2, CYP2C76, CYP2C43, CYP2D6, CYP3A or UGT1A1. The ds-siRNA itself undergoes exonuclease degradation over time. Following 24h patisiran-LNP post-exposure, there was 62-71% intact siRNA in rodent serum, 42-44% in cynomolgus monkey serum and ~100% in human serum. Out of six DLin-MC3-DMA metabolites detected in rat, one hydrolysis metabolite (4-Dimethylaminobutyric acid or DMBA) was also detected in cynomolgus monkeys and humans. Regarding PEG_{2000}-C-DMG, a total of 6 O-detetradecylation metabolites were detected in cynomolgus monkey and human but none in rodents. Overall, for both in-vivo rat and monkey, no plasma metabolites of >10% of the total dose were detected.

In both rats and monkeys, patisiran-LNP radioactivity (radiolabelled DLin-MC3-DMA) was primarily recovered in the urine (~49% in rats and ~50% in cynomolgus monkeys) with some excreted in the faeces (~24% in rats and ~10% in monkeys). No intact patisiran was recovered in either urine or faeces of rats or monkeys. There are indications that some of the biliary excretion of radiolabelled DLin-MC3-DMA is reabsorbed and then excreted via the kidneys. PEG_{2000}-C-DMG was only detected in faeces but not in urine.

Toxicology: The non-clinical toxicological program was in line with the ICH M3(R2) guideline, covering repeat-dose toxicity, genotoxicity, carcinogenicity, and reproductive toxicity. Only the cynomolgus monkey was a 'pharmacologically active' animal model for patisiran-LNP although some rodent studies (the DART studies) used rodent-specific ds-siRNA-LNP combinations. The latter allowed the investigation of the patisiran induced

secondary reductions of vitamin A during development in rat. Supplementary studies have been performed to evaluate excipients and immunostimulation. Overall, the liver is the main target organ to patisiran-LNP toxicity (hepatotoxicity defining the NOAELs/LOAELs in both rodents and non-rodents) which is in line with it having the greatest uptake. Hepatotoxicity signs in both rats and cynomolgus monkeys were hepatocyte single-cell necrosis, vacuolation and liver inflammation/infiltration. Based on studies using a sham-LNP (ds-siRNA against insect luciferase), the observed toxicities are to a large degree likely due to the LNP part ofpatisiran-LNP. Secondary to the liver, the spleen was also a target organ, demonstrating lymphoid depletion/atrophy/necrosis in rodents and red pulp hypocellularity in primates. An evaluation of immunotoxicity indicates that both primate and mouse-specific ds-siRNA-LNP administration induced transient complement activation within 15min to 24h of administration. Vascular/perivascular inflammation at the infusion site was a common finding across studies. No other associated clinical signs were detected. No genotoxicity was found for patisiran-LNP, and while there were some signs of increased number of neoplasms in transgene mice after 26w exposure, these were considered to be within the mouse strain's background occurrences of neoplasms. In the DART studies, no developmental toxicity besides some developmental delays was observed in the rat studies despite marked reductions in vitamin A and thyroxine. Some uncommon cardiac malformations were found in rabbits that were exposed to patisiran-LNP (i.e. non-rabbit specific ds-siRNA) but due to toxicokinetic considerations, it is at this point considered unlikely that these effects are linked to patisiran-LNP. No effects were observed in the pre-postnatal toxicity studies. There were also no signs of reductions in serum TTR, vitamin A, or thyroid hormones in the F1 offspring generation. Specific metabolite toxicity studies were not conducted. The excipients DLin-MC3-DMA and PEG_{2000}-CDMG were only evaluated in genotoxicity tests and measured in TK analyses. Impurities have been qualified or adequately controlled. Phototoxicity studies were not considered to be necessary.

Overall, and as noted in the RMP, the non-clinical data indicates that the toxicological effects of Onpattro seem to be dependent on the LNP part (although the non-clinical data and the literature does not fully rule out a contribution from nucleotide sequence-unspecific toxic effects) and that likely toxicological targets are the liver, the spleen and the infusion site (the latter also being observed clinically). Considering that the indication calls for life-long treatment, potential clinical effects on these organs from chronic exposure should be kept in mind.

An ERA for Patisiran-LNP was submitted in accordance with the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA/CHMP/SWP/4447/00 corr 2*), 2006. The $log10P_{ow}$ was determined below 4.5 therefore no further PBT studies were considered necessary. Based on the PEC_{SW} value < 0.01µg/L, a Phase II environmental fate and effects analysis was not required. It can thus be concluded that patisiran-LNP in the proposed use, does not pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

Overall, the non-clinical data were considered by the CHMP sufficient to support the application for a marketing authorisation of Onpattro in the treatment of adult patients with hereditary transthyretin amyloidosis (hATTR).

The CHMP furthermore concluded that Onpattro was not expected to pose a risk to the environment.

2.6. Clinical aspects

2.6.1. Introduction

GCP

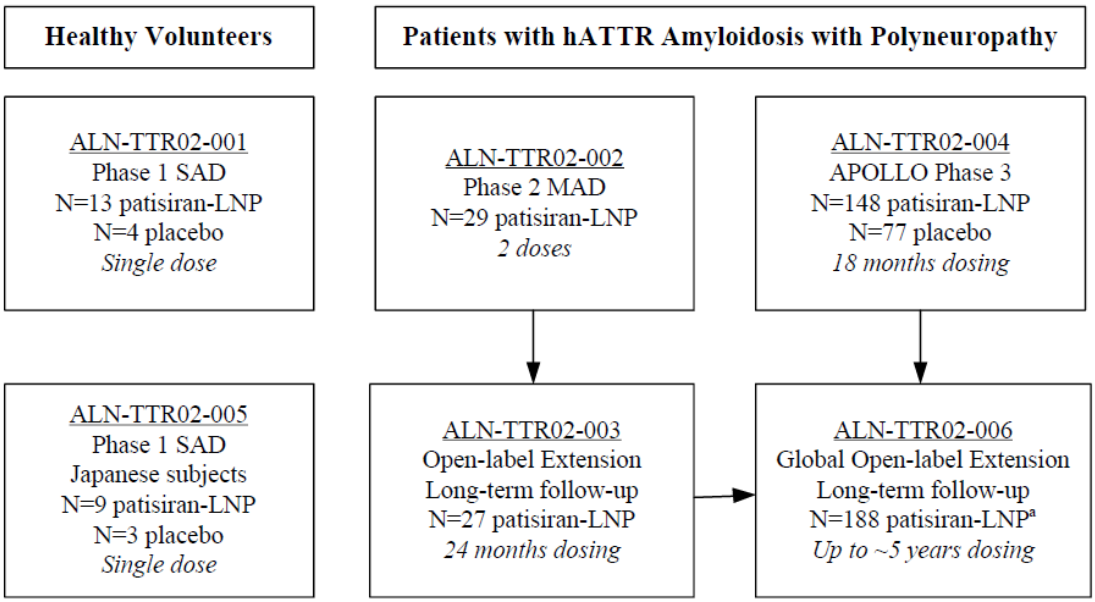
The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC. Following the routine GCP inspection (reference number INS/GCP/2018/001) conducted at one clinical investigator site in Spain and at the sponsor Alnylam Pharmaceuticals, Inc. USA, several deficiencies were noted in the preliminary inspection report. Concerns were raised on GCP compliance and data reliability and therefore a follow up inspection was requested to address issues identified in trial management; monitoring process and data management (systems set up and audit trail).

The inspectors in conjunction with the assessment team recommended that the CRO responsible for the study conduct (Medpace) and an additional clinical investigator site to be inspected. This would allow to follow up on the issues observed and to assess the impact of the findings on the overall study and the clinical data submitted in support of the MAA.

The triggered GCP inspection has resulted in the recommendation to exclude the data from Site 061 in Mallorca, Spain, from the analyses. At this site, 5 patients were enrolled for study 004. Further, as a Central Assessment Site (CAS), this site also assessed efficacy endpoints for 7 additional patients from other sites.The Applicant provided the results of MMRM and binary analyses excluding these 12 patients who had efficacy assessments performed at the Mallorca site. Overall, the MMRM analyses and binary analysis results of mNIS+7 and Norfolk QoL-DNafter excluding the 12 patients were consistent with the results from the whole mITT population.

- Tabular overview of clinical studies



Abbreviations: hATTR amyloidosis=hereditary transthyretin-mediated amyloidosis; MAD=multiple ascending dose; SAD=single ascending dose

[a] Study 006 is an ongoing study. An interim analysis was conducted with a cutoff date of 14 July 2017; as of this cutoff date, N=188 patients had enrolled and N=184 patients had received at least one dose of patisiran-LNP.

2.6.2. Pharmacokinetics

Methods

Analytical methods

Plasma, urine and plasma ultra-filtrate concentrations of ALN-18328 (drug substance) were determined with a liquid chromatography fluorescence detection assay method. The concentration of ALN-18328 was determined by annealing a complementary fluorescent RNA probe (Atto-probe) to the antisense strand of the duplex. Ion exchange HPLC was used to separate the Atto-probe: antisense complex from the antiprobe: sense complex and the excess Atto-probe and anti-probe reagents. ALN-18328 was monitored using an excitation wavelength of 615 nm and an emission wavelength of 640 nm.

Plasma concentrations of PEG2000-C-DMG were determined with an LC-MS/MS method using PEG2000-C-DMA as internal standard. Samples were processed by protein-precipitation extraction. PEG2000-C-DMG was monitored using the MS/MS transition of 893.2 → 726.1.

Plasma concentrations of DLin-MC3-DMA were determined with an LC-MS/MS method using DB-449-27-1 as internal standard. Samples were processed by protein-precipitation extraction. DLin-MC3-DMA was monitored using the MS/MS transition of 642.5 → 132.0. Two methods were developed and validated; low range and high range.

Immunogenicity Assays

The presence of anti-drug antibodies (ADA; defined as serum IgG/IgM antibodies specific to PEG_{2000}-C-DMG) were assessed in all clinical studies. An ADA assay that detected antibodies to PEG_{2000}-C-DMG was developed since PEG_{2000}-C-DMG is exposed on the surface of the LNP, and antibodies generated against PEG_{2000}-C-DMG may impact PK exposure of the patisiran-LNP components.

The immunogenicity testing strategy used in all clinical studies followed regulatory guidance. Serum samples were first assessed for the presence of ADA using a screening assay. The samples that tested positive for ADA in the screening assay were subject to further testing with a confirmatory assay. Titer analysis was performed on those samples that tested positive in the confirmatory assay.

In the anti-PEG antibody ELISA assay, PEG2000-C-DMG is coated onto the plate, and captured anti-PEG antibodies are detected using a bi-specific goat anti-human IgG+IgM antibody coupled to horseradish peroxidase. The validation parameters were established based on 50 individual healthy donors serum samples (25 males and 25 females).

A neutralizing assay for ADA was not developed because any clinically significant ADA response would be detected most readily and reliably by the reduced ability to lower serum TTR concentrations, which is a robust biomarker of patisiran-LNP activity in all clinical studies.

Evaluation and Qualification of Models

Population PK

Separate popPK models for ALN-18328 and lipids (DLin-MC3-DMA and PEG_{2000}-C-DMG) were developed. The models were fit to collected PK data from Phase 1, 2 and 3 studies (study 001, 002, 003, 004 and 005). A semi-mechanistic model for ALN-18328 was constructed with initial uptake clearance from plasma to liver, followed by redistribution between liver and plasma compartment, and elimination through hepatic clearance. Overall the updated PopPK model for ALN-18328 was considered acceptable. Final PK parameter estimates are provided in Table 6 below.

Table 6 ALN-18328 – Population PK Parameters Estimates – original full model and Updated model

PK Parameters	Original Full Model Population Estimates[Test]	Updated Model Population Estimates[Reference]	Percent Difference[(Test-Reference)/Reference]
CL12 (L/h)	1.82	1.79	1.65
Weight	$\times(\text{Weight}/66)^{0.774}$	$\times(\text{Weight}/66)^{0.543}$	29.8
Healthy	×1.56	×1.50	3.85
CL20 (L/h)	0.0752	0.0739	1.72
Age	$\times (\text{Age}/62)^{-0.00976}$	NA	NA
Weight	$\times (\text{Weight}/66)^{-0.175}$	NA	NA
eGFR*	$\times (\text{eGFR}/101)^{-0.0106}$	NA	NA
Mild HI**	× 1.09	NA	NA
Sex (if female)	× 1.01	NA	NA
Race (if non-Caucasian)	× 0.988	× 0.990	-0.202
Healthy	× 0.899	NA	NA
CL23 (L/h)	0.00278; fixed	0.00275; fixed	1.10
CL32 (L/h)	0.0249; fixed	0.0208; fixed	16.5
R1	0.224	0.135	39.7
R2	2.19	1.19	45.7
V1 (L)	2.27	1.95	14.1
Weight	$\times (\text{Weight}/66)^{0.258}$	$\times (\text{Weight}/66)^{0.292}$	-13.2
V2 (L)	1; fixed	1; fixed	NA
Frel			
Batch scale 2	1; fixed	1; fixed	
Batch scale 1	×0.733	×0.661	9.82
Batch scale 3	×0.867	NA	NA
Error Model			
Log Additive Error	0.521	0.459	11.9

CL12 = clearance from compartment 1 to compartment 2; CL20 =hepatic clearance; CL23 = clearance from compartment 2 to compartment 3; CL32 = clearance from compartment 3 to compartment 2; eGFR = estimated glomerular filtration rate (mL/min/1.73 m^2); Frel = bioavailability relative to batch scale 10 kg, hATTR = hereditary TTR-mediated amyloidosis; HI = hepatic impairment; F1= (1+R1) = accumulation ratio for the first phase; F2 = (1+R2) = accumulation ratio for the second phase; V1 = distribution volume of compartment 1; V2 = distribution volume of compartment 2; V3 = distribution volume of compartment 3
NA = not applicable since not significant in the original full model
* eGFR values were capped to 150 mL/min1.73 m^2.
** One subject with moderate hepatic impairment was pooled with subjects with mild hepatic impairment.

Two separate 3 compartment models were developed for DLin-MC3-DMa and PEG_{2000}-C-DMG. The PK models for both components are considered sufficient to describe the PK data for both components. Final PK parameter estimates of DLin-MC3-DMA and PEG2000-C-DMG are provided in the Table 7 and Table 8 below.

Table 7 DLin-MC3-DMA – Population PK parameter estimate comparison

PK Parameters	Full Model (Model 1)		Updated Model (Model 4)		
	Population Estimates	BSV (%) [Shrinkage (%)]	Population Estimates	BSV (%) [Shrinkage (%)]	IOV (%)
CL (L/h)	0.117	22.6 [8.24]	0.118	17.7 [25.7]	17.0
Age	$\times (Age/62)^{-0.0593}$		$\times (Age/62)^{-0.151}$		
Weight	$\times (Weight/66)^{0.437}$		$\times (Weight/66)^{0.315}$		
eGFR*	$\times (eGFR/101)^{-0.0162}$		$\times (eGFR/101)^{-0.0254}$		
Mild HI**	×1.15		×1.01		
Sex (if Female)	× 0.813		× 0.766		
Race (if non-Caucasian)	× 1.09		× 1.02		
Healthy	× 1.08		× 1.09		
CL2 (L/h)	1.28	49.4 [29.7]	1.28	73.1 [18.2]	
CL3 (L/h)	0.206	Fix (0)	0.186	Fix (0)	
V1 (L)	2.72	50.0 [11.7]	2.53	20.8 [43.0]	22.6
Weight	$\times (Weight/66)^{0.645}$		$\times (Weight/66)^{0.533}$		
V2 (L)	13.1	Fix (0)	19.5	85.7 [38.3]	
V3 (L)	162	30.2 [29.8]	168	Fix (0)	
Frel					
Batch scale 2	1; fixed	NA	1; fixed	NA	
Batch scale 1	×1.17		×1.13		
Batch scale 3	×0.770		×0.773		
Error Model					
Proportional Error (%)	27.8		25.2		

CL = systemic clearance; CL2 = inter-compartment clearance between the central and peripheral-1 compartments; CL3 = inter-compartment clearance between the central and peripheral-2 compartments; eGFR = estimated glomerular filtration rate (mL/min/1.73 m^2); Frel = bioavailability relative to batch scale 10 kg; PK = pharmacokinetic; V1 = central volume of distribution; V2 = volume of the peripheral-1 compartment; V3 = volume of the peripheral-2 compartment
NA = not applicable
* eGFR values were capped to 150 mL/min1.73 m^2.

** One subject with moderate hepatic impairment was pooled with subjects with mild hepatic impairment.

Table 8 PEG2000-C-DMG - Population PK Parameter Estimates – parameter comparison original model and Updated model

PK Parameters	Original Full Model (Model 1)		Updated Model (Model 5)		
	Population Estimates	BSV (%) [Shrinkage (%)]	Population Estimates	BSV (%) [Shrinkage (%)]	IOV (%)
CL (L/h)	0.131	12.9 [14.0]	0.126	10.2 [29.8]	8.9
Age	$\times (Age/62)^{-0.114}$		$\times (Age/62)^{-0.105}$		
Weight	$\times (Weight/66)^{0.662}$		$\times (Weight/66)^{0.714}$		
eGFR*	$\times (eGFR/101)^{0.146}$		$\times (eGFR/101)^{0.112}$		
Sex (if Female)	× 0.971		NA		
Race (if non- Caucasian)	× 0.989		× 0.989		
Healthy	× 1.14		× 1.12		
Mild HI**	×0.955		NA		
CL2 (L/h)	0.0288	26.4 [39.8]	0.0278	29.0 [37.1]	
CL3 (L/h)	0.0857	9.0 [90.2]	0.0809	NA	
V1 (L)	4.33	29.1 [4.9]	4.27	22.3 [21.4]	17.6
Weight	$\times (Weight/66)^{0.602}$		$\times (Weight/66)^{0.570}$		
V2 (L)	8.91	23.1 [44.6]	19.7	19.7 [42.1]	
V3 (L)	2.32	13.2 [74.0]	2.11	NA	
Frel					
Batch scale 2	1; fixed		1; fixed		
Batch scale 1	×1.06		NA		
Batch scale 3	×0.791		×0.795		
Error Model					
Proportional Error (%)	20.4		18.4		

CL = systemic clearance; CL2 = inter-compartment clearance between the central and peripheral-1 compartments; CL3 = inter-compartment clearance between the central and peripheral-2 compartments; eGFR = estimated glomerular filtration rate (mL/min/1.73 m^2); Frel = bioavailability relative to batch scale 10 kg; PK = pharmacokinetic; V1 = central volume of distribution; V2 = volume of the peripheral-1 compartment; V3 = volume of the peripheral-2 compartment
NA = not applicable
* eGFR values were capped to 150 mL/min1.73 m^2.

Absorption

The bioavailability of the drug is 100% as patisiran-LNP is administered via intravenous (IV) infusion.

Comparability

Patisiran-LNP was manufactured using 3 different buffers for the ALN-18328 solution. As the changes were made at the same time as manufacturing batch scale up, patisiranLNP produced by each process is hereafter described by the corresponding batch scale (Batch scale 1, 2 or 3).

The overall PK exposure for ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG as indicated by AUC_{last} appeared to be slightly lower for Batch scale 1 compared to the Batch scale 2.

For Batch scale 3 the PK exposure for ALN-18328, DLin-MC3-DMA and PEG2000-C-DMG as indicated by C_{trough} appears to be similar to compared to the Batch scale 2. However the number of patients receiving the Batch scale 3 is limited compared with the Batch scale 2.

Distribution

Mean volume of distribution at steady state (Vss) of ALN-18328 in healthy volunteers (0.342 to 2.10 L/kg) over the dose range studied were markedly higher than typical blood volume in human, suggesting distribution of ALN-18328 into tissues. Following patisiran-LNP 0.3 mg/kg q3w IV dosing in patients, mean Vss±SD was 0.255±0.198 L/kg. Mean±SD Vss of the lipids DLin-MC3-DMA, and PEG_{2000}-C-DMG at steady state was 0.470 ± 0.238, and 0.130±0.050 L/kg, respectively.

Patisiran LNP binding to plasma protein was investigated using FPLC (fast protein liquid chromatography) followed by SDS-PAGE quantification. Patisiran-LNP had a low binding to rat serum albumin (0.89%), human serum albumin (0.46%), and human a1-acid glycoprotein (2.07), however the accuracy of these results is difficult to determine due to the assay used.

Elimination, excretion and metabolism

Following patisiran-LNP 0.3 mg/kg IV in patients, mean ± SD steady state CL of ALN-18328, DLin-MC3-DMA, and PEG2000-C-DMG were 3.0±2.5, 2.1±0.8 and 2.1±0.6 mL/h/kg, respectively. The mean ±SD terminal elimination half-life ($t_{1/2\beta}$) of ALN-18328, DLin-MC3-DMA, and PEG2000-C-DMG were approximately 3.2±1.8, 10.9±1.8 and 3.8±1.1 days, respectively.

ALN-18328 concentrations in plasma filtrate were generally less than 5% of plasma concentrations. This low concentration of un-encapsulated “free” ALN-18328 confirms that the LNPs are stable and the siRNA remained encapsulated in the LNP formulation.

No human ADME study was performed because of the long terminal half-life of patisiran-LNP and the consequent health hazard from prolonged exposure to radioactivity in healthy subjects.

In study 001 and 002 after one or two doses of patisiran-LNP the mean urine f_e for ALN 18328 was less than 1%, indicating that renal excretion was a minor route of elimination for ALN 18328.

In healthy volunteers, 5.5% of DLin-MC3-DMA was recovered after 96 h as its metabolite, DMBA (dimethylamino butyric acid) in urine.

In rats and monkeys, PEG_{2000}C-DMG is eliminated unchanged in the bile. PEG_{2000}-C-DMG excretion in humans was not measured.

ALN-18328 and PEG2000-C-DMG was not metabolised in vitro by any of the tested CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5).

DLin-MC3-DMA was metabolized to a minor extent by human CYP CYP3A4, CYP2C8, CYP2A6, CYP2C9, and CYP2C19, but not by CYP1A2, CYP2B6, or CYP2D6. No in vivo data are available so the clinical consequence is unknown. The metabolites observed are formed by single- and multiple-oxidation and by carboxylation. Hydrolysis (non CYP pathway) appears to be involved in elimination of DLin_MC3-DMA to form DMBA.

No specific uptake for ALN-18328 and PEG2000-C-DMG in any of the investigated transporter P-gp, BCRP and BSEP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K was observed.

For DLin-MC3-DMA it was not possible to investigate the transport by P-gp, BCRP and BSEP. At 3 µM DLin-MC3-DMA did not appear to be a substrate of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K.

Dose proportionality and time dependencies

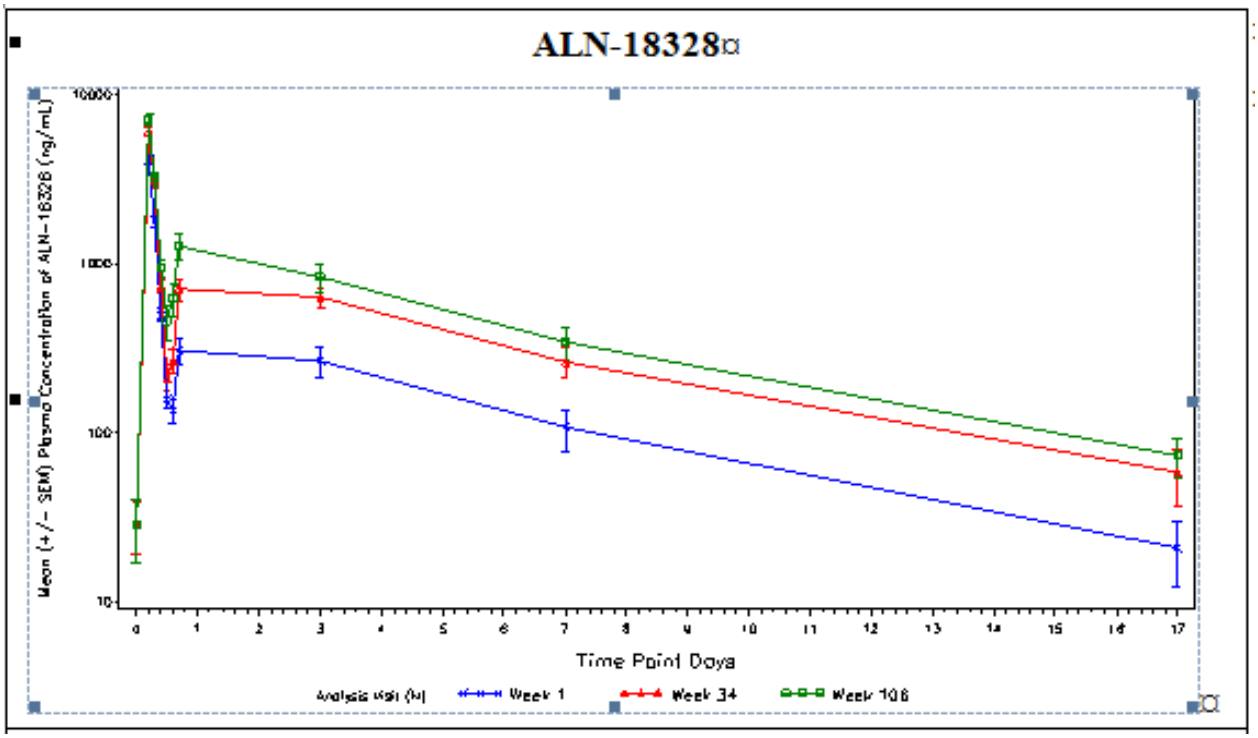
The exposure to ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG increased dose proportionally after single and repeated administration over the dose range 0.01-0.5 mg/kg.

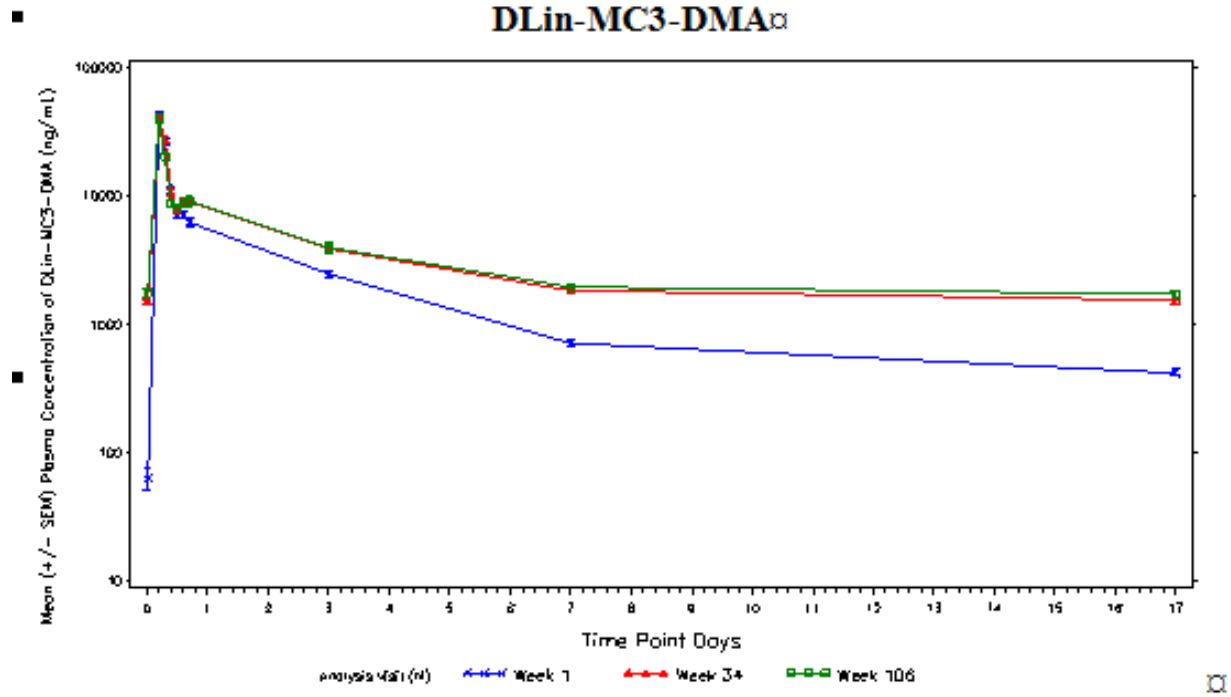
In patients at steady state the accumulation ratio (RAC) for ALN-18328 was 1.74 fold for Cmax, and 3.16 fold for AUC. For DLin-MC3-DMA, the RAC was for AUCT was 1.76-fold and C_{trough} was 2.79-fold compared to first dose. For PEG_{2000}-C-DMG accumulation was minimal.

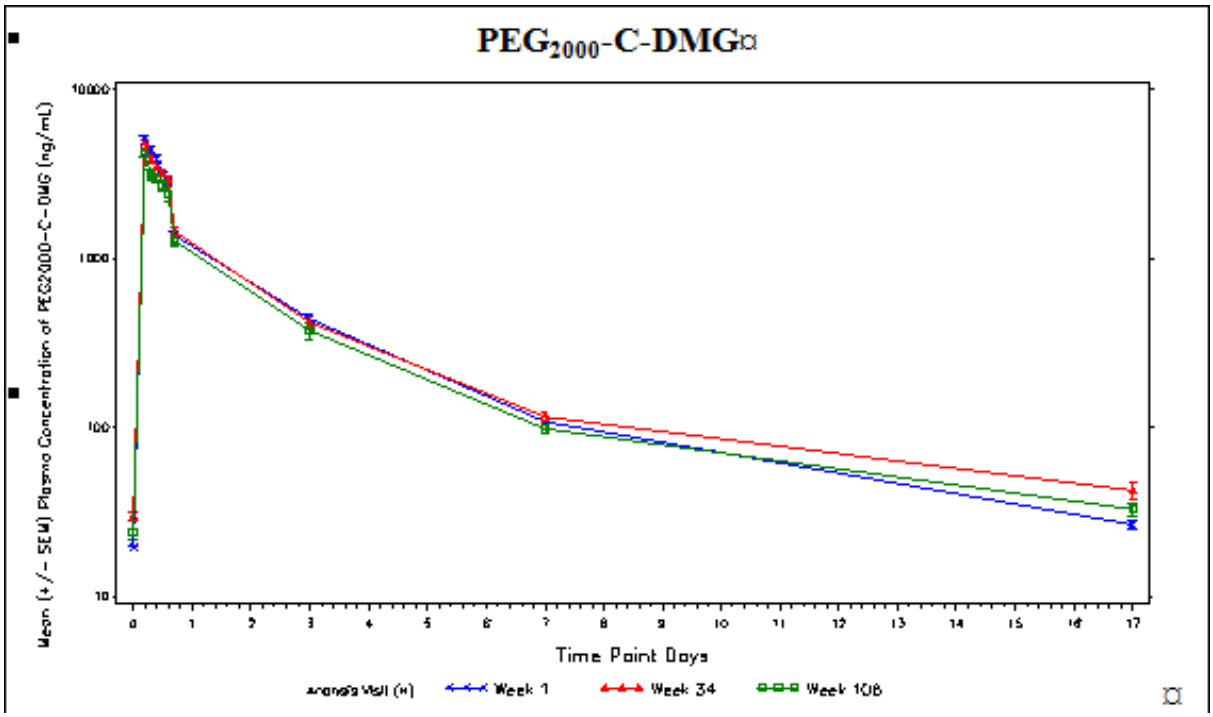
Pharmacokinetics in target population

After administration of 0.3 mg/kg qW3 in hATTR amyloidosis patients with polyneuropathy, the steady state was observed after week 24. All 3 compounds showed a rapid initial decline followed by a prolonged shallow phase with t 1/2β values of approximately 76 hours, 260 hours and 90 hours for ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG, respectively. The PK profile and PK parameters of ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG are shown in Figure 5 and Table 9 below.

Figure 5 FMean (SEM) Plasma Concentration-Time Profiles of ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG on Weeks 1, 34, and 106 After Administration of 0.3 mg/kg q3w Patisiran-LNP Over 24 Months in Patients







Abbreviations: q3w=once every 3 weeks; SEM=standard error of the mean

Source: CSR ALN-TTR02-003, Figure 14.2.3.1.1, Figure 14.2.3.2.1, Figure 14.2.3.3.1

Table 9 Mean±SD (%CV) Plasma PK Parameters of ALN-18328, DLin-MC3-DMA, and PEG2000-C-DMG After Administration of Patisiran-LNP 0.3 mg/kg q3w in Patients

PK Parameter	ALN-18328 N=27	DLin-MC3-DMA N=27	PEG_{2000}-C-DMG N=27
Week 1			
C_{max}, µg/mL	4.10±1.28 (31.2)	42.5 ±13.1 (30.9)	5.07±1.31 (25.9)
C_{trough}, µg/mL	0.0650±0.00281 (225)	0.628 ± 1.10 (176)	0.0179 ±0.00133
C_{max2}, µg/mL	0.367±0.30	7.94±2.30	NA
AUC_T, µg•h/mL	57.5±57.4 (99.8)	796 ±292 (36.7)	179 ±168 (93.7)
2nd phase AUC[d], %	81.7	71.0	NA
t_{max}[a], h	1.25 (1.03-2.67)	1.25 (1.03-2.67)	1.27 (1.03-3.42)
Week 34			
C_{max}, µg/mL	6.12 ±1.69 (27.7)	41.1 ±9.25 (22.5)	4.84±1.42 (29.3)
C_{trough}, µg/mL	0.0211±0.0377 (179)	1.51 ±0.367 (24.4)	0.0296 ± 0.0085 (28.7)
2nd phase AUC[d], %	91.5	82.2	NA
t_{max}[a], h	1.23 (1.15-2.10)	1.23 (1.15-2.10)	1.30 (1.15-3.00)
C_{max2}, µg/mL	0.781±0.492	9.65±2.49	NA
Week 106			
$C_{max,ss}$, µg/mL	7.15±2.14 (30.0)	40.2±11.5 (28.7)	4.22±1.22 (28.9)
$C_{trough,ss}$, µg/mL	0.0210 ± 0.0442 (210)	1.75±0.698 (39.9)	0.0236 ±0.00930
AUC_T, µg•h/mL	184 ±159 (86.4)	1403±105 (36.6)	145±64.7 (44.6)
2nd phase AUC[d], %	83.3	84.4	NA
$t_{max,ss}$[a], h	1.30 (1.17-2.10)	1.30 (1.17-2.10)	1.30 (1.17-3.10)
$C_{max2,ss}$ µg/mL	1.57 ± 2.04 (129)	9.90 ± 4.10 (41.4)	NA
$t_{½α}$, h	0.789 ±0.170 (21. 6)	0.875 ±0.112 (12.8)	17.1±5.35 (31.3)
$t_{½β}$, d	3.16 ±1.75 (55.3)	10.9 ±1.75 (72.7)	3.76±1.05 (28.0)
V_{ss}[b], L/kg	0.255±0.198 (77.9)	0.470 ±0.238 (50.5)	0.130±0.050 (38.7)
CL_{ss}[b], mL/kg/h	3.03±2.50 (82.6)	2.08±0.771 (37.1)	2.08±0.586 (28.2)
R_{AC}[c] for C_{trough}	3.23	2.79	1.32
R_{AC}[c] for C_{max}	1.74	0.95	0.812
R_{AC}[c] for AUC_T	3.20	1.76	0.832

Abbreviations: %CV=coefficient of variation; NC=not calculated; q3w=once every 3 weeks; SD=standard deviation

[a] Median (minimum - maximum). [b] Parameter derived using the actual dose of each component. [c] Calculated as Week 106/Week 1. [c] Calculated as Week 106/Week 1. [d] Calculated as (mean $AUC_{pTrough-last}$)/(mean $AUC_{0-CpTrough}$ + mean $AUC_{pTrough-last}$) x 100%.

Source: CSR ALN-TTR02-003, Table 14.2.5.6, Table 14.2.5.7, Table 14.2.5.8; CSR ALN-TTR02-003 Addendum 1/Erratum 1, Table 14.5.1.1.4, Table 14.5.1.1.5

Special populations

Several covariates were tested in the ALN-18328 popPK model. Based on the popPK analysis, age, gender, race, mild and moderate renal impairment, mild hepatic impairment were not significant covariates.

In the pivotal Phase 3 Study 004, a total of 137 (92.6%), 10 (6.8%) and 1 (0.7%) patients had normal hepatic function (BIL ≤ upper limit of normal (ULN) and AST ≤ ULN) and mild hepatic impairment (BIL ≤ ULN and AST > ULN) or (ULN < BIL ≤ 1.5 x ULN), and moderate hepatic impairment (1.5 ULN < BIL ≤ 3 x ULN), respectively. PK subgroup analysis indicated that there was no difference in steady state PK exposures (C_{max_ss}, $C_{p\_ss(30min)}$ and C_{trough_ss}) for patients with normal and mild hepatic impairment.

Most patients (141/199) studied had normal renal function while 40 and 18 patients, had mild or moderate renal impairment, respectively.

Subgroup analyses were performed to evaluate if dose adjustment was needed in Study 004. Results indicated that there were no meaningful differences in steady state plasma concentrations of ALN-18328 in any sub-group - age, gender, V30M mutation status, mild and moderate renal impairment, and mild hepatic impairment subgroups. PK exposures and TTR reduction was similar in patients regardless of age, gender, V30M mutation status, mild and moderate renal impairment, or mild hepatic impairment. Therefore, no dose adjustment was deemed necessary in any subgroup.

The risk for increased exposure in patients with hepatic impairment for DLIn-MC3-DMA was discussed and taken into consideration that the CYP enzymes are not involved in its metabolism it can be considered that there is no obvious reason to restrict the use in patients with hepatic impairment due to DLIn-MC3-DMA. However as PEG_{2000}-C-DMG might be eliminated via the bile it cannot be excluded that patients with moderate and severe hepatic impairment will have increased exposure of the PEG_{2000}-C-DMG part.

No dedicated study in subjects with renal and hepatic impairment was performed with patisiran–LNP. It was also noted the limited data for including hepatically impaired (all grades of impairment) and renally (moderate and severe) impaired patients as covariates in the popPK analyses in order to guide posology in these special populations.

Based on the above considerations, no formal contraindication for patients with moderate or severe hepatic impairment or severe to terminal renal impairment was deemed necessary to be included in the SmPC. However, Patisiran-LNP should not be used in these patients unless the anticipated clinical benefits outweigh the potential risks (see SmPC sections 4.2 and 5.2).

Pharmacokinetic interaction studies

As patisiran–LNP is given via IV infusion the initial drug-drug interaction assessment was made using the relevant cut off is Cmaxu* 50. Where protein binding was unknown the total concentration is used as a worst case scenario.

	Mw (g/mol)	**C_{max} (µg/mL)**	**C_{max} (µM)**	**C_{maxu}*50 (µM)**
ALN-18328	13424*	7.15	0.533	26.47
DLin-MC3-DMA	641.1	40.2	62.6	3130

PEG_{2000}-C-DMG	2650	4.22	1.59	79.5

*assuming 1Da=1g/mol

CYP inhibition

ALN-18328 did not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 activity using pooled human liver microsomes at concentrations up to 6.3 µM (~85 µg/mL). For CYP2C8 and CYP2B6 the IC_{50} was determined to >74.5 µM (1000 µg/mL) and 45.3 µM (~600 µg/mL); however, the risk of a clinical relevant DDI due to direct CYP inhibition is considered to be low. For ALN-18328 no time-dependent inhibition (TDI) was observed for any of the CYPs tested, except for CYP2B6. The effect on CYP2B6 in vivo is not known.

DLin-MC3-DMA and PEG2000-C-DMG did not show either direct or TDI of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5.

In vitro, DLin-MC3-DMA or PEG2000-C-DMG did not show time-dependent inhibition (TDI) for any of the CYP isoforms tested. For ALN-18328 no TDI was observed for any of the CYPs tested, except for CYP2B6. The effect in vivo is not known.

Induction

Patisiran-LNP did not induce CYP1A2 or CYP3A4. For CYP2B6 a concentration dependent and >2 fold increase of mRNA levels compared to vehicle control was observed in incubations in cryopreserved human hepatocytes. The effect on CYP2B6 in vivo is not known. DLin-MC3-DMA (up to 30 µM) and PEG2000-C-DMG (up to 75 µM) did not induce CYP1A2, CYP2B6 and CYP3A4 mRNA after incubation in cryopreserved human hepatocytes.

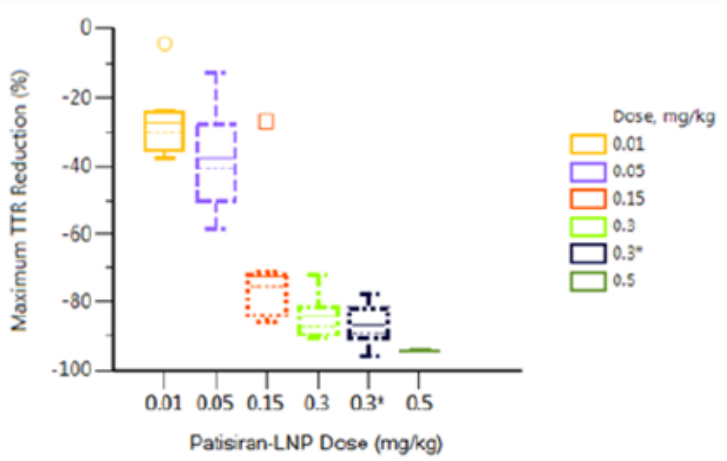
Transporter inhibition

ALN-18328 was tested at adequate concentrations and inhibition was observed for OATP1B1, OAT1 and OCT2 however no it was not possible to calculate an IC_{50} as the inhibition was <50%. It is considered unlikely that ALN-18328 is an in vivo relevant inhibitor of OATP1B1, OAT1 and OCT2 based on in vitro data. The highest tested concentration for DLin-MC3-DMA and was much lower compared to the calculated cut off concentration. For DLin-MC3-DMA there is probably not possible to do further experiments using higher concentrations with vesicles due to solubility issues. PEG_{2000}-C-DMG did inhibit the transporters P-gp, OAT1 and MATE1in vitro Considering the extensive protein binding (97% bound), a.clinical relevant inhibition of OAT1 and MATE1 is considered unlikely.

Dose/Exposure-Response

A PK/PD model was developed linking the plasma ALN-18328 predicted from the popPK model to serum TTR using an indirect response model. The dose-response simulations and the observed dose-response curve from 3 pooled studies indicate that choosing the 0.3 mg/kg dose for phase 3 was adequate, (see Figure 6 below for the observed data).

Figure 6 Maximum TTR reduction vs patisiran-LNP dose in dose escalation studies (001, 002 and 005)

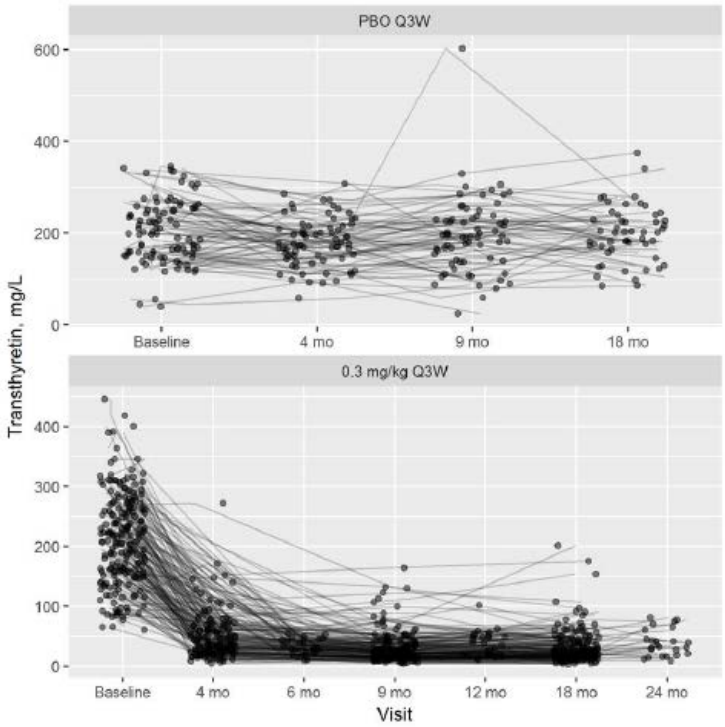


Abbreviations: TTR=transthyretin
Note: Studies pooled include 001, 002 (first dose) and 005. n=7, 9, 9, 13, 12 and 1 for 0.01, 0.05, 0.15, 0.3 mg/kg dose groups, respectively.
Note: * indicates that patients received reduced premedication.
Note: The ends of the box are the upper and lower quartiles, and the median and mean are marked by the dotted and solid line, respectively, inside the box. The top and lower lines are the range without outliers, and the symbols are outliers.
Source: 2.7.2, Figure 56

Disease progression modelling

The purpose of the disease-progression modelling was to evaluate the relationship between serum TTR and modified neuropathy impairment score +7 (mNIS+7) changes in hATTR patients with polyneuropathy. Repeated mNIS+7 data collected from patients in Study 003 (24 months follow up) and Study 004 (18 months follow up) following treatment with patisiran-LNP or placebo were pooled, Figure 7. A population mixed effects model of disease progression (i.e., mNIS+7 progression) was developed that related the progression of mNIS+7 as a function of on-treatment TTR levels in patients.

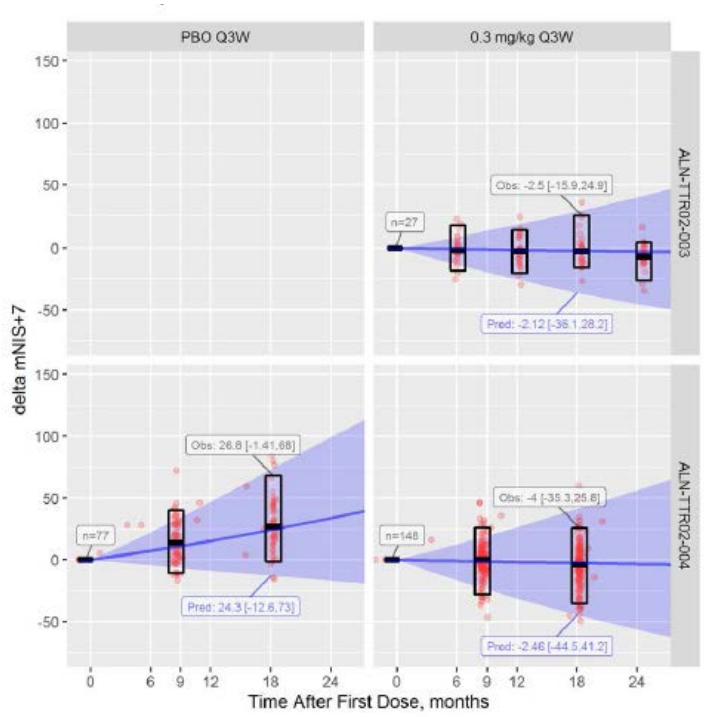
Figure 7 Serum TTR concentration by visit



0.3 mg/kg Q3W = Patisiran-LNP treatment; PBO Q3W = Placebo treatment; Q3W = Every three weeks

The time-course of mNIS+7 progression data was best described by a linear relationship after logit transformation of the mNIS+7. Visual predictive check based on 500 simulations is shown in Figure 8.

Figure 8 Simulated and observed Longitudinal Disease Progression by Treatment and Study



0.3 mg/kg Q3W = Patisiran-LNP treatment; LNP = Lipid nanoparticle; mNIS+7 = Modified neuropathy impairment score +7; PBO Q3W = Placebo treatment; Q3W = Every three weeks

2.6.3. Pharmacodynamics

Mechanism of action

ALN-18328 acts by binding to and activating the RNA-induced silencing complex (RISC) to inhibit the synthesis of wild-type (wt) and mutant TTR protein in hepatocytes expected to resulting in a reduction in circulating TTR levels.

According to the applicant the therapeutic hypothesis is that the reduction of liver-derived circulating amyloidogenic TTR protein by patisiran-LNP will reduce the deposition and promote the stabilization or clearance of TTR amyloid deposits, thereby stabilizing or improving the disease manifestations including polyneuropathy and cardiomyopathy and improving overall health. Supportive data referred to by the applicant for the hypothesis include: 1) the ability of OLT (which eliminates circulating mutant TTR) to slow disease progression in select patients with hATTR amyloidosis with polyneuropathy; 2) the patisiran-LNP Phase 3 trial in patients with hATTR amyloidosis with polyneuropathy, wherein patisiran-LNP-mediated reductions in serum TTR protein led to a statistically significant slowing of neuropathy progression and improvement in QoL relative to placebo; and 3) the demonstration that siRNA-mediated serum TTR reduction in a human V30M TTR transgenic mouse model resulted in reduction of TTR protein deposition and regression of established TTR protein deposits in nerves and gastrointestinal tract.

Primary and Secondary pharmacology

Two Phase 1 patisiran-LNP single ascending dose (SAD) studies in healthy volunteers (ALN-TTR02-001 and ALN-TTR02-005 referenced as “Study 001” and “Study 005”) contributed pharmacodynamic (PD) assessments (eg, TTR levels). Study ALN-TTR02-002 (referenced as "Study 002") was a Phase 2 multiple ascending dose (MAD) study that contributed PD in patients with hATTR amyloidosis with polyneuropathy; this study supported the selection of the patisiran-LNP dose and regimen for continued development. It included assessments of serum TTR levels.
In addition, ALN-TTR02-004 (the pivotal Phase 3, double-blind, randomized, placebo-controlled study), ALN-TTR02-003 (a Phase 2 open-label, single-arm, long-term follow-up extension study) and ALN-TTR02-006 (an ongoing open-label, single-arm, long-term follow-up extension study of patients who completed Studies 003 and 004) provided data on primary pharmacology.

Mean TTR concentrations at baseline were generally similar in healthy volunteers (mean: 241 to 336 µg/mL) (Study 001 and Study 005) and patients (mean: 197 to 276 µg/mL) (Study 002 and Study 004). Dose-dependent reductions in serum TTR concentrations were observed after single dose patisiran LNP in both healthy volunteers (Study 001 and Study 005) and in patients with hATTR amyloidosis with polyneuropathy (Study 002). In the pooled analysis of Studies 001, 005, and 002, the patisiran-LNP dose of 0.15 mg/kg was on the steep phase of the dose-response curve, resulting in less TTR reduction (75.4%) compared with 0.3 mg/kg and greater variability in PD response. The patisiran-LNP dose of 0.5 mg/kg (n=1) was on the plateau of the dose-response curve, resulting in only a marginal increase (4.7%) in TTR reduction compared with 0.3 mg/kg. The 0.5 mg/kg dose was not pursued in clinical studies after Study 001 because of the minimal observed difference in TTR reduction and the occurrence of an acute IRR at the start of infusion.
The maximum percent TTR reduction from baseline was observed within 10 to 14 days after the first dose. After a single dose of up to 0.5 mg/kg in healthy volunteers, TTR concentrations generally returned to baseline within 70 days. Sustained TTR reduction between doses was consistently achieved with 0.3 mg/kg q3w, where maximum TTR reductions of up to 96% were observed. A more sustained TTR reduction between doses was observed for a patisiran LNP dose of 0.3 mg/kg given q3w than q4w.

In the Phase 2 open-label extension Study 003, long term dosing with patisiran LNP 0.3 mg/kg q3w sustained a mean TTR reduction of approximately 80% over 2 years of treatment with an average maximum percent reduction of 92.5%. Similar profiles of sustained mean maximum TTR reduction were also observed in Phase 3 Study 004 (87.8% over 18 months) and the global open-label extension Study 006 (83.55% over 13 months for patients who were on placebo in Study 004 and initiated treatment with patisiran-LNP in Study 006). This PD effect supports long term dosing with patisiran LNP to consistently maintain low TTR concentrations.

Similar and consistent mean reductions in TTR levels over 18 months were achieved in patients with V30M and non V30M TTR genotypes, which is expected given the mechanism of action.

Serum TTR is a carrier of retinol binding protein (RBP), which facilitates transport of vitamin A in the blood. Secondary PD effects were assessed by measurement of vitamin A and RBP concentrations.
A dose-dependent reduction in vitamin A and RBP concentrations were observed in studies 001, 002 and 005. Over the course of the 18 month treatment period in the pivotal study 004, the mean percent reduction in vitamin A concentrations was 62.4% in the patisiran-LNP group and 0.2% in the placebo group. The mean percent reduction in RBP concentrations was 45.3% in the patisiran-LNP group and 0.48% in the placebo group. For patients who received patisiran-LNP, mean percent reductions in vitamin A and RBP concentrations over 18 months were also similar across the subgroups of age, sex, genotype, and prior tetramer stabilizer

use.
In study 003, overall over the 24-month treatment period serum vitamin A levels were reduced by >67% and RBP levels were reduced by >65%.

In study 006 baseline mean serum vitamin A levels in the 004 patisiran-LNP and 003 patisiran-LNP groups did not result in additional decreases in vitamin A over 52 weeks. In the 004 placebo group a mean vitamin A reduction of 63.91% at Week 52 was observed following administration of patisiran-LNP.

According to the Applicant, the impact of the reduced vitamin A or RBP levels is not anticipated to result in adverse events related to these reductions because transport and tissue uptake of vitamin A can occur through alternative mechanisms in the absence of RBP [Biesalski, H.K., et al., Biochemical but not clinical vitamin A deficiency results from mutations in the gene for retinol binding protein. Am J Clin Nutr, 1999, van Bennekum, A.M., et al., Biochemical basis for depressed serum retinol levels in transthyretin-deficient mice. J Biol Chem, 2001].

2.6.4. Discussion on clinical pharmacology

ADME

No labelled human mass-balance data is available, and this is acceptable, since patisiran-LNP drug substance ALN-18328 is a small interfering RNA and the fate of this type of molecules is well known. Further, it is reassuring that the excretion of ALN-18328 in urine has been measured and that this seems to be limited, less than 1% after single and repeated dosing of the product. Whether plasma exposure can be seen as surrogate for the local exposure in the hepatocytes is not entirely clear, however as “the second phase” of the PK profile probably reflects redistribution from the liver some association cannot be excluded.

For the elimination of DLin-MC3-DMA the Applicant refers to rat and monkey ADME studies, where a majority of DLin-MC3-DMA is stated to be eliminated as DMBA (dimethylamino butyric acid) in urine. DMBA is also found in human urine (5.5% of the dose), but was only collected for a short interval (up to 96 h, much shorter than plasma t½, while in monkey a plateau was reached after ca 10 weeks). In vitro DLin-MC3-DMA was metabolised by several CYP enzymes CYP3A4, CYP2C8, CYP2A6, CYP2C9, and CYP2C19, but to a minor extent. Hydrolysis (non CYP pathway) appears to be involved in the elimination of DLin-MC3-DMA to form DMBA. The fate of the remaining part, the lipid chain, is not known.

Based on nonclinical data PEG_{2000}-C-DMG seems not to be extensively metabolized and is suggested to be eliminated unchanged through the hepatobiliary tract to the faeces. Based on the presented data the elimination is similar in humans as well, i.e. with little to no metabolism and mainly biliary excretion.However it is still unknown whether transporters (but not substrate for P-gp, BCRP or BSEP) could be involved. Based on the preclinical findings it seems that the observed preclinical toxicology after administration of patisiran-LNP is linked to the LNP and not the siRNA.

The elimination in humans of the two new lipids DLin-MC3-DMA and PEG_{2000}-C-DMG is largely unknown. As a consequence of the uncertainty of the elimination pathways and narrow exposure margins to nonclinical adverse findings, caution when used in in special populations is warranted. Patisiran-LNP should not be used in patients with moderate or severe renal and/or hepatic impairment unless the anticipated clinical benefits outweigh the potential risks. This is appropriately reflected in SmPC section 4.2.

Population PK and PK/PD analysis

In the initial Population PK model there were apparent inconsistencies of the Population PK and PK/PD analysis. An updated popPK model was provided due to the raised issues regarding the structural PK model, weight allometric scaling, fixed PK parameters, covariates selection, estimated highly RUV (random and unexplained variability), its poor predictive performance and unreliable model derived secondary PK parameters.

A semi-mechanistic PK model was used to describe the typical PK profile of ALN-18328 with the observed two phases (rapid initial decline and second peak). To the three fixed (CL12, CL20, V1), four random (BSV on CL12, CL20, V1; RUV additive on the log-scale) effects, three random effects were added (inter-occasion variability (IOV) on CL12, CL20 and V1) and also estimated in the updated PK model. CL23 and CL32 fixed in the original PK model (according to applicant to stabilize the model) were here estimated in the updated model. In addition, allometric scaling has been tested and finally a standard reduction step (without using a full covariate model) has been used in the updated model. Based on the PopPK model discrimination, the -2LL (and the AIC (Akaike Information Criterion)) was considerably improved with 6315.2 (6355.2) vs 6806 (6848) for the updated vs original PK model. Allometric scaling by weight was not retained (increase of the -2LL, -twice the log-likelihood). Estimated PK parameters were similar (<20% difference) except for R1 and R2 and estimated exponent for weight on CL12 between original and updated PK model. Final covariates included were only weight and healthy status on CL12, Race on CL20 and Batch scale 1 on relative bioavailability (Frel). IOV have been estimated at 19.7%, 43.6% and 6.1% respectively on CL12, CL20 and V1. RUV have decreased from 52.1 to 45.9% and would not be reduced more. Predictive performance based on pcVPC (prediction-corrected Visual Predictive Check) was similar between the original and the updated model. Overall the updated PopPK model for ALN-18328 is considered acceptable. In addition, discrepancies were highlighted between post-hoc parameters and typical patients derived from the original PopPK model for both CL (0.464 vs 0.253 L/h) and Vd (46.7 vs 24.1 L). The differences were identified to be due to errors in calculation of steady state PK metrics. The steady state NCA derived PK parameters were actually derived from the first dose instead of steady state. The submitted updated results were in agreement with both CL (0.202 vs 0.253 L/h) and Vd (18.8 vs 24.1 L).

Two separates 3 compartment models were developed for DLin-MC3-DMa and PEG2000-C-DMG. For both updated reduced popPK model were provided with estimation of IOV terms. For both, PK parameters were similar (<20% difference) between original and updated PK model. Predictive performance based on pcVPC was similar between the original and the updated model. The updated PK model for both components was considered sufficient to capture both components PK.

The dose-response analysis supports that ALN-18328 affects TTR levels and that 0.3 mg/kg.

The 30 mg flat dose was chosen for safety reasons for patients weighing more than 100 kg. The highest body weight in patient studied was 110 kg. The popPK analysis was used by the applicant to show that a flat dose of 30 mg was adequate for patients weighing up to 120 kg, however the initial popPK model has concerns including the body weight based allometric scaling exponents. During the building of the updated popPK model, body weight allometric scaling with fixed coefficient of 0.75 on CL and 1 on V were tested. For safety reasons the flat dose approach with a maximum dose of 30 mg for patients weighing over 100 kg is supported. This was also supported by similar TTR lowering in the patients with body weight < 100 kg ≥ 100 kg in the Phase 3 Study 004.

Drug-drug Interactions

No clinically relevant direct CYP inhibition is expected to be caused by ALN-18328, DLin-MC3-DMA or PEG_{2000}-C-DMG. Also, DLin-MC3-DMA or PEG_{2000}-C-DMG did not show time-dependent inhibition (TDI) against any of

the CYP isoforms tested. For ALN-18328 no TDI was observed for any of the CYPs, except for CYP2B6. In general this should be followed up with further in vitro experiments to determine K_{inact} and K_I to be able to evaluate the potential risk for in vivo DDIs. This has not been done and thus it is not known whether there is an effect on the exposure of CYP2B6 substrates in vivo.

Patisiran-LNP did not cause induction of CYP1A2 or CYP3A4 and the change compared to positive controls was <20% and can therefore be concluded to be negative. For CYP2B6 there was a >2-fold increase of mRNA levels compared to vehicle control in all 3 human hepatocyte donors. In general an in vivo DDI study is warranted for a positive CYP induction in vitro signal. As ALN-18328 was also shown to be an in vitro time dependent CYP2B6 inhibitor and the net effect (TDI and induction) in vivo is not possible to predict from in vitro data. A clinical DDI study with a CYP2B6 probe would be needed for understanding of the clinical relevance of the in vitro signals. However, as there are a limited number of substrates metabolised by CYP2B6 it is considered acceptable to reflect the in vitro results in the SmPC Section 4.5, i.e. that the net effect, if any, on a CYP2B6 substrate co-medicated with Patisiran-LNP in vivo is not known and to include a list of examples of relevant CYP2B6 substrates.

Transthyretin and cytochrome P450s share common transcriptional regulatory pathways (such as Hepatic nuclear factors). This could lead to normalizing of CYP levels during the course of Patisiran-LNP administration and potentially change the exposure of drugs that are substrates of CYP enzymes. The Applicant has measured cytokine levels in the SAD study (001) as well in the patient study 002. No substantial changes were observed in any dose groups for the following cytokines: G-CSF, IFN-α, IFN-γ, IL-1b, and IL-12. Minor transient increases in mean IL-6, IL-1RA, IP-10, and TNF-α level were seen after the second dose for some dose groups. Even if this was observed only after one or two doses it indicates that the in vivo effect on CYP enzymes is likely limited.

Commercial Formulation

The majority of the clinical studies have been performed using development batch of drug product. There were very few patients administered the commercial formulation administered during phase III. Only ca. 18 patients, that also had plasma data, was administered the commercial formulation in the last 3 doses, i.e. a reasonable duration to consider that the plasma concentrations represents the commercial formulation. Although, no firm conclusion can be drawn based on PK, the point estimate for the exposure (C_{trough}) for patients administered the commercial batch is similar to patients receiving the development batch.

From a pharmaceutical perspective, the similarity of product manufactured with the two different manufacturing methods cannot be fully evaluated due to lack on data from the 10 kg batch size with regards to release of the drug substance from the nanoparticles. The limited data submitted with regards to in-vitro release of the drug substance and bioassay does not indicate any differences in quality between the batches, except for a possibly slightly higher stability of the 54 kg batch sizes.

From a PD perspective, the nadir values of TTR levels were compared. TTR levels were reached at day 10-24 post dosing, the TTR levels collected during the period 10-24 days post-dose were compared between the development and commercial batches. The long-term dosing of patisiran produced very similar mean TTR reductions of 77.28% and 75.91% for the development and commercial batches. PD data from 23 patients who had only received patisiran from the commercial batch showed a mean TTR reduction of 79.37%, further supporting the similar PD effects of the two batches.

The Applicant has compared the efficacy endpoints for patients who have received only the development batch and patients who have switched to the commercial batch. The change from baseline in a number of

endpoints was similar or slightly better for the commercial switch group, indicating that efficacy was maintained after switching to this new batch.

The Applicant has compared the subgroup of patients in studies 004 and 006 receiving only patisiran from the development batch (N=174) with the patients initially receiving the development batch, then switched to the commercial batch (N=177). The exposure times were different for the two batches: 18.6 months for the batch and 9.0 months for the commercial batch, so the incidence rate was chosen for comparison of AEs between the batches. The incidences of AEs and SAEs per 100 PYs were comparable between batches. The incidence of infusion reactions (21.3% vs 7.9%) and ADA (4.1% vs 0.9%) was higher in the development batch than the commercial batch, which is explained by the fact that IRRs occur after the 3 first infusions and patients tested positive for ADAs only transiently between day 21 to 126 in the 004 study. In study 006 there were 23 patients who had received placebo in the 004 study and only received patisiran from the commercial batch in the 006 study. The pattern of AEs and SAEs experienced by this cohort appeared comparable with the whole study population. From a clinical safety perspective, the development and commercial batches seem comparable.

Taken all data together the commercial formulation can be considered to be sufficiently similar to the formulation used during development.

Pharmacodynamics

The PD effect observed supports that long term dosing with patisiran LNP consistently maintain low TTR concentrations at the dose finally chosen.
The therapeutic hypothesis of patisiran-LNP is that the reduction in serum TTR will lead to a decrease in disease-causing amyloid deposits in tissues, which might include some clearance of TTR amyloid deposits through lowering of serum TTR. Results from pre-clinical mouse models, other forms of diseases with amyloidosis and liver transplanted hATTR patients give some support to the notion that treatment with patisiran may induce a reduction (“clearance”) of tissue deposits of TTR. However, the results from skin biopsies and LV wall thickness from the patisiran studies do not unanimously show that patisiran induces clearance of tissue amyloid deposits (see clinical section: results of exploratory efficacy endpoints and cardiac sub-population). Definite pharmacodynamic results on possible clearance remain to be shown from data collected from long-term efficacy follow up in post approval setting.

Low RBP and vitamin A levels were observed during the clinical development, as expected, as serum TTR is a carrier of retinol binding protein (RBP). Therefore the CHMP considered acceptable the recommendation for vitamin A supplementation to be included in section 4.2 of the SmPC, and the recommended daily intake of 2,500 IU (as used during the clinical studies) as a reference daily intake.
In addition, unbalance in vitamin A levels during the first trimester of the pregnancy may lead to teratogenic effects for the foetus; specific recommendations for vitamin A supplementation during an unplanned or planned pregnancy have also been included in the SmPC. All these risk minimisation measures were considered appropriate by the CHMP.

2.6.5. Conclusions on clinical pharmacology

Patisiran was shown to be an in vitro time dependent CYP2B6 inhibitor and inducer. The net effect (TDI and induction) in vivo is not possible to be predicted from in vitro data. In absence of a DDI study with a CYP2B6 probe and taken into consideration the limited number of substrates metabolized by CYP2B6, it is considered acceptable to reflect the in vitro results in the SmPC Section 4.5, i.e. that the net effect, if any, on a CYP2B6

substrate co-medicated with Patisiran-LNP in vivo is not known and to refer the prescriber to bupropion and efavirenz as as relevant CYP2B6 substrates.

In relation to the pharmacological effect of Onpattro which leads to a decrease in serum vitamin A (retinol) levels, the CHMP considers that the supplementation with 2500 IU vitamin A per day is an appropriate risk minimisation measure.

Overall the pharmacokinetics and pharmacodynamics of patisiran have been thoroughly investigated and well described. The results generally reflect the expected behaviour of a substance belonging to the chemical class of double-stranded oligonucleotides.

2.7. Clinical efficacy

The clinical development program of patisiran is based on 6 studies, three smaller studies (study 001, 005, study 002) assessing the dose, one clinical pivotal study (study 004) and two open extension clinical studies (study 003, study 006) assessing the efficacy and/or safety.

2.7.1. Dose response studies

Two Phase I patisiran-LNP single ascending dose (SAD) studies in healthy volunteers contributed PD assessments (eg, TTR levels)(See also PD section and PK). In addition, Study ALN-TTR02-002 was a Phase 2 multiple dose ascending (MAD) study that contributed PD (eg, TTR levels, safety) in patients with hATTR amyloidosis with polyneuropathy.
Dose-dependent reductions in serum TTR concentrations were observed in both healthy volunteers, and in patients with hATTR amyloidosis with polyneuropathy (Study 002). Sustained TTR reduction between doses was consistently achieved with 0.3 mg/kg q3w, where maximum TTR reductions of up to 96% were observed.

The 0.3mg/kg was selected as allowing a reduction of 80% of TTR levels, and the frequency of three-weekly leads to a more consistent fall over the treatment period without increase of adverse events over the 4-weekly frequency. Selection of the patisiran-LNP dose for the Phase 2 open label extension study (Study 003) and pivotal Phase 3 study (Study 004) was justified by the applicant based on what was known about amyloidogenic protein reduction and clinical benefit in other acquired and hereditary amyloidoses. In light chain (AL) amyloidosis, a >50% reduction in serum free light chains in response to chemotherapy was associated with a significant improvement in survival (Lachmann et al, 2003). In hereditary ApoA1 amyloidosis, where both the liver and small intestine contribute to circulating ApoA1 levels, a 50% reduction in variant ApoA1 levels following hepatorenal transplantation was shown to favourably alter the natural history of the disease (Gillmore et al, 2006). Serial SAP scintigraphy, used to assess visceral amyloid burden, has shown a correlation between the extent of serum amyloidogenic protein reduction and change in amyloid burden in AL and serum amyloid A protein (AA) amyloidosis (Gillmore et al, 2001; Lachmann et al, 2007; Lachmann et al, 2003). According to these publications, reductions of approximately 55-70% were associated with stabilization of amyloid deposits, whereas reductions of approximately 80% or greater were associated with regression of deposits. This is consistent with the findings in transgenic mice expressing human V30M TTR.

Results of disease and clinical efficacy modelling of Studies 003 and 004 indicated that there was a continuous relationship between TTR lowering and decrease in rate of mNIS+7 progression. Simulated median TTR reduction of ≥80% was associated with stabilization or improvement in mNIS+7 change from

baseline on a population basis. TTR reductions less than 80% were associated with an increase in mNIS+7 from baseline, although at a much slower rate compared to placebo.

Study ALN-TTR02-005 (Study 005, Japanese SAD study) (See also clinical PD section and PK)

Two of 12 subjects had anti-PEG antibodies and displayed reduced PD response measures as illustrated by the absence of TTR reduction. However, two subjects showed lower PK exposures explaining the corresponding lower PD response. Their doses of patisiran-LNP were low; 0.05 mg/kg and 0.15 mg/kg, respectively, with the 0.15 mg/kg dose being on the steep slope of the dose-response curve with high variability.

Study ALN-TTR02-002 (Study 002, MAD study) (See also clinical PD section and PK)

Two of 29 healthy subjects exposed to patisiran were anti-PEG positive on at least one time point. Of the 2 patients, one patient tested positive only once, and the other was repeatedly tested positive for ADA, although it diminished and finally disappeared during the extension study 003. The ADA status didn't affect the TTR titres. The data from these patients were compared to the PK and PD data from the overall study population with no differences shown.

2.7.2. Main study

APOLLO (ALN-TTR02-004): A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)

Methods

Study Participants

Study 004 was a multinational, randomized, double-blind, placebo-controlled, Phase 3 study designed to demonstrate the efficacy and safety of 0.3 mg/kg patisiran-LNP q3w in patients with hATTR amyloidosis.

The inclusion and exclusion criteria, as specified in the global protocol, are presented below:

- **Inclusion Criteria**

To be enrolled in the study, each patient must have met the following criteria at the Screening visit, except where specified:

1. Male or female of 18 to 85 years of age (inclusive);

2. Had a diagnosis of FAP with documented TTR mutation;

3. Had an NIS of 5 to 130 (inclusive) and a PND score of ≤3b (Note: This criterion must have been met at the Screening/Baseline visit);

4. Had an NCS sum of the sural sensory nerve action potential (SNAP), tibial compound muscle action potential (CMAP), ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥2 points (Note: This criterion must be met at the Screening/Baseline visit);

5. Had a Karnofsky performance status of ≥60%;

6. Had an absolute neutrophil count (ANC) ≥1500 cells/mm^3, and a platelet count ≥50,000 cells/mm^3;

7. Had aspartate transaminase (AST) and alanine transaminase (ALT) levels ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, international normalized ratio (INR) ≤2.0 (patients on anticoagulant therapy with an INR of ≤3.5 were allowed). Patients with total bilirubin ≤ 2 × ULN were eligible if the elevation was secondary to documented Gilbert's syndrome (elevation of unconjugated bilirubin with normal conjugated bilirubin) and the patient had ALT and AST levels within normal ranges;

8. Had a serum creatinine ≤2 × ULN;

9. No active infection with hepatitis B or hepatitis C by serology;

10. Women of child-bearing potential must have had a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 75 days after the last dose of study drug. Highly effective methods of birth control are defined in the protocol Section 4.7 (Appendix 16.1.1);

11. Males with partners of child-bearing potential, must have agreed to use 1 barrier method (eg, condom) and 1 additional method (eg, spermicide) of contraception throughout study participation and for 75 days after the last dose of study drug; males must have also abstain from sperm donation after the first dose of study drug through study participation and for 75 days after the last dose of study drug;

12. Must have been willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

- **Exclusion Criteria**

A patient was excluded if they met any of the following criteria at the time of the Screening visit:

1. Had a prior liver transplant or was planning to undergo liver transplant during the study period;

2. Had other known causes of sensorimotor or autonomic neuropathy (eg, autoimmune disease, monoclonal gammopathy, etc.);

3. Had known primary amyloidosis or leptomeningeal amyloidosis;

4. Had known type I diabetes;

5. Had had type II diabetes mellitus for ≥5 years;

6. Had vitamin B12 levels below the lower limit of normal (LLN);

7. Had untreated hypo- or hyperthyroidism;

8. Had a major surgery within the past 3 months or had a major surgery planned during any point of the study period;

9. Had known human immunodeficiency virus (HIV) infection;

10. Had an active infection requiring systemic antiviral or antimicrobial therapy that was not completed prior to the first dose of study drug administration;

11. Had a malignancy within 2 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that had been successfully treated;

12. Had a New York Heart Association heart failure classification >2;

13. Had acute coronary syndrome within the past 3 months;

14. Had uncontrolled cardiac arrhythmia or unstable angina;

15 Had a known history of alcohol abuse within the past 2 years or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = ½ pint of beer]);

16. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever was longer;

17. Participated in a clinical study with antisense oligonucleotide, must have had completed a 3-month wash-out prior to start of the study drug administration in this study;

18. Was currently taking tafamidis, doxycycline, or tauroursodeoxycholic acid (TUDCA); if previously on any of these agents, must have had completed a 14-day wash-out prior to start of study drug administration in this study;

19. Was currently taking diflunisal; if previously on this agent, must have had at least a 3-day wash-out prior to start of study drug administration in this study;

20. Had a prior severe reaction to a liposomal product or a known hypersensitivity to oligonucleotides or any component of patisiran-LNP;

21. Was unable to take the required premedications;

22. Anticipated survival was less than 2 years, in the opinion of the Investigator;

23. Was considered unfit for the study by the Investigator.

24. Was under legal protection (defined as “any person who becomes incapable of protecting his/her interests due to a medically diagnosed impairment of his/her mental faculties that may limit or prevent the expression of his/her will”).

Treatments

Patients who were randomized into the active treatment group received 0.3 mg/kg patisiran-LNP q3w diluted in 0.9% sodium chloride solution (normal saline). Patients who were randomized into the control group received placebo (normal saline) q3w.

The amount (in mg) of study drug to be administered was determined based on the patient's weight (kg). The body weight obtained during the previous visit was used to calculate the dose of study drug. Patients who weighed ≥105 kg were dosed based on assumption of body weight of 104 kg.

Study drug (either patisiran-LNP or placebo) was to be administered only through a secure and free-flowing venous access line, via a controlled infusion devise with an extension set containing a 1.2 micron filter, over approximately 70 minutes at an initial infusion rate of approximately 1 mL/min for the first 15 minutes, then increasing to approximately 3 mL/min for the remainder of the infusion. In the event of an IRR, the infusion time may have been extended as described below.

Premedication

All patients received premedication in order to reduce the potential of an IRR. The original premedication regimen, as outlined below, was implemented in the original protocol and in Amendment 1 of the protocol, which was instituted prior to any patient receiving treatment in the study. Subsequently, a subset of patients in Study ALN-TTR02-003 experienced AEs suspected to be related to steroids (eg, flushing) and were transitioned to a reduced premedication regimen, with a reduced dose of corticosteroid to mitigate these events. After observing that the subset of patients tolerated the lower corticosteroid dose with no consequent increase in IRRs, the protocols for Study ALN-TTR02-003 and ALN-TTR02-004 were amended (Global Amendment 6) to transition patients to the reduced premedication regimen described below.

The original premedication regimen was as follows:

On the evening prior to study drug administration, all patients received the following premedication:

- Oral dexamethasone (8 mg) or equivalent;
- Oral paracetamol (500 mg) or equivalent;
- Oral H2 blocker (eg, ranitidine 150 mg, famotidine 20 mg, or equivalent other H2 blocker dose);
- Oral H1 blocker, 10 mg cetirizine (hydroxyzine 25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine).

Prior to each dose of study drug, patients also received the following premedication at least 60 minutes prior to the infusion:

- Intravenous dexamethasone (20 mg) or equivalent;
- Oral paracetamol/acetaminophen (500 mg) or equivalent;
- Intravenous H2 blocker (eg, ranitidine 150 mg, famotidine 20 mg, or equivalent other
- H2 blocker dose); and
- Intravenous H1 blocker; diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site). Hydroxyzine or fexofenadine 25 mg per OS (PO, orally) or cetririzine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.

The reduced premedication regimen was as follows:

There were no premedication given in the evening prior to the infusion. Patients received the following premedication on the day of study drug administration at least 60 minutes prior to the infusion:

- Intravenous dexamethasone (10 mg) or equivalent;
- Oral paracetamol/acetaminophen (500 mg) or equivalent;
- Intravenous H2 blocker (eg, ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose);
- Intravenous H1 blocker: diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site). Hydroxyzine or fexofenadine 25 mg (PO, orally) or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.

Management of Infusion Related Reactions

The patient's infusion site was assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient remained at the study site for 1 hour following completion of dosing for observation and completion of assessments. Patients were instructed to call the Investigator if they experienced symptoms such as fever, chills, myalgia, or nausea/vomiting after being discharged from the site.

Signs and symptoms of IRRs were defined in the study protocol, and suggested guidelines for IRR management were provided including adjustment of the infusion rate or administration of additional concomitant medication, including adjustment of steroid dosing.

The severity of an IRR was categorized as follows depending on the actions taken to manage the IRR:

Categorization	Description
Mild	Mild reaction: infusion may be continued; if intervention is indicated it is minimal and additional treatment (other than paracetamol for delayed reactions) is not required.
Moderate	Moderate reaction: requires treatment including more intensive therapy (eg, IV fluids, nonsteroidal anti-inflammatory drugs) in addition to infusion interruption but responds promptly to medication. Treatment is indicated for ≤24 hours.
Severe	More than moderate reaction: not rapidly responsive to medication or to interruption of infusion; and/ or prolonged (treatment is indicated for >24 hours); recurrence of severe symptoms following initial improvement.

Objectives and endpoints

Primary objective / endpoint

The primary objective was to determine the efficacy of patisiran by evaluating the difference between the patisiran and placebo groups in the change from baseline of mNIS+7 score at 18 months. The mNIS+7 is a composite assessment that measures a range of motor, sensory, and autonomic neurologic impairment experienced by hATTR-PN patients.

Secondary objectives / endpoints

The secondary objective was to determine the effect of patisiran on various clinical parameters by assessing the difference between patisiran and placebo in the change from baseline in the following measurements at 18 months (see also more detailed description below under heading "Secondary and exploratory endpoint measures):

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire;
- Neurological impairment score (NIS)-weakness (NIS-W) score;
- Rasch-built Overall Disability Scale (R-ODS) score;

- Timed 10-meter walk test (10-MWT, gait speed);
- Modified body mass index (mBMI);
- Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS 31]).

Exploratory objectives / endpoints

The exploratory objectives of the study were:

- To determine the difference between the patisiran-LNP and placebo groups in the change from baseline in the following measurements at 18 months:
    - NIS+7 score;
    - Grip strength;
    - EuroQOL (EQ-5D) questionnaire;
    - Large vs small nerve fiber function including nerve conduction studies sum of 5 attributes (NCS Σ5), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), vibration detection threshold (VDT), heart rate variability to deep breathing (HRdb), postural blood pressure;
    - Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD),sweat gland nerve fiber density (SGNFD), and dermal amyloid content;
    - Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score;
    - Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels;
    - Pharmacodynamic (PD) biomarkers [TTR, retinol binding protein (RBP), vitamin A];
- To compare the proportion of patients in the patisiran-LNP and placebo groups who met the pre-defined criterion for rapid disease progression (defined as ≥24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months.
- To serially evaluate lower limb nerve injury via voluntary magnetic resonance (MR) neurography approximately every 6 months in patients receiving either patisiran-LNP or placebo. (This objective was added in country-specific amendments for France and Germany)

Sample size, randomisation and blinding (masking)

For the primary endpoint, a mean (±SD) mNIS+7 (see description of the variable below) progression rate in the placebo group of 24 ± 16 points over 18 months was estimated using natural history data from FAP patients. A sample size of 154 (2:1) was expected to yield a power of 90% based on a two-sided significance level of 0.05 and an assumption of a 8.95 point (37.5%) mean difference between treatment groups in the primary endpoint. Assuming a 25% discontinuation rate the expected total sample size was approximately 200.

On Visit Day 0 (pre dose) eligible patients were randomly assigned in a 2:1 ratio to receive either patisiran-LNP or placebo using IRS/IVRS. The randomisation was stratified by three factors: NIS (5-49 vs 50-130), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

This was a double-blind study, hence patients and all site personnel, except the pharmacist and the site personnel who set-up, dispensed, and prepared the infusion, were to be blinded to the study treatment. The procedures planned and steps taken to achieve masking of the treatments seem appropriate and included e.g. the use of covered infusion bags and lines since the colour of patisiran-LNP and placebo differed. To reduce the likelihood of infusion-related reactions, all patients received pre-medication before each study drug infusion.

Blinded study personnel who performed assessments related to efficacy endpoints were separate from personnel who monitored the administration of study drug and the well-being of the patient. The study personnel performing assessments related to the efficacy endpoints was also to be blinded to the results of any previous assessments. In this study there were two types of study centers. Patient Care Sites (PCS) could screen, dose, and manage the well-being of patients and collect safety assessments, but could not perform efficacy assessments. Central Assessment Sites (CAS) could perform efficacy assessments and perform the same assessments as at a PCS (screen, dose, and manage the well-being of patients). Assessors who performed efficacy assessments at a CAS were different from site personnel who monitored the administration of study drug during the study and monitored the well-being of the patient during the study. For patients who discontinued study drug at 9 months due to rapid disease progression, blinding was to be maintained; both patients and Investigators were to remain blinded throughout the study.

Statistical methods

The SAP was originally finalised on 31 March 2015 (SAP Version 1). Following regulatory feedback, the SAP was updated (SAP Version 2, 31 May 2017). Additional minor changes were incorporated to generate the final SAP Version 2.1 dated 23 August 2017, prior to database lock. The changes made to the SAP have been accounted for and also justified. However, the date for database lock and study treatment unblinding was not found and the applicant was requested to clarify. According to the applicant´s response the Electronic Data Capture (EDC) database was locked on 12 September 2017 followed by unblinding of that database on 12 September 2017. The overall database lock was completed on 14 September 2017, when all final data was available. The response raised concerns in that it was not clear what the differences between the two databases were and hence, what final data was not available at the first database lock 12 September. The Applicant explained that the first date (12 September 2017) i included the lock of all electronic Case Report Form (eCRF) data entered by clinical sites per the study protocol (e.g. demographics, medical history, adverse events, concomitant medications, exposure data, disposition data, etc.) and the finalization of all third party laboratory data which had not required blinding during the study (e.g. ERG and echocardiogram assessments). Following this, Alnylam authorized the unblinding of the study in accordance with Alnylam SOPs which then allowed third party providers of blinded laboratory data sources to transmit their final unblinded raw data transfers to the CRO on 13 September 2017 and 14 September 2017. The laboratory data sources included in this second transfer were considered assessments that even without treatment allocation, could lead to unblinding, e.g. retinol binding protein, vitamin A assessments. The Applicant stated that there was no impact or changes to the data previously locked on 12 September 2017 and refers to 14 September 2017 as the overall database lock date.

Overall, the analyses as planned seem appropriate. Formal statistical hypothesis testing was performed on the primary and secondary efficacy endpoints with all tests conducted using a 2-sided 0.05 level of significance. The primary population for efficacy analysis was a modified Intent-to-Treat (mITT) population including all randomised patients who had received at least one dose of patisiran or placebo. Patients were analysed according to randomised treatment.

Primary endpoint

The analysis of change from baseline of mNIS+7 score at 18 months, was based on a Mixed-Effects Model Repeated Measures (MMRM) model including baseline mNIS+7 score as a continuous covariate and fixed effect terms including treatment arm, visit (Month 9 or Month 18), treatment-by-visit interaction, genotype (V30M vs. non-V30M), age at hATTR symptom onset (<50; ≥50), region (North America, Western Europe, and Rest of World), and previous tetramer stabilizer use (yes vs. no). An unstructured covariance structure was used to model the within-subject errors. The Satterthwaite approximation was used to estimate the degrees of freedom. The analysis was implemented using SAS PROC MIXED.

For mNIS+7 two assessments were performed at each visit; each component contributing to the composite score was the average of the two assessments. A scoring algorithm, including methods for handling missing components of the mNIS+7 was pre-defined. For the primary endpoint, a number of sensitivity analyses were planned (see below Table 10). Most of them however relying on the assumption of missing at random (MAR). With the preferred estimate being that from the pattern mixture model (PMM), additional analyses were requested that were to be performed using a model including the data that were observed after discontinuation of study treatment and otherwise based on placebo-based multiple imputations for all missing or excluded data (i.e. a 'jump to reference', addressing the treatment effect if there is no benefit from treatment after treatment discontinuation; or for patients using alternative treatments). The additional analyses submitted were however not fully in line with what was requested. Instead of a 'jump to reference' approach (requested), a 'copy reference' approach was used as considered more appropriate by the Applicant. New analyses were requested and the Applicant provided the requested PMM J2R analyses based on a data set excluding in total 12 patients, 4 from the patisiran-LNP arm and 8 from the placebo arm, who had efficacy assessments performed at a site found to be GCP non-compliant (Site 061 in Mallorca).

In line with the earlier presented PMM 'copy reference' analyses (based on the full mITT population), the point estimates of the differences between patisiran-LNP and placebo as well as the corresponding 95% CIs from the PMM 'jump to reference' (J2R) analyses were very similar compared to the primary analysis although, as could be expected, implied slightly smaller differences between patisiran-LNP and placebo for the mNIS+7 and Norfolk QoL-DN endpoints respectively, as well as slightly bigger p-values. The same was true comparing the PMM J2R analyses with the newly performed MMRM analyses excluding patients who had efficacy assessments performed at Site 061 in Mallorca.

Table 10 Planned Analysis Methods for the Primary Endpoint, mNIS+7

Statistical Method	Analysis Population
Primary analysis: MMRM	mITT
Sensitivity analysis: • MI/ANCOVA • PMM • MMRM - including Data Post Alternative Treatment • MMRM - revised mNIS+7 Total Score Using a Different Algorithm to Handle Missing Components	mITT
Other analysis: MMRM	PP
Other analysis: Binary analysis using stratified CMH	mITT

Abbreviations: ANCOVA=Analysis of covariance; CMH=Cochran-Mantel-Haenszel test; mITT=modified Intent-to-Treat; MI=Multiple Imputation; MMRM=Mixed-effect Model Repeated Measures; PMM=Pattern-Mixture Model; PP=Per Protocol

Analyses of each of the component of mNIS+7, including NIS-W, NIS-R, QST, Σ5 NCS, and postural blood pressure, were also planned using MMRM models similar to those employed for the primary analysis. Given that the primary endpoint was a composite measure, these are considered to be of importance.

Secondary efficacy endpoints

Secondary efficacy endpoints were analysed using an MMRM model similar to the model described for the primary analysis of mNIS+7 while adjusting for baseline value of the endpoint being modeled. The planned analyses of the secondary endpoints are summarized in Table 11 below.

Table 11 Planned Analysis Method for the Secondary Endpoints

Endpoint	Statistical Method	Analysis Population	Special Notes
Norfolk QoL-DN total score	Primary analysis: MMRM	mITT	
	Sensitivity analysis: • MI/ANCOVA • MMRM - including Data Post Alternative Treatment	mITT	
	Other analysis: MMRM	PP	
NIS-W	MMRM	mITT	
R-ODS	MMRM	mITT	
10-meter walk test speed	MMRM	mITT	For patients unable to perform the walk, walk speed imputed as 0
mBMI	MMRM	mITT	Measured at baseline, Day 84, Day 189, Day 357, Day 462 and Day 546
COMPASS 31	MMRM	mITT	

Abbreviations: ANCOVA=ANalysis of CoVAriance; COMPASS 31=Composite Autonomic Symptom Score 31; mBMI=modified Body Mass Index; mITT=modified Intent-to-Treat; MI=Multiple Imputation; MMRM=Mixedeffect Model Repeated Measures; NIS-W=Neurologic Impairment Score-Weakness; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; PMM=Pattern-Mixture Model; PP=Per Protocol; R-ODS=Rasch-built Overall Disability Scale

Multiplicity

The approach to handle multiplicity implied that secondary endpoints were tested in a pre-specified hierarchy (1 Norfolk QOL-DN questionnaire [Total Score]; 2 NIS-W score; 3 R-ODS; 4 10-meter walk test speed; 5 mBMI; 6 COMPASS-31 total score) which is acceptable. Only if a comparison was significant at a 2-sided 0.05 significance level, the next endpoint in the hierarchy was formally tested; if a given comparison was not significant at a 2-sided 0.05 significance level, the subsequent tests were performed with nominal p values reported.

Cardiac subpopulation

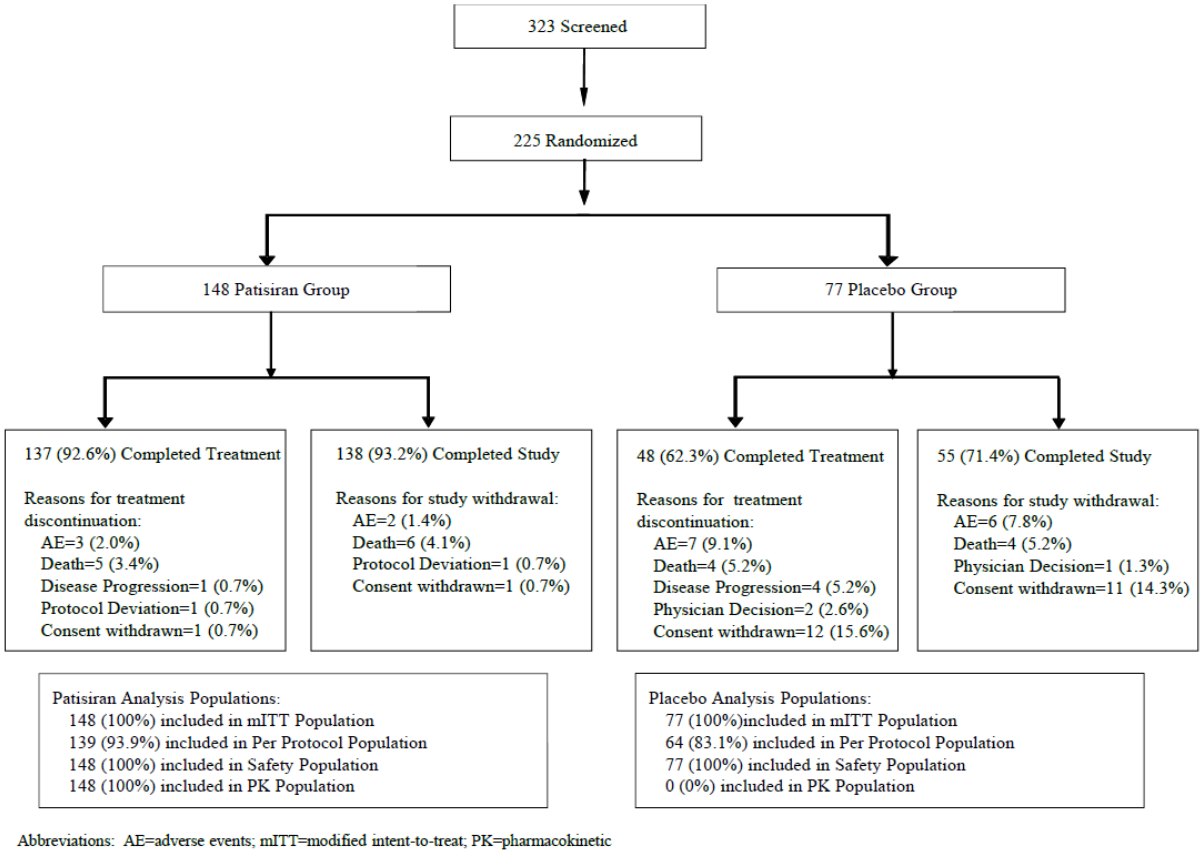
The cardiac subpopulation was defined within an amendment to the SAP (SAP V2.0, 31 May 2017) and included patients with evidence of pre-existing cardiac amyloid involvement, defined as patients with baseline left ventricular (LV) wall thickness ≥1.3 cm and no aortic valve disease or hypertension in medical history. For the Cardiac Subpopulation, the change from baseline to Month 18 in LV wall thickness, LV mass, LVEF, LV longitudinal strain, NT-proBNP and troponin I were analysed using MMRM (pre-specified analysis). Regarding these analyses, the cardiac subpopulation should preferable have been identified already from the start of the study with stratification at randomisation for subpopulation eligibility to ensure balance between the treatment arms. The analyses performed based on the cardiac subpopulation were however all considered to be exploratory.

Results

Recruitment and Participant flow

A total of 323 patients were screened for participation in the study; 225 patients were randomized.
A total of 225 patients were randomized [148 to the patisiran-LNP group and 77 to the placebo group] Table 12. All randomized patients were treated with study drug. Patients from 19 countries and 44 study centers were randomized and treated. Countries that randomized ⩾10 patients were: United States (33 patisiran-LNP, 9 placebo), France (23 patisiran-LNP, 12 placebo), Taiwan (8 patisiran-LNP, 10 placebo), Spain (7 patisiran-LNP, 10 placebo), Japan (7 patisiran-LNP, 9 placebo), Germany (13 patisiran-LNP, 2 placebo), Mexico (11 patisiran-LNP, 4 placebo), Portugal (6 patisiran-LNP, 4 placebo), and South Korea (8 patisiran-LNP, 2 placebo). The participant's flow is shown in Figure 9 below.

Figure 9 Patient Flow and Disposition Schematic



Abbreviations: AE=adverse events; mITT=modified intent-to-treat; PK=pharmacokinetic

Of special note from this figure is that a total of 185 (82.2%) patients completed study treatment, but there was a difference between the patisiran and placebo groups, 92.6% vs. 62.3% respectively. The most common reason for study treatment discontinuation overall was withdrawal of consent by the patient that was lower in the patisiran group compared with the placebo group (1 [0.7%] patisiran-LNP, 12 [15.6%] placebo). The reasons provided by patients in the placebo group who withdrew consent included “disease progression” or “worsening disease”. The next most common reason for study treatment discontinuation overall was AEs and less patients in the patisiran-LNP group discontinued treatment for this reason compared with the placebo group (3 [2.0%] patisiran-LNP, 7 [9.1%] placebo).

Conduct of the study

The original global protocol was finalized on 15 August 2013; one patient was enrolled under the original protocol. There were 5 amendments to the global protocol; a majority of patients enrolled in the study under these protocol amendments. Additionally, country-specific amendments were made for Italy, Portugal, Taiwan, France, Netherlands, Japan, Brazil, and Germany. In the applicant’s view these changes to the conduct of the study did not have any implications on the interpretation of the study results.

Demographic and baseline disease characteristics

Demographic and baseline disease characteristics are shown in the table below.

Table 12 Baseline Disease Characteristics (mITT Population)

Parameter	Statistic	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)	Overall (N=225)
Years since Diagnosis with hATTR Amyloidosis	N	77	148	225
	Mean	2.60	2.39	2.46
	SD	3.244	3.261	3.249
	Median	1.41	1.34	1.37
	Min, Max	0.0, 16.5	0.0, 21.0	0.0, 21.0
Age at hATTR Amyloidosis Symptom Onset (years)				
<50	N (%)	20 (26.0)	42 (28.4)	62 (27.6)
>=50	N (%)	57 (74.0)	106 (71.6)	163 (72.4)
Baseline NIS[a]				
<50	N (%)	35 (45.5)	62 (41.9)	97 (43.1)
>=50 - <100	N (%)	33 (42.9)	63 (42.6)	96 (42.7)
>=100	N (%)	9 (11.7)	23 (15.5)	32 (14.2)
Missing	N (%)	0	0	0
Baseline NIS	N	77	148	225
	Mean	57.02	60.50	59.31
	SD	32.042	34.512	33.656
	Median	53.88	57.88	57.00
	Min, Max	7.0, 125.5	6.0, 141.6	6.0, 141.6
Baseline mNIS+7	N	77	148	225
	Mean	74.61	80.93	78.77
	SD	37.041	41.507	40.064
	Median	71.50	76.94	75.16
	Min, Max	11.0, 153.5	8.0, 165.0	8.0, 165.0
Polyneuropathy Disability (PND) Score[a]				
I	N (%)	20 (26.0)	36 (24.3)	56 (24.9)
II	N (%)	23 (29.9)	43 (29.1)	66 (29.3)
IIIA	N (%)	22 (28.6)	41 (27.7)	63 (28.0)

IIIB	N (%)	11 (14.3)	28 (18.9)	39 (17.3)
IV[b]	N (%)	1 (1.3)	0	1 (0.4)
Familial Amyloidotic Polyneuropathy (FAP) Stage[a]				
0	N (%)	0	0	0
I	N (%)	37 (48.1)	67 (45.3)	104 (46.2)
II	N (%)	39 (50.6)	81 (54.7)	120 (53.3)
III	N (%)	1 (1.3)	0	1 (0.4)
Genotype				
V30M	N (%)	40 (51.9)	56 (37.8)	96 (42.7)
non-V30M	N (%)	37 (48.1)	92 (62.2)	129 (57.3)
Genotype Class[a]				
Early onset V30M (<50 years of age at onset)	N (%)	10 (13.0)	13 (8.8)	23 (10.2)
All other mutations (including late onset V30M)	N (%)	67 (87.0)	135 (91.2)	202 (89.8)
Previous Tetramer Stabilizer Use[a]				
No	N (%)	36 (46.8)	70 (47.3)	106 (47.1)
Yes	N (%)	41 (53.2)	78 (52.7)	119 (52.9)
Tafamidis	N (%)	27 (35.1)	47 (31.8)	74 (32.9)
Diflunisal	N (%)	14 (18.2)	31 (20.9)	45 (20.0)
Time from Discontinuation of Tetramer Stabilizer to Start of Study Drug (days)	Mean	31.4	54.2	46.4
	SD	29.32	124.94	102.94
	Median	22.0	27.0	23.0
	Min, Max	6, 148	4, 1037	4, 1037
Karnofsky Performance Status (KPS)				
60	N (%)	22 (28.6)	49 (33.1)	71 (31.6)
70-80	N (%)	45 (58.4)	80 (54.1)	125 (55.6)
90-100	N (%)	10 (13.0)	19 (12.8)	29 (12.9)
Cardiac Subpopulation[c]				
Yes	N (%)	36 (46.8)	90 (60.8)	126 (56.0)

No	N (%)	41 (53.2)	58 (39.2)	99 (44.0)
New York Heart Association (NYHA) Class				
I	N (%)	40 (51.9)	70 (47.3)	110 (48.9)
II	N (%)	36 (46.8)	77 (52.0)	113 (50.2)
III	N (%)	0	0	0
IV	N (%)	0	0	0
Missing	N (%)	1 (1.3)	1 (0.7)	2 (0.9)

Abbreviations: hATTR=hereditary amyloid transthyretin; LNP=lipid nanoparticle; max=maximum; min=minimum; mITT=modified Intent-to-Treat; mNIS+7=modified Neurologic Impairment Score +7; NIS=Neurologic Impairment score; SD=standard deviation
a A stratification factor at randomization; the data presented in this table is from clinical database. Previous tetramer stabilizer use includes tafamidis and diflunisal.
b Patient 020-0001 in the placebo group had a PND IV score at baseline. This patient enrolled in the study prior to protocol amendment 4.0 which added an inclusion criterion requiring a baseline PND score of ≤3b
c Patients with baseline LV wall thickness ≥ 1.3cm and no medical history of aortic valve disease or hypertension are included in the cardiac subpopulation.

In the overall mITT population, a majority of patients (72.4%) were White or Caucasian, 23.1% were Asian and five patients (2.2 %) were Black/African or African American. A majority of patients were from Western Europe (43.6%) followed by North America (20.9%) and Asia (19.6%). A total of 39 different TTR mutations were represented; 57.3% of patients had a non-V30M genotype and 10.2% of patients had early onset V30M. The V30M genotype was most frequent in Portugal (100% of the Portuguese patients), Sweden (89%), Spain (82%), France (71%), and Japan (63%). The most common non-V30M genotypes present in >5% patients were Ala97Ser (100 % of Taiwanese patients), Thr60Ala (33% of US patients), Glu89Gln (88% of Bulgarian patients), and Ser50Arg (89% of Mexican patients). The Val122Ile mutation (found in up to 4% of African Americans, with a cardiac-predominant phenotype) was found in 2 patients, 1 from Spain and 1 from US.

There is a notable difference between treatment groups of more than 10% regarding some baseline demographics/characteristics (Asian or not, V30M or other, cardiac subpopulation yes/no). E.g. a higher proportion of patients in the patisiran group compared with the placebo group had a non-V30M genotype 62.2% vs. 48.1%, respectively. Furthermore 12% more Asian population was recruited in the active group as compared to the placebo group which could explain the higher proportion of non-V30M and cardiac population with patisiran-LNP. Complementary analyses assessing response rates across subgroups (genotype, pre-specified cardiac subpopulation, race) within each arm were provided by the Applicant. They do not show heterogeneity of responses in these subgroups within both arms since a consistent clinical benefit across these specific subgroups was observed.

Additionally, the Applicant provided a safety analysis of these specific subgroups targeting the most frequent AEs (≥ 3%). Among the 11 AEs identified, no imbalance of incidences were noticed except for oedema peripheral for which there was a higher incidence rate in non 30VM and cardiac subgroups compared to their counterparts.

Cardiac Subpopulation

The pre-specified cardiac subpopulation was included to provide a more sensitive cohort for detection of patisiran-LNP treatment effects on cardiomyopathy, which might otherwise be difficult to discern.

A total of 56% of patients were included in the predefined cardiac subpopulation. The proportion of cardiac patients was however higher in the patisiran-LNP group than the placebo group; 60.8% (90/148) vs. 46.8% (36/77), respectively (see table above). Complementary analyses assessing response rates across subgroups, including the cardiac subpopulation within each arm were provided by the Applicant. They do not show heterogeneity of responses in these subgroups within both arms since a consistent clinical benefit across these specific subgroups was observed. In addition, in new post-hoc analyses assessing cardiac endpoints in subpopulations defined using alternate definitions for the cardiac population, no meaningful imbalance between the two treatment arms was observed in the number of patients with cardiac manifestations of the disease.

Among patients not included in the cardiac subpopulation, 55.6% had mean LV wall thickness ≥1.3cm, indicating potential cardiac amyloid involvement, but were excluded from the predefined cardiac subpopulation primarily due to medical history of hypertension. Thus, 80.4% of patients had evidence of cardiomyopathy based on left ventricular wall thickness ≥ 1.3 cm at baseline.

Numbers analysed

Of the 225 patients randomized and treated, all were included in the mITT and Safety Population (see Table 13); patients in the patisiran-LNP group were included in the PK Population.

A total of 203 (90.2%) patients were included in the PP population for efficacy analysis.

Of the twenty-two patients that were excluded from the PP population, 19 were due to absence of either baseline or both 9-month or 18-month mNIS+7 and Norfolk QOL assessments, and 3 were due to major protocol deviations impacting efficacy interpretation. A higher percentage of patients in the placebo group withdrew from the study prior to the 9-month visit, and were not included in PP population.

Table 13 Table Analysis Populations

Study Population	Statistic	Placebo	Patisiran-LNP 0.3 mg/kg	Overall
Modified Intent-to-treat (mITT)	N (%)	77 (100.0)	148 (100.0)	225 (100.0)
Per-protocol (PP)	N (%)	64 (83.1)	139 (93.9)	203 (90.2)
Safety	N (%)	77 (100.0)	148 (100.0)	225 (100.0)
Pharmacokinetic (PK)	N (%)	0 (0.0)	148 (100.0)	148 (65.8)

Outcomes and estimation

Efficacy assessments

Efficacy assessments are summarised for studies 003, 004 (the pivotal study) and 006 in table below. Study 006 enrolled patients who completed Studies 003 and 004. The efficacy endpoints evaluated in the parent study are also evaluated in this long-term extension study, except that mNIS+7 is being evaluated only at the end of the first year of treatment (Week 52, Month 12), after which NIS is performed annually. Norfolk-

QoL-DN was assessed in patients who enrolled from Study 004. All efficacy endpoints in Study 006 were descriptively summarized.

Table 14 Summary of efficacy assessments in studies 003, 004 and 006

Assessment	Brief Description	Interpretation of the Score	Schedule of Assessment by Study		
			004	003	006[(1)]
PRIMARY ENDPOINT IN STUDY 004 / DESCRIPTIVE ENDPOINT IN STUDIES 003 AND 006[a]					
mNIS+7	A composite neurologic impairment score that assesses motor, sensory and autonomic neurologic impairment.	Score range: 0 to 304 points Less neurologic impairment = **Lower score**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline and 12 months or EOS visit
SECONDARY ENDPOINT IN STUDY 004/ DESCRIPTIVE ENDPOINT IN STUDIES 003 AND 006[a]					
Norfolk QoL-DN	A standardized QoL questionnaire designed to measure the perception of the effects of polyneuropathy by the patient.	Score range: -4 to 136 points Better quality of life = **Lower score**	Baseline, 9 and 18 months	Not evaluated	Baseline, every 12 months, and End of Study (EOS) visit
NIS-W	A component of mNIS+7 that assesses motor strength	Score range: 0 to 192 points Less neurologic impairment = **Lower score**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Not evaluated as a separate endpoint but measured annually as a component of NIS
R-ODS	A patient-reported disability scale that assesses activity and social participation limitations and specific activities of daily living.	Score range: 0 to 48 points Less disability = **Higher score**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline and at 12 months
10-MWT	A measure of ambulation that assesses how fast a patient can walk a distance of 10 meters	Gait speed reported in meters/second. Faster/better gait speed = **Higher speed**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline and at 12 months
mBMI	A measure of nutritional status.	mBMI reported as BMI (kg/m^2) x albumin (g/L) Better nutritional status = **Higher mBMI**	Baseline, 3, 6, 12, 15, 18 months	Baseline, 6, 12, 18, 24 months	Baseline, every 12 months, and EOS visit

Assessment	Brief Description	Interpretation of the Score	Schedule of Assessment by Study		
			004	003	006[(1)]
COMPASS 31	Patient reported outcome that assesses autonomic neuropathy symptoms	Score range: 0 to 100 points Fewer autonomic neuropathy symptoms = **Lower score**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline and at 12 months
EXPLORATORY ENDPOINT IN STUDY 004/ DESCRIPTIVE ENDPOINT IN STUDIES 003 AND 006[a]					
NIS+7	A composite neurologic impairment score that assesses neuropathy. mNIS+7 is a modification of the NIS+7 score	Score range: 0 to 270 points Less neurologic impairment = **Lower score**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline and at 12 months
Large and small nerve fiber function	Composite neurologic impairment scores that uses subcomponents of the mNIS+7 and NIS+7 scores to evaluate large and small nerve fiber function	Large fiber score range: 0 to 52 points Small fiber score range: 0 to 44 points Less neurologic impairment = **Lower score**	Baseline, 9 and 18 months	Not evaluated	Not evaluated
Grip strength	Hand grip strength as a measure of motor strength	Measured in kg Better/stronger motor grip strength = **Higher grip strength**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline and at 12 months
FAP Stage and PND Score	Measures of neuropathy disease stage 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	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline, every 12 months, and EOS visit

Assessment	Brief Description	Interpretation of the Score	Schedule of Assessment by Study		
			004	003	006[(1)]
		the help of one stick or crutch • IIIB: Walking with the help of two sticks or crutches. • IV: Confined to a wheelchair or bedridden			
Cardiac assessments	Echocardiogram to evaluate cardiac structure (mean left ventricular [LV] wall thickness and LV mass), cardiac systolic function (longitudinal strain and left ventricular ejection fraction [LVEF]), and cardiac diastolic function (left ventricular end diastolic volume [LVEDV]). Cardiac biomarkers (NT-proBNP and troponin I) to evaluate cardiac stress and injury.	Less cardiac amyloid involvement = **Lower mean LV wall thickness or LV mass** Improved cardiac systolic function = **longitudinal strain further from 0 in the negative direction** (values increasingly abnormal as they approach 0); **Higher LVEF** Improved cardiac diastolic function = **increased LVEDV** (indicating a more distensible ventricle) Less cardiac stress = **Lower NT-proBNP** Less cardiac injury = **Lower troponin I**	Baseline, 9 and 18 months (all patients)	Baseline, 6, 12, 18, 24 months in the Cardiac Subpopulation only.	Baseline and at 12 months (all patients)
EQ-5D and EQ-VAS	General patient-reported QoL questionnaires	EQ-5D: score range 0 to 1 EQ-VAS: score range 1 to 100 Better quality of life = **Higher score**	Baseline, 9 and 18 months	Baseline, 6, 12, 18, 24 months	Baseline, every 12 months, and EOS visit
Dermal amyloid burden	The percent of dermal tissue with TTR amyloid deposits quantitated in the skin biopsy sample	Less amyloid in tissues = **Lower amyloid burden**	Baseline, 9 and 18 months (consenting patients only)	Baseline, 6, 12, 18, 24 months (consenting patients only)	Every 12 months and at EOS visit (consenting patients only)
IENFD and SGNFD	Intraepidermal nerve fiber density (IENFD) is a quantitative measure of sensory nerve pathology.	IENFD (reported in fibers/mm) and SGNFD (reported in m/mm3) can be decreased in patients	Baseline, 9 and 18 months (consenting patients	Baseline, 6, 12, 18, 24 months (consenting patients only)	Every 12 months and at EOS visit (consenting

Assessment	Brief Description	Interpretation of the Score	Schedule of Assessment by Study		
			004	003	006[(1)]
	Sweat gland nerve fiber density (SGNFD) is a measure of autonomic nerve fiber pathology.	with hATTR amyloidosis relative to healthy patients Less nerve injury= **Higher nerve fiber density**	only)		patients only)
TTR levels	A measure of the pharmacodynamic activity of patisiran-LNP.	Lower hepatic TTR production = **Lower circulating serum TTR levels**	Predose during Screening, predose on Day 0, Day 22, Day 126, Day 252, at month 9 (Day 253-272), Day 273, Day 399, Day 546, at 18 months (Day 553-560), and at EOS.	Predose on Day 0, 42, 84, 168, 182, 189, 231, 273, 357,371, 462, 546, 560, 651, 735, 749, 756, and 791 (if applicable). Postdose on Day 0, 3, 7, 17.	Predose on Day 0, at month 6, every 12 months, and EOS visit

Abbreviations: 10-MWT=ten meter walk test; BMI=body mass index; hATTR=hereditary ATTR; COMPASS 31=Composite Autonomic Symptom Score 31; EOS=end of study; EQ-5D=Euro Quality of Life-5 Dimension; EQ-VAS=Euro Quality of Life – Visual Analog Scale; FAP=Familial Amyloidotic Polyneuropathy; IENFD=intraepidermal nerve fiber density; LV=left ventricular; EF=ejection fraction; mNIS+7=Modified Neurologic Impairment Score +7; mBMI=modified Body Mass Index; NIS+7=Neurologic Impairment Score +7; NIS-W=Neurologic Impairment Score- Weakness; Norfolk QoL-DN=Norfolk Quality of Life – Diabetic Neuropathy; NT-proBNP=B-type natriuretic peptide; PND=polyneuropathy disability; R-ODS=Rasch-built-Overall Disability Scale; SGNFD=sensory nerve action potential; TTR=transthyretin.

[a] *In Study 003=the primary endpoint was safety and these efficacy assessments were either secondary or tertiary descriptive endpoints. In Study 006=the primary objective was long-term safety and efficacy=and these assessments were all descriptive efficacy endpoints.*

Primary outcome measure mNIS+7

As already mentioned, the modified Neuropathy Impairment Score +7 (mNIS+7), a composite neurologic impairment score, was the primary efficacy variable in the pivotal study 004. Composite neurologic impairment scores have been used previously as endpoints in clinical trials for the assessment of neuropathy progression. In particular, the Neuropathy Impairment Score (NIS) and NIS+7 (=NIS in addition with seven neurophysiological variables) have been used in clinical trials of diabetic polyneuropathy and CIDP (chronic inflammatory demyelinating polyneuropathy). These scores have been adopted in clinical studies of hATTR amyloidosis. NIS has been shown to correlate with disease stage and quality of life thereby supporting the clinical relevance of the NIS score in hATTR amyloidosis. NIS +7 has been validated in diabetic polyneuropathy and has been used in a Phase 3 trial in patients with hATTR amyloidosis with polyneuropathy.

The modified NIS+7 (mNIS+7) was developed specifically for monitoring progression of neurologic impairment in hATTR amyloidosis patients. The modifications address the limitations of the NIS+7 for the hATTR amyloidosis patient population and increase the sensitivity and dynamic range of the composite score in order to better measure progression of neurologic impairment in a heterogeneous group of patients with hATTR amyloidosis. The different components of the mNIS+7 used in the patisiran trials are shown in Table below.

Table 15 mNIS+7 Components, scoring and methodology

Component	Body regions/nerves evaluated	Methodology including Scoring
Motor strength/weakness Neurological impairment score-weakness [NIS-W]) (192 points)	48 muscle group in lower limb, upper limb and body and cranial nerve components (see Appendix 1) Assessments are performed separately for the right and left side of the body	Physical exam evaluating motor strength is performed. A score of 0 (normal), 1 (25% weak), 2 (50% weak), 3 (75% weak) to 4 (paralysis) is applied to muscle groups. The scores for individual muscle groups are summated to get the total NIS-W score
Quantitative sensory testing (QST) (80 points)	Up to 10 anatomical sites (see Appendix 2) Assessments are performed on one side of the body	Touch pressure by body surface area (QST-BSA_{TP}) and heat pain by body surface area (QST-BSA_{HP}) are assessed at up to 10 anatomical sites using CASE (Computer Aided Sensory Evaluator) IV. A score of 0 (< 95th percentile), 1 (>=95th and <99th percentile) or 2 (>= 99th percentile) is applied at each anatomical site, summated across all anatomical sites and then multiplied by 2 (for 2 sides of body).
Reflexes (NIS-reflexes or NIS-R) (20 points)	5 reflexes in lower limb, upper limb and body (see Appendix 1). Assessments are performed separately for the right- and left-hand side of the body	A physical exam evaluating 10 reflexes is performed. A score of 0 (normal), 1 (decreased) or 2 (absent) is applied to each reflex and summated to get the total NIS-R score.
∑5 Nerve Conduction Studies (∑5 NCS) (10 points)	Ulnar CMAP Ulnar SNAP Sural SNAP Tibial CMAP Peroneal CMAP (see Appendix 1)	Nerve conduction studies are performed on one side of the body. Values are transformed to percentile value correcting for applicable variables of age, gender, height, or weight based on earlier studies of a large healthy subject reference cohort and expressed as points (ie, > 5th percentile= 0 points; ≤ 5th percentile – > 1st= 1 point and ≤ 1st percentile= 2 points).
Postural blood pressure (2 points)	autonomic nerves	Postural blood pressure is performed and points assigned based on the change in blood pressure with standing (blood pressure decrease of <20 mmHg = 0 points, blood pressure decrease of 20 to <30 mmHg = 1 point, blood pressure decrease ≥30 mmHg = 2 points

Abbreviations: CMAP=compound muscle action potential; mNIS+7=Modified Neurological Impairment Score +7; SNAP=sensory nerve action potential; VDT=vibration detection threshold

Results of mNIS+7 in pivotal study 004

The global outcome measured in mNIS+7 at 18 months is shown in the table below. Improvement (change in mNIS+7 score <0) was seen in approximately half (56.1%) of patisiran-LNP treated patients at 18 months, compared to 4% in the placebo group. Relative to baseline, the patisiran-LNP group had a score decrease at

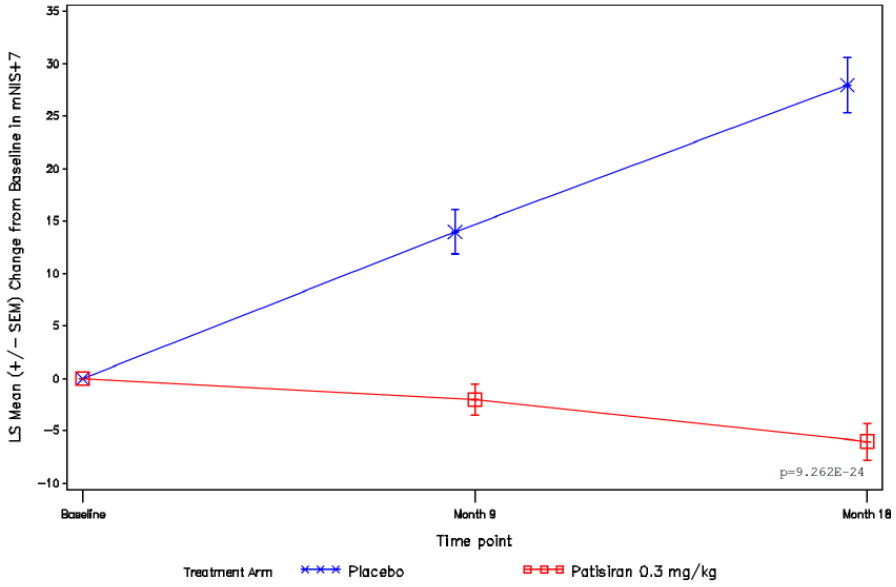
18 months (LS mean change from baseline: -6.03 points). In contrast, the placebo group had an increase of score indicating worsening of underlying neuropathy at 18 months relative (LS mean change from baseline: +27.96 points). The change in mNIS+7 over time is seen in Table 16 and in Figure 10 below.

Table 16 Primary efficacy endpoint mNIS+7 at 18 months

Statistic[a,b]	Placebo N = 77	Patisiran-LNP N = 148
Baseline Scores, Mean (SD)	74.61 (37.04)	80.93 (41.51)
Month 18 Scores, Mean (SD)	101.09 (45.35)	75.13 (43.18)
Change from Baseline, LS Mean (SEM) 95% CI	27.96 (2.602) 22.83, 33.09	-6.03 (1.739) -9.46, -2.60
LS Mean (SEM) Difference Treatment Difference (Patisiran-LNP – Placebo) **95% CI, p-value**	-	**-33.99 (2.974)** **-39.86, -28.13, P=9.262E-24**

Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; MMRM=mixed-effect model repeated measures; mNIS + 7=Modified Neurologic Impairment Score + 7; SD=standard deviation; SEM=standard error of the mean.
Note: In the MMRM model, the outcome variable is change from baseline in mNIS + 7. The model includes baseline mNIS + 7 score as covariate and fixed-effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region.
[a] Baseline and Month 18 are the averages of 2 assessments performed at least 24 hours but no more than 7 days apart.
[b] LS means, SEM, differences in LS means, 95% CIs, and Month 18 p-value from MMRM model

Figure 10 mNIS +7 change from baseline over time for placebo and patisiran



Abbreviations: LS=least square; mITT=modified intent-to-treat; MMRM=mixed-effect model repeated measures; mNIS + 7=Modified Neurologic Impairment Score + 7; SEM=standard error of the mean

As already noted the dropout rate in the placebo group was higher than in the patisiran-LNP group, and the reasons provided for withdrawal of consent in a majority of patients in the placebo group was “worsening of disease” or “disease progression”. Comparing dropouts vs completers within each group, the mNIS+7 median

change at Month 9 (based on last observation carried forward [LOCF]) was 28 vs 12 points for the placebo group, and -3.5 vs 0.5 points for the patisiran-LNP group, respectively. This suggests that placebo dropouts generally had a worse outcome compared with completers, while some patisiran-LNP dropouts derived benefit from treatment before withdrawal. All sensitivity analyses resulted in a consistent estimate of a positive treatment effect of patisiran-LNP compared to placebo on mNIS+7.

Binary analyses of mNIS+7

Table 17 Binary analyses of mNIS+7

	Placebo (N=77)	**Patisiran-LNP 0.3 mg/kg (N=148)**
< 0-point increase in mNIS + 7 score at Month 18 (N, %)	3 (3.9)	83 (56.1)
≥ 0-point increase in mNIS + 7 score at Month 18 (N, %)	48 (62.3)	54 (36.5)
Missing (No mNIS+7 value at month 18) (N, %)	26 (33.8)	11 (7.4)
Proportion of patients with < 0-point increase (95% CI)	3.9 (0.0, 8.2)	56.1 (48.1, 64.1)
Difference in proportions (Patisiran – Placebo) (95% CI) **Odds ratio (95% CI)a** **p-value**	- -	**52.2 (43.1, 61.3)** **39.9 (11.0, 144.4)** **1.823E-15**
< 10-point increase in mNIS + 7 at Month 18 (N, %)	11 (14.3)	110 (74.3)
≥ 10-point increase in mNIS + 7 at Month 18 (N, %)	40 (51.9)	27 (18.2)
Missing (No mNIS+7 value at month 18) (N, %)	26 (33.8)	11 (7.4)
Proportion of patients with < 10-point increase (95% CI)	14.3 (6.5, 22.1)	74.3 (67.3, 81.4)
Difference in proportions (Patisiran - Placebo) (95% CI) **Odds ratio (95% CI)a** **p-value**	- -	**60.0 (49.5, 70.6)** **18.4 (8.5, 39.7)** **8.240E-19**

Data collected post-alternative treatment are excluded from analysis
Notes: To determine the proportion of patients with <0- or <10-point increase, the denominator is the total number of patients, including patients with missing data.
a Odds ratio are from Cochran-Mantel-Haenszel test stratified by genotype.

A total of 27 patients on patisiran-LNP (18.2%) and 40 patients on placebo (51.9%) had an increase of mNIS+7 of 10 points or more. An supplementary analysis by the applicant of characteristics of patients on patisiran- LNP with an increase of mNIS+7 ≥ 10 points at 18 months identified no baseline demographics or patient characteristics that would lead to absence of treatment effect with patisiran-LNP. Furthermore, the applicant points out, in patients with an increase in mNIS+7 of ≥10 points on patisiran-LNP there was a difference in neuropathy (mNIS+7) and clinical benefit across multiple health measures compared to placebo patients, favouring patisiran-LNP.

mNIS+7 component analysis

Table 18 and Figures 11 and 12 below show the change from baseline to month 18 for the five different components of mNIS+7. Figure part a) shows actual numbers (mean, 95% confidence interval) and part b) shows standardised effect analysis which facilitates comparison between the different components.

Relative to baseline, all component scores worsened in placebo patients. Patients in the patisiran-LNP group showed an improvement relative to baseline in QST (-6.0 points) and postural blood pressure (-0.2 points); other components were stable.

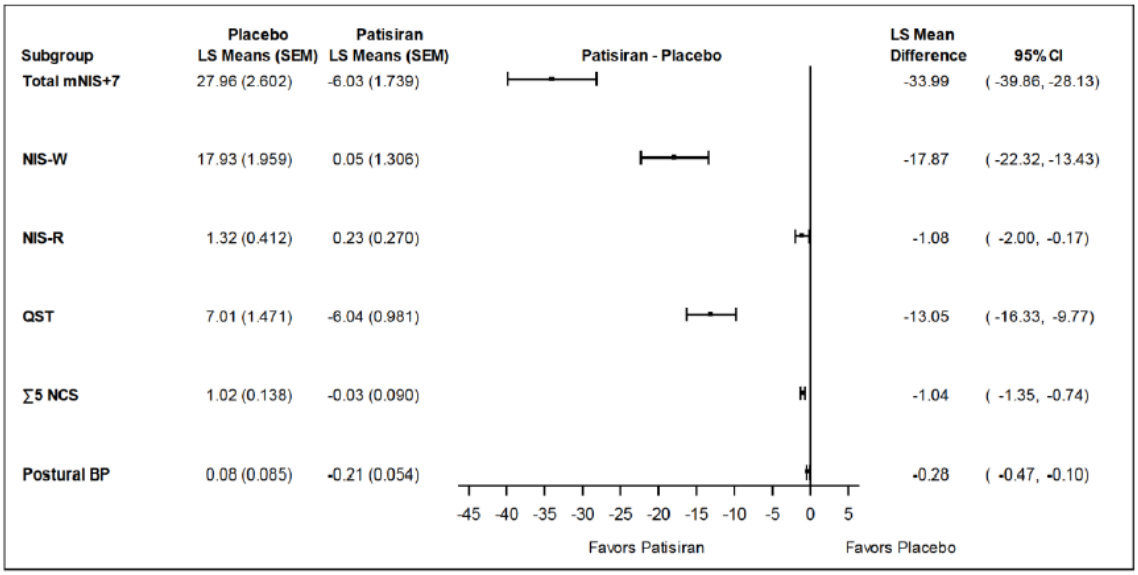
Table 18 mNIS+7 component change from baseline at month 18

mNIS+7 Subcomponent	Statistic	Placebo N=77	Patisiran-LNP 0.3 mg/kg N=148
Neurologic Impairment Score - Weakness (NIS-W) (192 points max. possible score)	Baseline Mean (SD)	29.03 (22.95)	32.69 (25.23)
	N	51	137
	LS Mean (SEM) change from Baseline at Month 18	17.93 (1.959)	0.05 (1.306)
	95% CI	14.07, 21.79	-2.52, 2.63
	LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-17.87 (2.254)**
	95% CI	-	**-22.32, -13.43**
Neurologic Impairment Score - Reflexes (NIS-R) (20 points max. possible score)	Baseline Mean (SD)	12.75 (5.90)	12.81 (6.07)
	N	61	141
	LS Mean (SEM) change from Baseline at Month 18	0.75 (0.376)	0.22 (0.267)
	95% CI	0.01, 1.49	-0.31, 0.74
	LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-0.54 (0.430)**
	95% CI	-	**-1.39, 0.31**
Quantitative Sensory Testing (QST) (80 points max. possible score)	Baseline Mean (SD)	24.8 (15.34)	27.2 (17.73)
	N	51	137
	LS Mean (SEM)	7.0 (1.47)	-6.0 (0.98)
	95% CI	4.1, 9.9	-8.0, -4.1
	LS Mean (SEM) Difference (Patisiran - Placebo)		**-13.05 (1.66)**
	95% CI	-	**-16.3, -9.8**
Nerve Conduction Studies Sum of Five Attributes (NCS ∑5) (10 points max. possible score)	Actual Mean (SD) at Baseline	7.43 (2.24)	7.58 (2.32)
	N	51	137
	LS Mean (SEM)	1.02 (0.138)	-0.03 (0.090)
	95% CI	0.75, 1.29	-0.21, 0.15
	LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-1.04 (0.157)**
	95% CI	-	**-1.35, -0.74**
Postural Blood	Baseline Mean (SD)	0.6 (0.74)	0.7 (0.79)

mNIS+7 Subcomponent	Statistic	Placebo N=77	Patisiran-LNP 0.3 mg/kg N=148
Pressure (BP) (2 points max. possible score)	N	51	137
	LS Mean (SEM)	0.1 (0.08)	-0.2 (0.05)
	95% CI	-0.1, 0.2	-0.3, -0.1
	LS Mean (SEM) Difference (Patisiran - Placebo)	-	**-0.3 (0.10)**
	95% CI	-	**-0.5, -0.1**

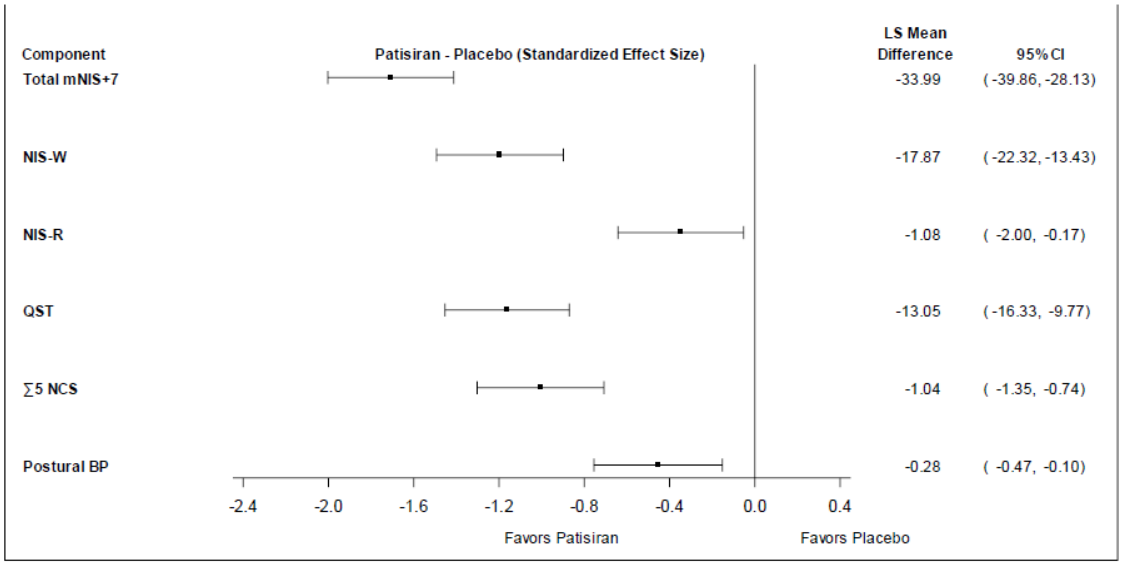
Abbreviations: CI=confidence interval; CMAP=compound muscle action potential; FAP= familial amyloidotic polyneuropathy; hATTR=hereditary transthyretin-mediated; LS=least squares; mITT=modified intent-to-treat; MMRM=mixed-effect model repeat measures; mNIS + 7 = Modified Neurologic Impairment Score + 7; SD=standard deviation; SEM=standard error of the mean.
Notes: In the MMRM model, the outcome variable is change from baseline in mNIS + 7 component score. The model includes baseline component score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use, and region. Data collected post-alternative treatment are excluded from analysis.
[a] NCS ∑5 includes ulnar CMAP, ulnar SNAP, sural SNAP, tibial CMAP, and peroneal CMAP.
[b] Baseline, Month 9, and Month 18 are the averages of 2 assessments performed at least 24 hours but no more than 7 days apart.
[c] LS means, SEM, differences in LS means, and 95% CIs from MMRM model.

Figure 11 (a) mNIS+7 components change from baseline to month 18



Abbreviations: NIS, Neuropathy Impairment Score; NIS-W, NIS Weakness; NIS-R, NIS Reflex; QST, Quantitative Sensory Testing; BP, Blood Pressure; NCS, Nerve Conduction Studies

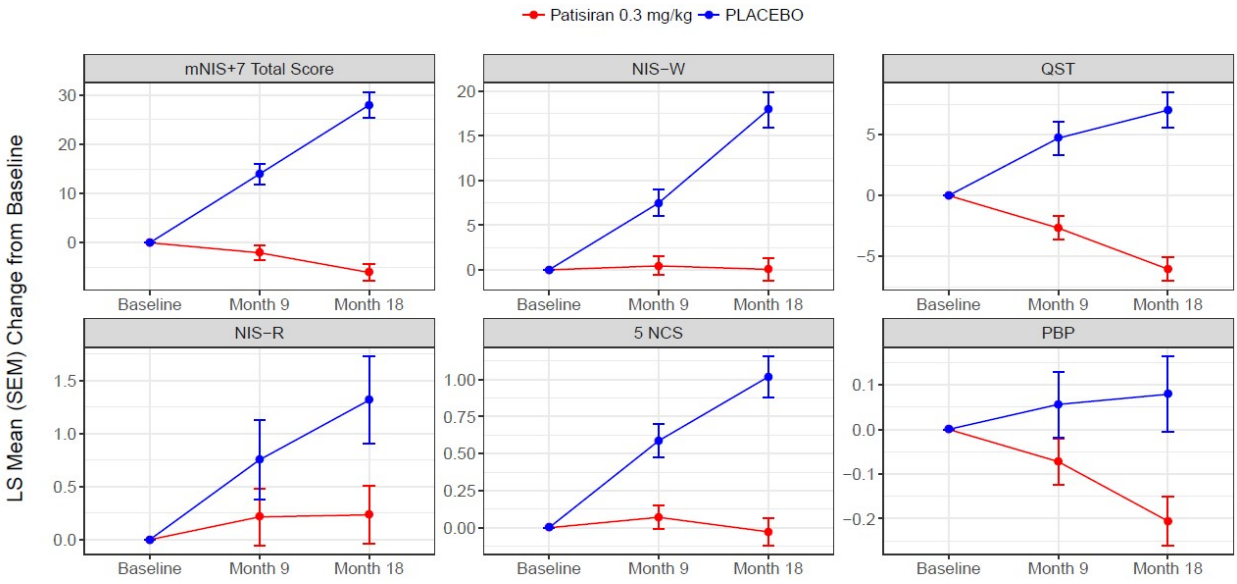
Figure 12 (b) mNIS+7 components standardized effect analysis



Abbreviation: NIS=Neuropathy Impairment Score, NIS-W=NIS Weakness, NIS-R=NIS Reflex, QST=Quantitative Sensory Testing, BP=Blood Pressure, NCS=Nerve Conduction Studies
Note: The confidence intervals are plotted after dividing 95% CIs of LS Mean Difference by estimated pooled standard deviation.

mNIS+7 is a composite score with five main parts. Motor skills are clearly dominating while autonomic function only contributes less than 1% of the total score. The electrophysiological tests may be less subjective, but don't add any new information than clinical motor examination and QST of touch pressure. Reflexes decline with increasing sensory and /or motor affection and do not represent any other specific function. Thus large myelinated nerve fibres are assessed several times which will amplify or reduce the total score. What appears as an improvement from baseline of mNIS+7 in the patisiran treated patients is completely dependent on the score change in QST component (see Figure 13 below).

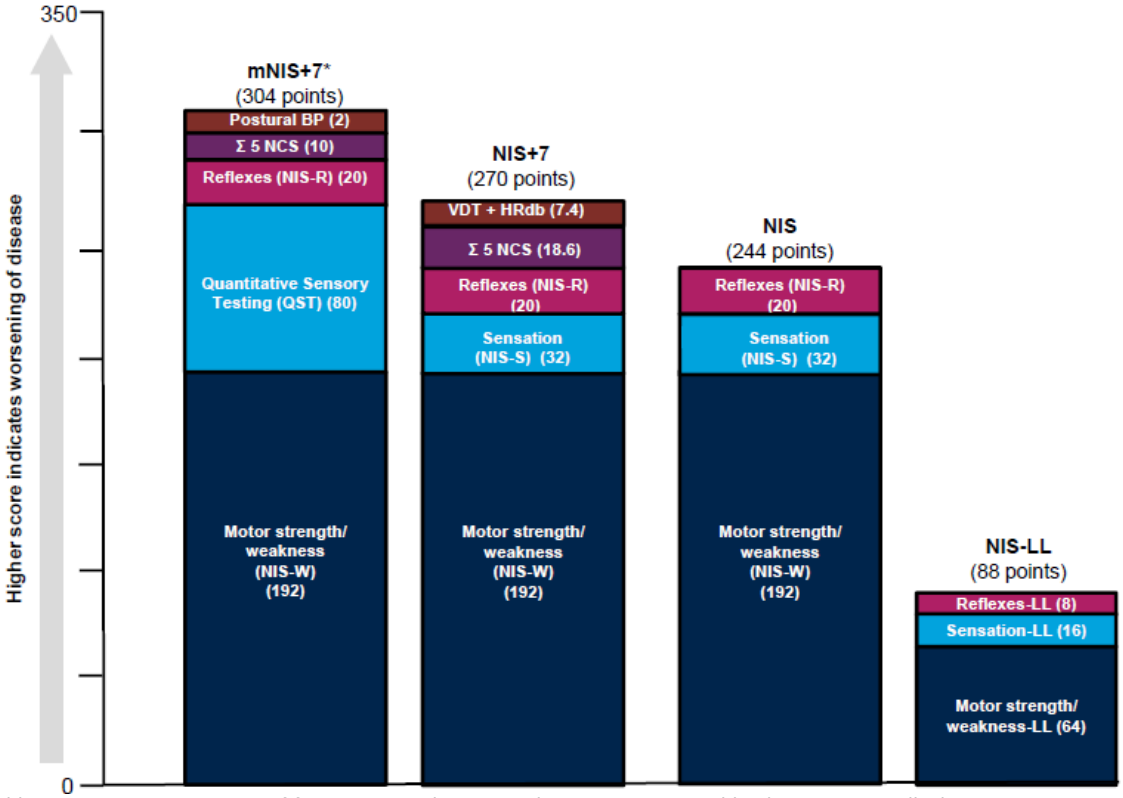
Figure 13 mNIS+7 Total Score and Component Score LS Mean Change Over Time, MMRM Analysis



There is a great variability in QST reliability. Other data than QST on sensory nerves and their function (nerve density in skin biopsies, SNAP data as presented by the applicant) as well as QoL assessments by the patients are not consistent with improvement in sensory function and symptoms. However, it is agreed that all 5 components of the mNIS+7 scales showed significant treatment effects compared to placebo. The mNIS+7 composite has not been used in clinical trials before and weighting is different for each component with NIS-W and QST driving the total scoring.

A Consensus report of the Peripheral Nerve Society suggested that a clinically significant change in neurological impairment as assessed by NIS score (a related predecessor of the mNIS+7) would be the degree of change that is twice (for the 2 sides of the body) the least degree of neurological impairment change that can be recognized on physical exam by an examining physician. In the case of change of muscle strength, strength of one muscle group on one side of the body is graded as normal (0 points) 25% weak (1 point), 50% weak (2 points), 75% weak (3 points) and paralyzed (4 points). A change of 25% of one muscle group times two body sides would be a change of 2 points. The Peripheral Nerve Society concluded that a 2-point change in NIS (scale range 0-244 points) would be the minimal clinically meaningful difference, as it reflects a detectable difference in muscle strength. Prior hATTR amyloidosis phase 3 trials (tafamidis and diflunisal) have used 2-point threshold for NIS-LL and NIS+7 scores therefore the CHMP considered appropriate that a threshold of 2 points could be applied to mNIS+7. A side-by-side alignment of NIS, NIS-LL, NIS+7 and mNIS+7 is presented in Figure 14 below. NIS, NIS+7 and mNIS+7 use the same NIS-W component which is the largest component of all the scales.

Figure 14 Components of various neurologic impairment scales



Abbreviations: Σ5 NCS=sum of five nerve conduction study parameters; BP=blood pressure; HRdb=heart rate variation with deep breathing; LL=lower limbs); mNIS+7=modified NIS+7; NIS=Neurologic Impairment Score; NIS+7=Neurologic Impairment score +7; NIS-LL=NIS-lower limbs; VDT=vibration detection threshold.

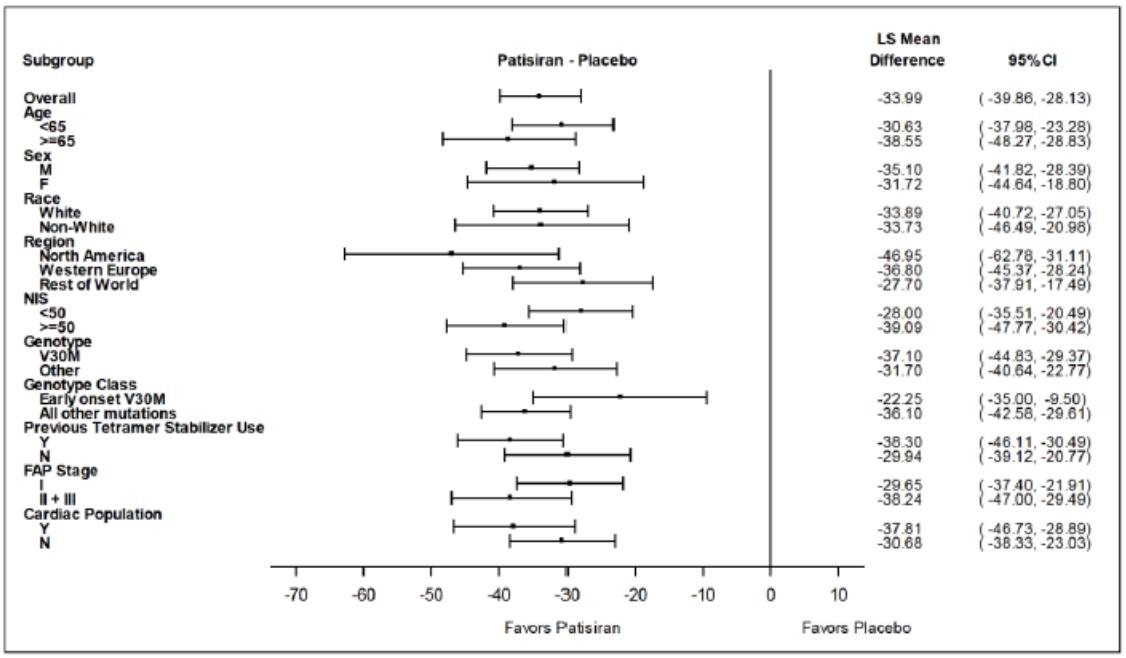
The baseline and change from baseline at 18 months for the 5 neurophysiological variables were examined by the applicant. The 5 neurophysiological variables include: ulnar CMAP, ulnar SNAP, sural SNAP, tibial CMAP, and peroneal CMAP. The results show baseline values for neurophysiological variables were generally similar in the placebo and patisiran-LNP groups.

mNIS+7 Subgroup Analysis

Baseline demographic and disease characteristics

A consistent treatment effect of patisiran-LNP on mNIS+7 was observed across all subgroups demonstrating reduced rate of neuropathy deterioration compared to placebo across a broad range of demographic and baseline disease characteristics. Subgroups evaluated included the following: age (<65; ≥65), gender, race (white, non-white), region (North America, Western Europe, Rest of World), NIS (< 50; ≥ 50), genotype (V30M; non-V30M), genotype class (Early onset V30M; Other), previous tetramer use, FAP stage (I; II & III) and cardiac subpopulation, see Figure 15 below.

Figure 15 Change from baseline in mNIS+7 compared to placebo by baseline demographic and disease subgroups



Abbreviations: F, female; FAP, familial amyloidosis with polyneuropathy; M, male; NIS, Neuropathy Impairment Score; V30M, valine to methionine mutation

The observed least improvement on the primary endpoint seen for early onset V30M as noted in the figure above could be a chance finding as early onset V30M is a very small subgroup. Also, the relatively lower baseline mNIS+7 score in the early onset placebo patients could potentially lead to the smaller magnitude of mNIS+7 progression compared with all other mutation patients (LS mean change 14.0 versus 31.5 points), which results in the numerical difference in treatment effects on the absolute change scale. Although a numerical difference is observed on the absolute change scale, the treatment difference on the percentage change scale appears to be similar for the two subgroups early onset V30M compared to all other mutation.

Since randomization was not stratified by region, some imbalance in prognostic factors between the two treatment groups in each region may occur, as exemplified by that the variation between North America and rest of the world was at least 20%. Also, the sample sizes per region are relatively small which can lead to some uncertainty with estimates of treatment effect. The applicant also points out that the North America and rest of the world regions demonstrated statistically significant and clinically meaningful treatment effects with patisiran compared with placebo.

The age by categories included patients <65 years which represents nearly 60% of the overall included patients. A more precise repartition of patients, <50 years and between 50 and 65 year has been provided, at the CHMP request, and analysed for the primary and secondary endpoints. The analyses provided show consistent treatment effect across class of age (<50, ≥50 – 65, ≥65) for the primary endpoint and the 6 secondary endpoint explored. There is no observed significant impact of these 3 age brackets on the treatment outcome regarding the considered endpoints.

The Applicant has provided Boxplots of mNIS+7 and Norfolk QoL-DN change at Month 18 by region, by country, and by study center. The number of patients recruited per center varied between 1 and 22. These Boxplots demonstrate consistent treatment effect across different regions, countries, and study centers.

Change from baseline in mNIS+7 compared to placebo for the baseline demographic and disease characteristics subgroups, shows a consistent difference in favour of patisiran, see table below.

The mNIS+7 values at baseline and change from baseline to Month 18 for the mITT population are presented for age, sex, race, region, NIS at baseline, genotype, genotype class, previous tetramer use, and FAP stage at baseline. It is agreed with the applicant that the data indicate a consistent and robust effect of patisiran-LNP on neuropathy impairment across all evaluated subgroups.

Table 19 Subgroup Analysis of Mean Change from Baseline to Month 18 in mNIS+7, MMRM Model (mITT Population)

Subgroup		Treatmenta	N		mNIS+7		LS Mean Difference (Patisiran-LNP - Placebo)
			Baseline	Month 18	Baseline (Mean)	Change from Month 18 (LS Mean)	
Age	<65 years	Patisiran-LNP	86	81	73.61	-6.33	-30.63
		Placebo	44	29	70.41	24.3	95% CI: -37.98, -23.28
	≥65 years	Patisiran-LNP	62	56	91.10	-2.26	-38.55
		Placebo	33	22	80.22	36.29	95% CI: -48.27, -28.83
Sex	Male	Patisiran-LNP	109	103	82.40	-5.63	-35.10
		Placebo	58	39	77.91	29.47	95% CI: -41.82, -28.39
	Female	Patisiran-LNP	39	34	76.85	-2.25	-31.72
		Placebo	19	12	64.55	29.47	95% CI: -44.64, -18.80
Race	White	Patisiran-LNP	113	107	80.71	-5.96	-33.89
		Placebo	50	36	73.26	27.93	95% CI: -40.72, -27.05
	Non-white	Patisiran-LNP	33	28	82.72	-1.88	-33.73

		Placebo	26	14	76.27	31.85	95% CI: -46.49, -20.98
Region	North America	Patisiran-LNP	37	34	65.93	-3.65	-46.95
		Placebo	10	5	86.43	43.30	95% CI: -62.78, -31.11
	Western Europe	Patisiran-LNP	62	61	88.29	-8.10	-36.80
		Placebo	36	27	74.98	28.70	95% CI: -45.37, -28.24

Subgroup		**Treatment[a]**	**N**		**mNIS+7**		**LS Mean Difference (Patisiran-LNP - Placebo)**
			Baseline	**Month 18**	**Baseline (Mean)**	**Change from Month 18 (LS Mean)**	
	ROW	Patisiran-LNP	49	42	82.96	-1.29	-27.70
		Placebo	31	19	70.38	26.41	95% CI: -37.91, -17.49
NIS	<50	Patisiran-LNP	62	59	43.23	-4.83	-28.0
		Placebo	35	24	41.24	23.16	95% CI: -35.51, -20.49
	≥50	Patisiran-LNP	86	78	108.12	-4.43	-39.09
		Placebo	42	27	102.43	34.46	95% CI: -47.77, -30.42
Genotype Class	Early-onset V30M	Patisiran-LNP	13	13	81.06	8.28	-22.25
		Placebo	10	8	68.89	13.97	95% CI: -35.00, -9.50
	Other	Patisiran-LNP	135	124	80.92	-4.58	-36.10
		Placebo	67	43	75.47	31.51	95% CI: -42.58, -29.61
Genotype	V30M	Patisiran-LNP	56	56	88.54	-9.97	-37.10
		Placebo	40	27	73.40	27.14	95% CI: -44.83, -29.37
	Non-V30M	Patisiran-LNP	92	81	76.30	-0.10	-31.70
		Placebo	37	24	75.93	31.60	95% CI: -40.64, -22.77
Previous Tetramer Use	Yes	Patisiran-LNP	78	76	79.51	-5.26	-38.30
		Placebo	41	25	73.15	33.04	95% CI: -46.11, -30.49
	No	Patisiran-LNP	70	61	82.52	-3.98	-29.94
		Placebo	36	26	76.28	25.96	95% CI: -39.12, -20.77
FAP Stage	I	Patisiran-LNP	67	64	52.02	-5.17	-29.65
		Placebo	37	25	45.47	24.48	95% CI: -37.40, -21.91
	II or III	Patisiran-LNP	81	73	104.85	-4.18	-38.24
		Placebo	40	26	101.58	34.06	95% CI: -47.0, -29.49

Abbreviations: CI=confidence interval; EU=Europe; FAP=familial amyloidosis with polyneuropathy; LNP=lipid nanoparticle; LS=lease square; mITT=modified intent-to-treat; mNIS+7=Modified Neurologic Impairment Score +7; MMRM=mixed-effect model repeated measures; N=number of patients; NIS=Neuropathy Impairment Score; ROW=rest of world; V30M=valine to methionine mutation a Patisiran-LNP 0.3 mg/kg. Notes:

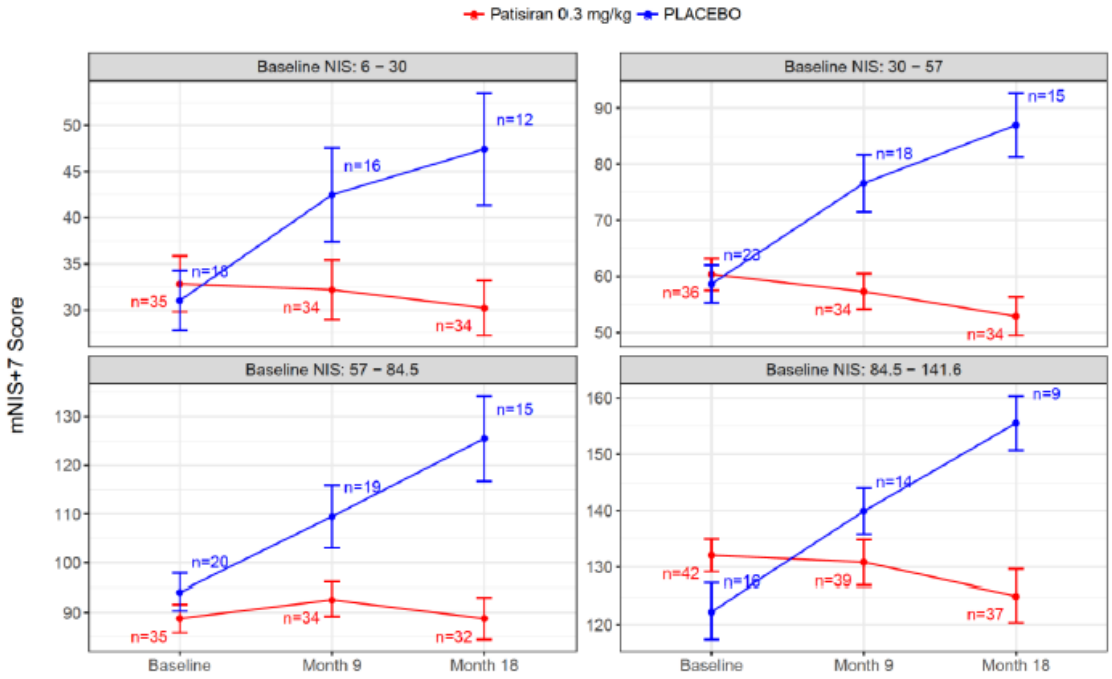
•In the MMRM model, the outcome variable was change from baseline in mNIS+7 total score.

•The model includes baseline mNIS+7 score as covariate and fixed effect terms including treatment group, visit, treatment-by- visit interaction and genotype.

Baseline neuropathy severity (NIS)

The effect of patisiran-LNP was further evaluated across quartiles of baseline neuropathy severity as assessed by baseline NIS. Within each quartile, the placebo patients had a worsening of neuropathy score while the patisiran-LNP patients had a numerical improvement of neuropathy score, see Figure 16 below.

Figure 16 mNIS+7 score at baseline, month 9 and month 18 by baseline NIS quartiles

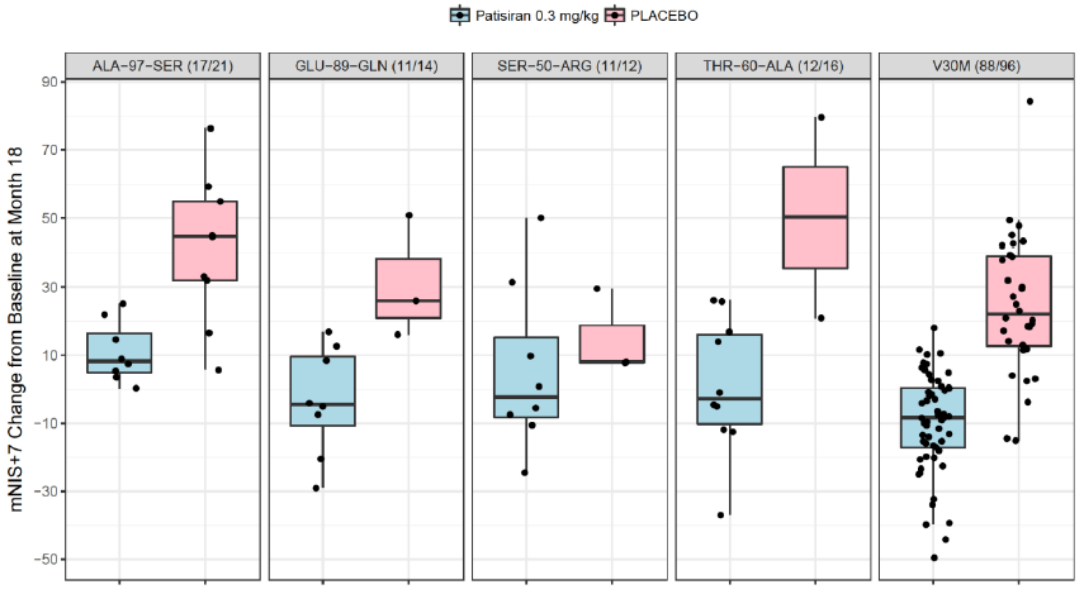


Abbreviations: mITT=modified intent-to-treat; mNIS + 7=modified Neurologic Impairment Score + 7; NIS=neurologic impairment score

Genotype

The effect of patisiran-LNP was further evaluated across different genotypes. Change in mNIS+7 at 18 months with patisiran-LNP compared to placebo was evaluated for TTR genotypes with at least 10 patients. The genotypes included: V30M, Thr-60Ala, Ser-50-Arg, Glu-89-Gln and Ala-97-Ser and the result is shown in Figure 17 below.

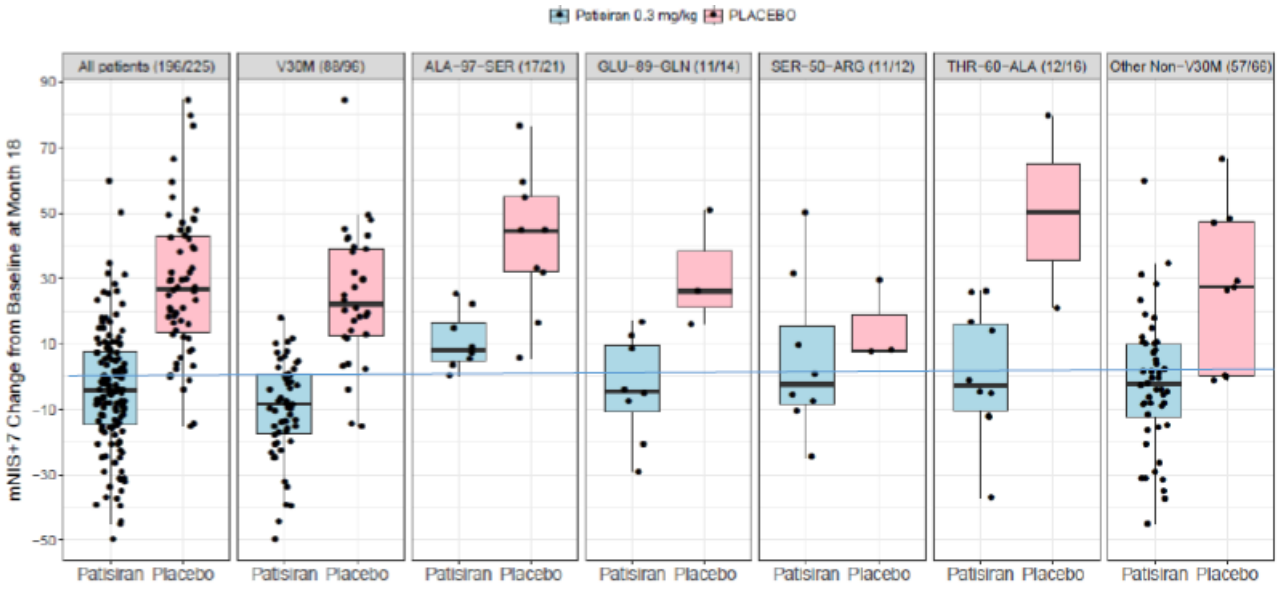
Figure 17 Boxplots of mNIS+7 change from baseline to month 18 in the five most common genotypes studied



Note: All data are presented including assessments post alternative FAP treatment

Boxplots of mNIS+7 change from baseline in the five most common genotypes included in the study show some variability for the placebo groups, possibly indicating different phenotypes with different progression speed. Four of the patisiran genotype groups show slight numerical decrease in mNIS+7 but one group, A97S, shows increase (worsening) in mNIS+7. As explained by the applicant, with respect to the Ala97Ser mutation, the total number of patients included with this mutation in study 004 was only 21, of which only 17 contributed with results. The Ala97Ser mutation is the only group with a patisiran mNIS+7 result >0, but the change in mNIS+7 in these patients is within the range of mNIS+7 change seen in the overall group of patients treated with patisiran. The change from baseline in mNIS+7 for all patients, individual genotypes with at least 10 patients, and all other non-V30M genotypes are shown as boxplots in Figure below.

Figure 18 Boxplot of mNIS+7 Change at Month 18 in All Genotypes (Number of Patients Completing Month 18 Assessments/Total Number of Patients with the Genotype)



Note: All data are presented including assessments post alternative FAP treatment

As shown in Figures 17 and 18 above, patisiran-LNP led to a consistent difference to placebo in neuropathy across all 6 TTR mutation subgroups. Based on the mechanism of action patisiran-LNP targeting the 3' UTR of TTR mRNA, consistent TTR reduction across a broad range of genotypes in Study 004, and consistent improvement mNIS+7 across a broad range of genotypes in Study 004, the applicant argue that the benefit in neuropathy can be extrapolated to all genotypes including those not evaluated in study 004.
The applicant has presented the frequencies of various genotypes in study 004 compared to published frequencies in various European countries, confirming that the genotype frequencies in study 004 were representative for Europe.

Secondary and exploratory endpoint measures

To control overall type I error, these secondary endpoints were tested in a hierarchical order, in the order listed below.

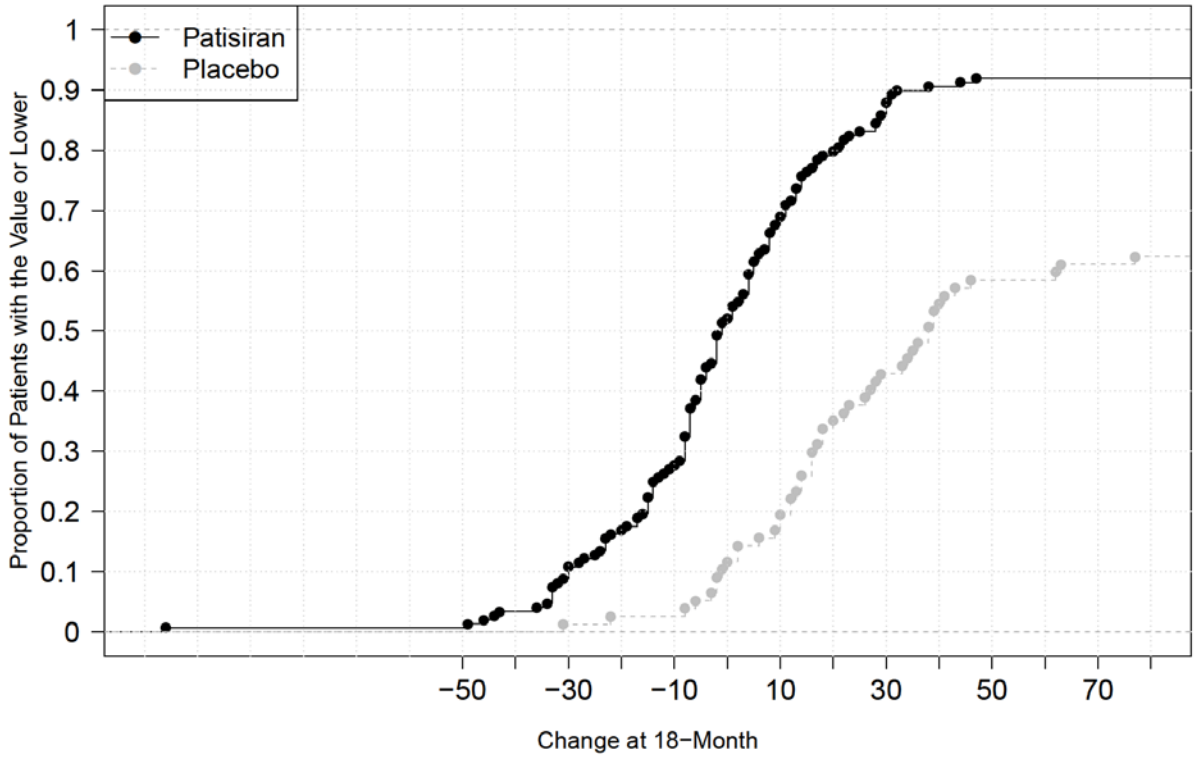
Norfolk QoL-DN Domains

The Norfolk QoL-DN is a nerve fiber specific QoL tool that was developed for patients with diabetic neuropathy. In an observational cross-sectional natural history study it was demonstrated that the Norfolk QoL-DN is reliable and valid in assessing quality of life in patients with hATTR amyloidosis. The Norfolk-QoL-DN uses 35 scored questions across five domains that comprise the total score. The total score ranges from -4 (best possible quality of life) to 136 points (worst possible quality of life). The domains measure quality of life pertinent to all aspects of polyneuropathy in hATTR amyloidosis, including large and small nerve fiber function, autonomic function, symptoms, and activities of daily living.

At baseline, the mean Norfolk QoL-DN was 55.5 points in the placebo group and 59.6 points in the patisiran-LNP group. There was a statistically significant difference in quality of life as assessed by Norfolk QoL-DN at 18 months favouring the patisiran-LNP group compared to placebo (LS mean patisiran -6.7, LS mean placebo 14.4, LS mean difference between groups: -21.3 points, P=1.103 ×10-10). The difference in quality of life

between treatment arms was observed already at 9 months (LS mean difference between groups: -15.0 points, 95% CI: -19.8, -10.2). The cumulative distribution of Norfolk QoL change from baseline at Month 18 is shown in Figure 19 below.

Figure 19 Cumulative distribution of Norfolk QoL change from baseline at Month 18 (mITT Population)



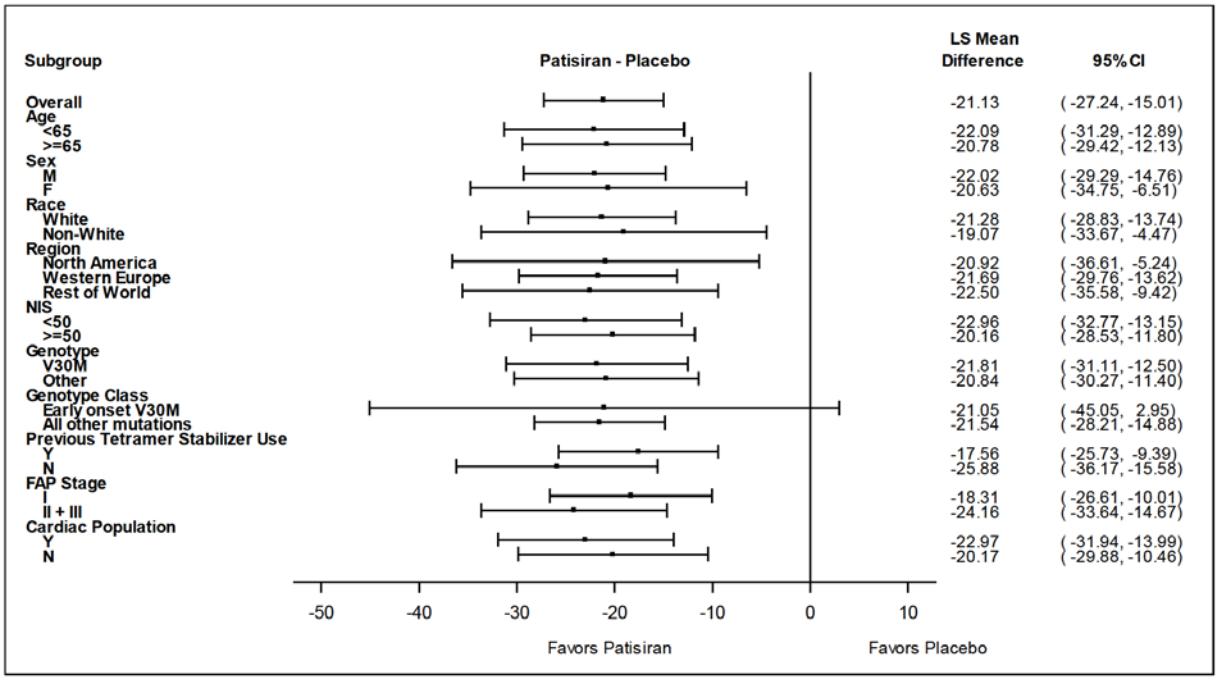
Norfolk QoL-DN Subgroup Analysis

Subgroup analysis for Norfolk QoL-DN included analysis by baseline demographic and disease characteristics, and by the most frequent TTR genotypes as described below.

Baseline demographic and disease characteristics

A consistent treatment effect of patisiran-LNP on Norfolk QoL-DN was observed across all subgroups, as demonstrated by a difference favouring patisiran-LNP compared to placebo in quality of life across a broad range of demographic and baseline disease characteristics (as shown in Figure 20 below). Subgroups evaluated included the following: age (<65; ≥65), gender, race (white, non-white), region (North America, Western Europe, Rest of World), NIS (< 50; ≥ 50), genotype (V30M; non-V30M), genotype class (Early onset V30M; Other), previous tetramer use, FAP stage (I; II & III) and cardiac subpopulation.

Figure 20 Norfolk QoL-DN Total Score Change from Baseline to Month 18 by Subgroup Analysis (mITT Population)



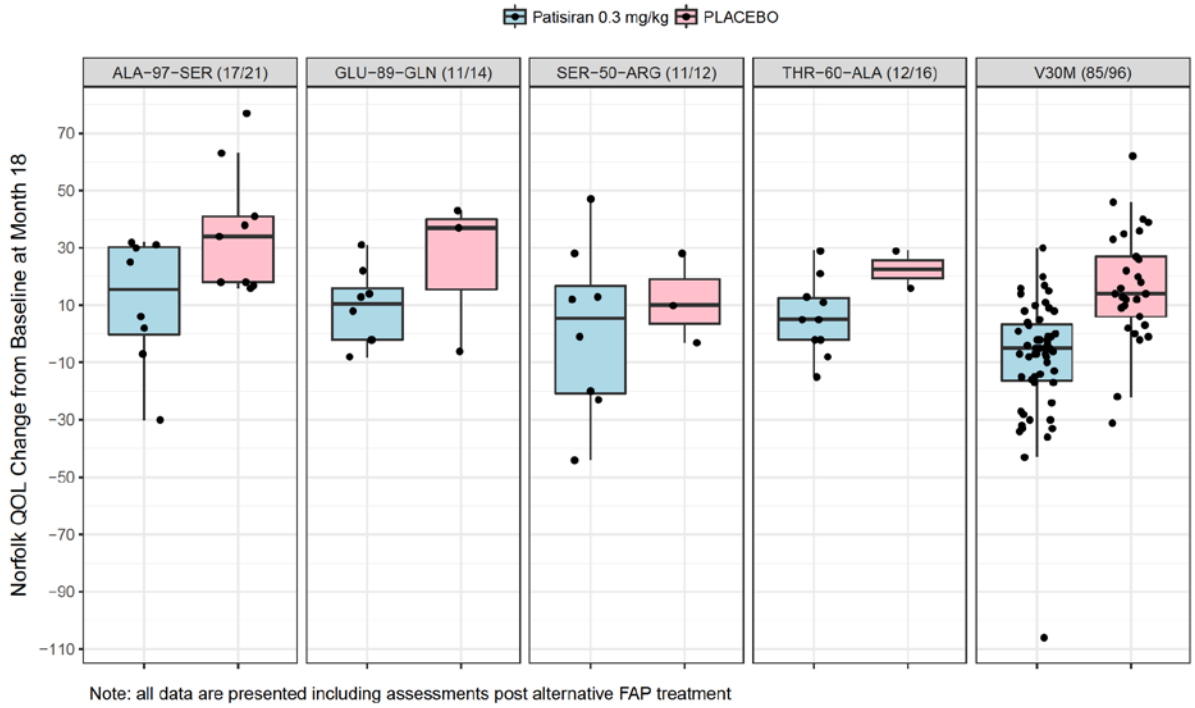
Abbreviations: F=female; FAP=familial amyloidosis with polyneuropathy; M=male; mITT=modified intent to treat; NIS=Neuropathy Impairment Score; V30M=valine to methionine mutation

Particularly with V30M early onset, there was a large dispersion in results, particularly on Norfolk QoL-DN. As explained by the applicant and based on literature there seems to be no evidence for the existence of triplet nucleotide expansion in the TTR gene itself nor in neighbouring regions despite efforts to identify such regions in patients with V30M (Soares, 1999, Soares, 2004). Since DNA samples were not collected, the analysis on trinucleotide repeats in the APOLLO patients cannot be conducted.

Genotype

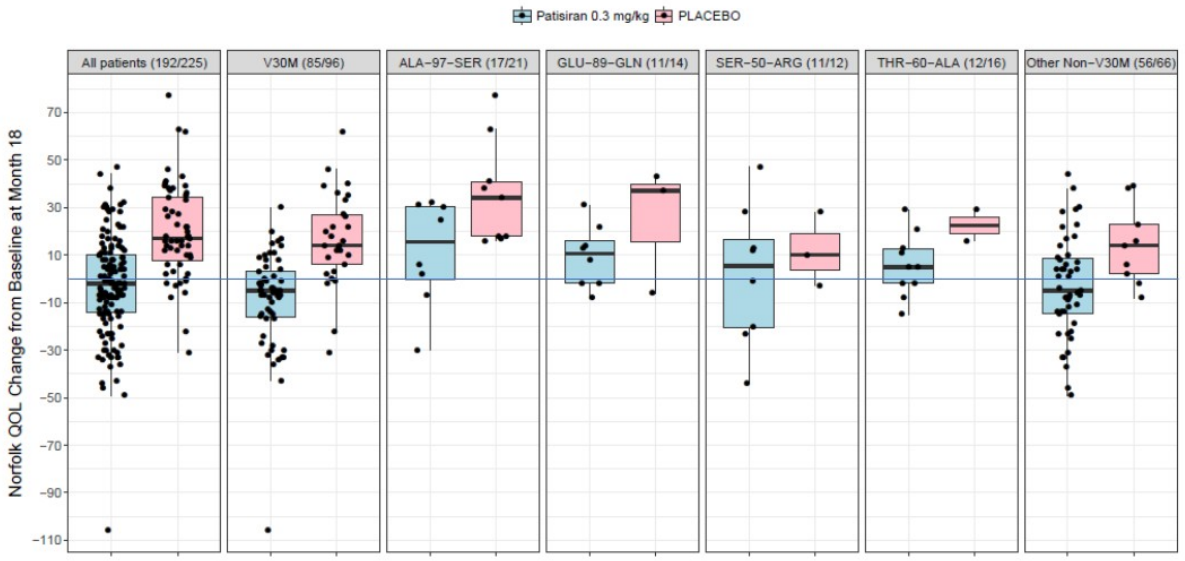
Change in Norfolk QoL-DN at 18 months with patisiran-LNP compared to placebo was evaluated for TTR genotypes with at least 10 patients: V30M, Thr-60Ala, Ser-50-Arg, Glu-89-Gln and Ala-97-Ser. As shown in Figure 21 below, differences in quality of life favouring patisiran-LNP compared to placebo were noted across all 5 genotypes.

Figure 21 Study 004: Boxplot of Norfolk QoL Change from Baseline at Month 18 in Genotypes with 10 Patients or More (No. of Patients Completing Month 18 Assessments/Total No. of Patients with the Genotype)



Patients with V30M mutation treated with patisiran in study 004 is the only subgroup that show a trend of improvement for the QoL score from baseline. The change from baseline in mNIS+7 for all patients, individual genotypes with at least 10 patients, and all other non-V30M genotypes are shown as boxplots in Figure 22 for additional analysis.

Figure 22 Boxplot of Norfolk QoL Change at Month 18 in All Patients, All Genotypes with at Least 10 Patients, and All Other Non-V30M Genotypes (Number of Patients Completing Month 18 Assessments/Total Number of Patients with the Genotype)



Note: All data are presented including assessments post alternative FAP treatment

As shown in the figure above, in the V30M and other non-V30M genotype groups, which represented 72% of the whole study population, there was a trend for improvement of Norfolk QoL relative to baseline, whereas for the other most common genotypes (Al97Ser, Glu89Gln, Ser50Arg, Thr60Ala) didn't show any improvement of Norfolk QoL relative to baseline, but only relative to placebo. The variability of the results for various genotypes was probably related to the small number of patients represented in these individual mutations in the setting of a large dispersion of interpatient results.

Study 004 Additional Secondary Endpoints (NIS-W, 10-meter Walk Test, R-ODS, mBMI, and COMPASS 31)

A statistical significant differences favouring Patisiran-LNP group compared to placebo at 18 months were observed in motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic symptoms (COMPASS 31) (see table below).

Table 20 Study 004: Secondary Efficacy Endpoints at 18 Months

Secondary Endpoint	**Statistic[a]**	**Placebo** N = 77	**Patisiran-LNP** N = 148
NIS-W	Baseline Scores, Mean (SD)	29.03 (22.95)	32.69 (25.23)
Score range: 0 to 192 points	Score at 18 months, Mean (SD)	46.32 (31.77)	33.72 (28.34)

Secondary Endpoint	**Statistic**[a]	**Placebo** N = 77	**Patisiran-LNP** N = 148
Less neurologic impairment = Lower score	Change from Baseline, LS Mean (SEM) 95% CI	17.93 (1.959) 14.07, 21.79	0.05 (1.306) -2.52, 2.63
	LS Mean (SEM) Difference Treatment Difference (Patisiran-LNP – Placebo) **95% CI, p-value**	-	**-17.87 (2.25)** **-22.32, -13.43, P=1.404E-13**
R-ODS Score range: 0 to 48 points Less disability = Higher score	Baseline Scores, Mean (SD)	29.8 (10.76)	29.7 (11.51)
	Actual at 18 months, Mean (SD)	21.0 (13.36)	29.5 (12.70)
	Change from Baseline, LS Mean (SEM) **95% CI**	-8.9 (0.88) -10.7, -7.2	0.0 (0.59) -1.1, 1.2
	LS Mean (SEM) Difference Treatment Difference (Patisiran-LNP – Placebo) 95% CI, p-value	**-**	**9.0 (1.01)** **7.0, 10.9, P=4.066E-16**
10-MWT Gait speed reported in meters/second. Faster/better gait speed = Higher speed	Baseline Mean (SD)	0.79 (0.32)	0.80 (0.40)
	Actual at 18 months, Mean (SD)	0.56 (0.39)	0.85 (0.50)
	Change from Baseline, LS Mean (SEM) 95% CI	-0.24 (0.04) -0.31, -0.16	0.08 (0.02) 0.03, 0.12
	LS Mean (SEM) Difference Treatment Difference (Patisiran-LNP – Placebo) **95% CI, p-value**	**-**	**0.31 (0.04)** **0.23, 0.39, P=1.875E-12**
mBMI	Baseline Mean (SD)	989.9 (214.19)	969.7 (210.45)
mBMI reported as BMI (kg/m^2) x albumin (g/L)	Actual at 18 months, Mean (SD)	892.7 (221.10)	975.4 (228.56)

Secondary Endpoint	Statistic[a]	Placebo N = 77	Patisiran-LNP N = 148
Better nutritional status = Higher mBMI	Change from Baseline, LS Mean (SEM) 95% CI	-119.4 (14.51) -148.0, -90.8	-3.7 (9.57) -22.6, 15.1
	LS Mean (SEM) Difference Treatment Difference (Patisiran-LNP – Placebo) **95% CI, p-value**	-	**115.7 (16.91)** **82.4, 149.0, P=8.832E-11**
COMPASS 31 Score range: 0 to 100 points Less autonomic neuropathy symptoms = Lower score	Baseline Scores, Mean (SD)	30.31 (16.37)	30.61 (17.58)
	Actual at 18 months, Mean (SD)	33.11 (17.58)	25.61 (17.05)
	Change from Baseline, LS Mean (SEM) 95% CI	2.24 (1.94) -1.59, 6.06	-5.29 (1.30) -7.85, -2.72
	LS Mean (SEM) Difference Treatment Difference (Patisiran-LNP – Placebo) **95% CI, p-value**	-	**-7.53 (2.21)** **-11.89, -3.16, P=0.0008**

Abbreviations: CI=confidence interval; LS=least squares; mBMI=modified body mass index; MMRM=mixed-effect model repeated measures; NIS=Neurologic Impairment Score; NIS-W=NIS-weakness; QoL-DN=Quality of Life-Diabetic Neuropathy; SD=standard deviation; SEM=standard error of the mean.
NIS-W: The model includes baseline NIS-W score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region. Data collected post alternative treatment are excluded from analysis.
R-ODS, 10-MWT, mBMI, COMPASS 31: The model includes baseline score as covariate and fixed effect terms including treatment group, visit, treatment-by-visit interaction, baseline NIS, genotype, age at hATTR symptom onset, previous tetramer stabilizer use and region.
a LS means, SEM, differences in LS means, 95% Cis and Month 18 p-value from MMRM model.

Rasch-built Overall Disability Scale, R-ODS, is a patient-reported disability scale that captures activity and social participation limitations.

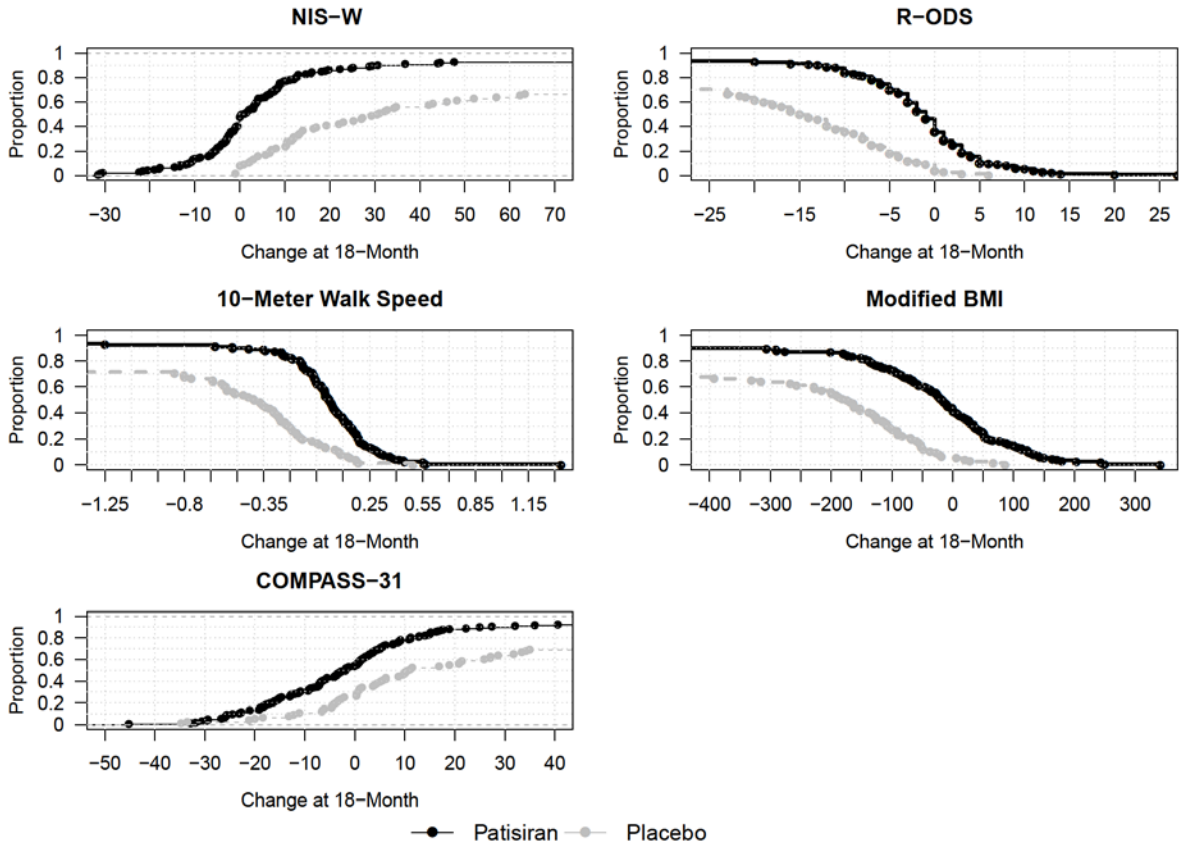
The 10-MWT measures the time it takes a patient to walk a distance of 10 meters. In performing this test, patients were allowed to use ambulatory aids such as a cane or walker.
Gait speed is commonly studied in patients with neuropathy and is anticipated to be associated primarily with muscle strength but also with sensation. The mean self-selected gait speed in healthy subjects ages 10 to 79 years ranged from approximately 1.1 to 1.3 m/s. A walking speed of 1.4 m/s is considered to represent an "extremely fit" individual whereas a walking speed of <0.8m/s suggests a patient with more limited community ambulation.

mBMI is a measure of nutritional status calculated as the product of BMI (weight in kilograms divided by the square of height in meters) and serum albumin (g/L). Patients with hATTR amyloidosis often have poor nutritional status and overall weight loss due in part to severe gastrointestinal manifestations and in part due to cardiac disease.

The COMPASS 31 has been used to assess autonomic symptoms in patients with diabetic neuropathy and small fiber polyneuropathy and is considered a valid instrument in these patients. Patients with hATTR amyloidosis often have debilitating autonomic dysfunction, COMPASS 31 was adopted for use in patisiran-LNP clinical studies.

Cumulative distribution curves of change from baseline at 18 months in all additional secondary endpoints shows separation between patisiran-LNP and placebo treated patients, favouring patisiran-LNP, across all response thresholds (Figure 23).

Figure 23 Study 004: Cumulative Distribution Plots of Secondary Efficacy Endpoints Change from Baseline at Month 18 (mITT Population)



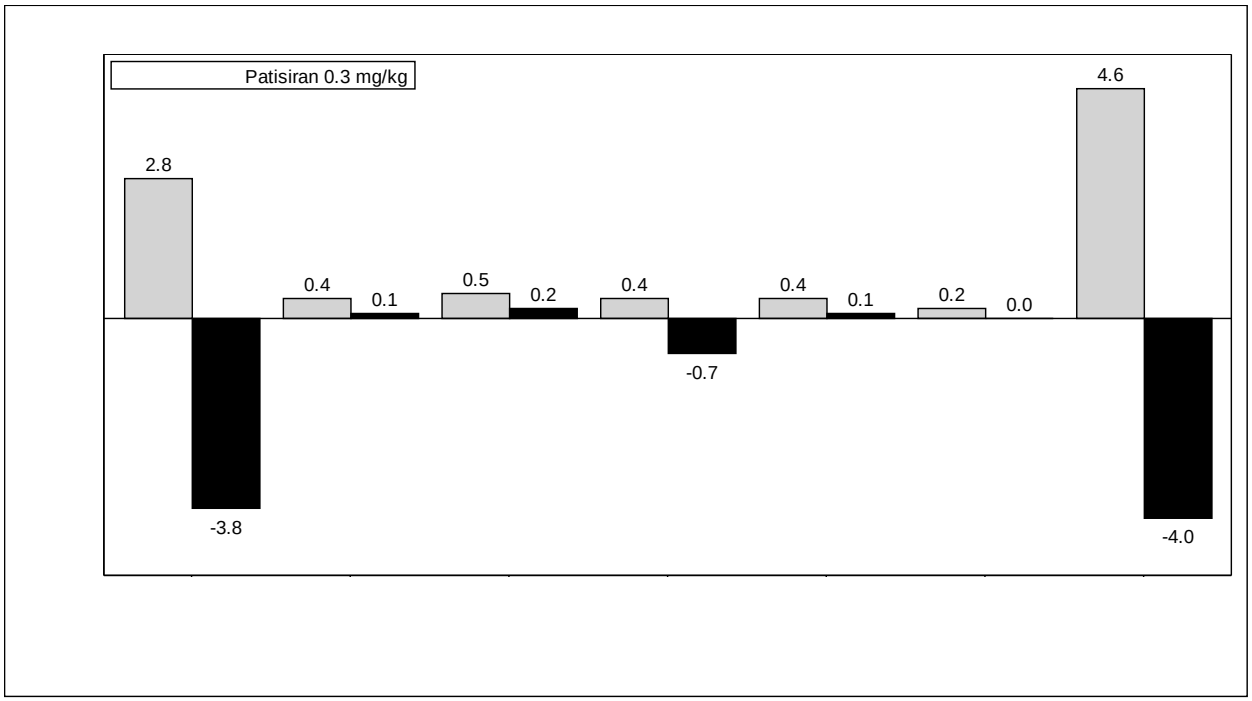
Abbreviations: mBMI=modified body mass index; mITT=modified intent to treat; NIS=Neurologic Impairment Score; NIS-W=NIS-weakness; QoL-DN=Quality of Life-Diabetic Neuropathy;

Relative to baseline, the patisiran-LNP group showed stabilization or improvement of motor strength, R-ODS, gait speed and COMPASS 31, in contrast the placebo group showed a worsening (for COMPASS only numerical) in these secondary end-points.

Relative to baseline, the patisiran-LNP group showed a limited numerical decrease from baseline in mBMI, in contrast the placebo group showed a significant decrease in mBMI at 18 months.

Patisiran-LNP appears to reduce the frequency and severity of diarrhea symptoms based on COMPASS 31 results. In addition, Patisiran-LNP reduces patient reported symptoms of moderate or severe diarrhoea and/or loss of bowel control based on Norfolk QoL-DN results.

Figure 24 Study 004: Mean Change from Baseline to Month 18 in COMPASS-31 Total Score and Domain Scores (mITT Population)



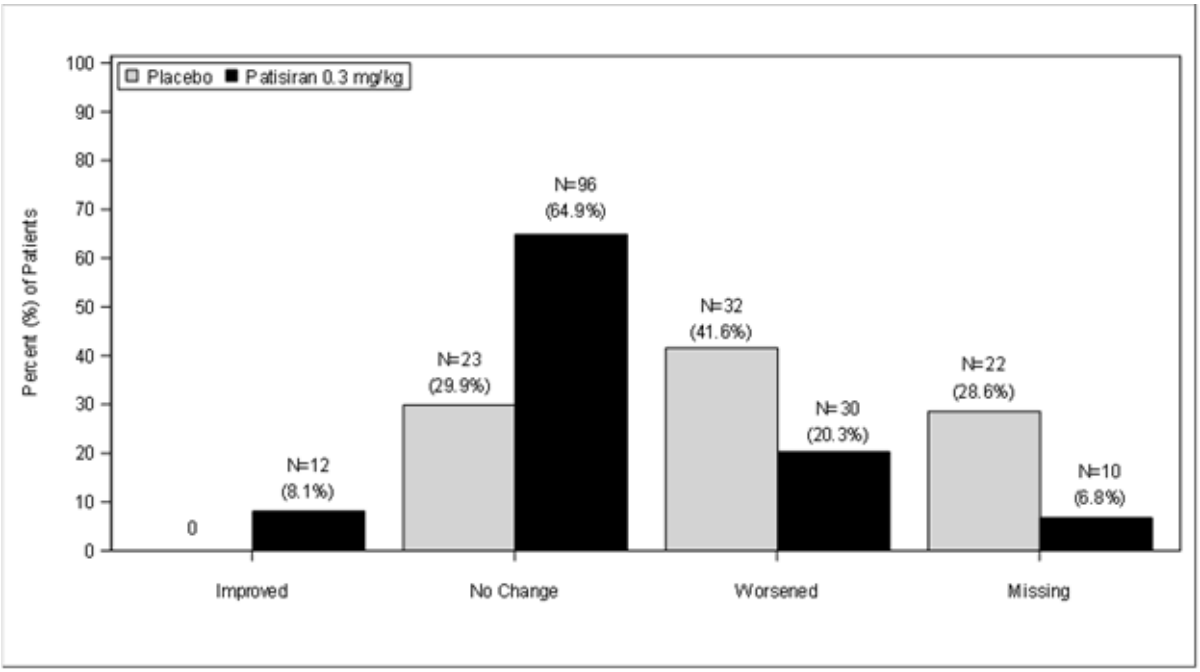
Abbreviations: COMPASS 31=composite autonomic symptom score; mITT=modified intent-to-treat.

Study 004: Exploratory Efficacy Endpoints

NIS+7 score, Grip strength, EuroQOL (EQ-5D) questionnaire, Large vs small nerve fiber function including nerve conduction studies sum of 5 attributes (NCS Σ5) (quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), vibration detection threshold (VDT), heart rate variability to deep breathing (HRdb), postural blood pressure), assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score and some additional exploratory endpoints were measured. Relative to baseline, patisiran-LNP treatment generally either improved or stabilized for these measures whereas patients in the placebo group showed worsening at 18 months.

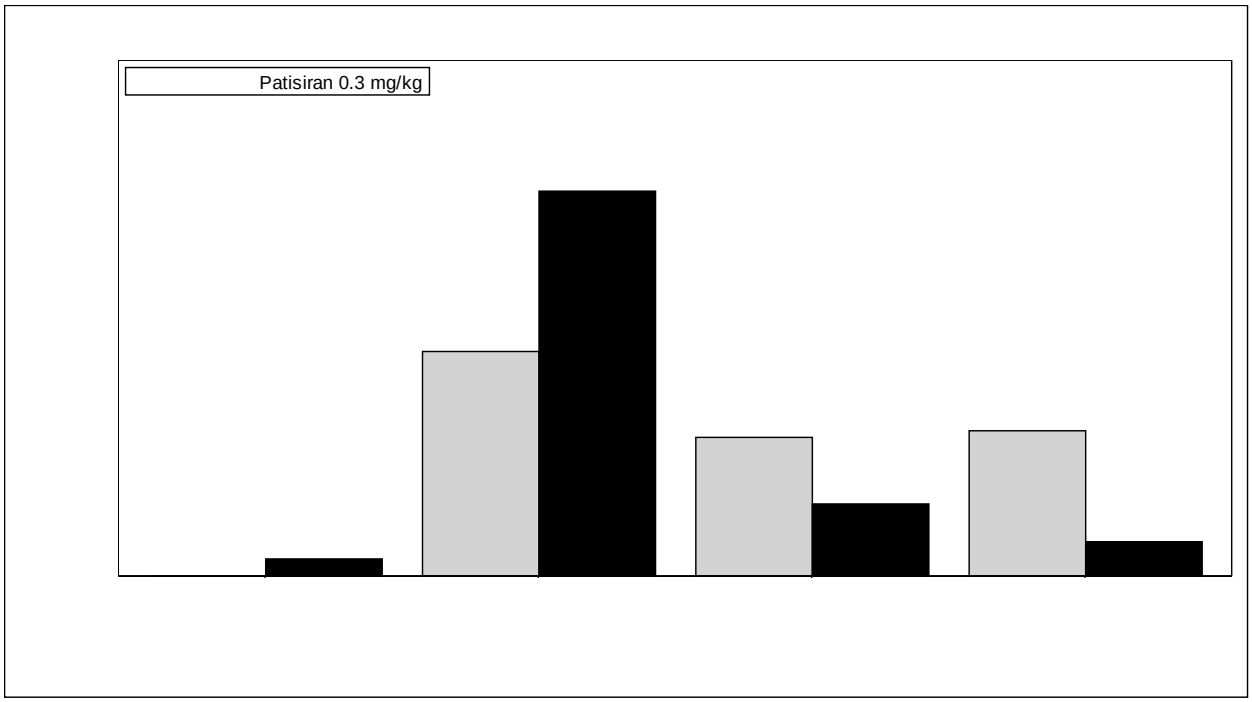
As can be seen in the figures below patients in the patisiran-LNP group were more likely to have stable or improved PND score and FAP Stage at 18 months compared with placebo patients. Conversely, patients in the placebo group were more likely to have progression or worsening in PND score and FAP Stage (Figure 25 and Figure 26 below). PND score and FAP stage are used clinically to characterize a patient's baseline neuropathy severity and changes in stage and score are reflective of changes in disease severity and ability to ambulate without assistance (Adams, 2015).

Figure 25 Study 004: Shift in PND Score from Baseline to Month 18 (mITT Population)



Abbreviations: mITT=modified intent-to-treat; PND=Polyneuropathy Disability.
Note: Percent includes number of patients with missing data.

Figure 26 Study 004: Shift in FAP Stage from Baseline to Month 18 (mITT Population)



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

Evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD), sweat gland nerve fiber density (SGNFD), and dermal amyloid

content were performed in a very limited number of patients. For intraepidermal nerve fiber density (IENFD), a difference favouring patisiran-LNP patients compared to placebo was noted at 18 months, but also the patisiran-LNP patients had less nerve fiber density. For sweat gland nerve fiber density (SGNFD), and dermal amyloid content no difference between patisiran-LNP patients and placebo were noted, there were generally no change from baseline.

Pharmacodynamic exploratory biomarkers [TTR, retinol binding protein (RBP), vitamin A] were included, and the results showed an expected substantial reduction in these PD markers.

Cardiac assessment

Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels were included as exploratory end-points. The analysis of the effect of patisiran-LNP on amyloid cardiomyopathy was focused on a pre-specified cardiac subpopulation (LV wall thickness ≥1.3 cm with no history of aortic valve disease or hypertension), which comprised 56% of the overall study population. Among patients in the cardiac subpopulation, 73.0% had non-V30M genotype. The majority (60.3%) had NYHA class II heart failure.

The analyses focused on echocardiographic parameters indicative of cardiac structure (mean LV wall thickness and LV mass) and systolic and diastolic cardiac function (longitudinal strain, left ventricular ejection fraction [LVEF], and Left ventricular end-diastolic volume [LVEDV]) and the cardiac biomarker NT pro-BNP; all assessed at baseline, Month 9, and Month 18.

Table 21 Results for Select Echocardiographic Parameters in Study 004 (Cardia Subpopulation)

Echocardiographic Parameter		Placebo (N=36)	Patisiran-LNP (N=90)	Treatment Difference (Patisiran-LNP – Placebo)	P value
LV Wall Thickness, cm	Baseline, mean	1.64	1.68		
	CFB to 18 months	-0.007	-0.100	-0.093	0.017
LV Mass, g	Baseline, mean	264.52	275.48		
	CFB to 18 months	0.63	-15.12	-15.75	0.150
Longitudinal Strain, %	Baseline, mean	-15.7	-15.1		
	CFB to 18 months	1.46	0.08	-1.37	0.0154
Cardiac Output, L/min	Baseline, mean	3.92	3.77		
	CFB to 18 months	-0.56	-0.18	0.38	0.0441
Ejection Fraction, %	Baseline, mean	62.21	60.00		
	CFB to 18 months	0.57	1.00	0.43	0.7852
LV End Diastolic Volume, mL	Baseline, mean	84.9	86.2		
	CFB to 18 months	-13.44	-5.13	8.34	0.036

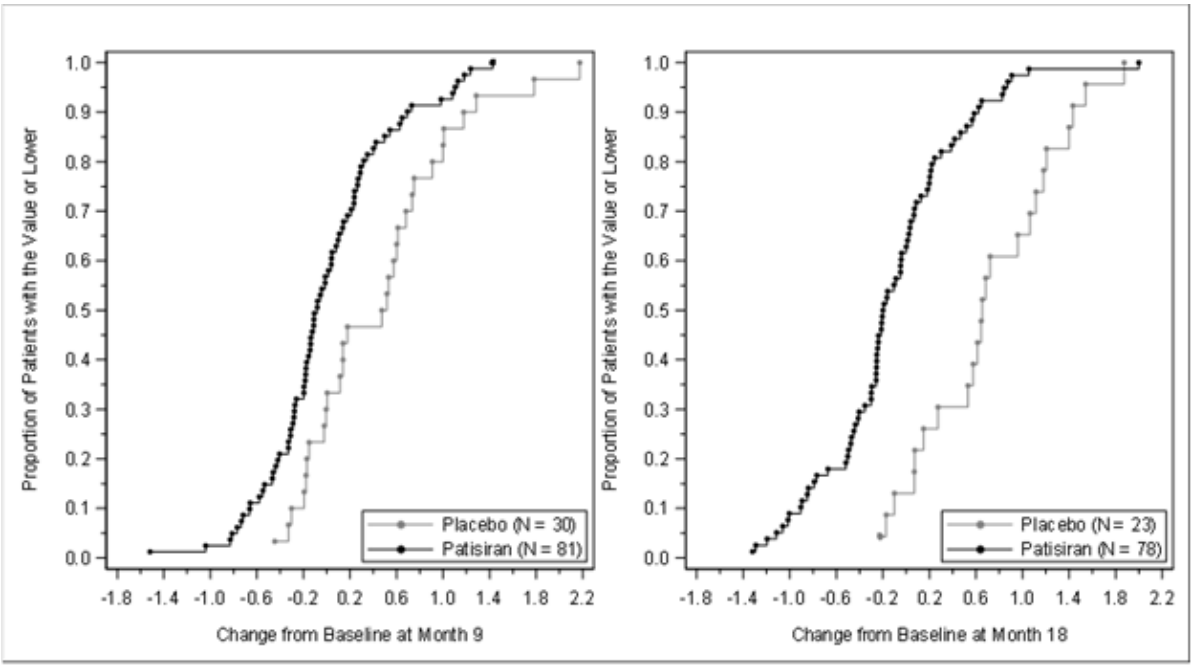
A post hoc analysis on the percentage of patients with > than 2 mm, or < 2 mm or in between change in left ventricular wall thickness and the proportion of patients that achieved an improvement of > absolute 2% for longitudinal strain was performed.

These thresholds were chosen to be greater than the values suggested as being predicative of outcomes based on published data (Quarta 2014).In the cardiac subpopulation, a greater proportion of evaluable (non-missing data at baseline and Month 18) patients in the patisiran-LNP group had a decrease in mean LV wall thickness >2 mm compared to placebo (29.1 % vs 4% respectively) and a greater proportion of patients in the patisiran-LNP group had an absolute improvement in longitudinal strain of >2% compared to placebo (21.3% vs. 8% respectively).

NT-proBNP

In the cardiac subpopulation, at baseline, geometric mean NT-proBNP levels were 726.92 and 711.10 ng/L in the patisiran-LNP and placebo groups, respectively. 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. Cumulative distribution curves demonstrate that the decrease in NT-proBNP compared to placebo was observed, at both 9 and 18 months, across all response thresholds (Figure 27).

Figure 27 Study 004: Cumulative Distribution Function of Change from baseline in Log-transformed NT-proBNP (ng/L) by Visit (Cardiac Subpopulation)



Abbreviations: NT-proBNP= B-type natriuretic peptide

Data on the relationship between NT-proBNP response to intervention and mortality outcomes in AL amyloidosis is available from 5 independent studies (Merlini 2016), (Palladini 2012), (Palladini 2010), (Palladini 2006), (Kastritis 2010), (Kastritis 2015). Data from one of these studies indicate that in patients with a baseline NT-proBNP > 650 ng/L, changes in NT-proBNP of >30% and >300 ng/L in response to interventions are predictive of mortality outcomes (Merlini 2016).

In the 004 Study, in the cardiac subpopulation, approximately one third of evaluable patients (baseline NT-proBNP ≥650 ng/L and non-missing 18 month data) on the patisiran-LNP arm demonstrated a decrease from baseline in NT-proBNP of >30% and >300 ng/L compared to 0% in the placebo arm.

Post-hoc analyses on hospitalizations and deaths

Table 22 Summary of Hospitalization and/or All-Cause Death (mITT Population)

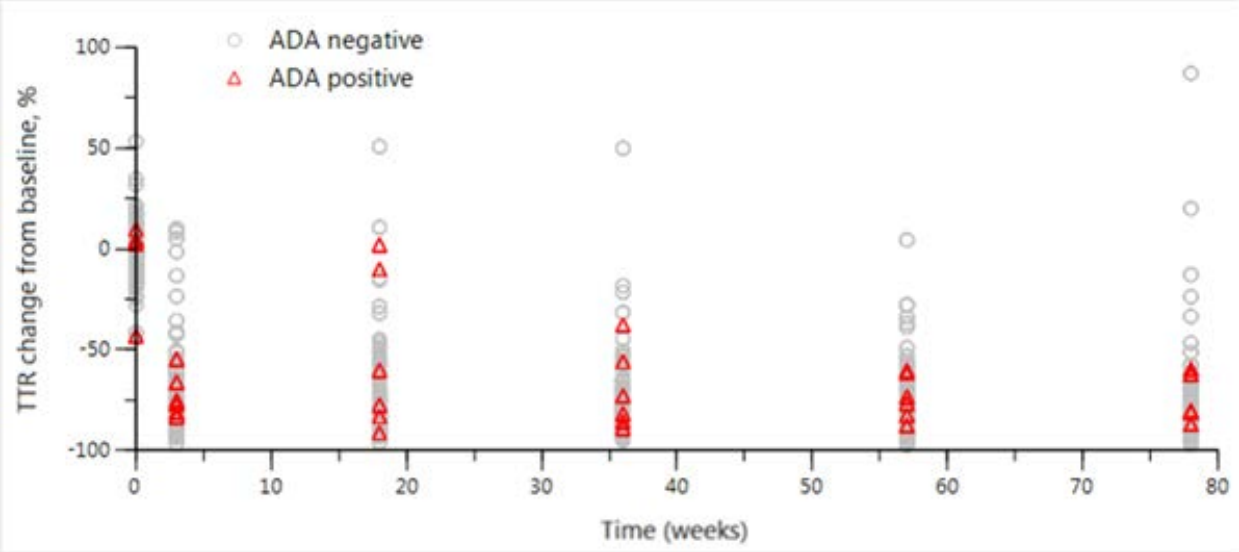
Category	Placebo (N=77)	Patisiran (N=148)
Total duration of exposure, years	96.1	218.9
Deaths, n (%)	6 (7.8)	7 (4.7)
Rate of death per 100 patient-years (95% CI)	6.2 (2.5–12.7)	3.2 (1.4–6.2)
Patients with all-cause hospitalization[a]	30 (39.0)	50 (33.8)
Patients with any hospitalization and/or death [a] n (%)[b]	31 (40.3)	51 (34.5)
Hospitalization and/or death per 100 patient-years (95% CI) [c]	71.8 (56.1–90.1)	34.7 (27.5–43.1)
Patients with cardiac hospitalizations[d]	10 (13 0)	18 (12.2)
Patients with cardiac hospitalization and/or death [d] n (%)[b]	12 (15.6)	20 (13.5)
Cardiac hospitalization and/or death per 100 patient-years (95% CI) [c]	18.7 (11.4–28.8)	10.1 (6.4–14.9)

Abbreviations: CI=confidence interval; mITT=modified intent to treat; N, n=number of patients
a Treatment-Emergent SAEs which required inpatient hospitalizations or prolongation of existing hospitalization (and/or resulted in death).
b Rate per 100-patient year (py) is defined as number of patients divided by cumulative follow-up time x 100, where the follow-up time for each patient is calculated as the minimum of the time when the first event occurred (years) or the total duration of exposure (years).
c Rate per 100-patient year (py) is defined as number of events divided by total duration of exposure x 100. 95% CIs are computed based on the profile likelihood function.
d Treatment-Emergent SAEs with SOC=Cardiac Disorders which required inpatient hospitalizations or prolongation of existing hospitalization (and/or Treatment-Emergent SAEs resulted in death).

Study 004: Anti-drug Antibodies (ADA) Impact on Efficacy

All patients provided ADA test results in the study, either at baseline or post baseline. Overall, 8 patients tested positive for ADA at any time (pre and/or post-treatment), 6 (4.1%) in the patisiran-LNP treatment group and 2 (2.6%) in the placebo group. There was no obvious impact of ADA on TTR reduction in the 6 patients in the patisiran-LNP group who tested positive for ADA compared to patients with negative ADA status (Figure 28).

Figure 28 Study 004: Individual Serum TTR (ELISA) Change from Baseline over Time by ADA status (Positive / Negative) for Patisiran-LNP Treatment Group



Abbreviations: ADA=antidrug antibodies; ELISA=Enzyme-linked immunosorbent assay; TTR=transthyretin

Table 23 Study 004: Summary for the Change from Baseline at Month 18 mNIS+7 Score by ADA Status in the Patisiran-LNP Group

	ADA Positive	ADA Negative
N	6	131
Mean (±SD) (min-max)	-2.9 (±18.1) (-25, 26)	-4.3 (±18.24) (-50, 60)

Abbreviations: ADA=antidrug antibodies; max=maximum; min=minimum; mNIS+7=Modified Neurologic Impairment Score +7; SD=standard deviation.
Note, total number of patients with treatment-emergent ADA = 5 patients. One additional non-treatment emergent patient is included who had a titer level that met the threshold level.

There was also no impact of ADA on change from baseline in mNIS+7 according to the results presented in table 23. Only 6 patients out of 148 were ADA positive in study 004. The effects on mNIS+7 were similar between ADA-positive and ADA-negative patients, keeping in mind that the low number of patients with ADA (N=6) yielded a high variability.

Summary of main study

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

Table 24 Summary of efficacy for the single pivotal trial ALN-TTR02-004

Title: APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)			
Study identifier	Pivotal trial ALN-TTR02-004 EudraCT Number: 2013-002987-17		
Design	A Phase 3 multicenter, double-blind, randomized, stratified, placebo controlled study of patisiran in subjects with a diagnosis of FAP with documented TTR mutation, with symptomatic polyneuropathy defined as Impairment Score (NIS) ≥10 and ≤130, PND score of ≤3b and a Karnofsky performance status of ≥60%. Patients with New York Heart Association heart failure classification >2 were excluded. Consented eligible patients will be randomized to receive either patisiran or placebo in a 2:1 ratio. Patients will have baseline efficacy assessments and efficacy assessments at 9 and 18 months.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		18 months not applicable Study 006: ongoing, up to 5 years
Hypothesis	Superiority to placebo		
Treatments groups	Patisiran		0.3 mg/kg IV patisiran every 3 weeks for 18 months, N=148
	Placebo		Placebo IV (with same premedication as patisiran), every 3 weeks for 18 months, N=77
Endpoints and definitions	Primary endpoint	mNIS+7	Compare change from Baseline to month 18 between treatment arms in the modified Neuropathy impairment score +7 (mNIS+7) (0-304 p)
	Secondary endpoint	Norfolk QoL-DN	Compare change from Baseline to Month 18 between treatment arms in the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire total score (-4 to 136 p)
	Secondary endpoint	NIS-W	Compare change from Baseline to Month 18 between treatment arms in the NIS-W (Neuropathy Impairment Score-Weakness Score)(range: 0 to 192 points). Less neurologic impairment = Lower score

	Secondary endpoint	R-ODS	Compare change from Baseline to Month 18 between treatment arms in the R-ODS (Rasch-Built Overall Disability Scale)(range: 0 to 48). Less disability = Higher score
	Secondary endpoint	Timed 10-meter walk test	Compare change from Baseline to Month 18 between treatment arms in the Timed 10-meter walk test. Faster/better gait speed = Higher speed.
	Secondary endpoint	mBMI	Compare change from Baseline to Month 18 between treatment arms in the modified Body mass index (kg/m^2 x albumin (g/L)). Better nutritional status = Higher mBMI.
	Secondary endpoint	COMPASS 31	Compare change from Baseline to Month 18 between treatment arms in the COMPASS 31 (Autonomic Symptoms Questionnaire [Composite Autonomic Symptom Score]) (0-100 p). Less autonomic neuropathy symptoms = Lower score.
Database lock	The ALN-TTR02-004 Electronic Data Capture (EDC) database was locked on 12 September 2017. This milestone included the lock of all electronic Case Report Form (eCRF) data entered by clinical sites per the study protocol (e.g. demographics, medical history, adverse events, concomitant medications, exposure data, disposition data, etc.) and the finalization of all third party laboratory data which had not required blinding during the study (e.g. ERG and echocardiogram assessments). Following the EDC lock on 12 September 2017, Alnylam authorized the unblinding of the study in accordance with Alnylam SOPs. This unblinding authorization allowed third party providers of blinded laboratory data sources to transmit their final unblinded raw data transfers to the CRO on 13 September 2017 and 14 September 2017. The laboratory data sources included in this second transfer were considered assessments that even without treatment allocation, could lead to unblinding, e.g. retinol binding protein, vitamin A assessments. The CRO integrated the final unblinded raw lab data into the study datasets on 14 September 2017. There was no impact or changes to the data previously locked on 12 September 2017.		
Results and Analysis			

Analysis description	**Primary Analysis**		
Analysis population and time point description	mITT, Month 18. The primary efficacy endpoint was the change in mNIS+7 from baseline to Month 18 between treatment arms.		
Descriptive statistics and estimate variability	Treatment group	Patisiran 0.3 mg/kg	Placebo
	Number of subjects (Baseline and mITT)	148	77
	mNIS+7 (MMRM, mITT) Change from baseline, LS mean (SEM)	-6.03 (1.74)	27.96 (2.60)
	95%CI	-9.46, -2.60	22.83, 33.09
	Norfolk QoL-DN (MMRM, mITT) Change from baseline, LS mean (SEM)	-6.7 (1.77)	14.4 (2.73)
	95%CI	-10.2, -3.3	9.0, 19.8
	NIS-W Change from baseline, LS mean (SEM) 95%CI	0.05 (1.31) -2.52, 2.63	17.93 (1.96) 14.07, 21.79

	R-ODS Change from baseline, LS mean (SEM) 95%CI	0.0 (0.59) -1.1, 1.2	-8.9 (0.88) -10.7, -7.2
	Timed 10-meter walk test Change from baseline, LS mean (SEM) 95%CI	0.08 (0.02) 0.03, 0.12	-0.24 (0.04) -0.31, -0.16
	mBMI Change from baseline, LS mean SEM 95%CI	-3.7 (9.57) -22.6, +15.1	-119.4 (14.51) -148.0, -90.8
	COMPASS 31 Change from baseline, LS mean SEM 95%CI	-5.29 (1.30) -7.85, -2.72	2.24 (1.94) -1.59, +6.06
Effect estimate per comparison	mNIS+7	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		LS Mean Difference	-33.99
		95% CI of difference	-39.86, -28.13
		P-value	9.262×10^{-24}
	Norfolk QoL-DN	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		LS Mean Difference	-21.1
		95% CI of difference	-27.2, -15.0

		P-value	1.103×10^{-10}
	NIS-W	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		LS Mean Difference	-17.87
		95% CI of difference	-22.32, -13.43
		P-value	1.404×10^{-13}
	R-ODS	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		LS Mean Difference	9.0
		95% CI of difference	7.0, 10.9
		P-value	4.01×10^{-16}
	Timed 10-meter walk test	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		LS Mean Difference	0.31
		95% CI of difference	0.23, 0.39
		P-value	1.88×10^{-12}
	mBMI	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		LS Mean Difference	115.7
		95% CI of difference	82.4, 149.0
		P-value	8.832×10^{-11}
	COMPASS 31	Comparison groups	Patisiran 0.3 mg/kg vs placebo
		Difference in LSM	-7.53
		95% CI of difference	-11.89, -3.16
		P-value	0.0008

Supportive studies

Study 003

Study 003 was a multinational, multicenter, Phase 2, open-label, extension study designed to provide long term (up to 2 years) patisiran-LNP dosing to patients with hATTR amyloidosis with polyneuropathy who received and tolerated patisiran-LNP in Study 002. Patients could continue treatment in Study 006 after completion of this study. All efficacy summaries were descriptive and no hypothesis testing was performed.

A total of 27 patients were enrolled and treated in Study 003. Twenty-five patients completed the study. Patients were enrolled from 7 countries. The mean age at screening was 57.9 years, and 66.7% of patients were male. All patients were Caucasian. Almost three quarters (74.1%) of patients had the V30M mutation. Patients in this study were allowed concomitant use of TTR tetramer stabilizers. A significant proportion of patients (N=11, 40.7%) were in the predefined cardiac subpopulation. The mean total duration of study drug exposure was 24.7 months (range 19-25 months).

Study 003 Primary efficacy endpoint mNIS+7

The mean mNIS+7 score at baseline was 53.02 points with a range from 2.0-122.5 points (possible range 0-maximum score of 304). Following administration of patisiran-LNP, the mean (SEM) change from baseline in the mNIS+7 at 24 months was -6.95 (2.03) points. mNIS+7 change at different time points is shown in Table 25 below. The decrease of the score is mainly due to a reduction in the QST score (-6.00 by 24 months).

Table 25 Study 003 Change from baseline in mNIS+7 over time by 6 months intervals, full analysis set

	Patisiran-LNP Treatment Overall			
	6 months (Week 27)	12 months (Week 54)	18 months (Week 81)	24 months (Week 108)
N	27	27	27	26
Mean change (SEM)	-1.33 (2.04)	-3.26 (2.29)	-0.88 (2.69)	-6.95 (2.03)
Median change (range)	-2.00 (-25.38, 22.00)	-2.50 (-29.75, 24.00)	-2.50 (-26.88, 35.75)	-7.00 (-34.63, 15.38)

Abbreviations: mNIS=modified Neuropathy Impairment Score; SEM=standard error of the mean

The mean (SEM) change from baseline in mNIS+7 at 24 months was similar between the following subgroups of patients who received patisiran-LNP:

- With or without a concomitant TTR stabilizer [-7.03 (2.11) vs. -6.75 (5.24), respectively],
- Age <65 years old or ≥65 years old [-5.12 (2.25) vs. -9.09 (3.55), respectively]
- Male or female patients [-7.31 (1.97) vs. –6.16 (5.16), respectively]
- In the predefined cardiac subpopulation, the mean (SEM) change from baseline in mNIS+7 at 24 months -9.98 (3.29) was similar to that in the non-cardiac subpopulation -4.73 (2.50).

Study 003: Additional Efficacy Endpoints

Overall, NIS and NIS+7, measures of disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI), autonomic function (COMPASS 31), motor strength (grip strength), and QoL (EQ-5D and EQ-VAS) were stable over the 24 month treatment period (Table 26).

Table 26 Study 003: Additional Neuropathy Endpoints, Quality of Life, 10-MWT, R ODS, mBMI, and COMPASS 31

Efficacy Endpoint:	Mean (SD) Baseline	6 months	12 months	18 months	24 months

		Mean Change from Baseline (SEM)			
Neurologic Impairment	N = 27	N = 27	N = 27	N = 27	N = 26
NIS	34.80 (28.05)	-0.69 (1.29)	0.21 (1.10)	2.56 (1.69)	1.92 (1.84)
NIS+7	48.08 (30.64)	-0.01 (1.28)	1.22 (1.17)	2.95 (1.56)	2.54 (1.84)
Disability	N = 26	N = 26	N = 26	N = 26	N = 25
R-ODS	38.1 (8.61)	-0.6 (0.77)	-1.5 (0.76)	-1.0 (0.84)	-1.8 (0.83)
Gait Speed	N = 22	N = 21	N = 22	N = 22	N = 21
10-meter Walk Test Speed (meters/second)	1.14 (0.43)	0.03 (0.05)	0.01 (0.05)	0.07 (0.05)	0.03 (0.04)
Nutritional status	N = 27	N = 26	N = 27	N = 25	N = 22
mBMI (BMI [kg/m^2] x albumin [g/L])	1030.49 (168.64)	-0.68 (14.14)	1.94 (21.02)	-32.08 (31.40)	-60.76 (34.86)
Autonomic neuropathy score	N = 27	N = 26	N = 27	N = 27	N = 26
COMPASS-31	15.85 (13.34)	1.47 (2.05)	-0.28 (2.0)	-2.12 (1.46)	1.32 (1.80)
Motor function	N = 27	N = 27	N = 26	N = 27	N = 26
Hand Grip Strength (kg)	25.81 (11.86)	-0.36 (0.85)	0.31 (0.51)	1.41 (0.62)	1.49 (1.23)
Quality of life	N = 27	N = 27	N = 27	N = 27	N = 26
EQ-5D Index	0.78 (0.14)	0.00 (0.01)	-0.03 (0.02)	-0.01 (0.02)	-0.01 (0.02)
EQ-VAS	67.9 (17.85)	3.1 (1.95)	4.4 (3.01)	0.9 (2.48)	1.7 (2.53)

Abbreviations: 10-MWT=10 Meter Walk Test; Compass 31=Composite Autonomic Symptom Score ; EQ-5D=European Quality of Life-5 Dimensions; EQ-CAS=EuroQoL Visual Analogue Scale Index; mBMI=Modified body mass index; NIS=Neurologic Impairment Score; SEM=standard error of the mean

The mean (SD) dermal amyloid burden at baseline was 10.91% (9.49%) and 15.78% (14.93%) for distal thigh and distal leg, respectively, and the mean absolute (SEM) change from baseline to 24 months (Week 110) was -3.81% (1.31%) for distal thigh and -6.38% (2.81%) for distal leg that reached nominal statistical significance.

Overall, IENFD was stable throughout the 2-year treatment period; SGNFD in both distal thigh and distal leg increased over time relative to baseline.

Study 003 Measures of Cardiac Manifestations in Cardiac Subgroup

Echocardiogram measures (LV mass, LV wall thickness, longitudinal strain and LVEF) and cardiac biomarkers (NT-proBNP and troponin I) performed in the predefined cardiac subgroup were stable over time (Table 27).

Table 27 Study 003: Change from Baseline in Echocardiogram Results by 6-month Intervals, Cardiac Subgroup

	Baseline	**Change from Baseline**			
		6 months (Week 27)	**12 months (Week 54)**	**18 months (Week 81)**	**24 months (Week 108)**
LVEF (%)	N =11	N = 11	N = 10	N = 11	N = 10
Mean (SEM)	62.46 (2.63)	-1.67 (2.05)	-2.84 (1.55)	1.57 (1.28)	0.63 (1.45)
Median (range)	63.33 (40.71, 75.66)	-0.55 (-19.85, 6.49)	-3.12 (-8.90, 6.78)	1.70 (-4.91, 7.93)	0.73 (-8.15, 6.84)
LV Wall Thickness (cm)	N = 11	N = 11	N = 11	N = 11	N = 10
Mean (SEM)	1.58 (0.06)	-0.01 (0.02)	-0.03 (0.03)	-0.02 (0.04)	-0.08 (0.05)
Median (range)	1.58 (1.34, 1.92)	-0.02 (-0.10, 0.16)	-0.05 (-0.17, 0.11)	-0.03 (-0.26, 0.13)	-0.04 (-0.41, 0.08)
LV Mass (g)	N = 11	N = 11	N = 11	N = 11	N = 11
Mean (SEM)	278.05 (23.20)	-5.19 (6.33)	-13.22 (9.81)	-7.47 (10.05)	-16.74 (11.66)
Median (range)	296.47 (154.98, 423.29)	-9.34 (-35.38, 33.10)	-14.33 (-93.19, 36.72)	-5.32 (-68.02, 39.67)	-9.145 (-69.55, 27.39)
Average Peak Longitudinal Strain (%)	N = 11	N = 11	N = 11	N = 11	N = 10
Mean (SEM)	-16.64 (1.32)	-0.45 (1.08)	-0.37 (0.67)	0.26 (0.90)	0.85 (0.89)
Median (range)	-18.10 (-23.0, -9.2)	-0.30 (-5.9, 8.2)	-0.30 (-4.8, 2.7)	-1.00 (-3.2, 4.9)	0.65 (-4.1, 5.1)

Abbreviations: EF=ejection fraction, LV=left ventricular, SEM=standard error of the mean

Table 28 Study 003: Change from Baseline in Serum Troponin I and NT-proBNP by 6 month Intervals, Cardiac Subgroup

	Baseline	Change from baseline			
		6 months (Week 25)	12 months (Week 52)	18 months (Week 94)	24 months (Week 106)
Troponin I (µg/L)	N = 8	N = 8	N = 8	N = 7	N = 8
Mean (SEM)	0.14 (0.08)	-0.12 (0.10)	-0.11 (0.08)	-0.11 (0.08)	-0.09 (0.08)
Median (range)	0.050 (0.03, 0.69)	-0.020 (-0.64, 0.00)	-0.01 (-0.66, 0.01)	-0.02 (-0.59, 0.00)	-0.01 (-0.66, 0.03)
NT-proBNP (ng/L)	N = 9	N = 7	N = 9	N = 8	N =8
Mean (SEM)	809.8 (246.68)	165.4 (60.22)	244.8 (229.06)	-184.5 (112.44)	-49.6 (170.83)
Median (range)	604.0 105, 2070	241.0 (-120, 318)	-35.0 (-136, 2028)	-76.5 (-869, 75)	-47.0 (-986, 807)

Abbreviations: NT-proBNP=N-terminal B-type natriuretic peptide; SEM=standard error of the mean

Study 003: Anti-drug Antibodies (ADA) Impact on Efficacy

ADA was detected in one patient. This patient tested positive for ADA at baseline (titer 1:80) and up to Week 13 (maximum titer 1:80) following which ADA tested negative. Baseline ADA positivity in this patient was likely due to previous exposure to patisiran-LNP in the parent Study 002. There was no observed impact of ADA on PK of ALN-18328 or TTR reduction.

Study 006

Study 006 is an ongoing multicentre, multinational, open-label extension study. Eligible patients who completed either of the two parent Studies, 003 or 004, were given the option to participate in this study.

At the time of the interim Study 006 data cut-off date (14 July 2017), Study 003 was completed and all 25 patients who completed Study 003 had enrolled in Study 006. Study 004 was ongoing; 163 of the 169 patients who completed Study 004 by that time had enrolled in Study 006. Patients from Study 004 remained blinded to Study 004 treatment assignment during participation in Study 006 at the time of the interim data cut. Efficacy assessment is presented for the smaller subset of approximately 64 patients who had efficacy assessment at Week 52.

Overall, the mean age at baseline was 61.3 years, 73.9% were male, 79.3% were Caucasian/White. 46.3% of patients had V30M mutations, and 53.7% of patients had non V30M mutations that included 30 different mutations.

A total of 184 patients were dosed with patisiran-LNP in this study for a mean (SD) of 9 (5.976) months (range 0.7 to 24.6 months).

Study 006: Efficacy Endpoint mNIS+7

Of the 184 patients who had been treated in this study at the time of the interim data cut, Week 52 mNIS+7 efficacy data were available for 64 patients (n=10 from 004 placebo, n=30 from 004 patisiran-LNP, and n=24 from 003 patisiran-LNP). The results are shown in Table 29 below.

Table 29 Study 006 mNIS+7 at week 52

Visit	Actual/ Change	Statistic	004 Placebo (N=43)	004 Patisiran-LNP (N=120)	003 Patisiran-LNP (N=25)
Baseline[a]	Actual	N	43	116	25
		Mean	100.08	77.74	45.66
		SD	43.739	43.695	31.640
		Median	93.88	73.94	40.00
		Min, Max	21.5, 190.1	8.0, 198.9	3.0, 127.8
Week 52	Actual	N	10	30	24
		Mean	99.65	81.53	48.49
		SD	44.389	39.167	37.965
		Median	107.13	78.75	41.75
		Min, Max	15.0, 173.0	17.0, 163.0	1.5, 164.4
	Change	N	10	30	24
		Mean	-1.31	1.48	2.47
		SEM	3.116	2.560	2.751
		Median	-1.13	2.38	2.25
		Min, Max	-17.1, 11.3	-29.8, 44.4	-34.0, 36.6

Abbreviations: mNIS+7=Modified Neuropathy Impairment Score; SD=standard deviation; SEM=standard error of the mean.
Note: At each visit the total scores are calculated as the mean of the two independent assessments.
[a] The last non-missing measurement on or prior to the first dose of study drug in this study. Per protocol, the last testing visit in the parent study will serve as the baseline, unless more than 45 days have elapsed in which case baseline is the Day 1 value.

Norfolk QoL-DN

Mean (SD) Norfolk QOL-DN at baseline were 73.5 (27.69) and 56.0 (30.87) points in the 004 placebo and 004 patisiran-LNP groups, respectively. At Week 52, mean change from baseline Norfolk QOL-DN was -10.2 points in the 004 placebo and -1.8 points in the 004 patisiran-LNP groups. Only 1 patient from the 003-patisiran-LNP group performed a Norfolk-QOL-DN assessment at baseline (not required in study 003), therefore the change over time cannot be evaluated.

Study 006: R-ODS, 10-meter walk test, mBMI, COMPASS 31, NIS, NIS+7, Grip Strength, EQ-5D, EQ-VAS, PND Score and FAP Stage

Clinical endpoints assessing disability (R-ODS), ambulatory ability (10 meter walk test), nutritional status (mBMI), autonomic symptoms (COMPASS 31), neuropathy (NIS, NIS+7), motor strength (grip strength),

quality of life (EQ-5D) and overall health (EQ-VAS) were generally stable or improved at Week 52 relative to Study 006 baseline.

Study 006: Cardiac Manifestations

All patients enrolled in Study 006 are included in the analysis of cardiac assessments regardless of whether they met the cardiac subpopulation criteria in the parent study.

At Week 52, echocardiogram results were available for only 9 patients in 004 placebo group, 27 patients (23 patient for LV ejection fraction) in the 004 patisiran-LNP group and 24 patients in the 003 patisiran-LNP group. Among the group of patients from the 004 placebo group with Week 52 assessments, there was evidence of stabilization of cardiac structure and function relative to baseline following transitioning to patisiran-LNP on Study 006 according to the applicant. In patients from the 004 patisiran-LNP and 003 patisiran-LNP groups who received treatment with patisiran-LNP on the parent studies, continued treatment with patisiran-LNP in Study 006 resulted in maintenance of efficacy according to the applicant.

At Week 52, median change from baseline in NT-proBNP levels were -12.01 ng/L in the 004 placebo, -15.01 ng/L in 004 patisiran-LNP, and 3.93 ng/L in the 003 patisiran-LNP group.

Overall, there were no notable changes in serum Troponin I levels over 52 weeks in any study group.

Study 006: Anti-drug Antibodies (ADA) Impact on Efficacy

None of the patients tested positive for ADA up to the interim analysis time point.

2.7.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The pivotal study 004 was a double-blind, placebo-controlled, Phase 3 study designed to demonstrate the efficacy and safety of patisiran-LNP in patients with hATTR amyloidosis with polyneuropathy, and the used design is overall adequate when focusing on the neuropathic manifestations of the disease. The study population was limited to subjects with Karnofsky performance status of ≥60 and Polyneuropathy Disability (PND) Scores of 0-IIIB hATTR disease with polyneuropathy (hATTR-PN), i.e had at least mild polyneuropathy at baseline and were ambulant. Study 004 population included subjects with different TTR mutations and patients with some cardiac involvement, but excluded subjects with severe cardiac involvement. Patisiran-LNP was administered at a dose of 0.3 mg/kg q3w as an IV infusion based on phase 1-2 study data. The dose selection and the administration schedule is supported by the phase 1-2 data.

The Applicant justified that the study did not use the already approved tafamidis (which is indicated for the treatment of transthyretin amyloidosis in adult patients with stage 1 symptomatic polyneuropathy) as an active comparator because the target population for patisiran differs from the population for which tafamidis is approved and moreover it is not universally part of standard of care. The CHMP agreed with the justifications and considered that it was acceptable not to include tafamidis as a comparator arm.

The primary efficacy variable selected was the modified Neuropathy Impairment Score +7 (mNIS+7). It is a composite neurologic impairment score that was developed specifically for monitoring progression of neurologic impairment in hATTR amyloidosis patients, and was considered acceptable, even though not formally validated. The clinical relevance of the mNIS+7 is supported by the use of the Neuropathy Impairment Score (NIS) and NIS+7 (=NIS in addition with seven neurophysiological variables) which have

been used in clinical trials of diabetic polyneuropathy and CIDP (chronic inflammatory demyelinating polyneuropathy). NIS has been shown to correlate with disease stage and quality of life thereby supporting the clinical relevance of the NIS score in hATTR amyloidosis. NIS +7 has been validated in diabetic polyneuropathy and has been used in a Phase 3 trial in patients with hATTR amyloidosis with polyneuropathy.

Also the secondary endpoints capturing QoL(R-ODS), walking speed (10-MWT), mBMI, autonomic dysfunction (COMPASS-31) were considered adequate and clinical relevant. Exploratory efficacy variables for e.g. serum TTR concentration and cardiac function were also evaluated in the study.

Overall, the analyses as planned were considered appropriate.

A total of 56% of patients were included in the predefined cardiac subpopulation. The proportion of patients was however higher in the patisiran-LNP group than in the placebo group; 60.8% (90/148) vs. 46.8% (36/77), respectively. Complementary analyses assessing response rates across subgroups within each arm were provided. They do not show heterogeneity of responses in these subgroups within both arms since a consistent clinical benefit across these specific subgroups was observed.

Additionally, the MAH provided a safety analysis of these specific subgroups targeting the most frequent AEs (≥ 3%). Among the 11 AEs identified, no imbalance of incidences were noticed except for oedema peripheral for which there was a higher incidence rate in non 30VM and cardiac subgroups compared to their counterparts. However, at the planning stage, focus was seemingly on neuropathy progression alone and the analyses performed based on the cardiac subpopulation were all considered to be exploratory. Among patients in the cardiac subpopulation, 73.0% had non-V30M genotype; 68/90 (75.6%) on patisiran and 24/36 (66.7%) on placebo. The majority (60.3%) had NYHA class II heart failure, also fairly well balanced between the treatment groups (56/90 (62.2%); patisiran, 20/36 (55.6%); placebo) and, medical history of terms mapping to the cardiac disorders system organ class including terms related to heart failure, cardiac conduction disorders and arrhythmias were commonly reported. Looking at cardiac disorders overall; there was an imbalance between placebo and patisiran with 58.9% (53/90) and 75.0% (27/36) in the patisiran and placebo treated patients respectively.

Among patients *not* included in the cardiac subpopulation, 55.6% had mean LV wall thickness ≥1.3cm, indicating potential cardiac amyloid involvement, but were excluded from the predefined cardiac subpopulation primarily due to medical history of hypertension. Thus, 80.4% of patients had evidence of cardiomyopathy based on LV wall thickness ≥ 1.3 cm at baseline. Based on that the cardiac subpopulation were considered to likely under-represent cardiac amyloid involvement in the study, key analyses were also performed for the mITT population (all randomised). The relevance of the analyses based on the cardiac subpopulation could hence be questioned and besides additional analyses based on all randomised patients, the Applicant was invited to discuss a (new) cardiac subgroup defined not only by the cut-off value of left ventricular wall thickness, but also on e.g. cardiac medical history deemed related to hATTR cardiomyopathy and a NT-proBNP cut-off value. The applicant has examined two alternate definitions of the cardiac subpopulation:

1. the originally used cardiac subgroup criteria OR mean LV wall thickness ≥17mm OR medical history of cardiac amyloidosis and NT-proBNP>650ng/L

2. the originally used cardiac subgroup criteria OR NT-proBNP>650ng/L

The newly defined cardiac subgroups included more patients (alternative 1: 50 placebo (64.9% of the mITT population), 102 patisiran (68.9%), alternative 2: 50 placebo (64.9%), 104 patisiran (70.3%)) than the

original cardiac subgroup (36 placebo (46%), 90 patisiran (60.8%)). (The mITT population was 77 placebo, 148 patisiran.)

The results in the two alternate definitions of cardiac subgroups and the pre-specified cardiac subpopulation were compared. Echocardiogram results were almost identical in terms of level of p-value, the treatment effects on NT-proBNP were highly significant in all three subgroups and the results on deaths and hospitalizations didn't differ either.

Patisiran treatment effects were very similar in all three alternate definitions of cardiac subgroups. Since the newer definitions of the cardiac subgroup included a higher percentage of the mITT population, this could indicate that more than half of the total study population actually had cardiac involvement of their hATTR disease, and that the exact definition of the cardiac subgroup didn't affect the results on cardiac endpoints. It should also be kept in mind that also the mITT population showed significant results for many of the cardiac endpoints.

A limited number of subjects discontinued from the study, a total of 82.2% of the 225 patients completed study treatment, but there was a difference between the patisiran and placebo groups with more subjects having discontinued treatment in the placebo group 29/77 (37.7%) versus 11/148 (7.4%) in the patisiran group. The most common reason for study treatment discontinuation overall was withdrawal of consent and the dominating causes for withdrawal of consent in the placebo group were lack of efficacy or worsening of disease. The discontinuation pattern is not supporting a bias favouring the patisiran group as a worsening is the expected natural course of hATTR amyloidosis.

Two adequately designed Phase 1 patisiran-LNP SAD studies in healthy volunteers contributed towrds the pharmacodynamic (PD) assessments (eg, TTR levels), pharmacokinetic (PK) data, and safety data. Study ALN-TTR02-002 was an adequate Phase 2 multiple ascending dose (MAD) study that contributed PD, PK, and safety data in patients with hATTR amyloidosis with polyneuropathy; this study supported the selection of the patisiran-LNP dose and regimen for continued development.

Efficacy data and additional analyses

Dose-dependent reductions in serum TTR concentrations with patisiran LNP were observed in both healthy volunteers and in patients with hATTR amyloidosis with polyneuropathy. Long term dosing with patisiran LNP sustained a mean TTR reduction of approximately 80% over 2 years of treatment. Overall, the expected effect of patisiran LNP on serum TTR concentrations has been convincingly shown in the clinical studies.
The difference in neurologic impairment, as measured by mNIS+7, was highly statistically significant: -33.99 [-39.86; -28.13] points (LS mean (SEM) favouring the patisiran group. Furthermore, in the study 004 the mean mNIS+7 change from baseline at 18 months for the placebo group was +27.96 points. This is in line with the estimated change per year (+17.8) found in a multinational study of 282 patients with fATTR amyloidosis. The patisiran patients mean change at 18 months was -6.03 mNIS+7 points, which is a subtle numerical improvement instead of the expected worsening. The favourable effect of patisiran-LNP shown by mNIS+7 results was already observed at 9 month and it further improved leading to a large effect size at 18 months. It showed consistent improvement across motor, sensory, and autonomic components of neuropathy confirming a clinical relevant effect.

Statistically significant differences favouring Patisiran-LNP group compared to placebo at 18 months were also observed for all secondary endpoints, as illustrated by quality of life as assessed by Norfolk QoL-DN at 18 months group (LS mean difference between Patisiran-LNP – Placebo groups, [95% CI, p-value]): (-21.3

points [-27.2; -15.0, P=1.103 $\times 10^{-10}$]), motor strength (NIS-W)(-17.87 points [-22.32, -13.43, P=1.404E-13]), disability (R-ODS)(+9.0 points [7.0, 10.9, P=4.066E-16]), gait speed (10-MWT)(0.311 m/sec [0.23, 0.39, P=1.875E-12]), nutritional status (mBMI)(+115.7 kg/m2 × albumin g/L [82.4, 149.0, P=8.832E-11]) and autonomic symptoms (COMPASS 31)(-7.53 points [-11.89, -3.16, P=0.0008]), supporting the statistically significant differences between Patisiran-LNP group and placebo for the primary endpoint. Secondary endpoints reflect clinically relevant oucomes which account for a broad range of clinical manifestations of hATTR amyloidosis, including improved quality of life (Norfolk QoL-DN), motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic symptoms (COMPASS 31). The consistent favourable results on all these secondary endpoints support the results of the primary end-point and further confirm that patisiran treatment achieves clinically meaningful benefits in hATTR amyloidosis patients.

For the primary endpoint, a number of sensitivity analyses were planned. Most of them however relying on the assumption of missing at random (MAR). With the preferred estimate being that from the pattern mixture model (PMM), additional analyses were requested that were to be performed using a model including the data that were observed after discontinuation of study treatment and otherwise based on placebo-based multiple imputations for all missing or excluded data (i.e. a 'jump to reference', addressing the treatment effect if there is no benefit from treatment after treatment discontinuation; or for patients using alternative treatments). The additional analyses submitted were performed using instead of a 'jump to reference' approach (requested), a 'copy reference' approach was used. This was considered acceptable by the CHMP. New analyses were requested and the Applicant provided the requested PMM J2R analyses based on a data set excluding in total 12 patients, 4 from the patisiran-LNP arm and 8 from the placebo arm, who had efficacy assessments performed at a site found to be GCP non-compliant (Site 061 in Mallorca). In line with the earlier presented PMM 'copy reference' analyses (based on the full mITT population), the point estimates of the differences between patisiran-LNP and placebo as well as the corresponding 95% CIs from the PMM 'jump to reference' (J2R) analyses were very similar compared to the primary analysis although, as could be expected, implied slightly smaller differences between patisiran-LNP and placebo for the mNIS+7 and Norfolk QoL-DN endpoints respectively, as well as slightly bigger p-values. The same was true comparing the PMM J2R analyses with the newly performed MMRM analyses excluding patients who had efficacy assessments performed at Site 061 in Mallorca. Given that the outcomes between the PMM J2R analyses were very similar the results from the MMRM analyses could be acceptable, the CHMP considered that the latter results are suitable to reflect the treatment effects in the SmPC.

Change from baseline in mNIS+7 compared to placebo across all subgroups (age [<65; ≥65], gender, race [white, non-white], region [North America, Western Europe, Rest of World], NIS [< 50; ≥ 50], genotype [V30M; non-V30M], genotype class [Early onset V30M; Other], previous tetramer use, FAP stage [I& II] and cardiac subpopulation) shows a consistent difference in favour of patisiran.
In addition, the same trend favouring patisiran group compared to placebo were seen for mNIS+7 and Norfolk QoL-DN for all the different genotypes with 10 or more patients included.
Overall, the effects measured on neuropathy in the subgroups clearly indicate that patisiran is effective in the different subgroups analysed.

For the cardiac exploratory echocardiographic end-points in the cardiac subpopulation, patisiran-LNP treatment resulted in an LS mean statistical significant decrease of 0.093 cm (95% CI: -0.169, -0.017) in mean LV wall thickness compared to placebo.

A difference in longitudinal strain, a measure of cardiac function, was also observed in the cardiac subpopulation favouring patisiran-LNP compared to placebo, with an LS mean treatment difference in

absolute change from baseline at 18 months of -1.37% (95% CI: -2.48%, -0.27%).
For LVEDV, a parameter of ventricular stiffness and distensibility, a difference favouring patisiran-LNP treatment compared to placebo was also observed.

To further describe the magnitude of the treatment effect of patisiran-LNP on cardiac structure and function, the Applicant has identified the proportion of patients that achieved a decrease (change from baseline) of >2 mm in mean LV wall thickness and the proportion of patients that achieved an improvement of > absolute 2% for longitudinal strain, as presented in the Study 004 CSR. These thresholds were chosen to be greater than the values suggested as being predicative of outcomes based on published data noted above (Quarta 2014). However, the results from this study show only a limited association between each incremental 1% in longitudinal strain and risk of death (HR 1.1 95%CI 1.01-1.19) and half of the study population had AL amyloidosis which has a worse prognosis. Further the predictive value of this surrogate marker is not known in the context of interventional therapy.

Patisiran-LNP treatment was associated with larger effects on NT-proBNP compared to placebo. In the cardiac subpopulation, at baseline, geometric mean NT-proBNP levels were 726.92 and 711.10 ng/L in the patisiran-LNP and placebo groups, respectively. 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. Data on the relationship between NT-proBNP response to intervention and mortality outcomes in AL amyloidosis is available from 5 large, independent studies (Merlini 2016). Data from these studies show that, in patients with a baseline NT-proBNP > 650 ng/L, changes in NT-proBNP of >30% and >300 ng/L in response to interventions are predictive of mortality outcomes (albeit that these patients have a worse prognosis compared to the current target population).

In the 004 Study, in the cardiac subpopulation, approximately one third of evaluable patients (baseline NT-proBNP ≥650 ng/L and non-missing 18 month data) on the patisiran-LNP arm demonstrated a decrease from baseline in NT-proBNP of >30% and >300 ng/L compared to 0% in the placebo arm.

All predefined exploratory analyses of cardiac endpoints intended to compare the two treatment groups were performed using MMRM. Considering the number of subjects excluded and given the underlying assumption of missing at random, sensitivity analyses of cardiac endpoints were requested to be based on methods for imputation of missing data that could be considered sufficiently conservative. These additional analyses were to be based on efficacy data sets excluding data from the (GCP non-compliant) site in Mallorca Spain. The additional analyses based on pattern mixture models using a 'Jump to reference' (PMM J2R) approach were provided and were based on the cardiac subpopulation excluding those patients who had efficacy assessments performed at Site 061. The cardiac subpopulation included 87 and 35 patients of those randomised to the patisiran-LNP arm and the placebo arm respectively. Overall, 31.4%-37.1% of the patients on placebo included in the cardiac subpopulation had missing data compared to 11.4%-16.1% on patisiran-LNP, depending on endpoint. The outcomes from the PMM J2R analyses were similar to the outcomes from analyses based on MMRM with nominally significant differences between patisiran-LNP treated patients and placebo patients for three cardiac endpoints as discussed above (NTproBNP, LV wall thickness, and LV longitudinal strain) of the six cardiac endpoints as pre-defined in the SAP (LV wall thickness, LV mass, LVEF, LV longitudinal strain, NT-proBNP and troponin I).

Similar changes over time were also observed in the mITT population for the cardiac end-points.
In addition, change from baseline in NT-proBNP compared to placebo across all subgroups (age [<65; ≥65], gender, race [white, non-white], region [North America, Western Europe, Rest of World], NIS [< 50; ≥ 50], genotype [V30M; non-V30M], genotype class [Early onset V30M; Other], previous tetramer use, FAP stage [I & II] and cardiac subpopulation) shows a difference in favour of patisiran.

2.7.4. Conclusions on the clinical efficacy

Hereditary ATTR amyloidosis is a autosomal dominant disease caused by mutations in the transthyretin (TTR) The formation of amyloid fibrils and plaques from mutant TTR in the extracellular space of various tissues, including the peripheral nervous system, heart, and gastrointestinal tract is the main characteristic of hATTR. The patisiran-mediated inhibition of the synthesis of wild-type (wt) and mutant TTR proteins resulted in significant reduction in circulating TTR levels.

The study population in the pivotal study 004 was limited to subjects with Polyneuropathy Disability (PND) Scores of 0-IIIB hATTR/FDP stage 1 and 2. It is acknowledged that the clinical data from study 004 shows a convincing difference between patisiran and placebo regardless of baseline disease stages at inclusion for the studied population. The favourable effect of patisiran-LNP shown by mNIS+7 results was already observed at 9 month and it further improved leading to a large effect size at 18 months. It showed consistent improvement across motor, sensory, and autonomic components of neuropathy though suggesting a clinical relevant effect. Statistically significant differences favouring Patisiran-LNP group compared to placebo at 18 months were also observed for all secondary endpoints. Secondary endpoints reflect clinically relevant outcomes which account for a broad range of clinical manifestations of hATTR amyloidosis, including improved quality of life (Norfolk QoL-DN), motor strength (NIS-W), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic symptoms (COMPASS 31). The internal consistency of the primary and secondary endpoints efficacy results and the large effect sizes obtained across all endpoints are supportive of the clinically meaningful effect of patisiran treatment.

The development programme included cardiac endpoints as exploratory surrogate endpoints in a cardiac subpopulation which comprised of 56% of the study population. The results of the pivotal study show a difference compared to placebo for several of the measured echocardiographic variables as well as on NT-proBNP, favoring patisiran. Even though some of endpoints may have a prognostic value, it is unclear if these surrogate markers (as well as the magnitude of the documented differences) are predictive of clinically relevant outcomes in the context of therapeutic interventions. It is however acknowledged that literature data (Merlini 2016) supports the importance of changes in NT-proBNP of >30% and >300 ng/L in response to interventions which may be predictive of mortality outcomes in patients with AL amyloidosis. (Ultimately, the results indicate that patisiran may have an effect on the heart (which is considered plausible from a biological perspective) even though the importance of this effect is not fully understood.

The study population in the pivotal study 004 was limited to subjects with Polyneuropathy Disability (PND) Scores of 0-IIIB hATTR/FAP stage 1 and 2. It is acknowledged that the clinical data from study 004 shows a convincing difference between patisiran and placebo regardless of baseline disease stages at inclusion for the studied population. However, there is only very limited data in patients with more severe disease and it is therefore not found adequate to extrapolate the results from less severe to more advanced stages of the disease (PND stage IV/FAP stage 3 = non ambulatory patients).

For the claim “treatment of adult patients with hATTR”, the CHMP was of the opinion that an effect of treatment on both neurological and cardiac manifestations of the disease should be documented. The effect on neurological symptoms have been shown while, there were several uncertainties concerning the clinical relevance of the data supporting an effect of patisiran on cardiac manifestations. However, considering that the sought indication is not an explicit claim with respect to treatment of cardiomyopathy, the fact that patisiran treatment seems to have positive impact on cardiac parameters the CHMP considers that the presented data supports the following indication; *Onpattro is indicated for the treatment of hereditary*

transthyretin-mediated amyloidosis (hATTR amyloidosis) in adult patients with stage 1 or stage 2 polyneuropathy". The applicant accepted the CHMP position and amended the sought indication accordingly.

2.8. Clinical safety

Patient exposure

Overall, the integrated safety database includes 218 patients with hATTR amyloidosis with polyneuropathy exposed to patisiran-LNP for a period of up to 3.74 years. These data represent a total of 412.3 patient-years of exposure.

In addition to the clinical studies described above, patisiran-LNP is also currently being administered to patients with hATTR amyloidosis with polyneuropathy via an ongoing Expanded Access Protocol (EAP) in the United States and compassionate use programs in the EU. As of the data cutoff of 14 July 2017, a total of 48 patients were treated in the EAP, representing 11.5 person-years of exposure; at the time of this cutoff, no patients had been dosed in the compassionate use program.

Safety pools:

"Placebo-controlled experience" (Double-blind, placebo-controlled pool)

Study 004

According to the protocol, patients were to receive study drug for 78 weeks (total of 27 doses).

The mean duration of study drug was 17.7 months in the patisiran-LNP group (N= 148) and 15.0 months in the placebo group (N=77), with 218.9 person-years of exposure in the patisiran-LNP group and 96.1 person-years in the placebo group. In the patisiran-LNP and placebo groups, respectively, there were 96.6% and 87.0% of patients who had received study drug for at least 6 months, 94.6% and 71.4% of patients who had received study drug for at least 12 months, and 93.2% and 66.2% of patients who had received study drug for at least 15 months.

"Overall pooled experience" (Long-term safety pool)

Studies 003, 004, 006

In the overall pooled experience across studies 003, 004, and 006, 218 patients with hATTR amyloidosis with polyneuropathy were treated with patisiran-LNP for durations of up to 44.9 months, with 412.3 person-years of exposure. Overall, 179 patients (82.1%) were treated for ≥12 months, 101 patients (46.3%) were treated for ≥24 months and 32 patients (14.7%) were treated for ≥36 months. The mean duration of patisiran-LNP exposure was 22.69 months (range: 0.7 to 44.9). The mean number of doses received was 31.9 (range: 1 to 65)

The primary factors that were considered in the strategy for selecting studies in the integrated analysis were to include studies that utilized the same dose, dose regimen and infusion regimen that is proposed to be used commercially, and which were evaluated in the target patient population. All 3 studies were multicenter, enrolled patients with hATTR amyloidosis with polyneuropathy and documented TTR mutation, dosed patients with patisiran-LNP at 0.3 mg/kg once every 3 weeks, administered study drug as an approximately 80-minute IV infusion, and collected a similar battery of safety assessments.

Adverse events

In Study 004, the overall proportion of patients experiencing AEs was similar in the patisiran-LNP (96.6%) and placebo (97.4%) groups.

Table 30 Placebo-controlled Experience: Overview of Adverse Events (ALN-TTR02-004 Safety Population)

Category	**Statistic**	**Placebo (N=77)**	**Patisiran-LNP 0.3 mg/kg (N=148)**
At Least 1 AE	N (%)	75 (97.4)	143 (96.6)
At Least 1 AE Related to Study Drug	N (%)	30 (39.0)	73 (49.3)
At Least 1 Severe AE	N (%)	28 (36.4)	42 (28.4)
At Least 1 Severe AE Related to Study Drug	N (%)	2 (2.6)	3 (2.0)
At Least 1 Serious Adverse Event (SAE)	N (%)	31 (40.3)	54 (36.5)
At Least 1 SAE Related to Study Drug	N (%)	0	4 (2.7)
At Least 1 AE Leading to Treatment Discontinuation	N (%)	11 (14.3)	7 (4.7)
At Least 1 Study Drug-Related AE Leading to Treatment Discontinuation	N (%)	0	2 (1.4)
At Least 1 AE Leading to Study Withdrawal	N (%)	9 (11.7)	7 (4.7)
At Least 1 Study Drug-Related AE Leading to Study Withdrawal	N (%)	0	2 (1.4)
Death	N (%)	6 (7.8)	7 (4.7)

Abbreviations*: AE=adverse event; LNP=lipid nanoparticle; SAE=serious adverse event*
Notes:

- *TEAEs are those with onset during or after the first dose through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related is considered a TEAE.*
- *Related AEs are AEs that are deemed to be either 'definitely related' or 'possibly related' to study drug by the Investigator.*
- *If a patient experienced more than 1 event in a given category, that patient is counted only once in that category.*
- *Patients who discontinued study treatment could remain in the study and were considered to have completed the study if they completed the 18 Month visit*

All deaths are summarized, including deaths due to AEs that are not treatment-emergent. An additional placebo patient who withdrew from the study due to a deterioration of health status and worsening cardiac failure was reported to have died shortly after the end of the study.

A greater proportion of patients on patisiran-LNP (≥5 percentage point difference) experienced an AE in the following SOC: general disorders and administration site conditions (patisiran-LNP 58.8%, placebo 51.9%) with the difference mostly in peripheral edema; immune system disorders (patisiran-LNP 20.3%, placebo

10.4%) with the difference largely due to IRRs; and ear and labyrinth disorders (patisiran-LNP 9.5%, placebo 2.6%) accounted for mostly by vertigo.

A greater proportion of patients in the placebo group (≥5 percentage point difference) experienced AEs in the following SOC: nervous system disorders (patisiran-LNP 50.7%, placebo 58.4%); injury, poisoning and procedural complications (patisiran-LNP 37.8%, placebo 49.4%); cardiac disorders (patisiran-LNP 28.4%, placebo 36.4%,); renal and urinary disorders (patisiran-LNP 13.5%, placebo 29.9%); metabolic disorders (patisiran-LNP 22.3%, placebo 29.9%); psychiatric disorders (patisiran-LNP 18.9%, placebo 26.0%); and blood and lymphatic system disorders (patisiran-LNP 6.1%, placebo 15.6%).

AEs that were reported in ≥10% of patients in any group are presented in table below. AEs reported in ≥15% of patients in the patisiran-LNP group included diarrhea (37.2%), peripheral edema (29.7%), IRR; 18.9%, and fall (16.9%). AEs reported in ≥15% of patients in the placebo group included diarrhea (37.7%), fall (28.6%), peripheral edema (22.1%), nausea (20.8%), urinary tract infection (18.2%), and constipation (16.9%).

Table 31 Adverse Events in ≥10% of Patients in Any Group by Preferred Term, (ALN-TTR02-004 Safety Population)

	Number of Patients (%)[a]/Events[b]	
Preferred Term	**Placebo (N=77)**	**Patisiran-LNP 0.3 mg/kg (N=148)**
At Least 1 AE	75 (97.4)/1231	143 (96.6)/2078
Diarrhea	29 (37.7)/95	55 (37.2)/165
Peripheral edema	17 (22.1)/35	44 (29.7)/69
Infusion-related reaction	7 (9.1)/79	28 (18.9)/145
Fall	22 (28.6)/43	25 (16.9)/47
Constipation	13 (16.9)/19	22 (14.9)/29
Nausea	16 (20.8)/22	22 (14.9)/50
Dizziness	11 (14.3)/37	19 (12.8)/24
Urinary tract infection	14 (18.2)/23	19 (12.8)/40
Fatigue	8 (10.4)/18	18 (12.2)/27
Headache	9 (11.7)/10	16 (10.8)/25
Cough	9 (11.7)/11	15 (10.1)/18
Insomnia	7 (9.1)/12	15 (10.1)/24
Nasopharyngitis	6 (7.8)/11	15 (10.1)/26
Vomiting	8 (10.4)/30	15 (10.1)/21

Preferred Term	Number of Patients (%)[a]/Events[b]	
	Placebo (N=77)	**Patisiran-LNP 0.3 mg/kg (N=148)**
Asthenia	9 (11.7)/15	14 (9.5)/25
Pain in extremity	8 (10.4)/12	10 (6.8)/13
Muscular weakness	11 (14.3)/17	5 (3.4)/8
Anemia	8 (10.4)/12	3 (2.0)/3
Syncope	8 (10.4)/9	3 (2.0)/3

***Abbreviations**: PT=Preferred Term*
[a] If a patient experienced more than 1 event with a given PT, that patient is counted only once for that PT. Percentages are based out of the total number of patients (N) who were on study at the start of the indicated exposure duration category.
[b] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.

AEs that were reported at a higher frequency (≥3 percentage point difference) in the patisiran-LNP group compared with the placebo group are presented in the table below. Of the AEs presented in the table, AEs with a ≥5 percentage point higher frequency in the patisiran-LNP group than the placebo group included peripheral edema, IRRs, arthralgia, muscle spasms and dyspnoea.

Peripheral edema was reported in 44 patients (29.7%, 69 events) in the patisiran-LNP group and 17 patients (22.1%, 35 events) in the placebo group. The majority of these patients had a medical history of cardiac disorders. Among patients in the protocol-specified cardiac subpopulation; peripheral edema was reported in 29 patients (32.2%, 51 events) in the patisiran-LNP group and 9 patients (25.0%, 21 events) in the placebo group. When analyzed over time, the proportion of patients with AEs of peripheral edema and the number of peripheral edema events in the patisiran-LNP group decreased over the course of the study (first 9 months: range 7.6% to 10.8%, 49 events; second 9 months: range 2.1% to 6.5%, 20 events). In contrast, in the placebo group, the proportion of patients and number of events showed an increasing trend (first 9 months: range 4.0% to 7.5%; 15 events; second 9 months: range 5.9% to 12.5%, 20 events). All of the AEs of peripheral edema were mild or moderate in severity. Of the 29.7% of patients in the patisiran-LNP group with peripheral edema, mild and moderate events by maximum severity were experienced by 18.9% and 10.8% of patients, respectively; of the 22.1% of placebo patients with peripheral edema, mild and moderate events by maximum severity were experienced by 10.4% and 11.7% of patients, respectively. Peripheral edema was considered related to study drug in 1 patient (0.7%, 1 event) in the patisiran-LNP group and 5 patients (6.5%, 8 events) in the placebo group. One patient in the patisiran-LNP group was admitted to the hospital (SAE) to have cardiac testing performed (echocardiogram and right cardiac catheterization) to investigate chronic inferior limb edema considered related to the patient's amyloidosis. The event was considered not related to study drug. No patients discontinued treatment due to peripheral edema, and 1 patient in the placebo group had an infusion cycle delay.

Dyspnoea was reported in 10 patients (6.8%, 14 events) in the patisiran-LNP group and no patients in the placebo group. The majority of these patients had a medical history of cardiac disorders, with dyspnoea reported by 6 patients (6.7%, 8 events) in the patisiran-LNP group in the cardiac subpopulation. When dyspnoea was analyzed over time, the proportion of patients and number of events decreased over time (first 9 months: 2.0-2.1%, 10 events; second 9 months: 0.7-1.4%, 4 events). Most of the events were mild or moderate in severity. Two patients in the patisiran-LNP group had single events of dyspnoea that were

considered severe. None of the events led to treatment discontinuation or were considered IRRs and no action was taken with the study drug. All of the events were considered not related or unlikely related to study drug by the investigators. One patient who had been having worsening dyspnoea due to congestive heart failure and associated chest discomfort was admitted to the hospital (SAE) for evaluation and treatment of worsening orthostatic hypotension. The patient's medications were adjusted and the event resolved. The event was considered not related to study drug.

Arthralgia, mostly as pain in the knee, hip, or ankle, was reported in 11 patients (7.4%, 13 events) in the patisiran-LNP group and no patients in the placebo group. All of the events were mild or moderate in severity. None of the events led to treatment discontinuation or were considered IRRs, and no action was taken with study drug. All of the events were considered not related or unlikely related to study drug by the investigators.

Muscle spasms were reported in 12 patients (8.1%, 18 events) in the patisiran-LNP group and 1 patient (1.3%, 1 event) in the placebo group. Most of the events were mild in severity except for 1 transient event that was moderate in severity. None of the events led to treatment discontinuation or were considered IRRs, and no action was taken with study drug. Most of the events were considered not related or unlikely related to study drug except for 1 mild event which was possibly related.

In the patisiran-LNP group, for the other AEs listed in Table 32 (dyspepsia, erythema, vertigo, rhinitis, sinusitis, and bronchitis), most of the patients had AEs that were mild or moderate in severity. There were 2 patients with severe events: 1 patient with dyspepsia and 1 patient with vertigo. Both severe events were transient, considered unlikely or not related to study drug, and did not result in any change of dose or action with drug. Overall, none of the events cited above led to discontinuation of treatment or had any action taken with study drug.

Table 32 Adverse Events with a Higher Frequency (≥3 Percentage Points) in Patisiran-LNP versus Placebo Groups by System Organ Class and Preferred Term (ALN-TTR02-004 Safety Population)

System Organ Class/ Preferred Term	Number of patients (%)		Difference (Patisiran-LNP – Placebo)
	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)	
Ear and labyrinth disorders			
Vertigo	1 (1.3)	8 (5.4)	4.1
Gastrointestinal disorders			
Dyspepsia	3 (3.9)	12 (8.1)	4.2
General disorders and administration site conditions			
Peripheral edema	17 (22.1)	44 (29.7)	7.7
Immune system disorders			
Infusion-related reaction	7 (9.1)	28 (18.9)	9.8

System Organ Class/ Preferred Term	Number of patients (%)		Difference (Patisiran-LNP – Placebo)
	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)	
Infections and infestations			
Rhinitis	0	6 (4.1)	4.1
Sinusitis	0	6 (4.1)	4.1
Bronchitis	2 (2.6)	9 (6.1)	3.5
Musculoskeletal and connective tissue disorders			
Arthralgia	0	11 (7.4)	7.4
Muscle spasms	1 (1.3)	12 (8.1)	6.8
Respiratory, thoracic and mediastinal disorders			
Dyspnoea	0	10 (6.8)	6.8
Skin and subcutaneous tissue disorders			
Erythema	2 (2.6)	10 (6.8)	4.2

Abbreviations: PT=preferred term; SOC=system organ class
Note: SOCs are sorted alphabetically; PTs within an SOC are sorted by decreasing order of the percentage difference.

AEs with a ≥5% higher frequency in the placebo group compared to the patisiran-LNP group included falls, nausea, urinary tract infections, muscular weakness, anemia, syncope, dehydration, hematuria, weight decreased, abdominal pain upper, hemorrhoids, hyponatremia, supraventricular extrasystole, and atrioventricular block first degree.

Adverse drug reactions for patisiran-LNP are defined to include those AEs that occurred with a ≥3% higher frequency in patisiran-LNP treated patients compared with placebo in the pivotal Study 004 and other potentially relevant AEs based on other studies with patisiran-LNP (Studies 001, 002, 003, 005, and 006). Evidence for causality can be based on the mechanism of action of a drug, medical review of individual or aggregate AEs, analysis of laboratory data, and other information.

AEs of special interest for patisiran-LNP

The following AEs (IRR, liver, cardiac, renal, ocular, thyroid) are considered AEs of special interest for patisiran-LNP given the mechanism of action-specific concerns related to patisiran-LNP, the class- and platform-related adverse effects seen with siRNAs and lipid nanoparticles and the non-clinical toxicology findings and the disease-related pathophysiology of hATTR amyloidosis. AEs and related laboratory findings are presented together to facilitate the reading.

Infusion-related AEs (Infusion-related reactions, IRR) incl infusion interruptions

Infusion-related reactions (IRR)

In the SAD study with healthy volunteers, there was only 1 IRR reported, in the single subjects who received patisiran at the 500 µg/kg dose.

In the MAD study 002, IRRs occurred in 3 (10.3%) patients, all of whom were in the 300 µg/kg Q4W dose group, and all of whom received the original premedication regimen. There were no IRRs reported among the 12 patients in the 300 µg/kg Q3W dose group, 9 of who had received patisiran as a 70-minute infusion with the alternative premedication regimen.

In Study 004, in which all patients received premedication prior to receiving patisiran-LNP or normal saline placebo, a total of 28 (18.9%) patients in the patisiran-LNP group and 7 (9.1%) patients in the placebo group reported at least 1 IRR. All IRRs were mild or moderate in severity and resolved. There were no severe IRRs, and no IRRs were reported as SAEs. One patient in the patisiran-LNP group discontinued study treatment due to an IRR of moderate flushing that resolved within 15 minutes. None of the IRRs in the placebo group led to discontinuation of study treatment or led to the interruption of infusion.

In the patisiran-LNP group, of the patients with IRRs, the majority (78.6%, 22 of 28 patients) had their first IRR within the first 2 doses and >82.1% by the third dose.

Infusion related reaction signs and symptoms reported in ≥2% of patients in the patisiran- LNP group were back pain (6.1%), flushing (4.1%), nausea (3.4%), headache (2.7%), and arthralgia and dyspnoea (2.0% each). The only IRR sign or symptom reported in ≥2% of patients in the placebo group was flushing (7.8%). Flushing occurred in both groups and was most likely due to premedication or other causes.

Table 33: Infusion-Related Reactions Signs and Symptoms in 2 or More Patients in Any Group (ALN-TTR02-004 Safety Population)

	Number of Patients (%)[a]/Events[b]	
System Organ Class/ Preferred Term	**Placebo (N=77)**	**Patisiran-LNP 0.3 mg/kg (N=148)**
Number of patients with at least 1 IRR and number of IRRs (AEs)	7 (9.1) /79	28 (18.9) /145
Number of IRR symptoms (events)	105	223
Gastrointestinal disorders	0	9 (6.1) /15
Abdominal pain	0	2 (1.4) /2
Abdominal pain lower	0	2 (1.4) /2
Abdominal pain upper	0	2 (1.4) /4
Nausea	0	5 (3.4) /5
General disorders and administration site conditions	2 (2.6) /25	10 (6.8) /46
Chest discomfort	0	2 (1.4) /6

Table 33: Infusion-Related Reactions Signs and Symptoms in 2 or More Patients in Any Group (ALN-TTR02-004 Safety Population)

System Organ Class/ Preferred Term	Number of Patients (%)[a]/Events[b]	
	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
Chest pain	0	2 (1.4) /8
Chills	1 (1.3) /1	2 (1.4) /10
Fatigue	0	2 (1.4) /9
Injection site erythema	0	2 (1.4) /2
Injection site swelling	0	2 (1.4) /2
Pain	0	2 (1.4) /6
Musculoskeletal and connective tissue disorders	1 (1.3) /2	9 (6.1) /74
Arthralgia	0	3 (2.0) /8
Back pain	0	9 (6.1) /52
Nervous system disorders	1 (1.3) /1	7 (4.7) /11
Headache	1 (1.3) /1	4 (2.7) /7
Respiratory, thoracic and mediastinal disorders	0	5 (3.4) /11
Cough	0	2 (1.4) /2
Dyspnoea	0	3 (2.0) /3
Skin and subcutaneous tissue disorders	1 (1.3) /14	5 (3.4) /16
Skin warm	0	2 (1.4) /2
Vascular disorders	6 (7.8) /36	9 (6.1) /40
Flushing	6 (7.8) /36	6 (4.1) /34
Hypotension	0	2 (1.4) /4

Abbreviations: AE=adverse event; IRR=infusion-related reaction; PT=preferred term; SOC=system organ class.
[a.] If a patient experienced more than 1 event in a given SOC, that patient is counted once for the SOC. If a patient experienced more than 1 event with a given PT, that patient is counted only once for that PT. Percentages are based out of the total number of patients (N) who were on study at the start of the indicated exposure duration category.
[b.] The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.

Extravasation

In Study 004, 12 patients had 12 infusions with AEs that were potentially associated with or possible symptoms of extravasation (11 patients in the patisiran-LNP group with 11 events and 1 patient in the placebo group with 1 event). Adverse events associated with possible events of extravasation included

phlebitis, superficial thrombophlebitis, cellulitis, dermatitis (subcutaneous inflammation at infusion site), and infusion site swelling, erythema, pain, burning sensation, or extravasation. One of the events in the patisiran-LNP group occurred after administration of premedication during the saline flush and prior to administration of study drug. Two patients in the patisiran-LNP group had events of superficial thrombophlebitis/post procedural cellulitis and dermatitis (subcutaneous inflammation)/phlebitis/infusion site extravasation that were reported as SAEs. All patients, including the 2 patients with SAEs, had events that were mild or moderate in severity. All events resolved without sequelae. Dosing was interrupted in 4 of the 11 patients in the patisiran-LNP group and in the 1 patient in the placebo group who had a moderate event. None of the patients in either group were discontinued due to extravasation. All patients in the patisiran-LNP and placebo groups received a complete dose and went on to complete study treatment.

Similar results were seen in Study 003. Five patients had 6 events of possible extravasation. Adverse events associated with possible events extravasation included: 1 event of phlebitis in 1 patient, 2 events of infusion site irritation in 1 patient, and 3 events of infusion site extravasation in 3 patients, of which one event was associated with infusion site erythema, pain, and fever All events were non-serious, mild in severity, and resolved. Study drug was interrupted in 4 cases, and the patients received complete doses.

In Study 006, 4 patients had 4 events of possible extravasation. Adverse events associated with possible events of extravasation included 2 events of injection site swelling in 2 patients, 1 patient with phlebitis, and 1 patient with an SAE of phlebitis/cellulitis/hypotension. The events were mild or moderate in severity, including the SAE, and resolved. There were no infusion interruptions and the patients received complete doses.

Overall, potential events of extravasation have been reported in 0.3% of infusions (21 events in 6954 administrations) in patients administered patisiran-LNP and characterized by symptoms of phlebitis, or localized pain, burning sensation, swelling, tenderness, or redness at or near the infusion site. There have been no reports of tissue injury or damage such as blistering, exfoliation, or skin necrosis around or near the infusion site.

Infusion Interruption

Overall, in the patisiran-LNP group, based on the cumulative number of doses administered across all patients, the number of infusions with AEs leading to infusion interruption (17 of 3740 doses) and number of patients with AEs leading to infusion interruption who received partial doses (2 of 3740 doses) was low.

Of the 6954 doses of patisiran-LNP administered across the pooled experience, there were 114 events of infusion interruption; in 109 of these interruptions, the complete dose of patisiran-LNP was received. A total of 18 (8.3%) patients across the pooled experience reported an AE leading to an infusion interruption. AEs that led to an infusion interruption reported in ≥1% of patients were IRRs (6.0%) and infusion site extravasation (2.3%).

Premedication and IRRs

All patients in the patisiran-LNP and placebo groups received a premedication regimen including corticosteroids prior to each dose of study drug to reduce the potential for an IRR. During the study, 2 premedication regimens were used: the original premedication regimen and the reduced premedication regimen. The reduced regimen was amended to the protocol to mitigate potential AEs suspected to be related to corticosteroids, such as flushing. At the time of the amendment, patients who were receiving study drug were transitioned to the reduced regimen and newly enrolled patients started and received all infusions with the reduced regimen.

With respect to IRRs, analysis of IRRs following the original and reduced regimens demonstrated that there was no increase in IRRs when patients received the reduced regimen compared to the original regimen, and that patisiran-LNP was well tolerated on both regimens. On the original premedication regimen, there were 22 patients (17.9%) in the patisiran-LNP group and 7 patients (10.1%) in the placebo group with at least 1 IRR; on the reduced premedication regimen at least 1 IRR occurred in 9 patients in the patisiran-LNP group (6.9%) and in 3 patients in the placebo group (5.1%). Furthermore, in an evaluation of IRR signs and symptoms by premedication regimen, no notable differences were observed after the change in premedication regimen.

Adverse events related to premedication

Investigators were asked to assess whether an AE was considered related to premedication.

In the placebo-controlled experience, the proportion of patients with AEs considered related to premedications by the investigators was similar across the patisiran-LNP (36.5%) and placebo (36.4%) groups. Adverse events reported in ≥3% of patients in either treatment group included osteoporosis, dizziness, somnolence, and insomnia. Serious AEs considered related to premedications were reported in a total of 4 patients: 1 patient with deafness unilateral in the patisiran-LNP group; and 3 patients in the placebo group (1 patient with oesophagitis, pulmonary oedema and urinary retention; 1 patient with urinary tract infections; and 1 patient with erysipelas and skin ulcer).

In the placebo-controlled experience, an analysis of AEs related to the premedication regimen comparing the original and reduced regimens demonstrated that there was a decrease in the proportion of patients and number of events with AEs considered related to premedications reported after the switch to the reduced premedication regimen. The proportion of patients with AEs considered related to the original regimen was 29.3% (106 events) and 37.7% (77 events) in the patisiran-LNP and placebo groups, respectively, and AEs considered related to the reduced regimen was 19.1% (54 events) and 20.3% (28 events) in the patisiran - LNP and placebo groups, respectively. In Study 004, the cumulative study drug exposure for the two 2 regimens was similar.

Adverse events of selected events associated with glucocorticoids such as Cushingoid (1patient), hyperglycemia (3 patients), glycosuria urine present (2 patients), glucose tolerance impaired (1 patient), and diabetes (0 patients) were limited. In the pooled experience, osteopenia and osteoporosis were reported in 9 and 12 patients, respectively. When evaluated over time, the reports of osteopenia and osteoporosis were scattered with the majority reported during the first year and did not increase over time.

Hepatobiliary events and liver function tests

Adverse events

Because patisiran-LNP targets the liver and because nonclinical studies of patisiran-LNP revealed changes in serum liver markers and liver histopathology, the frequency of hepatic AEs was evaluated by performing an analysis of events mapping to the Drug-related hepatic disorders standardized MedDRA query (SMQ).

In Study 004, the proportion of patients with hepatic events mapping to the SMQ was similar across the patisiran-LNP (5.4%) and placebo (9.1%) groups. Within the SMQ, the frequency of AEs in the Hepatobiliary disorders SOC (1.4% patisiran-LNP, 1.3% placebo) and Investigations SOC (3.4% patisiran-LNP, 3.9% placebo,) were similar across the 2 treatment groups. In the placebo group, hypoalbuminemia was reported in 2 (2.6%) of patients, and 1 patient underwent a liver transplant.

Most of the hepatic AEs were mild or moderate in severity, and considered not or unlikely related to study drug. In the placebo group, 1 patient had a severe AE of hypoalbuminemia considered unlikely related to study drug. Hepatic AEs considered possibly related to study drug were hepatic enzyme increased and blood ALP increased in the patisiran--LNP group (1 patient [0.7%] each) and hypoalbuminemia in the placebo group (1 patient [1.3%]), all of which were mild in severity. Hepatic AEs that were SAEs were reported in 3 patients in the placebo group (LFTs abnormal, hypoalbuminemia, and liver transplant; 1 patient each) and in 1 patient in the patisiran-LNP group (ascites). All hepatic SAEs were considered unlikely or not related to study drug.

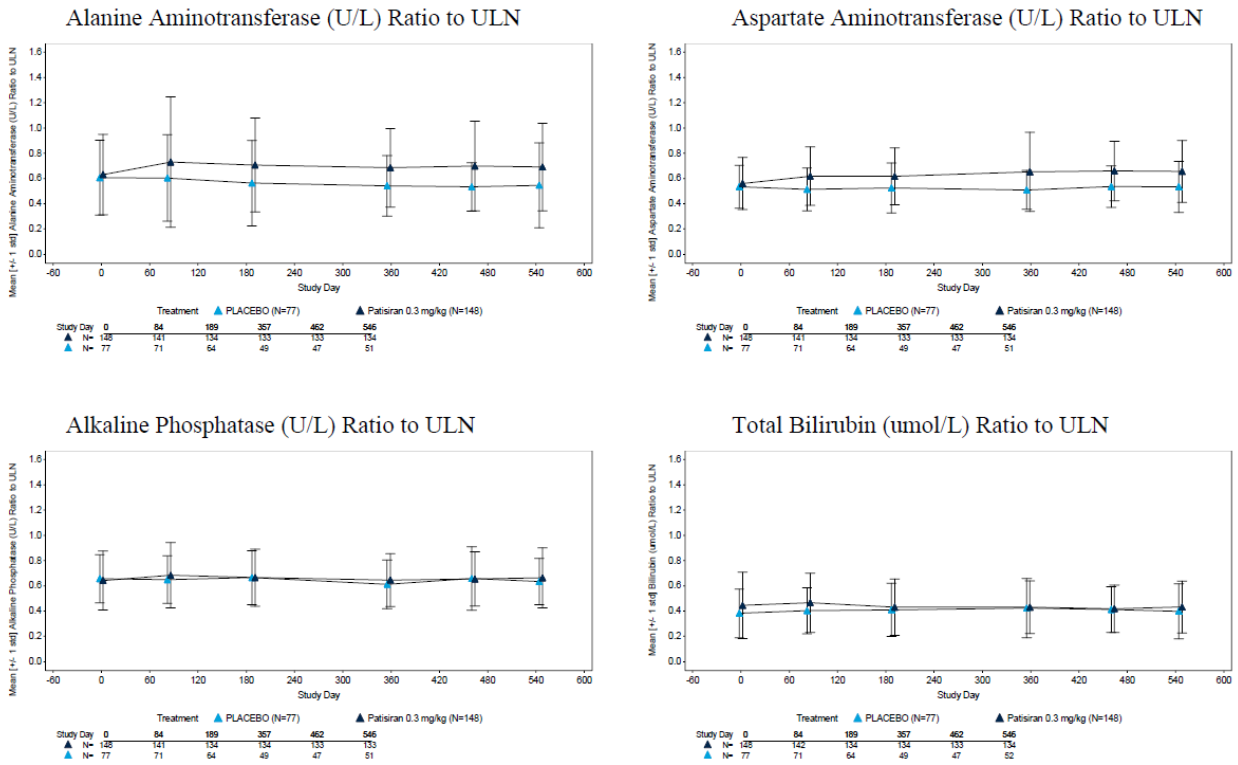
In the overall pooled analysis, the proportion of patients with hepatic events mapping to the Drug-related hepatic disorders SMQ was 4.6% and similar to the placebo-controlled experience. Most hepatic events reported were laboratory elevations within the investigations SOC, which occurred in 3.2% of subjects. Hepatic events reported in ≥1% of patients were ALT increased (1.4%) and ALP increased (1.4%); in all patients in whom these AEs appeared, the AEs were consistently mild in severity and, in most cases, not considered related to study treatment.

Liver function tests

Mean ALT and AST levels remained relatively constant over the course of the MAD study (study ALN-TTR02-002) for all dose groups, except for the 300 µg/kg Q3W dose group at Day 7. In this group, the highest individual ALT and AST values observed were 99 U/L and 110 U/L, respectively, and resolved to normal by Day 14.

In Study 004, mean absolute values and mean percent changes from baseline for LFT parameters, including ALT, AST, total bilirubin, and ALP, were generally stable and similar across the patisiran-LNP and placebo groups. Shifts from baseline to worst post-baseline values for LFTs in the patisiran-LNP group compared with the placebo group did not show any clinically significant patterns.

Figure 29 Study 004 Mean Liver Function Test Ratio to ULN (±1 Standard Deviation) Over Time by Treatment Group (Safety Population)



Note: mean and standard deviation are presented for each parameter in each panel.

A summary of abnormalities in worst post-baseline LFTs is presented in the table below. The majority of patients in Study 004 had ALT and AST values within normal limits. Overall, 12 (15.6%) patients in the placebo group and 47 (31.8%) patients in the patisiran-LNP group had at least 1 ALT or AST value >ULN. Most of these elevations were ≤3 × ULN. One patient (0.7%) in the patisiran-LNP group had a transient elevation of ALT >3 × ULN and is described below. No patients had ALT or AST values >3×ULN with concurrent elevation of total bilirubin >2×ULN at any time during Study 004.

Table 34 Summary of Worst Post-baseline Abnormalities in Liver Function Test Results (ALN-TTR02-004 Safety Population)

Parameter	Criterion	Placebo (N=77)	Patisiran-LNP 0.3 mg/kg (N=148)
ALT	≤ULN	66 (85.7)	105 (70.9)
	>ULN and ≤ 3 × ULN	10 (13.0)	39 (26.4)
	>3 × ULN and ≤ 5 × ULN	0	1 (0.7)
	>5 × ULN and ≤ 10 × ULN	0	0

	Missing	1 (1.3)	3 (2.0)
AST	≤ULN	72 (93.5)	114 (77.0)
	>ULN and ≤3 × ULN	4 (5.2)	31 (20.9)
	>3 × ULN and ≤5 × ULN	0	0
	>5 × ULN and ≤10 × ULN	0	0
	Missing	1 (1.3)	3 (2.0)
ALT or AST	≤ULN	64 (83.1)	97 (65.5)
	>ULN and ≤3 × ULN	12 (15.6)	47 (31.8)
	>3 × ULN and ≤5 × ULN	0	1 (0.7)
	>5 × ULN and ≤10 × ULN	0	0
	Missing	1 (1.3)	3 (2.0)
TBili	≤ULN	73 (94.8)	135 (91.2)
	>ULN and ≤1.5 × ULN	2 (2.6)	9 (6.1)
	>1.5 × ULN ≤2 × ULN	1 (1.3)	1 (0.7)
	>2 × ULN ≤3 × ULN	0	0
	Missing	1 (1.3)	3 (2.0)
ALP	>1.5 × ULN	1 (1.3)	3 (2.0)
AST or ALT and concurrent TBili	ALT or AST >3 × ULN and TBili >2 × ULN	0	0

Abbreviations: *ALP= alkaline phosphatase; ALT=alanine aminotransferase; AST= aspartate aminotransferase; TBili=total bilirubin; ULN= upper limit of normal.*

One patient had a transient, asymptomatic elevation of ALT >3 × ULN that was not associated with elevation of bilirubin and is discussed below. There were no patients with elevations of ALT or AST >3 × ULN and total bilirubin >2 × ULN, i.e. no cases fulfilling the Hy's Law criteria.

Results for LFT evaluations in Study 003 and Study 006 were similar to the placebo-controlled experience. In Study 003, few patients had shifts in liver function parameters. One patient (3.7%) had a shift in ALT >3×ULN and ≤5×ULN (3.13×ULN) that was not associated with an elevation in bilirubin. All other shifts in liver function parameters were minor (>ULN to ≤3 × ULN for ALT and AST, >ULN to ≤1.5×ULN for total bilirubin, and >1.5×ULN for alkaline phosphatase). No patients in Study 003 had elevations of AST or ALT >3 × ULN and total bilirubin >2 × ULN.

In Study 006, most patients did not exhibit shifts in liver function parameters, and shifts in LFTs were generally considered not clinically relevant. No patients in the clinical database had ALT or AST values > 3× ULN and no patients had total bilirubin values >2×ULN. There was 1 patient who had an SAE of transaminase increased in the setting of a cellulitis of the lower leg. The event was considered related to an inflammatory response due to the cellulitis and not related to study drug. The patient had elevation of ALT and AST >3 × ULN that resolved quickly when the cellulitis improved.

Cardiac events, ECG and blood pressure

As patients with hATTR amyloidosis often have cardiac involvement, an evaluation of cardiac AEs based on the Cardiac disorders SOC was conducted.

At baseline in Study 004, 63.5% of patients in the patisiran-LNP group and 72.7% of patients in the placebo group had a medical history of conditions with terms that mapped within to the Cardiac disorders SOC. A higher proportion of patients in the placebo group (24.7%) were noted to have in their medical history terms that mapped to the Cardiac device therapeutic procedures HLT (including cardiac pacemaker insertion, implantable defibrillator insertion, and pacemaker generated rhythm) compared to the patisiran-LNP group (14.2%), as well as to the HLT Myocardial disorders NEC (27.0% patisiran-LNP, 46.8% placebo), driven by the PT term cardiac amyloidosis (21.6% patisiran-LNP, 36% placebo). In contrast, a higher proportion of patients in the patisiran-LNP group (7.4%) had medical history terms that mapped to the HLT Ventricular arrhythmias and cardiac arrest compared to the placebo group (3.9%).

During Study 004, the proportion of patients with AEs (28.4% patisiran-LNP; 36.4% placebo) and SAEs (13.5% patisiran-LNP; 13.0% placebo) within the Cardiac disorders SOC was similar across the 2 treatment groups. Cardiac AEs reported in ≥5% of patients in either group were atrial fibrillation (8.8% patisiran-LNP, 6.5% placebo), atrioventricular block first degree (0% patisiran--LNP, 5.2% placebo), cardiac failure (4.7% patisiran-LNP, 5.2% placebo), and supraventricular tachycardia extrasystole (1.4% patisiran-LNP, 6.5% placebo). Cardiac SAEs reported in ≥2% of patients in the patisiran-LNP group were atrioventricular block complete, cardiac failure, and cardiac failure congestive in 2.0% (3 patients) each. In the placebo group, cardiac SAEs reported in ≥2% of patients were cardiac failure and cardiac failure congestive in 2.6% (2 patients) each.

An additional analysis of cardiac SAEs that resulted in hospitalizations or death was performed. A total of 18 patients (12.2%) in the patisiran-LNP group and 10 patients (13.0%) in the placebo group had at least 1 hospitalization or death due to SAEs in the Cardiac disorders SOC, with mean number of events of 1.0 in the patisiran-LNP group and 1.5 in the placebo group. The proportion of patients with 2 or more hospitalizations or deaths in the Cardiac SOC was higher in the placebo group than the patisiran-LNP group. The rate of hospitalizations or death in the Cardiac SOC was lower in the patisiran-LNP group (9.6 per 100 patient-years) than in the placebo group (15.6 per 100 patient-years).

As patients with hATTR amyloidosis frequently have conduction defects, an analysis of AEs within the HLGT of Cardiac arrhythmia was performed. The proportion of patients with AEs that mapped within the Cardiac arrhythmias HLGT was lower in the patisiran-LNP group (28 patients [18.9%]) than in the placebo group (22 patients [28.6%]). This difference was also notable across the various HLTs, including the Cardiac conduction disorders HLT (6.8% patisiran-LNP, 9.1% placebo), Supraventricular arrhythmias HLT (10.1% patisiran-LNP, 16.9% placebo), and Ventricular arrhythmias and cardiac arrest HLT (2.7% patisiran-LNP, 7.8% placebo). The only imbalance was in the Rate and rhythm disorders NEC HLT (3.4% [5 patients] patisiran-LNP, 0% placebo), which was predominantly due to 3 patients (2.0%) with bradycardia (reported in patients with history of cardiomyopathy, or conduction disorders such as atrioventricular block, atrial fibrillation and atrial flutter, and all considered unlikely or not related to study drug). An analysis of AEs within the Torsade de pointes SMQ was also performed to search for possible events of QTc prolongation. The proportion of patients with AEs mapping to this SMQ was lower in the patisiran-LNP group (5.4%) compared to the placebo group (18.2%) No events of Torsade de pointes were reported.

As patients with cardiac amyloid involvement frequently develop progressive heart failure, an analysis of AE terms mapping to the Cardiac failure SMQ using both a narrow and comprehensive search was performed.

The proportion of patients with AEs mapping within the Cardiac failure SMQ using both a narrow search (9.5% patisiran-LNP, 10.4% placebo) and a comprehensive search (34.5% patisiran-LNP, 33.8% placebo) was similar across the two 2 treatment groups.

In the overall pooled analysis, 148 (67.9%) patients had a medical history of conditions within the Cardiac disorders SOC at baseline.

Overall, the proportion of patients with AEs in the Cardiac disorders SOC was 28.0%. Cardiac events reported in ≥3% of patients were atrial fibrillation (7.3%), cardiac failure (4.6%), cardiac failure congestive (3.2%), and cardiac amyloidosis (3.2%). For the majority of patients who experienced cardiac AEs, the maximum severity was mild or moderate (62.3%). The proportion of patients experiencing cardiac AEs remained stable over the course of the study.

In the overall pooled experience, a total of 37 patients (17.0%) had SAEs within the Cardiac disorders SOC; none were considered related to the study treatment. Serious cardiac events reported in 3 or more patients were cardiac amyloidosis, cardiac failure, and cardiac failure congestive (5 patients each, 2.3%); cardiac arrest (4 patients, 1.8%), and conduction disorder (3 patients, 1.4%).

In the overall pooled experience, there were 40 patients (18.3%) with AEs that mapped within the Cardiac arrhythmias HLGT, consistent with the patisiran-LNP group in the placebo-controlled study. A total of 13 patients (6.0%), 21 patients (9.6%), 6 patients (2.8%), and 9 patients (4.1%) of patients had AEs mapping within the HLTs of Cardiac conduction disorders, Supraventricular arrhythmias, Ventricular arrhythmias and cardiac arrest, and Rate and rhythm disorder NEC, respectively. The proportion of patients with AEs mapping to the Torsade de pointes SMQ in the overall pooled experience was 15 patients (6.9%), with no events of Torsade de pointes reported. In the overall pooled experience, the proportion of patients with AEs mapping within the Cardiac failure SMQ using both a narrow search and a comprehensive search was 9.2% (20 patients) and 32.6% (71 patients), respectively. These proportions are consistent with those observed in Study 004.

In the clinical studies, the type and nature of cardiac events reported were consistent with those expected in patients with hATTR amyloidosis and there were no safety concerns considered related to patisiran-LNP. Of note, the rate of hospitalizations or deaths in the Cardiac SOC was lower in the patisiran-LNP group compared to placebo. Similarly, the proportion of patients with AEs mapping within the Cardiac arrhythmias HLGT and Torsade de pointes SMQ were lower in the patisiran-LNP group compared to placebo. The proportion of patients with AEs mapping with the Cardiac failure SMQ was comparable across the groups. Similar results were observed in the overall pooled experience.

Blood pressure

The proportion of patients with increases and/or decreases in pulse rate, systolic blood pressure, and diastolic blood pressure were similar across the placebo and patisiran-LNP groups.

Vital signs results were similar in the open-label experience, with no patterns of clinical relevance noted.

ECG

No clinically relevant changes from baseline were observed in the Phase 1 healthy volunteer studies and the Phase 2 study.

Over the course of Study 004, mean ECG parameters, including QTc interval and all other ECG parameters, appeared to be stable, with no clinically relevant changes observed. Electrocardiogram parameters were

similar across the treatment groups in both the safety population and the cardiac subpopulation. No events of Torsade de pointes were reported.

Electrocardiogram results were similar for Study 003, with no patterns of clinical relevance noted.

In Study 006, ECGs were not collected throughout the study.

Renal events and kidney function tests

In Study 004, the proportion of patients with renal AEs using the Acute renal failure SMQ was lower in the patisiran-LNP group compared to the placebo group, occurring in 6.1% and 11.7% of patients, respectively. Renal AEs observed in ≥2% of patients in the patisiran-LNP group included blood creatinine increased (2.0%) and creatinine renal clearance decreased (2.7%). AEs observed in ≥2% of patients in the placebo group included acute kidney injury (5.2%) and proteinuria (3.9%).

The proportion of patients with serious renal AEs in the SMQ was higher in the placebo group and included 4 patients (5.2%) with SAEs of acute kidney injury, compared with 1 patient (0.7%) in the patisiran-LNP group. All events were considered unlikely or not related to study drug. In the placebo group, 2 patients discontinued study treatment due to SAEs of acute kidney injury, of which one 1 patient died. No patient in the patisiran LNP group discontinued study treatment due to a renal AE.

In the overall pooled analysis, the proportion of patients with renal AEs mapping to the Acute renal failure SMQ was 5.0%. Renal AEs observed in 2 or more patients were creatinine renal clearance decreased (4 patients; 1.8%), blood creatinine increased (3 patients; 1.4%), renal impairment (3 patients; 1.4%), and acute kidney injury (2 patients; 0.9%).

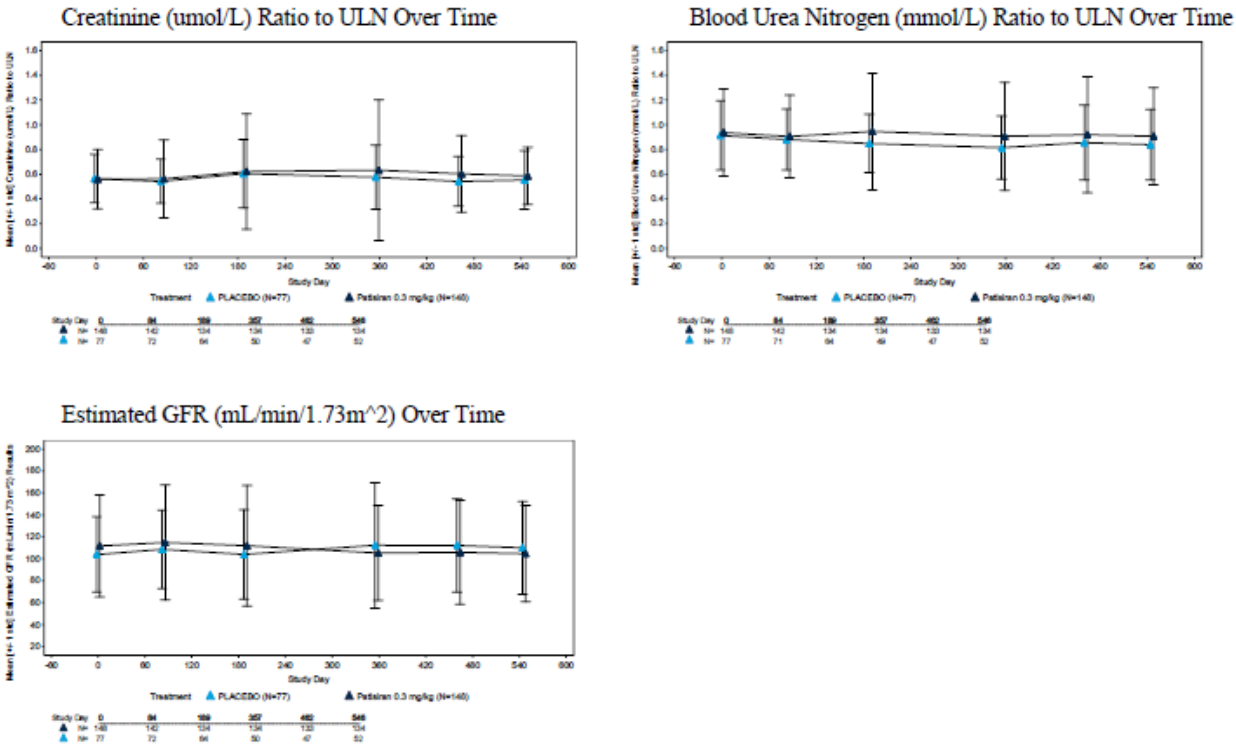
A total of 2 patients in the overall pooled experience had serious renal AEs in the Acute renal failure SMQ: the 1 patisiran-LNP treated patient with acute kidney injury from Study 004 and 1 patient with 2 events of acute prerenal failure from the 003 Pati/006 Pati group.

Within the Renal and urinary disorders SOC, there were 2 additional patients with SAEs of chronic kidney disease that were reported in Study 006: 1 in a 004 Pbo/006 Pati patient and 1 in a 004 Pati/006 Pati patient. Both patients had a long history of renal impairment and hATTR amyloidosis. Both patients had progression of their disease while in Study 004 and in Study 006. For the 004 Pati/006 Pati patient, the exacerbation of the patient's chronic renal failure also occurred in the setting of worsening cardiac disease with 2 events of atrial fibrillation and worsening congestive heart failure. Both patients discontinued study treatment and withdrew from the study. In both cases, the results were considered not related to study drug.

Renal Function Parameters over Time

In Study 004, mean absolute values and mean percent changes from baseline for blood urea nitrogen (BUN), creatinine, and estimated glomerular filtration rate (eGFR; (mL/min/1.73 m^2) were relatively consistent throughout the study in the patisiran-LNP and placebo groups, see Figure 30 below.

Figure 30 Study ALN-TTRT02-004 Mean (±1 Standard Deviation) Renal Function Tests Over Time by Treatment Group



Note: mean and standard deviation are presented for each parameter in each panel. Creatinine values were used to calculate the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) study equation.

Table 35 Study ALN-TTR02-004 Summary of Analyses of Urine Protein (mg/dL)

Parameter	Category	Placebo (N=77)	Patisiran-LNP (N=148)
Summary of Worst Post-Baseline Urine Protein (mg/dL)[a]			
Protein (mg/dL), Urine	Negative	31 (40.3)	80 (54.1)
	Trace	12 (15.6)	29 (19.6)
	30	12 (15.6)	20 (13.5)
	100	9 (11.7)	8 (5.4)
	300	1 (1.3)	4 (2.7)
	≥2000	3 (3.9)	1 (0.7)
	Missing	9 (11.7)	6 (4.1)
Summary of Patients with Two Consecutive Positive Tests for Urine Protein[b]			
Patients with at least 2 consecutively positive tests for urine protein	At any time during the study[c]	N=69 14 (20.3)	N=143 13 (9.8)
	At any time post-baseline[d]	N=55 11 (20.0)	N=124 9 (7.3)

a Baseline is defined as the measurement closest to and prior to the first dose of study drug.
b Positive Test Result on urinalysis is defined as a value of >= 30 mg/dL. Only patients (N) with at least 2 test results were included in the analysis.
c Includes patients with ≥2 positive tests including results at baseline.

d Includes Patients with ≥2 positive tests results collected post-baseline.

In the 2 treatment groups, a similar proportion of patients had worst post-baseline shifts values for BUN and creatinine that were high.

Changes in Renal Function Parameters

In general, the proportion of patients who had worst post-baseline values for eGFR that were <90 mL/min/1.73 m^2 was similar between the 2 groups, but there were 5 patients in the patisiran-LNP group who had a worst post-baseline shift to <15 mL/min/1.73 m^2 compared with none in the placebo group. A review of the eGFR values for these 5 patients showed that these were very transient shifts in eGFR and that subsequent values had returned to the values the patients had at baseline. Four of the 5 patients were from a single site and the other patient was from another site, but showed the same pattern. All 5 patients had transient, concurrent creatinine values >3 × baseline or >4 mg/dL. Three of the patients had baseline eGFRs of >90 mL/min/1.73 m^2 and 2 patients had baseline eGFRs of 30 to 59 mL/min/1.73 m^2.

A similar proportion of patients in the patisiran-LNP and placebo groups had worst post-baseline shifts of eGFRs to 15-29 mL/min/1.73 m^2, 5 patients (3.4%) and 3 patients (3.9%), respectively. In the patisiran-LNP group, 3 of the 5 patients were from the same site and showed the same pattern of transient shifts with return to baseline values as noted before. The other 2 patients had impaired renal function at baseline. In the placebo group, 1 patient had a transient shift with return to baseline as noted above in the patisiran-LNP groups. The other 2 patients had significant impaired renal function at baseline and showed a persistent decline in function.

Results for Study 003 and Study 006

Renal function results in Study 003 and Study 006 were similar to the placebo-controlled experience. In Study 003, shifts in eGFR from 60 to 89 mL/min/1.73 m^2 to ≤59 mL/min/1.73 m^2 were observed in 2 patients (refer to CSR ALN-TTR02-003, Table 14.3.5.12). In both patients, the shifts were transient, and the eGFR values at Week 109 were consistent with those at entry into the study. Additionally, there was 1 patient with a baseline eGFR of 30 to 59 mL/min/1.73 m^2 and the lowest on-study eGFR level was 30 mL/min/1.73 m^2. Clinically significant values for renal parameters, including BUN and creatinine, were seen in 2 patients. (refer to CSR ALN-TTR02-003, Table 14.3.4.1)

In Study 006, the majority of patients had worst post-baseline categories that were similar to their baseline eGFR category, with a small proportion of patients showing a shift of either 1 category improved or 1 category worsened. Of these patients, there were 2 patients, 1 in the 004 placebo group and 1 in the 004 patisiran-LNP group who had an eGFR of 15 to 29 mL/min/1.73 m^2 at baseline in Study 006 that shifted 1 category to <15 mL/min/1.73 m^2. At the time of the shift, both patients had a concurrent creatinine values >3 × baseline or >4 mg/dL. Both patients had a long history of renal impairment and hATTR amyloidosis. In 1 patient, the shift occurred in the setting of worsening of congestive heart failure. Both patients discontinued study drug due to worsening of renal impairment.

Urinalysis

In Study 003, Study 004, and Study 006, there did not appear to be any pattern of abnormalities in urinalyses observed overall. The majority of patients in all studies had urinalyses that were negative for protein. In the patients who had a urinalysis positive for protein at any time, the majority of the values was intermittent and showed no trend.

Ocular

Patients with hATTR amyloidosis may suffer from disease-related visual impairment due to accumulation of amyloid in the vitreous body of the eye. Most of the TTR protein in the body is synthesised by the liver (>95%), but the retina also synthesises its own TTR. The production of mutant TTR by the retina may not be affected by patisiran treatment, since patisiran targets mainly the liver. Glaucoma and corneal wounds, the latter related to dry eyes, are also more common among these patients. Further, TTR reduction in patients treated with patisiran-LNP is associated with concomitant reduction in circulating serum levels of RBP and vitamin A. Thus, patients were administered ophthalmology exams throughout the studies and an evaluation of ocular disorder AEs was conducted.

During Study 004, the proportion of patients with AEs in the Eye disorder SOC was similar across the treatment groups and observed in 27.7% of patients in the patisiran-LNP group and 26.0% of patients in the placebo group. Ocular events reported in 2% or more of patients in either group included cataract, (including the PTs cataract, cataract subcapsular, and cataract nuclear) in 7.4% of patients in the patisiran-LNP group and 6.5% of the placebo group; conjunctival hemorrhage in 0.7% of patisiran-LNP and 3.9% of placebo groups; dry eye, in 4.7% of patisiran-LNP and 2.6% of placebo groups; vision blurred in 2.7% of patisiran-LNP and 1.3% of placebo groups; and visual acuity reduced in 0.7% of patisiran-LNP and 2.6% of placebo groups.

In the overall pooled analysis, 61 (28.0%) patients reported AEs in the Eye disorders SOC. The type of ocular events reported were consistent with those expected in the general hATTR amyloidosis patient population. Ocular events reported in ≥2% of patients were cataract (5.5%), dry eye (3.7%), and vision blurred (2.3%).

Overall, in both the placebo-controlled study (Study 004) and the pooled patisiran-LNP experience, the type of ocular AEs reported was consistent with those expected in patients with hATTR amyloidosis and was consistent with the age of the patients in the study. ERG results were similar across the treatment groups and studies and were not suggestive of vitamin A deficiency.

In the placebo-controlled Study 004, evaluations of visual acuity, intraocular pressure, visual field deviation, slit lamp biomicroscopy, and dilated indirect ophthalmoscopy were performed. No clinically notable trends were observed in the patisiran-LNP or placebo groups. Mean values of visual acuity, intraocular pressure, and visual field deviation remained stable over the course of the study in both treatment groups. Overall, few patients had clinically significant findings in slit lamp biomicroscopy and dilated indirect ophthalmoscopy results over the course of the study for both treatment groups; of the patients who had clinically significant findings, the findings appeared to be transient. Electroretinography results remained stable over the course of the study for both treatment groups.

Mean intraocular pressure remained stable over the course of the study for both treatment groups, and no patient had a value <5 or >30 mm Hg during the study Mean visual field mean deviation results remained stable over the course of the study for both treatment groups.

Ophthalmology results were similar in the open-label experience, with few and transient abnormal findings observed. Electroretinography was not collected in Study 003. Electroretinography was protocol-mandated in Study 004 but was only collected as clinically indicated in Study 006. Electroretinography results in Study 004 remained normal over the course of the study for both treatment groups. In 2 patients in Study 006 with ERG results, there were no clinically significant findings.

Thyroid

Transthyretin is a minor transporter of thyroxine in humans. In patisiran-LNP nonclinical studies, expected pharmacologic decreases in total thyroxine (up to 50%) were observed. Thus, an evaluation of thyroid related events was conducted.

Overall, the proportion of patients with hypothyroidism and other thyroid disorders was low and consistent with those expected in patients with hATTR amyloidosis. There was no safety concern considered related to treatment with patisiran-LNP.

Thyroid parameters

In Study 004, mean absolute values and mean percent change from baseline for thyroid parameters were relatively consistent and remained proportionate throughout the study in both the patisiran-LNP and placebo groups.

Results for thyroid evaluations in Study 003 and Study 006 were similar to the placebo-controlled experience.

Malignancies

Patisiran-LNP is considered to have a low risk for carcinogenicity in humans based on its mechanism of action. In nonclinical studies, there was no evidence of any carcinogenic or genotoxic potential. As the median age at entry into Study 004 was 62 years of age, a comprehensive search was performed based on the subordinate SMQ of Malignant or unspecified tumors under the SMQ of Malignancies to identify potential malignant neoplasms. Each identified case was medically reviewed to validate the presence of a malignancy based on case details.

In Study 004, a total of 7 patients reported malignancies, 3 (2.0%) in the patisiran-LNP group and 4 (5.2%) in the placebo group; none of these cases were considered related to study treatment by the applicant. Of the 3 patients in the patisiran-LNP group with malignancies, 2 patients with a medical history of malignant skin neoplasms (basal cell carcinoma and atypical fibroxanthoma) had recurrences of their neoplasms. The third patient had a newly diagnosed case of bladder cancer that resolved on treatment.

Of the 4 patients in the placebo group with malignancies, 2 patients had malignant skin neoplasms (basal cell carcinoma and malignant melanoma in situ), and 3 patients had solid organ malignancies (2 colon cancers and 1 prostate cancer). One of the patients with metastatic colorectal cancer had a prior history of Stage 3 colon cancer. This patient died due to metastatic disease. The other patient had a poor prognosis and was receiving palliative chemotherapy.

Overall in the pooled analysis, 10 patients (4.6%) reported malignancies (Table 36). There were 3 patients with malignancies in Study 004 (noted above), 2 patients with malignancies in Study 003, and 8 patients with malignancies in Study 006 of whom 3 had prior malignancies in Study 004.

Table 36 Overall Pooled Experience: Incidence of Malignancies by High Level Term and Preferred Term

	Total (N=218)
High Level Term / Preferred Term	**Patients[a] n (%) / events**

At Least 1 AE of Malignancy	10 (4.6) /17
Bladder neoplasms malignant Bladder cancer Bladder cancer recurrent	1 (0.5) /4 1 (0.5) /2 1 (0.5) /2
Breast and nipple neoplasms malignant Intraductal proliferative breast lesion Invasive ductal breast carcinoma	2 (0.9) /2 1 (0.5) /1 1 (0.5) /1
Endocrine neoplasms malignant and unspecified NEC Thyroid neoplasm.	1 (0.5) /1 1 (0.5) /1
Gastric neoplasms malignant Gastric cancer	1 (0.5) /1 1 (0.5) /1
Hypopharyngeal neoplasms malignant and unspecified Hypopharyngeal cancer.	1 (0.5) /1 1 (0.5) /1
Oesophageal neoplasms malignant Oesophageal carcinoma	1 (0.5) /1 1 (0.5) /1
Skin neoplasms malignant and unspecified (excluding melanoma) Atypical fibroxanthoma Basal cell carcinoma	4 (1.8) /7 1 (0.5) /2 3 (1.4) /5

Note: All AEs are included from the Malignant or unspecified tumors standardized MedDRA query.
Note: If a patient experienced more than 1 event in a given high level term, that patient is counted once for the high level term. If a patient experienced more than 1 event with a given preferred term, that patient is counted only once for that preferred term
[a] *The total number of events for all patients; a patient can be counted more than once if the patient has multiple events.*

Serious adverse event / deaths / other significant events

SAEs

In the placebo-controlled study (Study 004), SAEs occurred in 36.5% of the patisiran-LNP group and 40.3% of the placebo group. Differences in the SAE profile between the patisiran-LNP and placebo groups that occurred with a ≥5 percentage point difference by SOC were infections and infestations (5.4% patisiran-LNP; 11.7% placebo), metabolism and nutrition disorders (2.7% patisiran-LNP; 7.8% placebo) and renal and urinary disorders (patisiran-LNP 0.7%; placebo 6.5%). The proportion of patients with cardiac SAEs was similar between the patisiran-LNP (13.5%) and placebo (13.0%) treatment groups.

In the patisiran-LNP group, SAEs reported in ≥2% of patients included diarrhea (5.4%), atrioventricular block complete, cardiac failure, cardiac failure congestive, pneumonia and orthostatic hypotension (reported in 2.0% each). All other SAEs were reported in <2% of patients.

Diarrhea was reported as an SAE in 8 patients (5.4%) in the patisiran-LNP group and 1 patient (1.3%) in the placebo group. The majority of the diarrhea SAEs (6 events in 5 patients) in the patisiran group occurred at one study site; at this site, the standard of care for diarrhea treatment mandates that patients are hospitalization for the initiation of anti-diarrheal medications, such as sandostatin. All the SAEs of diarrhea resolved in all affected patients in both treatment groups, and did not lead to treatment discontinuation. In

one patient (in the patisiran-LNP group), diarrhea was considered possibly related to study treatment; in all other patients, it was considered unlikely or not related.

In the placebo group, SAEs reported in ≥2% of patients were acute kidney injury and urinary tract infection (both reported in 5.2%); dehydration, pneumonia and vomiting (reported in 3.9% each); cardiac failure, cardiac failure congestive, constipation, hereditary neuropathic amyloidosis, hyponatremia, and pneumonia aspiration (reported in 2.6% each). All other SAEs were reported in <2% of patients.

In the overall pooled patisiran-LNP experience, SAEs occurred in 40.4% of patients. System organ classes with a frequency of SAEs of ≥5% were cardiac disorders (17.0%), gastrointestinal disorders (7.3%), infections and infestations (6.4%), and vascular disorders (5.5%). SAEs reported in ≥2% of patients were diarrhea (4.1%), cardiac amyloidosis (2.3%), cardiac failure (2.3%), and cardiac failure congestive (2.3%).

An additional analysis of all SAEs that resulted in hospitalization and/or death for any cause was performed. A total of 50 patients (33.8%) in the patisiran-LNP group and 30 patients (39%) in the placebo group had at least 1 hospitalization and/or death for any cause, with mean number of events of 1.4 in the patisiran-LNP group and 2.3 in the placebo group. The proportion of patients with 2 or more hospitalizations was lower in the patisiran-LNP group than the placebo group. The rate of hospitalizations and/or death for any cause was lower in the patisiran-LNP group (35.2 per 100 patient-years) than in the placebo group (72.8 per 100 patient-years).

Deaths

In total, there were 21 deaths reported in the patisiran development programme, all of which occurred in hATTR patients participating in the pivotal studies 003, 004 and 006.

None of the 21 deaths was considered related to study treatment by the investigators.

The 21 deaths reported in patisiran-LNP clinical studies were evaluated by an external, independent adjudication committee to classify whether the deaths were of cardiovascular (CV), non-cardiovascular (non-CV), or unknown origin.

The majority of deaths were cardiovascular in nature and consistent with those expected in this patient population. As further described below, the overall mortality observed in patisiran-treated patients was at the low-end of the range reported in literature for hATTR patients, including those with less severe disease. The majority of patients in both treatment groups also had known risk factors for poor prognosis, which can include increasing age, history or diagnosis of cardiomyopathy, non-Val30Met genotype (eg, Thr60Ala, Val122Ile, Glu89Gln), Val30Met genotype with late onset disease (>50 years), increased N-terminal pro b-type natriuretic peptide (NT-pro-BNP) levels, increased interventricular septum (IVS) thickness, poor diastolic function, as well as increased left ventricular posterior wall thickness and left atrial diameter, among others.

Table 37 Summary of all deaths in Patisiran-LNO clinical trials

Patient Number Genotype	Cardiac Sub- group	Serious Adverse Event reported as Fatal	Adjudication (Subgroup)[a]	Study day(s) to Last dose of Study Drug	Study day (s) to Date of Death	Relevant Current and Historical Medical Condition

Study 004: Placebo-Controlled Study						
Patisiran-LNP (n=7; 4.7%)						
Ala97Ser	Yes	Cardiac arrest, Cardiac failure congestive	CV (heart failure)	191	194	004 Baseline: FAP stage I, PND score II; NYHA class II, NT-proBNP 647.35 pmol/L, MLVWT 2.32cm Med Hx: congestive cardiac failure, cardiac amyloidosis, diabetes mellitus, atrial flutter, atrioventricular block Worsening CHF. Autopsy: Death due to complications of systemic TTR amyloidosis with extensive cardiac involvement.
Thr60Ala	Yes	Sudden cardiac death	CV (presumed sudden death)	64	169[b]	004 Baseline: FAP stage II, PND score IIIA; NYHA class I, NT-proBNP 876.15 pmol/L, MLVWT 2.08cm Med Hx: congestive cardiac failure, atrial fibrillation, atrial flutter, cardiac ablation, first degree atrioventricular block, cardiac amyloidosis, chronic kidney disease Patient with course complicated by heel ulcer, osteomyelitis, vancomycin toxicity resulting in acute kidney injury, CHF exacerbation, UTI, CVA, acute heart failure.

Thr60Ala	Yes	Sudden cardiac death	CV (sudden death)	378	381	004 Baseline: FAP stage II, PND score II; NYHA class II, NT-proBNP 326.03 pmol/L, MLVWT 2.06cm Med. Hx: cardiomyopathy, congestive cardiac failure, mitral valve disease, atrial fibrillation, atrial flutter Patient had shortness of breath while climbing stairs and then collapsed. Sudden death with amyloidosis, restrictive cardiomyopathy, atrial flutter, malnutrition and anemia as contributory conditions.
Ser50Arg	Yes	Cardiac failure, acute pulmonary edema	CV (sudden death)	356	378	004 Baseline: FAP stage II, PND score II; NYHA class II, NT-proBNP 503.39 pmol/L, MLVWT 1.87cm Med Hx: cardiomyopathy, cardiac failure Patient with 2 episodes respiratory arrest and cardiopulmonary resuscitation on same day. Cause of death listed as acute pulmonary edema secondary to heart failure and amyloidosis.
Ser77Phe	No	Cardiac arrest	CV (presumed CV)	547	565	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, NT-proBNP 1168.55 pmol/L, MLVWT 1.67cm Med Hx: atrial fibrillation, atrial flutter, hypertension, right bundle branch block During prolonged hospitalization for infected decubital ulcers, patient had cardiac arrest.

Glu89Gln	Yes	Pulseless electrical activity	CV (sudden death)	169	172	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 482.86 pmol/L, MLVWT 2.33cm Med Hx: cardiomyopathy, palpitations, left bundle branch block Prolonged episode of chest pain and palpitations at home; developed difficulty breathing and pulselessness. Resuscitation unsuccessful.
Glu89Gln	No	Cardiac failure	CV (heart failure)	526	529	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, MLVWT: 1.88cm; Med Hx: cardiac insufficiency, paroxysmal atrial fibrillation; hypotension, obstructive pulmonary disease, metabolic syndrome-hypercholesterolemia, obesity Worsening of cardiac insufficiency.
<u>Placebo (n=6; 7.8%)</u>						
V30 M	No	Subarachnoid hemorrhage	CV (fatal stroke, hemorrhagic)	547	558	004 Baseline: FAP stage II, PND score IIIA; NYHA class I, NT-proBNP 11.56 pmol/L, MLVWT 1.21cm Med Hx: ischemic stroke, right carotid stenosis, right retinal embolism, reactive hypertension, tobacco abuse Patient with sudden fall and cardio-respiratory arrest. Computed tomography of head showed subarachnoid hemorrhage

V30 M	Yes	Staphylococcal sepsis	Non-CV	380	407	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, NT-proBNP 416.42 pmol/L, MLVWT 1.98cm Med Hx: restrictive cardiomyopathy, congestive heart failure, pacemaker, hypotension Patient with cardiac arrest, and course complicated by cardiac failure, acute on chronic kidney injury, bacterial pneumonia, methicillin susceptible staphylococcus aureas septicemia and UTI. Multiple vegetations and erosions of cardiac valves consistent with staphylococcal endocarditis.
Thr59Lys	Yes	Anemia, gastrointestinal hemorrhage	CV (heart failure)	338	422[b]	004 Baseline: FAP stage II, PND score IIIB; NYHA class I, NT-proBNP 600.74 pmol/L, MLVWT 1.63cm Med. Hx: restrictive cardiomyopathy, congestive heart failure, atrial fibrillation, pulmonary fibrosis, orthostatic hypotension, scleroderma, hypothyroidism Several hospitalizations for CHF exacerbations during study. Recent worsening of cardiac symptoms. Developed melena with anemia. Received transfusion 1 PRBC. Based on overall poor health and prognosis, transferred to palliative care.

Ser52Pro	Yes	Acute kidney failure, urinary tract infection, bacteremia	Unknown	274	298	004 Baseline: FAP stage II, PND score IIIA; NYHA class I, NT-proBNP 69.38 pmol/L, MLVWT 1.32cm Med Hx: peripheral sensorimotor neuropathy UTI, acute renal failure (oliguria and anuria), and *E. coli* sepsis.
Ala97Ser	Yes	Colorectal cancer metastatic	Non-CV	379	558[b]	004 Baseline: FAP stage II, PND score IIIB; NYHA class I, MLVWT 1.48cm Med Hx: colectomy for Stage 3 colon cancer in (767 days before start in the study) Metastatic recurrence of colorectal cancer
Glu89Gln	No	Ischemic stroke	CV (fatal stroke, ischemic)	108	134	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 703.52 pmol/L, MLVWT 1.67 cm Med Hx: hypertension, hypotension, cardiomyopathy, atrial fibrillation, atrial flutter Ischemic stroke complicated by exacerbation of CHF, and pneumonia.
Study 003: Open-Label Extension Study						

Val30Met	No	Esophageal carcinoma	Non-CV	568	656	003 Baseline: FAP stage I, PND score I; NHYA class I Med Hx: Atrioventricular block first degree, left bundle branch block, upper abdominal pain, irritable bowel syndrome, ankylosing spondylitis, asthma. Developed stomach pain, worsening constipation, swallowing difficulties. Endoscopy showed tumor at gastroesophageal junction. Computed tomography showed metastases.
Val30Met	Yes	Myocardial infarction	CV (fatal MI)	735	757	003 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 1758 ng/L, MLVWT 1.74 cm Med Hx: atrial fibrillation, hypertension, chronic renal disease, hx prostate cancer Had ECG changes and positive cardiac enzymes consistent with MI. Not a candidate for stent. Palliative care.
Study 006: Global Open-Label Extension Study						
004 Placebo/006 Patisiran-LNP Group						

Val30Met	No	Cardiac Arrest	CV (sudden death)	64	82/649[c]	004 Baseline: FAP stage II, PND score IIIa; NYHA class II, NT-proBNP 69.50 pmol/L, MLVWT 2.04 cm 006 Baseline: FAP stage III, PND score IV; NYHA class II, NT-proBNP 360.84 pmol/L, MLVWT 1.94 cm Med Hx: AV block, restrictive cardiomyopathy, oropharyngeal squamous cell carcinoma, with dysphagia and weight loss Cardiac arrest: Patient found without vital signs, no treatment or resuscitation administered, no autopsy performed.
Val30Met	Yes	Cardiac Arrest	CV (presumed CV death)	106	125/706[c]	004 Baseline: FAP stage II, PND score II; NYHA class I, NT-proBNP 438.84 pmol/L, MLVWT 1.59 cm 006 Baseline: FAP stage II, PND score IIIB; NYHA class I, NT-proBNP 256.41 pmol/L, MLVWT 1.51 cm Med Hx: heart failure, atrial fibrillation, pacemaker placement, chronic deep vein thrombosis, acute myelogenous leukemia Cardiac arrest: pulseless event in setting of multiple fentanyl IV injections (2 doses of 50 µg followed by 1 dose of 100 µg) for pain control of acute hip fracture
Ile84Thr	No	Acute Respiratory Distress Syndrome Hemorrhagic Shock	Non- CV	106	111/678[c]	004 Baseline: FAP stage I, PND score II; NYHA class I, NT-proBNP 107.62 pmol/L, MLVWT 1.11 cm 006 Baseline: FAP stage II, PND score II; NYHA

						class I, NT-proBNP 157.65 pmol/L, MLVWT 1.23 cm Med Hx: cardiac amyloidosis, heart transplant, hypertension, pulmonary edema, hypothyroidism, UTI, cyclosporine therapy, ARDS (parainfluenza), hemorrhagic shock Hemorrhagic shock was a fatal complication of ARDS: No autopsy performed, death certificate listed bleeding due to coagulopathy and respiratory failure due to parainfluenza as direct causes of death.
Ala97Ser	Yes	Cardiogenic Shock	CV (presumed CV death)	22	39/605 [c]	004 Baseline: FAP stage II, PND score IIIB; NYHA class II, NT-proBNP 102.90 pmol/L, MLVWT 1.52 cm 006 Baseline: FAP stage III, PND score IV; NYHA class III, NT-proBNP 211.69 pmol/L, MLVWT 1.37 cm Med Hx: cardiac failure, limbs edema, AV block-1, left bundle branch block, sensorimotor polyneuropathy, autonomic dysfunction Cardiogenic shock: Death occurred in a context of food aspiration, unknown if an autopsy was performed, death certificate stated cause was of natural origin and complication of amyloidosis
004 Patisiran-LNP/006 Patisiran-LNP Group						

Thr60Ala	Yes	Invasive ductal breast carcinoma	Non-CV	19/590	262/832[b, c]	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 282.73 pmol/L, MLVWT 1.98 cm 006 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 526.99 pmol/L, MLVWT 1.69 cm Med Hx: atrial fibrillation; cardiac ablation, family history of breast cancer In Study 004, an undifferentiated right breast mass was identified. In Study 006, the diagnosis of invasive ductal breast carcinoma was confirmed. Patient withdrew from study and died 6.5 months after withdrawal from study
Thr60Ala	Yes	Worsening Amyloidosis	CV (heart failure)	149/737	158/746[c]	004 Baseline: FAP stage II, PND score IIIA; NYHA class II, NT-proBNP 931.61 pmol/L; MLVWT 1.86 cm 006 Baseline: FAP stage III, PND score IV; NYHA class IV, NT-proBNP 726.64 pmol/L; MLVWT 1.66 cm Med Hx: hypothyroidism, QT prolongation Amyloidosis complication: death due to cardiopulmonary insufficiency, no autopsy completed; death certificate listed immediate cause of death as amyloidosis.

Abbreviations: ARDS=Adult respiratory distress syndrome; CHF=congestive heart failure; CV=Cardiovascular, ECG=electrocardiogram; F=female,
FAP=familial amyloidosis polyneuropathy; M=male, Med Hx=medical history, MI=myocardial infarction; MLVWT=mean left ventricular wall thickness, NTproBNP=
B-type natriuretic peptide, NYHA=New York Heart Association, PND= polyneuropathy disability score; UTI=urinary tract infection.
aDefinitions of classifications and sub-classifications are provided in the adjudication charter in CSR ALN-TTR02-004, Section 16.1.9.3.
b Patient off treatment for more than 30 days.
c Study day in Study 006/Study day for overall observation time from baseline in Study 004

Laboratory findings

Haematology

The non-clinical and clinical experience so far with therapeutic oligonucleotides (mainly of the ASO (DNA-based) class) has shown that these may affect haematology values due to immune stimulation.

In Study 004, in the patisiran-LNP and placebo groups, mean hematology values for red blood cell parameters (including mean hemoglobin, hematocrit, corpuscular hemoglobin [MCH], corpuscular hemoglobin concentration, and corpuscular volume [MCV]) were generally stable and throughout the course of the study.

Mean platelet parameters (including platelet counts and mean platelet volume) were also stable in both the patisiran-LNP and placebo groups throughout the course of the study. No patients had platelet counts <50 $x10^9$/L (CTCAE Grade 3). One patient in the patisiran-LNP group had a transient mild AE of thrombocytopenia. In the placebo group, 1 patient had a transient moderate AE of thrombocytopenia and 1 patient had a mild AE of platelet count decreased (intermittent, not recovered/resolved).

Certain other laboratory tests—namely, WBC, eosinophil, basophil, lymphocyte, monocyte, and neutrophil counts were expected to show the effects of corticosteroids administered as premedication approximately 12 hours before baseline laboratory sample collection.

Overall, as anticipated, there was some effect of corticosteroid administration on mean values for the various WBC parameters such as lymphocytes, monocytes, neutrophils, and WBCs. At the Day 252 study visit, absolute mean lymphocyte, basophils, eosinophils and monocytes counts showed a decreased from baseline. At the Day 546 study visit, the corticosteroid effect was less pronounced, and values were trending back to baseline at the end of the study. The inverse pattern was seen with neutrophils and WBC, which showed some increase from baseline at the Day 252 study visit, and trended back to baseline at the end of the study. As all patients received premedication, a similar pattern was seen in the placebo group. Beyond the effect of corticosteroids, the treatment with patisiran-LNP did not appear to have an additional effect on hematology values, as the values were similar to the placebo group.

Results for hematology evaluations in Study 003 and Study 006 were similar to the placebo-controlled experience. In Study 003, 2 patients had hemoglobin values of <100 g/L: 1 patient with a transient Grade 4 decrease and 1 patient with a Grade 2 decrease. A total of 5 patients had at least 1 lymphocyte count <0.5 $\times 10^9$/L (Grade 3). The Grade 3 shifts in lymphocyte counts were transient and consistent with glucocorticoid administration.

In Study 006, a total of 7 patients had at least 1 hemoglobin value of <100 g/L, all were Grade 1 or 2 except for 1 patient in the 004 patisiran-LNP group had a Grade 3 value in hemoglobin. The patient had a decreased hemoglobin value of 79 g/L (Grade 3) at baseline in Study 006. The patient's hemoglobin was stable and increased during Study 006 with a value of 89 g/L at Week 52. The low hemoglobin was not considered an AE and was part of the patient's medical history; in Study 004, the patient had an ongoing AE of worsening anemia that started on Day 71 that was considered not related to study drug. All values for 7 patients were either transient or stable with respect to the patients' baselines values.

In Study 006, a total of 7 patients had at least 1 post-baseline lymphocyte count of <0.8 × 10^9/L (Grade 2), of which 1 patient in the 003 patisiran-LNP group had a single lymphocyte count of <0.5 × 10^9/L (Grade 3). In general, these Grade 2 and Grade 3 lymphocyte counts were transient and consistent with the timing of

administration of corticosteroids There was 1 patient in the 004 placebo group with a single neutrophil count $<1.5 \times 10^9$/L (Grade 2) and WBC count $<3.0 \times 10^9$/L (Grade 2) at Week 12. There was 1 patient in the 004 placebo group who had a single platelet count $<75 \times 10^9$/L (Grade 2).

Coagulation parameters

The non-clinical and clinical experience so far with therapeutic oligonucleotides (mainly of the ASO (DNA-based) class) has shown that these may lead to inhibition of the coagulation cascade.

In Study 004, mean coagulation parameters appeared to be stable over the course of the study and generally similar for both the patisiran-LNP and placebo groups. There were no Grade 3 or Grade 4 shifts in activated partial thromboplastin time (aPTT) or prothrombin time during the study.

Similar results for coagulation parameters were observed in Study 003 and Study 006. In Study 003, 2 patients had clinically significant elevated coagulation parameter levels (1 patient had clinically significant aPTT and prothrombin International Normalized Ratio (INR) levels and 1 patient had clinically significant prothrombin time, aPTT, and INR levels). In Study 006, no patients had prothrombin time values that were Grade 3 or 4.

Complement

“Class” or platform-related effects such as activation of C’ alternative complement pathway and immune stimulation have been described for other therapeutic oligonucleotides (mainly of the ASO (DNA-based) class).

Complement factors were analysed in the single-dose dose-escalation study in healthy volunteers (study 001) and in the open-label dose-escalation study in patients with TTR amyloidosis (study 002), in which each patient received two doses 4 or 3 weeks apart.

Transient increases (up to 7-fold) in complement factor Bb were observed from 30 min to 2 h post-dose from the 50 µg/kg dose, and had returned to baseline within 24 h. There were no associated clinical signs or AEs.

Cytokines and CRP

Cytokines were analysed in the single-dose dose-escalation study in healthy volunteers (study 001) and in the open-label dose-escalation study in patients with TTR amyloidosis (study 002), in which each patient received two doses 4 or 3 weeks apart.

No substantial changes were observed in any dose groups for the following cytokines: G-CSF, IFN-α, IFN-γ, IL-1b, and IL-12. Minor transient increases in mean IL-6, IL-1RA, IP-10, and TNF-α levels were seen after the second dose for some dose groups. Of note, most time points, cytokine values were below the lower limit of detection for most patients.

In study 001, mean CRP remained relatively constant through 6 hours post infusion. Twenty-four hours after the infusion, slight increases were observed in the 3 highest doses of ALN-TTR02 (150, 300, and 500 µg/kg); placebo and the 10 and 50 µg/kg cohorts remained constant throughout the study.

In study 002, notable changes from baseline in CRP levels included a 14.1-fold increase seen 24 hours after the second infusion (Day 28) in the 300 µg/kg (Q4W) dose group and a 10.2-fold increase seen 24 hours after the second infusion (Day 21) in the 300 µg/kg (Q3W) dose group. The changes in CRP were transient and not associated with TEAEs. No clear dose-related changes in CRP were observed. The increases in CRP are of unclear clinical significance.

Cytokines were neither analysed in the pivotal study 004, nor in the extension studies 003 and 006.

Immunogenicity

An anti-PEG antibody assay specific to the PEG2000-C-DMG surface component of the patisiran-LNP drug product was used for the assessment of immunogenicity in human serum. The validated cut-points were established based on serum from a healthy donor population rather from patisiran-LNP patient specific sera.

The incidence of treatment-emergent ADA was 3.4% (5 of 148 patients) in the patisiran-LNP group and 1.30% (1 of 77 patients) in the placebo group. In the 5 patients with ADA in the patisiran-LNP group, ADA titers were low and ranged from 40 to 80. In the placebo group, ADA titers ranged from 40 to 160. In general, ADA was transient with all patients in both treatment groups testing negative for ADA after Week 36 (Day 252). In the patisiran-LNP group, the patients with positive ADA status had a similar pattern of AEs as that observed in the overall population. There is no evidence to suggest an association between ADA and IRRs, anaphylactic reactions, or hypersensitivity events. Overall, a positive ADA status did not appear to affect the safety profiles of patisiran-LNP in Study 004.

In Study 003, 1 of 27 patients treated with patisiran-LNP had a positive ADA result. This patient tested positive for ADA at baseline and at several sampling time points up to Week 13. Baseline ADA positivity was likely to due to exposure to patisiran-LNP in the parent Study ALN-TTR02-002. There was no impact of ADA on the PK, PD, or safety profiles of patisiran-LNP.

None of the patients in Study 006 had a confirmed positive ADA result during the study.

Safety in special populations

The cardiac subgroup

The proportions of patients experiencing AEs and SAEs in the cardiac subpopulation were similar to those in the study population as a whole. In the cardiac subpopulation, the proportion of patients with AEs was similar between the patisiran-LNP (95.6%) and placebo (97.2%) groups, and the proportion of patients with SAEs was lower in the patisiran-LNP group (34.4%) group compared with the placebo (50.0%) group.

In study 004, 5 of the 90 (5.6%) patients in the patisiran-LNP cardiac subpopulation group and 4 of the 36 (11.1%) patients in the placebo cardiac subpopulation group died.

In the overall pooled experience, a total of 10 of 124 patients (8.1%) in the cardiac subpopulation died over a cumulative period of observation of 220.8 patient-years.

Age, sex, weight, race, genotype (V30M or non-V30M mutation), FAP stage

No clinically meaningful differences.

Use in Pregnancy and Lactation

Pregnant or lactating women were excluded from participation in all clinical studies, and women of childbearing potential were required to use acceptable methods of birth control throughout the study. As of the data cutoff date for Study 006of 14 July 2017, there have been no reported pregnancies in the patisiran-LNP clinical development program. Thus, the effect of patisiran LNP on pregnancy and lactation in humans is unknown.

Expanded access program in the US

An open-label EAP, under Protocol ALN-TTR02-007 [Study 007]) is currently ongoing in the United States; it is designed to allow patients with hATTR amyloidosis to receive patisiran-LNP. Patients in Study 007 are receiving patisiran-LNP at the dose and regimen proposed in labeling, including the proposed premedication regimen.

As of the data cutoff date of 14 July 2017, 48 patients were treated in Study 007. The mean age of patients at screening was 63.8 years (range 41-81 years), 81.3% were male, and 97.9% were White/Caucasian. Patients with at least 16 different TTR mutations have been enrolled, with the majority having non-V30M genotypes (79.2%). Baseline PND scores ranged from 0 to IIIB, with approximately half being earlier stages (PND score 0 [2.1%], I [41.7%], II [12.5%]) and half being later stages (PND score IIIA [25.0%] and IIIB [18.8%]).

The mean duration of patisiran-LNP exposure was 87.3 days (range, 21 to 191). The mean number of doses received was 4.1 (range, 1 to 9), with 19 patients (39.6%), receiving at least 3 to 6 months of patisiran-LNP dosing. Cumulative study drug exposure was 11.5 patient-years.

The proportion of patients with AEs was 43.8%. Adverse events that were reported in ≥5% of patients were restless legs syndrome (8.3%; 4 patients) and headache (6.3%, 3 patients).

The total of 6 (10.4%) of patients reported IRRs (reported as an IRR or as individual symptoms) Symptoms included facial oedema; dysphonia; flushing; pain in the back, neck, shoulder, hip, or body; fatigue; and peripheral edema. All were mild or moderate in severity except for one case with facial swelling and hoarse voice. Two patients had IRRs that led to discontinuation of patisiran-LNP (1 prior to the data cutoff and 1 after).

No deaths have been reported in Study 007. Six patients experienced SAEs. There were no SAEs by PT that were reported in more than one patient. Serious AEs in 2 of the patients with SAEs were assessed by the Investigators as possibly related to study drug (the IRR with facial swelling and a hoarse voice and .an SAE of small intestinal obstruction.

In addition, 2 patients had malignancies (malignant melanoma in situ and metastatic pancreatic carcinoma with mutation in the adenomatous polyposis coli (APC) gene) that were categorized as SAEs.

Overall, the open-label data available to date in EAP Study 007 suggest a consistent safety profile with that observed in the clinical studies.

Discontinuation due to adverse events

Discontinuation of study drug or withdrawal from study

In Study 004, a total of 7 (4.7%) patients in the patisiran-LNP group and 11 patients (14.3%) in the placebo group reported AEs that led to treatment discontinuation. AEs leading to treatment discontinuation reported in 2 or more patients were cardiac failure (2 patients, 1.4%) in the patisiran-LNP group and acute kidney injury (2 patients, 2.6%) in the placebo group. In both treatment groups, all other AEs leading to treatment discontinuation were reported in 1 patient each.

All patients who discontinued study treatment due to an AE also withdrew from the study.

A total of 13 (6.0%) patients across the overall pooled experience reported an AE leading to discontinuation of patisiran-LNP and study withdrawal. The only AE leading to discontinuation of patisiran-LNP and study withdrawal reported in 2 or more patients was cardiac failure (2 patients, 0.9%) as noted above in Study 004. All other AEs leading to treatment discontinuation were reported in 1 patient by preferred term.

2.8.1. Discussion on clinical safety

Based on the mechanism of action, i.e. reduction of both mutated and wild-type TTR produced by the liver, treatment with patisiran may lead to reduced levels of vitamin A, which may result in night blindness and retinal dystrophy. In addition, TTR can also (in addition to thyroid binding globulin) serve as a carrier for thyroxine (T4), thus thyroid hormone levels were analysed in the development programme.
In general, the non-clinical and clinical experience so far with therapeutic oligonucleotides (mainly of the ASO (DNA-based) class) has shown that these may lead to adverse effects related to immune stimulation, activation of C' alternative pathway and inhibition of the coagulation cascade, as well as tissue accumulation in the liver, kidneys, spleen and lymph nodes.
Patisiran is encapsulated in a lipid nanoparticle (LPN) in the circulation. Non-clinical studies have shown that the toxicity primarily is related to the liposomal formulation and not to the siRNA itself.
The safety database includes 218 patients with hATTR amyloidosis with polyneuropathy exposed to patisiran-LNP. 179 patients have been dosed for at least 12 months and 101 for at least 24 months. In addition, 48 patients have been exposed in an expanded access program in the US. Overall, the safety database is small, but taking into consideration the rarity of the disease, it is considered acceptable.
The studies that were selected for the integrated safety analysis used the intended therapeutic dose, dosing interval, and infusion rate. In addition, all patients, including those randomised to placebo, received the same infusion premedication. Two different premedication regimens were applied during the clinical studies, the original (premedication 12 h and 60 min prior to infusion) and the reduced premedication (only 60 min prior to infusion) regimens. The reduced premedication regimen is proposed to be used in clinical practice.
In the clinical studies, patients were instructed to take an oral supplemental dose of 2,500 IU per day of vitamin A (corresponding to approximately 833 retinol equivalents (RE) per day). This recommendation is appropriately reflected in the SmPC section 4.2 and a statement on the recommended dose of 2,500 IU of Vit A is provided.

AEs
The overall proportion of patients experiencing AEs was similar in the patisiran (96.6%) and the placebo (97.4%) groups.
The ADRs for the tabulated list of ADRs in SmPC 4.8 were selected based on the following three criteria:

- either TEAEs + relatedness + more common in patisiran group
- **OR** related to the mechanism of action
- **OR** at least 3 percentage point higher frequency in patisiran than placebo.

IRRs fulfilled all these criteria. All other listed ADRs were based on criterion c. above, i.e. at least 3 percentage points higher frequency in the patisiran group. There were a number of potential ADRs (asthenia, dizziness, UTI) that were dismissed due to lower frequency in the patisiran group. Erythema was related AEs with a 2 percentage point difference and overall AEs with a 4.2 percentage point difference and was also included.
Upper respiratory tract infections were reported with more than 3 percentage points higher frequency and thus were included in spite of no obvious clinical or pharmacological reason explaining why patisiran treatment would lead to these infections.

In contrast, there were some AEs with a higher frequency in the placebo group compared to the patisiran group (fall, nausea, pain in extremity, muscular weakness, urinary tract infection, syncope, anaemia), that probably reflected the higher disease activity in these patients.
Since the non-clinical and clinical experience so far with therapeutic oligonucleotides (mainly of the ASO (DNA-based) class) has shown that these may lead to class and platform related adverse effects which include immune stimulation, siRNA formulated as a lipid nanoparticle is directed to the liver, the heart and kidneys are affected by hATTR amyloidosis and the reduction of serum TTR leads to a risk for thyroid-related AEs through effects on retinol (vitamin A) and TTR is a minor transporter of thyroxine, the following AEs are considered AEs of special interest for patisiran treatment: IRR, liver, cardiac, renal, ocular and thyroid.

Infusion-related reactions and premedication

Infusion-related reactions were more common in the patisiran (18.9%) than in the placebo (9.1%) group. All IRRs were mild to moderate and resolved. In addition the IRRs are considered as an identified risk and reflected accordingly in the RMP.
Flushing occurred in both groups and was the most common IRR in the placebo group. This was considered probably related to the use of premedication.
Events potentially related to extravasation have been reported in 0.3% of infusions and symptoms consist of phlebitis, localized pain, burning sensation, swelling, tenderness, or redness at or near the infusion site.
In the patisiran group, amongst the patients reporting IRRs, the majority (78.6%) had their first IRR reported during the first 2 dose administrations and 82.1% by the third dose. In the open-label extension study 006, home infusions were permitted for patients in certain regions after the dose was administered in the clinic at least 3 times with no IRRs or other AEs observed. Home administration of premedication and infusion has been successfully performed in 25 patients and a cumulative total of 269 doses of patisiran-LNP in the open label extension study 006. In order to be a candidate for home infusion in Study 006, patients had to tolerate 3 infusions in the clinic without any infusion-related reactions (IRRs) or other adverse effects and be considered suitable for home infusion by their treating physician (based on the physician's clinical judgment). All infusions were administered in full without dose interruptions due to an IRR or a complication with premedication. Of the 25 patients, 24 patients have had no IRRs reported during the home infusions. One patient had mild symptoms (primarily flushing) that did not require any specific medical treatment. It is considered that home infusions are feasible when given by health care personnel who have received appropriate instructions. The instructions received in the study were similar to those included in the proposed SmPC. Section 4.2 of the SmPC recommends that the patient should have tolerated at least 3 infusions in the clinic before they can receive home infusions. This was considered acceptable by the CHMP.
While infusion reactions can produce signs that are observable, patients may experience subjective symptoms that are not immediately observable by a health care professional (HCP), for example dizziness, chest pain, nausea or back pain. In addition, in the event of an infusion reaction occurring in a geographically remote location, it will be important for it to be recognised and managed swiftly. Patients with hATTR amyloidosis may be frail, and they are notably also often elderly. Therefore, the CHMP considered that it would be helpful and prudent for patients to have immediately to hand a concise, focussed educational material detailing signs and symptoms that may indicate an infusion reaction is occurring and the advice to inform the HCP immediately should one occur, in order to mitigate any delay in implementing management. This would facilitate safe, and sustainable, home infusions in the post-marketing setting, which would benefit patients.

AEs deemed related to the premedication by the investigators were mainly related to corticosteroid treatment

and these AEs appeared to decrease in frequency once the reduced premedication regimen was introduced in the clinical trials. The applicant presented data suggesting that corticosteroid-related AEs didn't increase over time during long-term treatment with patisiran. It was noted that the reduced premedication included 10 mg dexamethasone every 3 weeks which raised the concern that such premedication regimen may lead to adverse effects related to long-term corticosteroid treatment. Long-term adverse effects of repeated use of high-dose corticosteroids as premedication may be a safety concern in patients with manifest diabetes or osteoporosis. Therefore the applicant proposed to decrease corticosteroid posology in patients to a minimal dose of 5 mg of intravenous dexamethasone or equivalent for patients who well tolerated their patisiran infusions. This risk minimisation measure was welcomed by the CHMP and it was considered that it alleviates the long term safety concerns related to long-term use of corticosteroids.

Vitamin A

Serum TTR is a carrier of retinol binding protein, which facilitates transport of vitamin A in the blood. Treatment with patisiran reduces serum TTR levels, which results in reduced levels of retinol binding protein and vitamin A in the serum. However, the transport and tissue uptake of vitamin A can occur through alternative mechanisms in the absence of retinol binding protein. As a result, laboratory tests for serum vitamin A do not reflect the total amount of vitamin A in the body and should not be used to guide vitamin A supplementation during treatment with patisiran. No negative findings, such as ocular toxicity, related to lower vitamin A levels have been observed in the clinical trials. However, a warning about the ocular signs and symptoms of vitamin A deficiency and recommendation for referral for ophthalmological assessment if such symptoms occur have been added to section 4.4 of the SmPC.

The liver

The liver is an organ of interest for safety reasons, since patisiran-LNP specifically targets the liver and nonclinical studies of patisiran-LNP revealed changes in serum liver markers and liver histopathology. In the MAD study (study 002), a transient increase in AST and ALT was seen during the first 14 days only in dose group 300 µg/kg Q3W, peaking at day 7, which normalised by day 14. This observation indicates that liver transaminases are affected acutely by patisiran-LNP treatment in a dose-dependent manner. In study 004, there was a trend for slightly elevated ALT (26.4% vs 13.0% >ULN and ≤3xULN), AST (20.9% vs 5.2% >ULN and ≤3xULN) and total bilirubin (6.1% vs 2.6%, >ULN and ≤1.5xULN) in the patisiran group vs the placebo group in the pivotal study 004. There were no Hy's Law cases observed (AST or ALT >3 × ULN and total bilirubin >2 × ULN). A small initial increase from baseline in AST and ALT compared to placebo was observed in study 004, but this increase remained stable over the 18 months of the placebo-controlled study. The applicant presented data showing that no clinically meaningful changes in liver function tests appear to occur with patisiran-LNP treatment relative to placebo for up to 18 months of treatment (study 004) and ALT and AST levels continued to remain stable in the open label study (study 006) for up to 49.5 months. No changes in APL or total bilirubin were observed. In the open label study 003, the percentage of increases in AST or ALT (>ULN and <3x ULN) during patisiran-LNP treatment was very similar to that in study 004 (33.3% vs 31.8%). Therefore the CHMP considered that no monitoring of liver function is warranted during treatment with patisiran-LNP. In addition the monitoring of hepatic events would be warranted during the ongoing open label study (006) and the planned prospective observational cohort study.

The heart

In hATTR, amyloid is accumulated in the heart tissue, leading to arrhythmias and congestive heart failure. Thus, cardiac AEs were expected in this patient population. The proportion of patients with AEs (28.4% patisiran; 36.4% placebo) and SAEs (13.5% patisiran; 13.0% placebo) within the Cardiac disorders SOC was

similar across the 2 treatment groups. Efficacy data indicate that treatment with patisiran inhibits the amyloidosis-related increase in left ventricular wall thickness and disease-related increase in NT-proBNP values. The cardiac safety data points in the same direction:
- The rate of hospitalizations or deaths in the Cardiac SOC was lower in the patisiran group compared to placebo (9.6/100 PYs for patisiran vs 15.6/100 PYs for placebo)
- A lower proportion of patients who had cardiac arrhythmias in the patisiran group (18.9% vs 28.6% in the placebo group).

The kidneys

hATTR disease-related amyloid accumulation in the kidneys lead to proteinuria and ultimately to renal failure. In contrast to ASOs, siRNA is not distributed to the kidneys and 90% of the patisiran-LNP dose is observed in the liver post-dose according to findings in non-clinical studies. In non-clinical toxicology studies, no findings related to the kidney related toxicity were observed.
The frequency of renal events observed in the clinical studies was lower in the patisiran-treated patients compared to placebo-treated patients and the types of events were consistent with those expected in patients with hATTR amyloidosis. In study 004, creatinine, BUN and estimated GFR remained stable over the entire 18 months treatment period.

The eyes

Eye disorders in patients with hATTR are known. Hence around half of patients, in both groups in study 004, had ocular diseases reported in their medical history. However, TTR reduction during patisiran treatment can equally lead to reduction of circulating serum level of retinol binding protein and vitamin A.
In the placebo-controlled study, no difference between the treatment arms was observed in patients reporting at least an AE in SOC eye disorders. Analysis per preferred term (PT) showed that a higher percentage of patients in the patisiran group reported AEs such as dry eye (4.7% vs 2.6%) and vision blurred (2.7% vs 1.3%).
No safety signal related to visual disorders, including visual impairment, visual acuity reduced or vision blurred, was observed in patients exposed to patisiran in clinical trials which could be attributed to vitamin A deficiency. However, as 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 referral for ophthalmological assessment if such symptoms occur have been included in SmPC section 4.4. In addition the CHMP considered that the warning should also give examples of ocular symptoms suggestive of vitamin A deficiency such as: reduced night vision or night blindness, persistent dry eyes, eye inflammation, corneal inflammation or ulceration, corneal thickening, corneal perforation.
Of note, cataract nuclear, eye irritation, keratitis and visual impairment were reported in two patients both in the patisiran arm whereas there is no patient reported these events in the placebo group. Three SAEs in SOC Eye disorders were reported in patients treated with patisiran, all in study 004: including maculopathy, vitreous hemorrhage, and vitreous opacities.
Ophthalmology testing results remained stable in both treatment groups over the course of the study, indicating that ocular disease-related progression is slow. However, the probability that patisiran may halter the disease-related visual impairment due to accumulation of amyloid in the vitreous body of the eye is considered low, since patisiran mainly targets the TTR production of the liver and the retina synthesises its own TTR.

Thyroid parameters

TTR is a minor transporter of thyroxine (T4). In patisiran nonclinical studies decreases in total T4 up to 50% were noted. However, in the clinical programme, no changes in thyroid parameters or thyroid disorders were observed.

Malignancies

The mechanism of action of patisiran doesn't suggest the risk for an increase in malignancies. In the nonclinical studies, there was no evidence of any carcinogenic or genotoxic potential. The types and rates of malignancies observed were consistent with the ones reported in the general population of the same age as included in the studies and malignancies were not more common in the patisiran arm compared to placebo.

SAEs including deaths

Frequencies of SAEs were comparable between patisiran- and placebo-treated patients. Most SAEs were events to be expected in the patient population due to the background disease. A few SAEs in the patisiran group (post-infusion cellulitis/superficial thrombophlebitis, dermatitis, procedure extravasation) were related to extravasation of the drug.

The prognosis and mortality risk in hATTR is related to the genetic mutation: among the three most common genetic variants, V30M mutation has less cardiac involvement and a better prognosis, whereas V122I and T60A mutations have a worse prognosis (Adelaide et al. (2013) Genotype, echocardiography, and survival in familial transthyretin amyloidosis. Amyloid, 20:4, 263-268). The most common death cause in patients with hATTR is cardiac related (cardiac failure, MI, arrhythmias, ischaemic stroke due to atrial fibrillation) and other common causes of death are renal failure, infection and cachexia.

Even though patients were subdivided into cardiac and non-cardiac subgroups, based on a cut-off for left ventricle wall thickness of 13 mm, many patients who didn't belong to the cardiac subgroup had a medical history of cardiac disease including cardiomyopathy and arrhythmia-related problems.

In total, there were 21 deaths reported in the patisiran development programme: 15 deaths in patisiran-treated patients and 6 in placebo-treated patients. During the placebo-controlled study 004, there were 7 (4.7%) deaths in the patisiran arm and 6 (7.8%) deaths in the placebo arm. During the open-label studies 003 and 006 there were an additional 7 deaths in patisiran-treated patients. 14 of the 21 patients who died were allocated to the cardiac subgroup at baseline. However, based on the existence of a medical history of cardiac disease, 18 out of the 21 patients who died had some degree of cardiac disease before death.

The SAEs reported as fatal were adjudicated to the cardiovascular (CV) subgroup in 12 out of 15 of the patisiran-treated patients and in 3 out of 6 of the placebo-treated patients. Of note, of the 3 cardiovascular deaths in the placebo-group, 1 was adjudicated as related to heart failure and two rather to cerebrovascular than cardiovascular events, since both were fatal strokes (one hemorrhagic, one ischaemic). However, ischaemic stroke is often caused by atrial fibrillation related to cardiac amyloidosis in hATTR patients. Non-CV fatal SAEs were esophageal carcinoma, acute respiratory distress syndrome (parainfluenza), and invasive ductal breast carcinoma in patisiran-treated patients and staphylococcal sepsis, metastatic colorectal cancer and one case adjudicated as unknown (UTI & acute kidney failure) in placebo-treated patients.

Five other patients died after the cut-off date: 4 patients treated with patisiran in study 006 (reported cause of death: arrhythmia, electrolyte imbalance/acute respiratory failure cardiopulmonary failure, and unknown) and one patient who received placebo (cause of death: worsening cardiac failure).

Despite sudden death imbalance between groups in study 004 with more sudden deaths reported in patients treated with patisiran, these cases could be related to cardiomyopathy due to hATTR. Provided electrocardiograms highlight abnormalities observed in patients with severe amyloidosis in all these cases.

Two deaths cases with haemorrhage (subarachnoidal and gastrointestinal, respectively) were reported in the

placebo group, both adjudicated to the CV subgroup. Haemorrhage is an adverse event that has occurred in patients with thrombocytopenia treated with other therapeutic oligonucleotides and thus interesting to analyse. However, since these deaths occurred in the placebo group, they were not related to treatment with patisiran.

The death rates in the pivotal study and overall pooled experience were compared to reported death rates from other studies in the hATTR patient population. Since risk factors for poor prognosis include the non-V30M genotype and the proportion of non-V30M was 57.3% in the pivotal study 004, which is similar to the proportion of non-V30M in the tafamidis (53%) and diflunisal trials (45%), it was agreed with the applicant that death rates per 100 patient-years (PY) are comparable with the rates reported in the tafamidis and diflunisal studies. The overall death rate in the patisiran group of study 004 was 3.2/100 PY and 6.4/100 PY in the placebo group. The overall death rate in the long-term treatment study of tafamidis was 6.6/100 PY, and in the placebo-controlled diflunisal study it was 3.1/100 PY in the diflunisal group and 6.8/100 PY in the placebo group. Thus, the death rates are comparable between these studies.

Haematology and coagulation parameters

There were minor effects on haematology parameters, which occurred in both treatment groups which could be related to corticosteroid premedication prior to blood sampling in the study. No effects on coagulation parameters were observed.

Complement factors and cytokines/CRP

Activations of the alternative complement pathway and immune stimulation have been described for other therapeutic oligonucleotides (mainly of the ASO (DNA-based) class). In the non-clinical and dose-response clinical studies, transient effects were seen on both complement factors and cytokines/CRP, which were normalised within 24 hours. Effects on cytokines, complement factors and CRP were not measured in the pivotal trial. However, it is considered that these are elevated during infusion related reactions, which were more common in patisiran-treated patients than in the placebo group. Since IRRs were manageable in the pivotal trial, there is no need to routinely monitor these IRR-related laboratory assessments during treatment with patisiran post-approval.

ADAs and safety

Development of treatment-emergent ADA to PEG2000- C –DMG was rare and occurred in both patirsiran- (3.4%) and placebo-treated patients (1.30%) in study 004. There was no correlation between ADA and IRRs, anaphylactic reactions, or hypersensitivity events.

PEG_{2000}-C-DMG is exposed on the surface of patisiran-LNP to the immune system. Anti-PEG-ADA have also been described in subjects treated with PEGylated protein drugs. The applicant has described their efforts to try to establish ADA assays also for the other components of the nano-particle delivery system and the active component. However, this has not been possible to achieve due to some components considered relatively “autologous” and other technical issues such as rapid degradation.

Although it cannot be fully excluded that no other types of ADA directed towards other LNP components or the siRNA exist, no clinical signs were observed, such as lower PK exposures or severe IRRs, suggestive of such additional ADAs.

There were no clinically meaningful differences observed in the safety related to intrinsic factors (age, sex, weight, race), genotype, FAP stage or cardiac disease severity (cardiac subgroup or not).

Pregnancy and contraception

There have been no reported pregnancies in the patisiran-LNP clinical development programme.
Additional text has been included in the SmPC 4.4 regarding vitamin A supplementation and pregnancy to stress the fact that too low and too high levels of vitamin A may be teratogenic during pregnancy to the foetus. This recommendation for monitoring the vitamin A level unbalances during pregnancy is considered appropriate by the CHMP.

Expanded access program

In the expanded access program in the US, 48 patients had been treated as of the cut-off date of 14 July 2017. The patient population appears generally to be in line with the population studied in the pivotal trial and the dosing regimen including the premedication was the same as the one proposed in the SmPC. The majority of patients (79.2%) had non-V30M genotypes compared to 57.3% in the pivotal study 004.
4 patients (8.3%) reported restless legs syndrome in the expanded access program versus only 6 patients in total in the main studies of the development programme (study 003, 004, 006), 5 (3.4%) in the 004/006 studies and 1 (3.7%) in the 003/006 studies. Restless legs syndrome (RLS) is known to often be secondary to PNP. Teodoro et al. (2015) reports that RLS frequency was increased in TTR-FAP patients (18.4% versus controls (4.8%) and that female sex (p=0.037) and obesity (p= 0.048) were associated with RLS in this patient population (Teodoro et al., A peripheral pathway to restless legs syndrome? Clues from familial amyloid polyneuropathy, Parkinsonism Relat Disord. 2015 Dec;21(12):1465-8). The apparent difference in frequency between the clinical development studies and the expanded access program may be related to the overall small sample sizes.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics

2.8.2. Conclusions on the clinical safety

In total 218 patients with hATTR have been exposed to patisiran during the clinical development programme. 179 have been dosed for at least 12 months and 101 for at least 24 months, which is considered as an acceptable size for a safety database of an orphan drug. However, long-term safety data are still limited. In this respect, the applicant has committed to conduct, post approval, a prospective observational study to monitor and assess the long-term safety of patisiran-LNP in hATTR amyloidosis patients. The main AEs were IRRs, which are manageable with premedication and slowing or temporary stopping of infusion if necessary. In addition consequences of vitamin A deficiency and hepatic disorders are considered potential risks and are appropriately captured in the RMP.

2.9. Risk Management Plan

Safety concerns

Summary table of the Safety Concerns

Important identified risks	• Infusion-related reactions (IRRs)
Important potential risks	• Consequences of vitamin A deficiency • Severe hypersensitivity • Hepatic disorders

Summary table of the Safety Concerns

Missing information	• Longer-term safety (>3 years) • Use in patients with moderate or severe hepatic impairment • Use in patients with severe renal impairment or end-stage renal disease • Use in patients with prior liver transplantation • Use in pregnancy and lactation

Pharmacovigilance plan

Ongoing and Planned Additional Pharmacovigilance Activities

Study Number Short Name Status	**Summary of Objectives**	**Safety Concerns Addressed**	**Milestones**	**Due Dates**
ALN-TTR02-006 (Study 006) Global Open-Label Interventional Extension Study Ongoing **Category 3**	The objective of this study is to assess the safety and efficacy of longer-term patisiran-LNP dosing in adult patients with hATTR amyloidosis with polyneuropathy who previously completed Study ALN-TTR02-004 (Study 004) or Study ALN-TTR02-003 (Study 003).	Longer-term safety (> 3 years)	Last patient's last visit: planned: Q3 2022	Final study report: planned: Q3 2023
Title: Prospective observational study to monitor and assess the safety of patisiran-LNP in a real-world cohort of hATTR amyloidosis patients **Category 3**	The primary objective of this study is to characterize the safety of patisiran-LNP in real-world conditions, including determining and comparing the incidence of selected adverse events (e.g. cardiac hepatic) in hATTR amyloidosis patients exposed to patisiran-LNP. An appropriate control group within the study cohort will be created to serve for comparative risk analyses.	Hepatic disorders Longer term safety (>3 years) Use in patients with moderate or severe hepatic impairment Use in patients with severe renal impairment or end-stage renal disease Use in patients with prior liver transplant	Submission of the study protocol for PRAC review within 3 months from the EC decision: Q4 2018 Protocol final: Q1 2019 Planned study starts: Q2 2019 Study end: Q3 2026	Study progress reports will be provided with each PSUR. Adverse event frequencies will be provided once a year within the corresponding PSUR, starting 2 years after study start and continuing until the end of study. Full interim analysis provided 4 years after study start. Final study report planned: Q3 2027

Ongoing and Planned Additional Pharmacovigilance Activities

Study Number Short Name Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Planned: Global Pregnancy Surveillance Program **Category 3**	The primary objective of this program is to collect and evaluate data on exposure during pregnancy and infant outcomes in hATTR amyloidosis patients exposed to patisiran-LNP	Use in pregnancy and lactation	Estimated start: Q4 2019 Estimated completion: Q4 2030	Final program report planned: Q4 2031

Risk minimisation measures

Summary Table of PV Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
Important Identified Risk:		
Infusion-related reactions	Routine risk minimization measures: • SmPC Section 4.2, 4.4, 4.8 • PL Section 2,3, 4 • Legal status: restricted medical prescription Additional risk minimization measures: • Educational materials for HCPs and patients to ensure the safe and sustainable administration of patisiran-LNP in the home	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • Specific targeted follow-up of IRRs Additional pharmacovigilance activities: • None
Important Potential Risk:		
• Consequences of vitamin A deficiency	Routine risk minimization measures: • SmPC Section 4.4, 4.5, 4.6, 5.1 • PL Section 2 • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • None

Summary Table of PV Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
Severe hypersensitivity	Routine risk minimization measures: • SmPC Section *4.3* • PL Section 2 • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • Specific targeted follow-up of severe and serious events of severe hypersensitivity Additional pharmacovigilance activities: • None
Hepatic disorders	Routine risk minimization measures: • None • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Evaluation of data from the ongoing open-label extension Study 006 • Evaluation of data from a planned prospective observational cohort study
Missing Information:		
Longer term safety (> 3 years)	Routine risk minimization measures: • SMPC Section *4.8* • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Evaluation of data from the ongoing open-label extension Study 006 • Evaluation of data from a planned prospective observational cohort study
Use in patients with moderate or severe hepatic impairment	Routine risk minimization measures: • SMPC Section *4.2*, *5.2* • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Evaluation of data from a planned prospective observational cohort study
Use in patients with severe renal impairment or end-stage renal disease	Routine risk minimization measures: • SMPC *Section 4.2*, *5.2* • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Evaluation of data from a planned prospective observational cohort study

Summary Table of PV Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
Use in patients with prior liver transplant	Routine risk minimization measures: • SmPC *Section 4.2*. • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Evaluation of data from a planned prospective observational cohort study
Use in pregnancy and lactation	Routine risk minimization measures: • SmPC *Section 4.6, 5.3* • PL *Section 2* • Legal status: restricted medical prescription Additional risk minimization measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Planned Global Pregnancy Surveillance Program to collect and evaluate data on pregnancy exposure and infant outcomes

Conclusion

The CHMP and PRAC considered that the RMP version 1 is acceptable.

2.10. Pharmacovigilance

Pharmacovigilance system

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

Periodic Safety Update Reports submission requirements

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

The Applicant noted the PRAC rapporteur's team request for a progress report on the Global Pregnancy Surveillance Program (currently under discussion with the FDA) within the first PSUR for Onpattro.

2.11. New Active Substance

The applicant compared the structure of patisiran with active substances contained in authorised medicinal

products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

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

2.12. Product information

2.12.1. 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.*

2.12.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Onpattro (patisiran) 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) is a rare, life-threatening, autosomal dominant multi-systemic disease caused by mutations in the TTR gene that results in rapidly progressive, debilitating morbidity and high mortality. It has a male predominance (approximately 3:1 male to female ratio) with diagnosis typically occurring in the seventh decade. The cardinal manifestations of hATTR amyloidosis are polyneuropathy and cardiomyopathy.

The estimated European prevalence of hATTR amyloidosis is 0.10 per 10,000, 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. In northern Sweden is as high as 50 per 100,000 inhabitants. The age of onset of disease varies between countries and regions due to differences in the most common mutation types, which is also related to founder effects. For example, in Portugal the median age of onset is 30-40 years. The progression rate appears somewhat faster in early onset disease.

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). In comparison, the Swedish National Board of Health and Welfare (Socialstyrelsen) states that the survival is 9-13 years from onset.

3.1.2. Available therapies and unmet medical need

There are currently limited treatment options that may alter the course of the hATTR disease, namely orthotopic liver transplant (OLT) and TTR tetramer stabilizers, exist for a small subset of patients. OLT essentially eliminates mutant TTR from the circulation but does not affect the hepatic production of wt TTR, which continues to be made by the transplanted liver. OLT is only effective in slowing the progression of disease in patients with an early age of onset (<50 years of age), especially for those with the V30M mutation and short disease duration before transplant. Consequently, almost two-thirds of patients with hATTR amyloidosis are not transplant-eligible. Even when OLT is possible, morbidity and mortality are substantial; patients require life-long immunosuppressive medications, with their attendant risks of infection and renal injury. One-year mortality rates of up to 10% have been reported.

TTR tetramer stabilizers (including tafamidis and diflunisal) act by binding to the T4-binding site on TTR to reduce its dissociation into misfolded amyloidogenic monomers. Tafamidis received approval from EMA under exceptional circumstances in November 2011 'for the treatment of transthyretin amyloidosis in adult patients with stage I symptomatic polyneuropathy to delay peripheral neurologic impairment'. Tafamidis has been reported to be generally well tolerated. Long-term follow-up of the early stage V30M patients who continued tafamidis treatment showed continued neuropathy progression over time. Diflunisal, a generic, oral nonsteroidal anti-inflammatory drug (NSAID) that has been demonstrated to bind to and stabilize the TTR tetramer in a manner similar to tafamidis, was shown to reduce neuropathy progression compared with placebo in V30M and non-V30M patients with hATTR amyloidosis with both early and late stage neuropathy in a Phase 3 United States (US) National Institutes of Health-sponsored study. Diflunisal is not an approved treatment for hATTR amyloidosis in any country. Overall, the data to date with TTR tetramer stabilizers indicate some slowing of neuropathy progression, with effects limited in the case of tafamidis to early stage V30M patients, and no effect on the cardiac manifestations of the disease.

3.1.3. Main clinical studies

The pivotal evidence of efficacy of Onpattro comes from a randomised, double-blind, placebo-controlled study conducted in 225 patients with hATTR amyloidosis with a TTR mutation and symptomatic polyneuropathy **(study 004**). The primary efficacy variable selected was the modified Neuropathy Impairment Score +7 (mNIS+7). It is a composite neurologic impairment score that was developed specifically for monitoring progression of neurologic impairment in hATTR amyloidosis patients. This endpoint is a composite measure of motor, sensory, and autonomic polyneuropathy including assessments of motor strength and reflexes, quantitative sensory testing, nerve conduction studies, and postural blood pressure, with the score ranging from 0 to 304 points, where a higher score indicates more pronounced impairment.

Patients were randomised 2:1 to receive IV 0.3 mg/kg patisiran or placebo once every 3 weeks for 18 months. All patients received premedication with a corticosteroid, paracetamol, and H1 and H2 blockers.

A total of 82.2% of the 225 patients completed study treatment More subjects discontinued treatment in the placebo group 29/77 (37.7%) versus 11/148 (7.4%) in the patisiran group. The most common reason for study treatment discontinuation overall was withdrawal of consent and the dominating causes for withdrawal of consent in the placebo group were lack of efficacy or worsening of disease.

A cardiac subpopulation was defined within an amendment to the SAP (SAP V2.0, 31 May 2017) and included patients with pre-existing 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. For the Cardiac Subpopulation, the change from baseline to Month 18 in LV wall thickness, LV mass, LVEF, LV longitudinal strain, NT-proBNP and troponin I were analysed. Being part of this population was not a stratification factor at randomization. The proportion of cardiac patients was higher in the patisiran-LNP group than the placebo group; 60.8% vs. 46.8%, respectively.

Additional data for the application were provided from the following studies:

Two Phase 1 patisiran-LNP single ascending dose (SAD) studies in healthy volunteers contributed pharmacodynamic (PD) assessments (eg, TTR levels), pharmacokinetic (PK) data, and safety data. Study ALN-TTR02**-002** was a Phase 2 multiple ascending dose (MAD) study that contributed PD, PK, and safety data in patients with hATTR amyloidosis with polyneuropathy; this study supported the selection of the patisiran-LNP dose and regimen for continued development.

Study 003 was a multinational, multicenter, Phase 2, open-label, extension study designed to provide long term (up to 2 years) patisiran-LNP dosing to patients with hATTR amyloidosis with polyneuropathy who received and tolerated patisiran-LNP in Study 002.

Study 006 is an ongoing multicenter, multinational, open-label extension study. Eligible patients who completed either of the two parent Studies, 003 or 004, were given the option to participate in this study.

Interim results from Study 006 were presented by the applicant with data cut-off date (14 July 2017). All 25 patients who completed Study 003 had enrolled in Study 006. 163 of the 169 patients who completed Study 004 had enrolled in Study 006. Patients from Study 004 remained blinded to Study 004 treatment assignment during participation in Study 006 at the time of the interim data cut. Efficacy assessment is presented for the smaller subset of approximately 64 patients who had the efficacy assessment performed at Week 52.

3.2. Favourable effects

Dose-dependent reductions in serum TTR concentrations with patisiran LNP were observed in both healthy volunteers and in patients with hATTR amyloidosis with polyneuropathy. Long term dosing with patisiran LNP sustained a mean TTR reduction of approximately 80% over 2 years of treatment.

In the pivotal study 004, neurologic impairment as assessed by mean mNIS+7 change from baseline at 18 months was +27.96 points for the placebo group, which is in line with the estimated worsening per year (+17.8) found in a multinational study of 282 patients with hATTR amyloidosis. The patisiran patients mean change at 18 months was -6.03 mNIS+7 points. The difference in neurologic impairment was statistically significant: -33.99 [-39.86; -28.13] points (LS mean (SEM) favouring the patisiran group. The observed effects are expected to translate into improvements of the motor, sensory and autonomic neurologic functioning of the patients.

Statistically significant differences favouring patisiran-LNP group compared to placebo at 18 months were observed for all secondary endpoints**,** as illustrated by quality of life as assessed by Norfolk QoL-DN at 18 months group (LS mean difference between patisiran-LNP – Placebo groups, [95% CI, p-value]): (-21.3 points [-27.2; -15.0, P=1.103 $\times 10^{-10}$]), motor strength (NIS-W)(-17.87 points [-22.32, -13.43, P=1.404E-13]), disability (R-ODS)(+9.0 points [7.0, 10.9, P=4.066E-16]), gait speed (10-MWT)(0.311 m/sec [0.23,

0.39, P=1.875E-12]), nutritional status (mBMI)(+115.7 kg/m2 × albumin g/L [82.4, 149.0, P=8.832E-11]) and autonomic symptoms (COMPASS 31)(7.53 points [-11.89, -3.16, P=0.0008]). These results obtained for the secondary endpoints are expected to translate into imnprovments of a broad range of clinical manifestations of hATTR amyloidosis which would allow patients achieve a better quality life.

A consistent treatment effect of patisiran on mNIS+7 and Norfolk QoL-DN was observed across subgroups (age [<65; ≥65], gender, race [white, non-white], region [North America, Western Europe, Rest of World], NIS [< 50; ≥ 50], genotype [V30M; non-V30M], genotype class [Early onset V30M; Other], previous tetramer use, FAP stage [I & II] and cardiac subpopulation.

3.3. Uncertainties and limitations about favourable effects

Stabilization or improvement in PND score is seen for patients who had PND I, PND II, PND IIIA or PND IIIB score at baseline, but patients with the most severe form of the disease (FAP stage III corresponding to PND stage IV) were excluded from the pivotal study.
The applicant has analysed data from the pivotal study 004 on placebo patients having progressed to reach PND IV at month 9 showing that all patients (n=5 plus one patient who was PND IV at baseline) had a consistent worsening in clinical measures. Of these 6 patients, 1 subsequently enrolled in Study 006 and had efficacy assessments 12 months after starting patisiran-LNP. The other 5 patients did not have efficacy data after starting patisiran-LNP due to study discontinuation (n=1), death (n=2) or had not reached the 12-month efficacy visit in Study 006 (n=2). The applicant also presented data from 6 patients who were PND IV at baseline in Study 006. Results in these patients, indicate a stabilization of clinical measures during patisiran-LNP treatment. However, 4 of these patients had been treated with patisiran-LNP in study 004 and thus, had progressed on active treatment before showing stabilisation of disease progression.

The pivotal study was designed to show efficacy on polyneuropathy while the effect on cardiac manifestations were investigated by exploratory surrogate endpoints with analyses focused on the cardiac subpopulation.

Of the studied patients in Study 004, 56% were included in the pre-defined cardiac subpopulation; i.e., they had evidence of cardiac amyloid involvement (mean LV wall thickness ≥1.3 cm in the absence of potentially confounding medical history [hypertension or aortic stenosis]). Patients with NYHA class III and IV were excluded from the study. In these patients, centrally-assessed echocardiograms showed decreases in LV wall thickness (LS mean difference: −0.9 mm [95% CI −1.7, −0.2]) and longitudinal strain (LS mean difference: −1.37% [95% CI -2.48, -0.27]) with Onpattro treatment relative to placebo. N-terminal pro-B type natriuretic peptide (NT-proBNP) was 727 ng/L and 711 ng/L at baseline (geometric mean) in Onpattro-treated and placebo-treated patients, respectively. 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), representing a 55% difference in favour of Onpattro.

3.4. Unfavourable effects

Infusion-related reactions (IRRs) appeared to be dose-dependent with IRR events not occurring in the lower dose groups (0.05 to 0.15 mg/kg) and only occurring in the 0.3 mg/kg and 0.5 mg/kg dose groups in the dose-response SAD and MAD studies. In the pivotal trial 004 with only the 0.3 mg/kg dose every 3 weeks compared to placebo, infusion-related reactions were more common in the patisiran (18.9%) than in the placebo (9.1%) group. All these IRRs were mild to moderate and resolved. In the patisiran group, of the

patients with IRRs, the majority (78.6%) had their first IRR within the first 2 doses and >82.1% by the third dose.

Events potentially related to extravasation have been reported in 0.3% of patisiran infusions and symptoms consist of phlebitis, localized pain, burning sensation, swelling, tenderness, or redness at or near the infusion site.

Other adverse events, which occurred in at least 3% more patients on patisiran treatment than on placebo in the pivotal trial, were:

Peripheral oedema (29.7% vs 22.1%)

Arthralgia (7.4% vs 0%)

Muscle spasms (8.1% vs 1.3%)

Dyspnoea (6.8% vs 0%)

In total, there were 21 deaths in the patisiran development programme; 15 deaths in patisiran-treated patients and 6 in placebo-treated patients. During the placebo-controlled study 004, there were 7 (4.7%) deaths in the patisiran arm and 6 (7.8%) deaths in the placebo arm. Expressed in number of deaths per 100 PYs, there were 3.2 deaths per 100 PYs in the patisiran group vs 6.24 deaths per 100 PYs in the placebo group. During the open-label studies 003 and 006 there were an additional 7 deaths in patisiran-treated patients. 14 of the 21 patients who died were allocated to the cardiac subgroup at baseline. The SAEs reported as fatal were adjudicated to the cardiovascular (CV) subgroup in 12 out of 15 of the patisiran-treated patients and in 3 out of 6 of the placebo-treated patients.

3.5. Uncertainties and limitations about unfavourable effects

There was a trend for slightly elevated ALT (26.4% vs 13.0% >ULN and ≤3xULN), AST (20.9% vs 5.2% >ULN and ≤3xULN) and total bilirubin (6.1% vs 2.6%, >ULN and ≤1.5xULN) in the patisiran group vs the placebo group in the pivotal study 004. There were no Hy's Law cases (AST or ALT >3 × ULN and total bilirubin >2 × ULN). No clinically meaningful changes in liver function tests occurred with patisiran-LNP treatment relative to placebo for up to 18 months of treatment (study 004) and ALT and AST levels have continued to remain stable in the open label study (study 006) for up to 49.5 months. There were no changes in APL or total bilirubin. It is considered that no monitoring of liver function is warranted during treatment with patisiran-LNP in the clinical setting. Although this small increase of hepatic transaminases seems stable over time, there is a need to further assess the hepatic effects of patisiran post-marketing as part of a prospective observational study to monitor and assess the safety of patisiran-LNP in a real-world cohort of hATTR amyloidosis patients. Hepatic disorders are included as an important potential risk in the RMP.

Premedication with corticosteroid, anti-H1, anti-H2 and acetaminophen induced adverse drug reactions, including serious ADRs, in patients treated in clinical studies. The applicant did not differentiate ADR by drug used as part of the premedication. However, osteoporosis and insomnia reported in more than 3% of patients can be related to corticosteroid. Other ADRs associated to corticosteroid use were also reported as 'Cushingoid', 'hyperglycemia', 'glycosuria urine present' and 'glucose tolerance impaired'. Therefore the CHMP has found appropriate the SmPC recommendation to decrease corticosteroid posology in patients to a minimal dose of 5 mg of intravenous dexamethasone or equivalent for patients who have tolerated their patisiran infusions well.

In addition, the long-term safety will be followed up in the above mentioned prospective observational study.

The nonclinical PK data indicate that there was no accumulation of the PEG lipid in the liver. Further, nonclinical and modelled clinical PK data suggest that there is no further liver accumulation of the DLin-MC3-DMA lipid after reaching steady state following 24 weeks of multiple dosing. However, the prediction of human liver concentration of PEG and DLin-MC3-DMA lipids based on allometric scaling from rat and monkey PK data doesn't provide a satisfactory level of certainty. It might be possible that steady state in the liver of the two lipids is reached at the same time in plasma and the liver if there is no rate determining step that might influence the half-life in the liver. However, in the absence of an established methodology to examine this in humans so the issue is not further pursued.

In the placebo-controlled study, no difference between the treatment arms was observed in patients reporting at least an AE in SOC eye disorders. Analysis per preferred term (PT) showed that a higher percentage of patients in the patisiran group reported AEs such as dry eye (4.7% vs 2.6%) and vision blurred (2.7% vs 1.3%). No safety signal related to visual disorders, including visual impairment, visual acuity reduced or vision blurred, was observed in patients exposed to patisiran in clinical trials which could be attributed to vitamin A deficiency. However, as 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 referral for ophthalmological assessment if such symptoms occur have been included in SmPC section 4.4.
Low RBP and vitamin A levels were observed during the clinical development, as expected, as serum TTR is a carrier of retinol binding protein (RBP). Therefore the CHMP considered that a recommendation for vitamin A supplementation to be included in section 4.2 of the SmPC, and the recommended daily intake of 2,500 IU (as used during the clinical studies) as a reference daily intake.
In addition, unbalance in vitamin A levels during the first trimester of the pregnancy may lead to teratogenic effects for the foetus; specific recommendations for vitamin A supplementation during an unplanned or planned pregnancy have also been included in the SmPC.

3.6. Effects Table

Table 1. Effects Table for Onpattro for hATTR (data cut-off 14 September 2017)

Effect	Short Description	Unit	Patisiran	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
mNIS+7	Polyneuropathy scale, 0 -304 p	points	-6.03	+27.96	$P=9.262x10^{-24}$	Study 004
Norfolk QoL-DN	Polyneuropathy quality of life questionnaire, -4 to 136 p	points	-6.7	+14.4	$P=1.103x10^{-10}$	Study 004
Compass 31	Autonomous symptoms questionnaire, 0-100 p.	points	-5.29	+2.24	P=0.0008	Study 004

Effect	Short Description	Unit	Patisiran	Placebo	Uncertainties/ Strength of evidence	References
mBMI	BMI (kg/m^2) x albumin (g/L)		-3.7	-119.4	$P=8.832 \times 10^{-11}$	Study 004
NT-proBNP	Biomarker for cardiac failure	ng/L	544.06	1116.75	No statistics	Study 004
Unfavourable Effects						
IRRs	Infusion-related reactions	%	18.9%	9.1%	Placebo-controlled	Study 004
peripheral oedema		%	29.7%	22.1%	Placebo-controlled	Study 004
arthralgia		%	7.4%	0%	Placebo-controlled	Study 004
muscle spasms		%	8.1%	1.3%	Placebo-controlled	Study 004
dyspnoea		%	6.8%	0%	Placebo-controlled	Study 004
ALT	>ULN and $\leq$ 3 × ULN (worst post-baseline)	%	26.4%	13.0%	Placebo-controlled	Study 004
AST	>ULN and $\leq$ 3 × ULN (worst post-baseline)	%	20.9%	5.2%	Placebo-controlled	Study 004
eGFR	<15 $mL/min/1.73\ m^2$ (worst post-baseline)	#	5	0	Placebo-controlled	Study 004
deaths	Deaths per 100 PYs	#	3.2 (95%-CI: 1.37-6.18)	6.24 (95%-CI: 2.48-12.65)	Placebo-controlled	Study 004

Abbreviations: PYs = patient-years, ULN = upper limit of normal

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Overall, a substantial effect of patisiran LNP on reducing the serum TTR concentrations has been convincingly shown in the clinical studies. Furthermore, in the pivotal study 004 the worsening from baseline at 18 months in the primary end-point for the placebo group was in line with the estimated change per year, in contrast to the patisiran patients with a subtle numerical improvement instead of the expected worsening. The results from the secondary endpoints in study 004, and the results from the open labelled studies 003 and 006, strongly support a positive effect on the neuropathy progression in the studied population of hATTR patients.

The results were consistent in subgroups based on e.g. age, gender, race, region, age at diagnosis, genotype, previous tetramer use, FAP stage [I & II] and cardiac subpopulation.

The study population in the pivotal study 004 was limited to subjects with Polyneuropathy Disability (PND) Scores of 0-IIIB hATTR/FAP stages 1 and 2. It is acknowledged that the clinical data from study 004 shows a convincing difference between patisiran and placebo regardless of baseline disease stages at inclusion for the studied population. However, there is only very limited data in patients with more severe disease and it is therefore not found adequate to extrapolate the results from less severe to more advanced stages of the disease (PND stage IV/FAP stage 3 = non ambulatory patients).

The pivotal study was primarily designed to show an effect on hATTR disease with polyneuropathy and the cardiac endpoints were included as exploratory surrogate endpoints in a cardiac subpopulation including 56% of the study population. Since the definition of the cardiac population was included as a study amendment, inclusion in the study was not stratified based on this. Further, patients with NYHA III and IV were not included. The results of the pivotal study show a difference compared to placebo for several of the measured echocardiographic variables as well as on the biomarker NT-proBNP, favoring Patisiran.

The applicant has employed in the pivotal study a surrogate marker (NT-proBNP) that has not been shown to correlate with clinically relevant outcomes in the context of therapeutic interventions. Despite the limitations, the results indicate that patisiran treatment seems to have positive impact on cardiac parameters (which is plausible from a biological perspective) even though the importance of this effect is not fully understood.

With respect to safety, the most serious issue is the risk of IRR which occurred in the pivotal study despite premedication with e.g. corticosteroids. Most reactions were mild to moderate, but may be serious problem in the context of home infusion. However, infusion of Onpattro at home may be considered for patients who have tolerated at least 3 infusions well in the clinic. The decision for a patient to receive home infusions should be made after evaluation and recommendation by the treating physician. Home infusions should be performed by a healthcare professional.

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 referral for ophthalmological assessment if such symptoms occur have been included in SmPC section 4.4. Consequently, the recommendation for vitamin A supplementation is warranted up to a daily intake of 2,500 IU. In addition, as vitamin A is a potent teratogen at both low and high amounts, maintenance of balanced vitamin A levels during pregnancy is essential for normal foetal development.

3.7.2. Balance of benefits and risks

Overall the favorable effects on neuropathy clearly outweigh the unfavorable effects in the population studied, i.e. hATTR amyloidosis patients with FAP stage I and II. Even though there are uncertainties associated with the clinical relevance of results on cardiac parameters (see above), considering the pronounced effect of patisiran on neurological endpoints and the biological plausibility of an effect also on other manifestations of the disease (supported by the mechanism of action of patisiran and the pathophysiology of the condition), the presented results are considered sufficient to support the claim of '*treatment of hereditary transthyretin-mediated amyloidosis in patients with stage 1 or stage 2 polyneuropathy'.*

3.8. Conclusions

The overall B/R of Onpattro is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Onpattro is not similar to Vyndaqel and Tegsedi within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

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

Onpattro is indicated for the 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 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.

Additional risk minimisation measures

Prior to the launch of Onpattro in each Member State (MS), the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational materials, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority (NCA).

The MAH shall ensure that in each MS where Onpattro is marketed, all health care professionals (HCPs) and patients are provided with educational materials in order to ensure the safe and sustainable administration of the product in the home setting, aiming at preventing and/or minimising the important identified risk of Infusion Related Reactions (IRRs).

The educational material for HCPs should include information about:

- Suitability of the patient for home infusion;
- Requirements for home infusion, including availability and timely administration of the appropriate premedication;
- The appropriate infusion rate;
- Signs and symptoms of IRRs;
- Action to take in the event of an IRRs and in case of emergency;
- Steps to consider to prevent further IRRs;
- Reasons triggering HCPs to consider whether the patient should stop home infusions and return to the clinic to receive the infusions.

The educational material for patients (a home infusion guide detailing the steps to undertake during home infusion) should include information about:

- How the infusion is given;
- The potential for IRRs to occur;
- Signs and symptoms of IRRs;
- Patients to inform immediately the HCP if they experience any of the signs and symptoms of IRRs.

Obligation to conduct post-authorisation measures

Not applicable.

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 patisiran is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.