EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

31 May 2018
EMA/411876/2018
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Tegsedi

International non-proprietary name: inotersen

Procedure No. EMEA/H/C/004782/0000

Note

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

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List of abbreviations

A/C	Urine albumin/creatinine ratio
ADA	Antidrug antibodies
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
aPTT	Activated partial thromboplastin time
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
ATTR	Transthyretin amyloidosis
AUC/ $AUC_{0\text{-}168h}$	Area under the curve / Area under the curve baseline to 168 hours
BMI	Body mass index
BCRP	Human breast cancer resistance protein
CFH	Complement factor H
CI	Confidence interval
CKD	Chronic kidney disease
$CL/F_{0\text{-}24hr}$	Mean plasma clearance value
CL_{ss}/F	Steady-state apparent total body clearance
CPN	Chronic progressive nephropathy
CS1	Clinical Study 1
CS2	Parent Study (ISIS 420915-CS2)
CS3	OLE Study (ISIS 420915-CS3)
CSR	Clinical study report
C-SSRS	Columbia Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
C_{trough}	Trough plasma concentration
ECHO	Echocardiogram
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EOT	End of treatment
ERG	Electroretinogram
FAC	Familial amyloid cardiomyopathy
FAP	Familial amyloid polyneuropathy
FAS	Full Analysis Set
GLS	Global longitudinal strain
hATTR	Hereditary transthyretin amyloidosis
hATTR-CM	Hereditary transthyretin amyloidosis with cardiomyopathy
hATTR-PN	Hereditary transthyretin amyloidosis with polyneuropathy
HED	Human equivalent dose
HRDB	Heart rate to deep breathing
I_{max}	Maximum inhibitory effect
IVS	Interventricular septum

IXRS	Interactive voice/web-response system
LBM	Lean body mass
LCRIS	Local cutaneous reaction at the injection site
LLN	Lower limit of normal
LSM	Least squares mean
LV	Left ventricular
2'-MOE	2'-O-(2-methoxyethyl)
mBMI	Modified body mass index
MMRM	Mixed Effects Model with Repeated Measures
mNIS+7	Modified Neuropathy Impairment Score+7
mRNA	Messenger ribonucleic acid
NIS	Neuropathy Impairment Score
NIS-C	NIS-cranial nerve muscle strength
NIS-LL	Neuropathy Impairment Score-Lower Limb
NIS-R	NIS-reflexes
NIS-S	NIS-sensation of big toe and index finger
NIS-W	NIS-muscle strength
n/N	Number of subjects
Norfolk QoL-DN	Norfolk Quality of Life-Diabetic Neuropathy
ns	Not significant
NSC	Neuropathy Symptoms and Change
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OAEI	Other adverse events of interest
OAT	Organic anion transporter
OATP1	Organic anion transporter protein
OCT	Organic cation transporter
OLE	Open-label extension
OLT	Orthotopic liver transplant
P/C	Urine protein/creatinine ratio
P-gp	p-glycoprotein
PND	Polyneuropathy Disability Score
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate calculated using Fridericia's formula
RBP4	Retinol binding protein 4
RDA	Recommended daily allowance
SAE	Serious adverse event
SAP	Statistical analysis plan
SF-36	Short Form 36 Health Survey
SMQ	Standardized MedDRA query
SOC	System organ class
TEAE	Treatment-emergent adverse event
TTR	Transthyretin
ULN	Upper limit of normal
3'-UTR	3'-untranslated region
V30M	Val30Met

V_{ss}/F	Steady state apparent volume of distribution
wtATTR	Wild-type transthyretin amyloidosis
+7	SUM 7 Test

1. Background information on the procedure

1.1. Submission of the dossier

The applicant IONIS USA Ltd submitted on 3 November 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for Tegsedi, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 23 March 2017.

Tegsedi, was designated as an orphan medicinal product EU/3/14/1250 on 23 March 2014 in the following condition: *Treatment of ATTR amyloidosis*.

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

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).

The applicant applied for the following indication: *Inotersen is indicated for treatment of patients with hereditary TTR amyloidosis (hATTR) to delay disease progression and improve quality of life*.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0181/2015 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.

Applicant's request(s) for consideration

Accelerated assessment

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

New active Substance status

The applicant requested the active substance inotersen 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:

Scientific advice	**date**	**Area of dossier the advice pertained to**
EMEA/H/SA/2286/1/2012/III	19 April 2012	non-clinical and clinical
EMEA/H/SA/2286/3/2017/PA/I	20 July 2017	quality

1.2. Steps taken for the assessment of the product

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

Rapporteur: Martina Weise Co-Rapporteur: Tuomo Lapveteläinen

The application was received by the EMA on	3 November 2017
Accelerated Assessment procedure was agreed-upon by CHMP on	12 October 2017
The procedure started on	23 November 2017
The Rapporteur's first Assessment Report was circulated to all CHMP members on	22 January 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	23 January 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	30 January 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	8 February 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	20 February 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	23 March 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	12 April 2018
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	24 April 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	30 April 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	17 May 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 Tegsedi on	31 May 2018
The CHMP adopted a report on similarity with Vyndaqel (Appendix 1)	20 February 2018

2. Scientific discussion

2.1. Problem statement

Hereditary transthyretin amyloidosis (hATTR) is a progressive, irreversible, and life-threatening disease (please see below). There are very few therapeutic options, which have clear limitations.

2.1.1. Disease or condition

Hereditary transthyretin amyloidosis (hATTR) is a rare, progressive and fatal disease caused by mutations in the TTR gene. Single point mutations destabilize the tetrameric structure of the TTR protein, causing its dissociation into free misfolded monomers. These monomers subsequently aggregate to insoluble, extracellular amyloid fibril deposits resulting in cellular degeneration and death [Quintas, 2001; Plante Bordeneuve, 2011]. Amyloid deposits accumulate in multiple organs, particularly the peripheral nervous system, gastrointestinal tract, kidney, and heart, which manifests in progressive polyneuropathy including sensorimotor neuropathy and autonomic neuropathy. Cardiomyopathy, nephropathy, and gastrointestinal dysfunction frequently develop simultaneously. The phenotypic presentation of the disease is dependent on the pattern of affected organs. Historically, hATTR has been phenotypically divided into hATTR with polyneuropathy (hATTR-PN) with predominant involvement of peripheral nerves and gastrointestinal (GI) tract and hATTR with cardiomyopathy (hATTR-CM). However, due to the multiple organ deposition of amyloid, the disease has a wide clinical spectrum with the majority of patients exhibiting many of these manifestations to some degree.

The disease nomenclature used within this report has been updated to be consistent with more recently defined standards of classification [Sipe, 2016], i.e., use of hATTR-PN instead of familial amyloid polyneuropathy (FAP), and hATTR-CM instead of familial amyloid cardiomyopathy (FAC), except for historic references to orphan drug designation, study title, and previous regulatory and scientific discussions.

2.1.2. Epidemiology

The worldwide prevalence of hATTR-PN has been estimated at approximately 10,000 patients [Coelho, 2008]. In Europe, the incidence is estimated as 0.003 cases per 10,000 per year (or 0.3 new cases per year per 1 million inhabitants), with a prevalence estimate of 0.052 per 10,000 (or 5.2 cases per 1 million inhabitants) [Coelho et al., *"A Guide to Transthyretin Amyloidosis"*, Amyloidosis Foundation, 2016 Edition].

2.1.3. Biologic features, aetiology and pathogenesis

So far, 119 point mutations in the TTR gene have been reported with Val30Met (V30M) being the most prevalent. Each TTR mutation results in widely different clinical manifestation, age of symptom onset and rate of hATTR disease progression, even in patients with the same underlying mutation.

Early onset of hATTR symptoms between the third to fourth decades of life leads to rapid deterioration of the patients' health due to progression of autonomic and sensory-motor deficits, whereas the polyneuropathy slowly develops after late onset of the disease between the sixth and eighth decades of life. The average life expectancy is 3 to 15 years after diagnosis [Gertz, 2017]. Presence of significant

cardiomyopathy is associated with poorer prognosis. Patients typically die from malnutrition and cachexia, renal failure and cardiac disease [Coelho, 2008].

2.1.4. Clinical presentation, diagnosis and stage/prognosis

The main clinical manifestations of hATTR-PN are progressive peripheral sensorimotor and autonomic neuropathy [Plante Bordeneuve, 2011]. Non-specific and symmetrical numbness, pain, and temperature sensitivity typically begins in the lower extremities, progressing distal to proximal. Motor neuropathy follows within a few years, which affects ambulatory status [Coutinho, 1980; Sekijima, 2009; Plante Bordeneuve, 2011]. Hereditary ATTR-PN is classified into 3 stages based on ambulatory status of the hATTR patient [Coutinho, 1980]: in Stage 1, the patients present with weaknesses in the lower limbs and do not require assistance with ambulation, while they show gait dysfunctions, distal amyotrophies and hand involvement in Stage 2 and depend on assistance with ambulation, and are either wheel-chair bound or bedridden with generalised weakness and areflexia in Stage 3. This staging system was used to classify severity of disease in patients being considered for enrolment in the pivotal clinical study of inotersen (CS2). Disease severity can be also assessed using the Polyneuropathy Disability (PND) score, which is a 5-stage scoring system [Suhr, 1996].

Life-threatening autonomic dysfunction develops in many patients, affecting the cardiocirculatory, gastrointestinal and genitourinary systems. Symptoms include orthostatic hypotension, which can lead to dizziness and frequent falls. Gastrointestinal symptoms include diarrhoea, severe constipation, alternating diarrhoea/constipation, vomiting, and gastroparesis, all leading to progressive weight loss. Urinary symptoms, fecal incontinence, and, in men, erectile dysfunction may be present [Plante Bordeneuve, 2011].

Amyloid deposits in the kidney are common and can result in microalbuminuria with progression to renal failure in a subset of patients. Symptoms of chronic kidney disease may include lower extremity edema, anemia, fatigue and weakness, and decrease in appetite.

Cardiac involvement has been estimated to occur in 80% of ATTR [Plante Bordeneuve, 2011]. In subjects with hATTR-CM, mutant TTR amyloid fibrils infiltrate the myocardium, with resultant diastolic dysfunction progressing to restrictive cardiomyopathy and heart failure [Castańo, 2015]. Conduction abnormalities and arrhythmias are also common and many patients require pacemaker and/or defibrillator insertion.

Given the severity of hATTR, there is a significant impact on patients' and caregivers' quality of life [Gertz, 2017; Stewart, 2013]. Caregivers have moderate to high levels of fatigue and spend a significant amount of time caring for patients. Hereditary ATTR is associated with a substantial disruption in employment rates and work productivity. There is also a large mental health burden on both caregivers and patients.

2.1.5. Management

Current therapeutic strategies to treat hATTR include orthotopic liver transplant (OLT) or pharmacotherapy with tafamidis or off-label use of diflunisal, both of which are TTR stabilizers that work by preventing dissociation of the tetramer into amyloid-forming monomers [Adams, 2016]. Because most of the amyloidogenic mutated TTR is secreted by the liver, OLT results in rapid disappearance of mutant TTR protein from the serum. However, wild-type TTR protein continues to be produced by the donor liver and can aggregate with pre-existing amyloid deposits in the tissues after transplantation, leading to continuous disease progression and, in some cases, accelerating heart

disease [Liepnieks, 2007; Liepnieks, 2010; Yazaki, 2000; Yazaki, 2007]. Younger patients with early disease, V30M mutation, and mild symptoms (typically Stage 1) generally experience better outcomes with OLT. Stage 2 patients are often not candidates for OLT due to advanced age, cardiac involvement, or other health reasons [Herlenius, 2004; Stangou, 2004].

Since 2011, tafamidis (*"Vyndaqel"*; EMEA/H/C/2294) is approved across the EU for the treatment of ATTR in adult subjects with Stage 1 symptomatic polyneuropathy to delay peripheral neurological impairment and has also been licensed in Japan and several other countries. Diflunisal is a non-steroidal anti-inflammatory drug (NSAID) that is presently used off-label in subjects with Stage 1 and Stage 2 disease [Adams, 2016]; however, the cardiovascular and renal side effects associated with the NSAID class limit the use of this drug in older patients with hATTR-PN or patients with hATTR CM.

Consequently, there continues to be an unmet medical need for effective and well tolerated treatments for subjects with ATTR (both hereditary and wild type) in European Union and worldwide.

About the product

Inotersen (also known as ISIS 420915) is a chimeric 20-mer phosphorothioate ASO consisting of ten central 2'-deoxyribonucleotides that are flanked by five 2'-O-(2-methoxyethyl) (2'-MOE) ribonucleotides at each of the 5'- and 3'-termini (5-10-5 gapmer structure). These chemical modifications aim to enhance stability against nuclease-mediated degradation and reduce potential side effects associated with the polyanionic nature of phosphorothioate ASOs. Inotersen was designed to bind to the 3'-untranslated region (3'-UTR) of human TTR mRNA, which promotes RNase H1-mediated cleavage of the mRNA. This prevents production of the TTR protein.

Inotersen has been formulated as a sterile 200 mg/ml nonadecasodium salt solution (equivalent to 189 mg inotersen) in pre-filled syringes for single use. Each pre-filled syringe contains the recommended once-weekly subcutaneous (s.c.) dose of 284 mg inotersen in 1.5 ml.

Inotersen is not approved for use for any indication anywhere in the world. The clinical development program for inotersen for hATTR was designed based on feedback from regulatory authorities in the European Union and United States.

Type of Application and aspects on development

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

- The fact that familial ATTR is a rare disorder, which, with continuous progression over time, leads to complete disability, bedridden status, and ultimately death usually within 10 to 15 years from symptoms onset. There are only two treatment options currently available for hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN), liver transplantation and tafamidis, which both have considerable limitations.
- A clear unmet medical need, especially for patients with Stage 2 and Stage 3 hATTR-PN.
- A mechanism of action, which appears to be mediated via an inhibitory action and not via a TTR stabilization action.
- Promising efficacy results for the two co-primary endpoints (the modified Neuropathy Impairment Score +7 (mNIS +7) and Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)), which are supportive of the efficacy of inotersen demonstrating significant changes from

baseline compared to placebo.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as solution for injection in pre-filled syringe containing 284 mg/1.5 ml of inotersen (free acid), as 300 mg of inotersen sodium, as active substance.

Other ingredients are: sodium hydroxide, hydrochloric acid and water for injection.

The finished product is available in a clear Type 1 glass prefilled syringe (PFS).

Inotersen Solution for Injection is administered as a once weekly, single-use subcutaneous injection. No dilution is required prior to administration. The deliverable volume is 1.5 mL.

2.2.2. Active Substance

General information

The active substance, inotersen sodium, is a single stranded synthetic oligonucleotide with an antisense mechanism of action. It comprises 20 nucleotides connected via 19 phosphorothioate linkages that are fully ionised as the sodium salt.

The chemical name of inotersen sodium is *2′ -O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methyl-Pthiouridylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-Pthioguanylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thioguanylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thiothymidylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thiothymidylyl-(3′ -O→5′ -O)-2′ -deoxy-Pthioadenylyl-(3′ -O→5′ -O)-2′ -deoxy-5-methyl-Pthiocytidylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thioadenylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thiothymidylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thioguanylyl-(3′ -O→5′ -O)-2′ -deoxy-Pthioadenylyl-(3′ -O→5′ -O)-2′ -deoxy-P-thioadenylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-P-thioadenylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methyl-Pthiocytidylyl-(3′ -O→5′ -O)-2′ -O-(2-methoxyethyl)-5-methylcytidine, nonadecasodium salt* corresponding to the molecular formula $C_{230}H_{299}N_{69}O_{121}P_{19}S_{19}Na_{19}$. It has a relative molecular mass of 7600.8 Da (nonadecasodium salt) and the following structure (Figure 1).

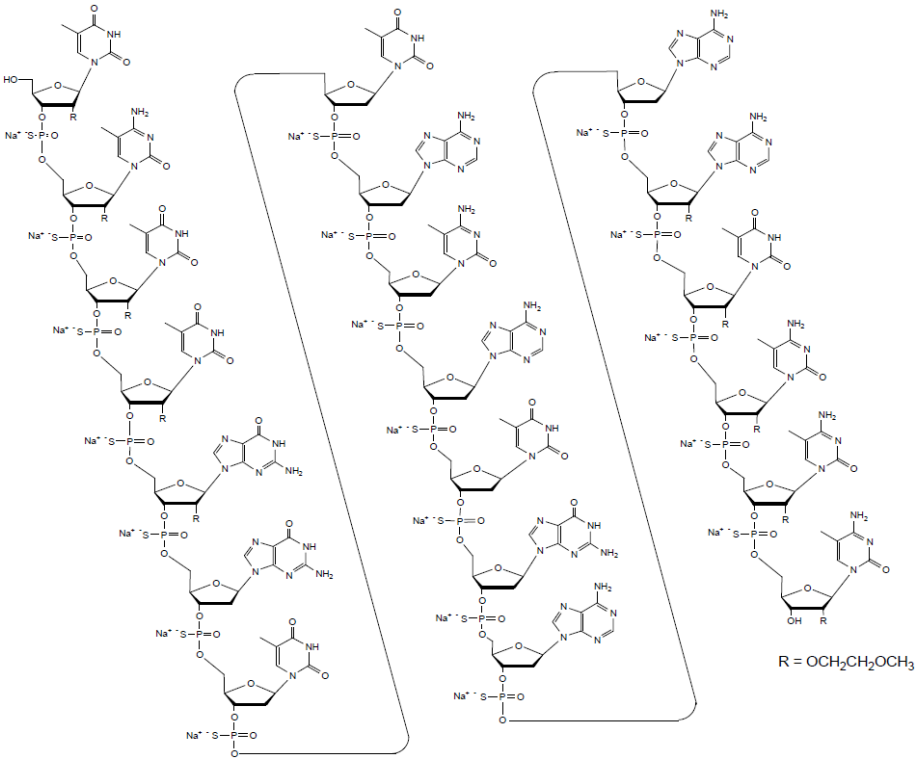


Figure 1: active substance structure

The sequence of the oligonucleotide is:

5'-$^{Me}\underline{U}^{Me}\underline{C}^{Me}\underline{U}^{Me}\underline{UG}GTTA^{Me}CATGAAA^{Me}\underline{U}^{Me}\underline{C}^{Me}\underline{C}^{Me}\underline{C}$-3'

Underlined letters are 2'-O-(2-methoxyethyl)ribonucleotides; non-underlined letters are 2'-deoxyribonucleotides; all pyrimidines are 5-methylated; all linkages are phosphorothioates; the compound is the nonadecasodium salt.

The absolute configuration of each 2-deoxy-D-ribose unit is (1R, 3S, 4R). The absolute configuration of each 2-O-(2-methoxyethyl)-D-ribose unit is (1R, 2R, 3R, 4R). The absolute configuration at each phosphorus atom is undefined. As other phosphorothioate oligonucleotides, inotersen is a mixture of 2^n diastereoisomers hence inotersen (where n=19) is a mixture of 2^{19} (524,288) diastereoisomers.

Inotersen sodium exists as a white to pale yellow amorphous solid. It is freely soluble in water, phosphate buffer pH 7.5 and phosphate buffer pH 8.5.

The chemical structure and sequence of the active substance has been adequately characterised and confirmed. Results of IR, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectroscopy, elemental analysis, mass spectrometry and sequencing data have been provided. The measured masses and the theoretical masses for the fragments are in compliance. Thermodynamic characterisation data (thermogravimetry and DSC), solid state form and X-ray powder diffraction, crystallinity, melting point to a complementary strand, solubility, UV spectrum and molar absorption coefficient have been provided.

Adequate information on stereochemistry has been provided. Because the factors that impact internucleotide linkage stereochemistry, i.e., the identities of the coupling partners in each cycle and the choice of activator (DCI), are fixed, the synthesis of inotersen sodium proceeds in a stereo-controlled manner to furnish a reproducible diastereoisomeric mixture.

Manufacture, characterisation and process controls

Inotersen sodium is synthesised by solid phase synthesis in five main steps using well defined starting materials with acceptable specifications. It is manufactured in one manufacturing site.

Step 1. Synthesis Stage

Step 2. Cleavage and Deprotection Stage

Step 3. Purification Stage

Step 4. Tangential Flow Filtration Stage

Step 5. Lyophilisation Stage

A single synthesis is processed through to a single active substance lot.

The manufacturing process of inotersen sodium has been developed using a combination of conventional univariate studies and elements of Quality by Design (QbD), in line with ICH Q8, Q9, Q10, and Q11. Consistent with ICH Q11, a systematic, science, and risk-based approach was applied to evaluate, understand and improve the manufacturing process for inotersen sodium. A comprehensive description of the development has been provided. The described quality target product profile (QTPP) is reasonable and the selection of the Critical Quality Attributes (CQAs) has been adequately justified. Quality risk management is applied throughout the product lifecycle to drive product development and continuous improvement. High level sufficient information on quality risk management has been provided.

Critical process parameters (CPP) and process parameters (PP) have been defined and are included in the description of the manufacturing process. Design spaces and proven acceptable ranges (PARs) have been defined for certain steps of the commercial manufacturing process. Good process understanding has been demonstrated and the classification of CPPs and PPs is acceptable. Summaries of the risk assessments, the description of initial screening and scoping experiments to identify potential CPPs and interactions between parameters and attributes and the design of experiments for the development of the design spaces are conclusive. Parameters that are defined as PARs or are part of a design space were explored at the limits of their ranges. The process scalability has been demonstrated to be valid for the commercial scale. The defined design spaces, PARs and the classification of PPs and CPPs are acceptable. The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed design spaces and PARs.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented. Suitable starting materials for this synthetic oligonucleotide have been selected. The suppliers of the starting materials are stated in the dossier. Detailed information on starting material impurities and their classification has been provided. This classification is adequately justified and the impact on the active substance impurity profile has been evaluated. The overall control strategy is acceptable and its development using an enhanced approach has been intensively described and justified.

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. A risk assessment of the commercial route of synthesis for potential mutagenic impurities was conducted in accordance with ICH M7. The result of the risk assessment that no mutagenic impurities are identified as inotersen sodium CQAs is acceptable.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program.–The comparability of inotersen sodium manufactured by development and commercial routes has been demonstrated by analytical testing.

The active substance is packaged in HDPE bottles with polypropylene closures which comply with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The active substance specification is based on the identified CQAs and includes tests for description, identification (accurate mass measurement by MS and molecular sequencing by mass spectrometry fragmentation (MS-MS)), sodium counter ion content (ICP-OES), full length product content, inotersen content (purity) and product related impurities (all measured by IP-HPLC-UV-MS), water (KF), microbiological purity (Ph. Eur.) and bacterial endotoxins (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 grouping of impurities as proposed by the Applicant in the specification is acceptable and reflects the specific properties of impurity profiles for synthetic antisense oligonucleotides. Impurities limits were qualified by toxicological and clinical studies and appropriate specifications have been set.

Batch analysis data of the active substance were provided. All results were within the currently proposed specifications.

Process adaptions made in the manufacturing process have resulted in higher purity for the most recent batches. Consequently the acceptance criteria for impurities, full length product content, inotersen content and the sum of impurities were tightened during the procedure. The CHMP recommends that while these revised acceptance criteria in the active substance specification are acceptable, in order to take account of scientific and technical progress, and to optimise purity, the applicant should submit a variation to update the specifications after additional drug substance batches are manufactured so that the proposed specifications are based on data from a total of 12 batches manufactured with the current process (see List of Recommendations).

Stability

Stability data from 3 batches of inotersen sodium active substance manufactured at production-scale by Route A2 at Nitto Denko Avecia, the proposed commercial manufacturer, stored in the intended commercial packaging for up to 15 months at long term conditions (-20°C) for up to 6 months at intermediate conditions (5°C), and on for up to 3 months at accelerated conditions (30°C / 75% RH) according to ICH guidelines were provided .

Additionally, supportive stability from 3 batches of inotersen sodium active substance manufactured at production-scale by Route A1 (demonstrated to be comparable to Route A2) at Ionis Pharmaceuticals stored in the intended commercial packaging for up to 24 months at long term conditions (-20°C), 6 months at intermediate conditions (5°C) and for 3 months at long term conditions (30°C / 75% RH) were provided.

The parameters tested are the same as for release. The analytical methods used were the same as for release and were stability indicating. No significant change was observed for any parameter at any storage conditions. All results complied with the specification limits.

Results of photostability testing following the ICH guideline Q1B and forced degradation under stress conditions (60°C, acidic, basic, oxidation) were also provided on one batch. Degradation to varying degrees was observed under all stressed conditions. Mass balance was achieved for all stressed samples.

According to ICH Q1E guideline extrapolation of stability data is not acceptable for new drug substances intended for storage in a freezer and the re-test period should be based on long-term data. Nevertheless, the proposed re-test period of 24 months if stored protected from light at -20°C is acceptable since it has been demonstrated that the results from the supportive stability studies are representative also for the primary stability batches. The comparability of the active substance produced via Routes A1 and A2 and have been demonstrated; the differences between specifications and analytical methods applicable for stability studies during the stages of development have been adequately described and justified.

The stability results indicate that the active substance manufactured by the proposed supplier(s) is sufficiently stable and support the proposed retest period of 24 months if stored protected from light at -20°C in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

Inotersen Solution for Injection is a sterile, clear, colourless to pale yellow solution, essentially free from visible particles, containing 284 mg/1.5 mL (189 mg/mL) of inotersen (free acid basis).

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

The QTPP was defined as a sterile, low endotoxin, product for subcutaneous injection, containing 284 mg / 1.5 ml inotersen free acid base with a high assurance of product purity, quality and stability. The CQAs and their relationship to the QTPP were defined.

The formulation used during clinical studies is the same as that intended for marketing. Initially, Inotersen Solution for Injection, 189 mg/mL, was supplied to the clinic in a vial configuration. The first vial configuration contained a 1.0 mL nominal volume in a 2 mL vial. As development proceeded and the target dose was established, the configuration was changed to a 1.5 mL nominal volume in a 3 mL vial. During Phase 3 clinical testing, a prefilled syringe (PFS) presentation was introduced for patient safety and convenience. The PFS delivers a dose of 284 mg in a delivered volume of 1.5 mL via subcutaneous injection.

In the course of development and clinical trials, the finished product was manufactured at different manufacturing sites however, the manufacturing process comprising dissolving the active substance in WFI, pH adjustment, sterile filtration and filling of the container closure system remained basically the same. The QTPP, CQAs and CPPs have been identified and investigated in a risk assessment. The defined QTPP, CQAs and CPPs are in accordance with general expectations concerning the development and manufacture of an aqueous solution for subcutaneous injection in a pre-filled syringe.

The finished product is aseptically prepared and sterilised by filtration. The provided justification for not applying heat sterilisation in the final container is accepted, as degradation products increased significantly after a treatment of 8 minutes at 121°C.

A risk assessment for metal impurities in accordance with ICH Q3D was provided. Compatibility studies with other medicinal products were not performed and are not necessary as the Tegsedi is a solution not co-administered with any other product.

The finished product is supplied in a Type 1 glass prefilled syringe (PFS) with a staked needle and needle shield assembled into a Safety Syringe Device (SSD). The SSD consists of a needle guard subassembly with extended finger flange and plunger rod. Relevant extractables and leachables studies have been performed. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. Suitability of the PFS is demonstrated by container closure integrity testing and stability results. The SSD was developed according to industry standards and guidance documents pertaining to medical devices and combination products.

Manufacture of the product and process controls

The manufacturing process consists of five main steps: active substance equilibration, formulation and mixing, bioburden reduction filtration, sterilizing filtration, and filling and stoppering. This is followed by safety syringe device assembly and inspection. The in-process controls are adequate for this type of manufacturing process / pharmaceutical form.

Due to the limited finished product manufacturing history the Applicant committed to evaluate the unfiltered bulk bioburden results for the first 10 finished product lots with the intent of establishing a tightened acceptance criterion (see List of Recommendations). The current acceptance criterion is accepted because the crucial limit of the bulk solution is subsequently controlled as an in-process control prior to the sterile filtration step.

The process is considered to be a non-standard manufacturing process. During the initial assessment, as only a process validation protocol, media fill and filter validation results was provided, the absence of full scale process validation data was raised as a major objection. In response, validation results for three consecutive commercial size batches comprising all unit operations and hold times were provided. The process qualification batches met all requirements outlined in the validation protocol and all specification limits demonstrating that the manufacturing process of inotersen finished product is under control. 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 specifications shown in Tables 5 & 6 include appropriate tests for this kind of dosage form; description, identification (by accurate mass measurement by MS and by duplex melting temperature), inotersen content and product related impurities (IP-HPLC-UV-MS), deliverable volume (Ph. Eur.), uniformity of dosage units (Ph. Eur.), particulate matter (Ph. Eur.), pH (Ph. Eur.), osmolality (USP), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), container closure integrity (validated dye ingress) and syringe performance testing (ISO).

The acceptance criteria for product related impurities were tightened during the procedure. A justification for not including a biological activity test was accepted. The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for batches manufactured in the prefilled syringe (PFS) configuration and used as part of stability studies and/or clinical trials. The batch analysis results

confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data was provided from 3 batches of finished product stored for up to 12 months under long term conditions (2-8°C) and 6 months under accelerated conditions (30°C/75%RH) according to the ICH guidelines were provided. These batches of Tegsedi are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing; type I glass pre-filled syringes with safety device and thermoform tray.

Supportive stability data was provided for 3 batches of finished product stored for up to 12 months under long term conditions (2-8°C) and 6 months under accelerated conditions (30°C/75%RH) in pre-filled syringes without safety device and thermoform tray; 3 batches of finished product stored for up to 18 months under long term conditions (2-8°C) and 6 months under accelerated conditions (30°C/75%RH) in pre-filled syringes where a different finished product manufacturing process and different active substance manufacturer were used and 2 batches of finished product stored for up to 36 and 60 months under long term conditions (2-8°C) and 6 months under accelerated conditions (30°C/75%RH) in glass vials.

Samples were tested in line with the shelf life specifications. The analytical procedures used were stability indicating. In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products.

No significant change in any attribute has been observed during the stability studies of the pre-filled syringes and the vials under long-term or accelerated storage conditions. The finished product is susceptible to light exposure (degradation observed) which is reflected in the SmPC with the advice "Store in the original package in order to protect from light".

Based on available stability data, the proposed shelf-life of 18 months and *"Store in a refrigerator (2 °C – 8 °C); Do not freeze; Store in the original package in order to protect from light"* as stated in the SmPC (section 6.3) are acceptable.

The statement in the SmPC "*Inotersen-Ionis may be stored unrefrigerated for up to 6 weeks at a temperature not above 30°C. If not used within 6 weeks, it should be discarded."* is considered acceptable based on the provided data.

Adventitious agents

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

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The 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. The applicant has applied QbD principles in the development of the active substance and finished product and their manufacturing process. Design spaces have been proposed for several steps in the manufacture of the active substance. The design spaces have been adequately verified.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.2.6. Recommendation(s) for future quality development

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

1. The applicant should submit a variation to update the specifications after additional drug substance batches are manufactured so that the proposed specifications are based on data from a total of 12 batches manufactured with the current process.
2. Due to the limited drug product manufacturing history, the Applicant commits to evaluate the unfiltered bulk bioburden results for the first 10 drug product lots with the intent of establishing a tightened acceptance criterion.

2.3. Non-clinical aspects

2.3.1. Introduction

The nonclinical testing strategy for inotersen followed a development pathway typical for a new chemical entity (Olejniczak et al. 2001) rather than the developmental programme more recently recommended for oligonucleotide drugs.

The doses selected for the nonclinical pharmacodynamic studies were intended to evaluate a full range of dose-response activity. Inotersen is 100% complementary to the human and Cynomolgus monkey TTR mRNA and, hence, exhibits pharmacological activity in humans and monkeys. However, the inotersen sequence contains several mismatches to the TTR mRNA of rodents and rabbits, which preclude its pharmacological activity in these species. For this reason, mouse- and rat-specific analogues of inotersen were included in the toxicology evaluations in order to distinguish toxicity associated with reduction of the target (TTR) from toxicities related to the chemistry of the oligonucleotide.

The pharmacologic evaluation of inotersen included demonstration of sequence-specific reduction of human TTR *in vitro* and in monkeys as well as in transgenic mice expressing mutant human TTR *in vivo*. The predicted absence of off-target RNA suppression was confirmed *in vitro* . Safety pharmacology studies using inotersen were conducted to evaluate the potential effects of inotersen on cardiovascular, respiratory, and central nervous system (CNS) function.

Studies were also performed to characterize the absorption, tissue distribution, metabolism and elimination of inotersen. The pharmacokinetic assessment included a complete characterization of plasma and tissue pharmacokinetics following single and repeated SC injections. The bioanalytical methods used allowed for the sensitive detection of the plasma levels as well as for the full characterization of tissue distribution, metabolism and clearance.

The comprehensive toxicology program of inotersen was conducted in accordance with pertinent ICH guidelines and exceeded in part prevailing recommendations for oligonucleotide drugs (Cavagnaro

et al., 2014; Berman et al., 2016): 1) repeat dose studies for up to 26 weeks in mice and rats, and for up to 39 weeks in monkeys; 2) the "core battery" of safety pharmacology studies; 3) standard in vitro and in vivo genotoxicity studies; 4) reproductive and developmental studies in mice and rabbits; 5) carcinogenicity studies in Tg.rasH2 mice; and 6) an in vitro platelet activation study, in vivo antigenicity in mice and monkeys as well as immunotoxicity and impurity qualification studies in mice. The Cynomolgus monkey is regarded as the most relevant nonclinical species for evaluation of inotersen, because inotersen is pharmacologically active in this species and elicited toxicities that are largely known for the class of oligonucleotide drugs containing the same backbone chemistry with stabilising modifications.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Inotersen was identified in a screen of more than 400 candidate ASOs that had been designed to selectively inhibit translation of human TTR mRNA. Inotersen binds to the 3'-UTR of human TTR mRNA.

The specificity of inotersen was confirmed *in silico*, which revealed 100 % complementary to the cognate TTR mRNA of humans and monkeys, whereas the analogous sequence contains 8 mismatches in mice and rats and 4 mismatches in rabbits that preclude efficient formation of a thermodynamically stable nucleic acid hybrid complex. Therefore, inotersen is only pharmacodynamically active in humans and monkeys.

Accordingly, inotersen concentration-dependently inhibited TTR mRNA translation in human HepG2 cells, in primary hepatocytes of transgenic mice overexpressing the mutated I84S variant of the human TTR gene (I84S transgenic mice) and in primary hepatocytes of monkeys.

In vivo, twice weekly s.c. injections of 10 to 100 mg/kg inotersen for four weeks dose-dependently reduced the levels of human TTR mRNA in liver and circulating TTR protein in plasma of I84S transgenic mice. TTR mRNA and protein reductions were maximal (≥70 %) between two to four days post dosing and persisted for 1 to 2 weeks before gradual recovery to pre-treatment levels. The ED_{50} values for human TTR mRNA and protein reductions in I84S transgenic mice were about 16.6 and 22.7 mg/kg/week and were reverse proportional to hepatic inotersen EC_{50} values of 132 and 184 µg/g, respectively.

Likewise, s.c. injections of 25 mg/kg inotersen for 12 weeks significantly inhibited hepatic TTR mRNA and TTR protein in plasma of monkeys by 78 % and 76 % in a time-dependent-manner. Concomitantly, the plasma level of Retinol Binding Protein 4 (RBP4), which is normally prevented from renal elimination by complexing with TTR, was time-dependently reduced in the monkeys by approximately 60 %. However, no significant effects on thyroid hormone levels and the histology of thyroid and retinol-dependent tissues were detected in these animals, albeit the TTR carrier protein normally transports about 15 % of thyroxine.

Secondary pharmacodynamic studies

Secondary pharmacology studies have not been conducted with inotersen. ASOs are predicted to be highly selective to their target sequences and secondary off-target actions are expected to be minimal as demonstrated by an in silico analysis conducted as part of the design of inotersen. In addition, inotersen proved not to suppress any potential off-target transcript with contiguous matches

≥10 nucleotides in vitro and no unexpected off-target pharmacologic effects were noted in the nonclinical toxicity studies.

Safety pharmacology programme

In safety pharmacology studies, inotersen did not inhibit hERG currents *in vitro* up to 300 µM and did not affect cardiovascular or respiratory function of telemetered monkeys following s.c. injections up to 40 mg/kg. Moreover, inotersen did not change neurobehavioural parameters in a modified Irwin test in mice after s.c. doses of up to 300 mg/kg corresponding to a maximum inotersen liver concentration of 175 µg/g.

Pharmacodynamic drug interactions

No pharmacodynamic studies have been performed to evaluate possible interactions of inotersen with other drugs that may be co-administered. Since inotersen inhibits the translation of the TTR mRNA by promoting its degradation with high selectivity, the likelihood of pharmacological action on other transcripts is very small.

As such, the probability of pharmacological drug-drug interactions in ATTR patients receiving inotersen in combination with other co-medications is very low due to the specificity of mode of action of inotersen.

2.3.3. Pharmacokinetics

The pharmacokinetic properties were investigated after single s.c. injection of a ^{3}H-labelled inotersen analogue in rats as well as following repeated s.c. administration of inotersen for 6 weeks in mice. These *in vivo* data were complemented with inotersen determinations in plasma, urine and tissue samples collected in repeated-dose toxicity, carcinogenicity, reproductive and developmental toxicity studies in mice, rats, rabbits and monkeys. Part of these analyses also included evaluations of the metabolic profile of inotersen.

Inotersen was readily absorbed after s.c. injection showing high plasma protein binding >94 % in monkeys and humans and dose-dependently increased plasma exposures (C_{max}, $AUC_{0\text{-}48h}$) with t_{max} of 0.5 to 2 h in rodents and up to 6 h in monkeys. Upon multiple dosing, no accumulation in plasma was observed, but inotersen rapidly and widely distributed from plasma into tissues as indicated by the rather short plasma $MRT_{0\text{-}48h}$ values of 2.1 to 4 h in mice, 3.23 to 8.01 h in rats and 4 to 9 h in monkeys. High concentrations of inotersen were detected in kidneys, liver, mesenteric lymph nodes, injection site and bone marrow.

However, anti-inotersen antibodies (ADAs) developed in some monkeys of the 39 weeks toxicity study, which increased the median plasma trough concentrations of inotersen at approximately 3 months or beyond from ~2- to 12-fold compared to those monkeys that did not develop antibodies (see section 3.2.3 below). Of note, plasma exposure and elimination half-life of inotersen were not altered and efficacy or toxicity parameters in chronic toxicity studies remained unaffected. This is attributed to the two-compartmental pharmacokinetics of inotersen due to rapid clearance from plasma and distribution into tissues and the limited binding capacity of ADAs (picomolar range) relative to plasma albumin or tissue proteins.

In kidney and liver, inotersen concentrations remained largely comparable after subchronic and chronic treatment of mice and monkeys indicating that steady-state levels were achieved within 13 weeks of dosing. No differences between genders were confirmed in mice, rats or monkeys.

Compared to maternal liver concentrations of inotersen in pregnant mice and rabbits, <10 % were determined in the placenta and the inotersen levels in the milk of mice were at least >660-fold lower. Given this minimal milk transfer and the generally low oral bioavailability of 2'-MOE ASOs, the systemic exposure of nursing pups appears negligible. Inotersen was not detectable in foetal livers, which can be attributed to its macromolecular size and hydrophilicity that presumably interferes with efficient permeation across the placenta.

Full length inotersen constituted <70 to 92 % of total oligonucleotides in kidneys and liver of mice, rats and monkeys compared to <10 % of metabolites of shorter chain length. Inotersen persisted in kidneys and liver until the end of the recovery periods of toxicity studies in mice, rats and monkeys suggesting slow tissue clearance. The estimated tissue half-lives of inotersen in kidney and liver consistently ranged from 13.5 to 19.3 days in mice and monkeys, whereas long terminal elimination half-lives of 17 to 29.7 days were determined in plasma of monkeys. Accordingly, unchanged inotersen was the major circulating component in plasma of mice, monkeys and humans accounting for >74 % of total oligonucleotides, whereas 13 metabolites of shorter chain length (mainly 3'-deletion metabolites) comprised ≤7 %.

The elimination of inotersen was delayed indicating slow metabolism of the ASO within tissues. Approximately 13 % of the s.c. injected radiolabelled dose was determined within 24 h post-dosing in the excreta of the mass-balance study in rats. Nevertheless, more than 90% of the originally administered radioactivity was recovered in excreta and tissues/carcass in the following 55 days. The majority of inotersen was excreted by the renal route (45.2 %).

Inotersen concentrations up to 100 µM did neither induce, nor interfere with the mRNA level or enzymatic activity of various members of the cytochrome P450 family in primary human hepatocytes. Inotersen also did not significantly inhibit or serve as a substrate of human drug transporters in MDCK-II cells.

2.3.4. Toxicology

Single dose toxicity

In accordance with the current guidance on single-dose toxicity (EMA/CHMP/SWP/81714/2010), no specific single-dose/acute toxicity studies were conducted for inotersen.

Repeat dose toxicity

The toxicity of repeated s.c. injections of inotersen was investigated for up to 26 weeks in mice and rats and for up to 39 weeks in monkeys using comparable drug substance as tested in the clinical program. Minimal safety margins with regard to the proposed therapeutic exposure are derived from these repeat-dose toxicity studies. As mismatches in the inotersen sequence preclude effective TTR mRNA translational inhibition in rodents and rabbits, mouse- (ISIS 401724) and rat-specific analogues (ISIS 594799) were additionally tested to discriminate between toxicities related to TTR inhibition from non-specific effects associated with the ASO administration.

The pharmacodynamic activity of inotersen was confirmed in monkeys by dose-dependent reductions of hepatic TTR mRNA (≤70 %) and of TTR and RBP4 protein in plasma (≤61 % and ≤56 %, respectively). Similarly, ISIS 401724 reduced TTR mRNA levels by up to 70 % in liver of mice, whereas ISIS 594799 reduced hepatic TTR protein levels by up to 34 % in rats.

Pro-inflammatory effects

Inotersen dose-dependently accumulated as cytoplasmic basophilic vacuolated granules within monocytes/macrophages throughout multiple organs of all animal species, including proximal tubular kidney epithelia, hypertrophied Kupffer cells of the liver, histiocytic infiltrates of lymph nodes and injection sites. In addition, lymphocyte depletion resulted in reduced thymus weights in mice and rats. Moreover, sporadic minimal to moderate mixed cell perivascular infiltration of multiple organs (liver, kidney, gallbladder, intestine and reproductive tract) with neutrophils, macrophages and lymphocytes was observed in monkeys. These pro-inflammatory effects in inotersen-treated animals were accompanied by decreased albumin levels, decreased albumin/globulin ratio and inter-individually variable increases of different inflammatory cytokines and chemokines.

Kidney toxicity

The kidneys constitute a target organ of toxicity, because inotersen mainly accumulates in this tissue and is excreted by the renal route. The kidney changes were more severe in rats than in other species and comprised increased glomerular cellularity, minimal to moderate increases in glomerular matrix and occasionally adhesion of the glomerular tuft to the Bowman's capsule (synechia formation). In monkeys, renal findings were limited to the 40 mg/kg/week high dose inotersen level of the 13 weeks subchronic toxicity study and involved mild multifocal tubular epithelial degeneration/regeneration, renal tubular dilatation, erythrocytic tubular casts, fibroconnective tissue proliferation and interstitial haemorrhage. In both rats and monkeys, interstitial mononuclear cell infiltration and signs of proteinuria were additionally apparent. No comparable kidney alterations were noted after subchronic or chronic dosing of mice.

Liver abnormalities

As inotersen also accumulates in the liver, adverse effects were expected. Mononuclear cell infiltrates were detected in the liver of inotersen-treated mice and rats, whereas the increased liver weights of rats were associated with sinusoidal dilatation and granular macrophages, bile duct hyperplasia, individual hepatocellular necrosis and oval cell hyperplasia. Furthermore, minor elevations of AST and ALT in mice (1.9- and 3-fold each) and of ALT in monkeys (<2-fold) were determined.

Platelet declines and thrombocytopenia

Inotersen clearly reduced platelet counts in chronic toxicity studies in mice, rats and monkeys. Of note, platelet reductions were not observed after treatment with the mouse- and rat-specific ASO analogues ISIS 401724 and ISIS 594799 indicating that the declines were not related to TTR inhibition.

Platelet decreases were moderate (-36 to -41%) at inotersen doses ≥40 mg/kg/week in mice, while in rats, mild reductions were determined at ≥15 mg/kg/week inotersen with compensatory increased megakaryopoiesis. In monkeys, platelet counts were reduced after chronic administration in one male monkey of the 3 mg/kg/week low dose group, 1 male and 1 female injected with 6 mg/kg/week and in 1 female of the 10 mg/kg/week group. Severe thrombocytopenia (platelet counts $<10x10^3$/µl; ~90 % platelet decrease from baseline) was evident between 11 and 13 weeks in two male monkeys administered 10 and 20 mg/kg/week inotersen, respectively. Platelet counts recovered when the dosing of the high dose animal was temporarily suspended, but dropped again to even lower levels

when the treatment was resumed. In view of bruising and petechiae, both monkeys were prematurely euthanized.

These platelet decreases were roughly dose-dependent with increased severity at inotersen dosages ≥10 mg/kg/week in monkeys, showed inter-individual variability and obviously required time to develop, which is consistent with the onset of thrombocytopenia in the clinical program of inotersen (see clinical section). No cytotoxicity was noted up to the highest dosages in the bone marrow or other haematopoietic centres. A direct influence of inotersen or TTR protein on platelet activation was also excluded. Likewise, the complement system is presumably not involved in the platelet decline, since it is usually non-specifically activated after ASO administration in monkeys (see below). The Applicant further confirmed that anti-inotersen antibodies did not affect the marked platelet decreases, because one of the prematurely sacrificed monkeys with severe thrombocytopenia was even negative for anti-inotersen antibodies, whereas several antibody-positive monkeys had normal platelet counts.

With respect to the lack of a clear dose-relationship and the considerable inter-individually variable platelet counts between monkeys of the same dose group, the Applicant hypothesises that the pro-inflammatory effects triggered by inotersen could induce the proliferation of pre-existing immunoglobulins that cross-react with platelets, hence accelerating platelet clearance.

For example, the positively charged chemokine platelet factor 4 (PF4), which is released during platelet activation, forms highly immunogenic complexes with many polyanions like heparin and nucleic acids and has been involved in heparin-induced thrombocytopenia. However, analysis of a subset of monkeys with decreased platelet counts revealed that anti-PF4 IgG antibodies were only elevated in two monkeys with platelet reductions from $400x10^3$/µl to either $124x10^3$/µl, or $141x10^3$/µl (monkeys of the 6 mg/kg/week group), but were not affected in the two prematurely sacrificed monkeys with severe thrombocytopenia (platelet count $<10x10^3$/µl). Instead, a combination of increased anti-platelet IgG and IgM together with anti-PF4 IgM antibodies were elevated in the two prematurely euthanized monkeys. Still, the analytic results of another monkey with the same antibody profile and only moderate platelet decreases (platelet counts $\sim230x10^3$/µl) suggests that antibodies against other platelet surface epitopes are additionally involved. This is also implicated by comparable antibody profiles in patients with thrombocytopenia or low platelet counts in the clinical Phase 2/3 and open-label extension studies (see clinical section below). Hence, induction of a combination of antibodies against several platelet antigens is apparently required to produce the observed individual cases of dramatic reductions in platelet counts.

Haematological alterations

Inotersen mildly reduced red blood cells in subchronic and chronic toxicity studies in mice, rats and monkeys. These signs of anaemia were obviously compensated by increased haematopoiesis in the spleen and bone marrow of mice and rats. Comparable effects were noted with the rat-specific analogue ISIS 594799. Inotersen was also found to activate the complement system in monkeys.

Genotoxicity and carcinogenicity

Inotersen lacked a genotoxic potential in a standard battery of *in vitro* and *in vivo* tests and shows negligible risk for triplex formation with genomic DNA. No carcinogenic potential was identified in a short-term carcinogenicity study in transgenic rasH2 mice. A 2 year carcinogenicity study in rats is currently ongoing and results will be submitted post approval.

Reproduction and developmental toxicity

The reproduction toxicity of inotersen was investigated in a combined fertility and embryonic development study as well as in a pre-/postnatal development study in mice. Effects on embryo-foetal development were also tested in rabbits.

Inotersen did not affect fertility, embryonic or pre- and postnatal development in mice and rabbits, respectively. This is consistent with the low placental and foetal liver inotersen exposure. The decreased foetal weights in rabbits at the inotersen high dose level were attributable to maternal toxicity. The mouse-specific ASO analogue ISIS 401724 did not evoke clinical pathology, reproductive or embryo-foetal developmental changes despite reductions of TTR mRNA levels in maternal liver by ~60 %.

TTR plays an important role as carrier of retinol, which is crucial for normal embryonic development, because deficiency as well as excess in retinol may result in teratogenicity. Moreover, TTR is synthesised, secreted and taken up by human placenta and influences human receptivity and normal pregnancy.

Local Tolerance

The local tolerability of the inotersen formulation was evaluated in repeat-dose toxicity studies in accordance with ICH M3(R2) recommendations (EMA/CPMP/ICH/286/1995). Accumulation of macrophages containing basophilic granules and mononuclear cell infiltrates were found in all species. Chronic inotersen administration induced occasionally minimal to mild subcutaneous fibrosis and/or oedema in rats or infrequent minor irritation and/or bruises, scabs, crusts in monkeys. Other findings were attributable to the s.c. injection procedure.

Other toxicity studies

Antigenicity/immunotoxicity

The development of anti-inotersen antibodies was investigated in the chronic toxicity studies in mice and monkeys. Except one mouse of the 3 mg/kg inotersen low dose group and one control, no anti-inotersen antibodies were detected in mice. In contrast, highly variable titres of anti-inotersen antibodies developed across all dose groups in 28.6 to 50 % of monkeys with a median onset of 185 days. The TTR levels did not return to normal at the end of the recovery period in the 13 weeks repeat dose toxicity study, which contrasts the reversibility confirmed in the 39 weeks chronic toxicity study. This apparent difference is explained by different dose levels used in these two studies and consequent difference in clearance of inotersen from the liver. Although there was a trend towards lower liver TTR mRNA levels in the ADA+ animals compared with the ADA- animals after a 6 months recovery period, the available nonclinical data do not suggest that immunogenicity would have a significant impact on safety or efficacy.

Inotersen or its mouse-specific analogue ISIS 401724 did not induce immunosuppression or immunotoxicity in the influenza host-resistance model at any dose level.

Impurities

Mixtures of starting material-derived impurities, or impurities arising from side or incomplete reactions during drug substance production were qualified in a 13 week repeated-dose toxicity study in mice.

2.3.5. Ecotoxicity/environmental risk assessment

The Applicant has provided a Phase I Environmental Risk Assessment in accordance with the pertinent guideline (EMEA/CHMP/SWP/4447/00 corr.21*) including an assessment for Persistence, Bioaccumulation and Toxicity as well as a Calculation of Market Penetration (F_{pen}) and Predicted Environmental Concentration (PEC). The $PEC_{SURFACEWATER}$ is below the action limit of 0.01 µg/l. The $LogK_{ow}$ value was experimentally determined for inotersen sodium using a validated shake-flask method. Inotersen sodium is not likely to be highly lipophilic, as demonstrated by the log K_{OW} values of -1.57, -1.58, and -1.58 in pH 5, 7, and 9 solutions, respectively. Therefore, it does not meet criteria for additional testing and evaluation for persistence, bioaccumulation and toxicity (PBT).

Table 1. Summary of main study results

Substance (INN/Invented Name): Inotersen			
CAS-number : 1492984-65-2 (free base), 1432726-13-0 (sodium)			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log *K*ow	OECD107	pH 5: -1.57 pH 7: -1.58 pH 9: -1.58	Potential PBT (N)
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log *K*ow	pH 5: -1.57 pH 7: -1.58 pH 9: -1.58	not B
	BCF	n/a	n/a
Persistence	DT50 or ready biodegradability	n/a	n/a
Toxicity	NOEC or CMR	n/a	n/a
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)	0.0021	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)	n/a	n/a	(N)

2.3.6. Discussion on non-clinical aspects

Inotersen was specifically designed to bind to the 3'-UTR of human TTR mRNA, which lacks widespread polymorphisms and appears free of point mutations in humans. Therefore, inotersen can be reasonably expected to inhibit the expression of wildtype and all presently known mutant variants of the TTR

gene. The target sequence of inotersen is conserved between monkeys and humans, but contains 8 mismatches in rodents and 4 mismatches in rabbits. Inotersen is therefore not pharmacologically active in these species. This has been previously accepted during scientific advice given by the CHMP as rationale for inclusion of non-human primates in the toxicology program. Nevertheless, an early analysis of the sequence homology in further animal species would have been desirable to evaluate if other non-rodent species could have served to replace the use of monkeys in non-clinical development (see European Dir. 2010/63/EU).

"Proof-of-concept" of the pharmacodynamic activity of inotersen was adequately demonstrated by the inhibition of TTR mRNA translation in hepatic cells of humans and monkeys *in vitro* as well as in monkeys and in transgenic mice overexpressing a mutated human TTR variant *in vivo*. The reduction of TTR protein in monkeys was associated with about 60 % lower levels of RBP4 that is normally prevented from renal clearance by complexing with TTR. Thus, inotersen treatment is expected to reduce the transport of vitamin A. However, no signs of vitamin A deficiency were observed after long-term administration of inotersen or its mouse- and rat-specific ASO analogues.

The available nonclinical data indicate that the presence of ADAs does not seem to have a significant impact on the TTR mRNA levels and consequently on the safety or efficacy of the product.

Repeated inotersen dosing did not significantly alter the levels of thyroid-stimulating hormone, thyroxine and triiodothyronine as well as the histology of thyroid-dependent tissues in monkeys after 12 weeks. This might be ascribed to the minimal transport of total thyroxine by TTR (~15 %).

The lack of relevant effects of inotersen in safety pharmacology studies of cardiac, respiratory and CNS function coincides with published observations of seven other 2'-MOE phosphorothioate ASOs in animals (Kim *et al*., 2014) and the absence of an arrhythmogenic potential reported for 8 ASOs in clinical trials (Rabinovich-Guilatt *et al.*, 2015; Yu *et al.*, 2017). The large molecular size of ASOs most probably precludes significant inhibition of cardiac ion channels or permeation of the blood-brain-barrier.

Inotersen did not suppress the expression of any *"off-target"* transcripts with contiguous matching of ≥10 nucleotides *in vitro*. As non-target mRNAs additionally need to be transcribed in a specific site- and time-dependent manner and must be also critical for cellular function, the manifestation of potential *"off-target"* effects appears unlikely. In view of the specific mechanism of action of inotersen, the CHMP agrees that secondary pharmacodynamic investigations and pharmacodynamic drug interaction studies are not required. However, inotersen potentially decreases platelet counts, which may culminate in thrombocytopenia. For this reason, anticoagulant agents or drugs that lower platelet levels should be used with caution in hATTR patients after administration of inotersen as reflected in section 4.4 of the approved SmPC.

Consistent with experience gained with other 2'-MOE phosphorothioate ASOs (Yu *et al*., 2007; Geary, 2009), subcutaneously administered inotersen was found to be rapidly absorbed with subsequent wide distribution from plasma into tissues. Thereafter, slow metabolism of inotersen was noted, which involves initial endonucleolytic cleavage between the central 2'-deoxy nucleotides followed by exonuclease-mediated degradation of the unprotected termini of the fragments. As metabolites of shorter chain length do no longer significantly interact with proteins, they are quickly eliminated, primarily via urine.

The rapid clearance of inotersen from plasma following subcutaneous administration and its distribution into tissues, the limited binding capacity of ADAs relative to plasma albumin or tissue proteins as well as constant intracellular nuclease-mediated metabolism provide the rationale for unchanged plasma pharmacokinetic parameters in 39-week toxicity study in Cynomolgus monkeys.

In long-term toxicity studies for up to 26 weeks in mice and rats and up to 39 weeks in monkeys, the observed toxicities of inotersen have been also described for other phosphorothioate ASOs and were predominantly related to its backbone chemistry, its extensive tissue distribution and its enhanced stability against enzymatic degradation. These toxicities comprise kidney, liver and red blood cell alterations across species as well as perivascular inflammation of multiple organs and complement activation in monkeys and, hence, constitute typical class-effects of treatment with phosphorothioate ASOs. A contribution of sequence-specific TTR inhibition to these toxicities was reliably excluded by testing of mouse- and rat-specific analogues in parallel to inotersen in mice and rats. These findings are appropriately addressed through precautions for monitoring of renal and hepatic function which are included in section 4.4 of the approved SmPC. Likewise, and supported by clinical findings anaemia is listed as very common adverse event of inotersen therapy in section 4.8 of the approved SmPC.

The potential for complement system activation appears to predominate in monkeys, because the binding of ASOs to complement factor H (CFH) has been demonstrated, which releases the inhibition of constitutive activation of the alternative complement pathway (Henry *et al*., 2014). Monkeys are more sensitive than humans to this stimulation because of the ~3-fold higher inhibitory capacity of 2'-MOE ASOs to monkey CFH compared to the CFH of other species (Shen *et al*., 2014). As the inotersen exposure was at least 3-fold higher in monkeys than in patients at the recommended therapeutic dose, the CHMP considers that the potential for complement system activation in humans is minor.

The most prominent toxicity of inotersen is the reduction of platelet counts in mice, rats and monkeys over time, which was not observed with the mouse- and rat-specific ASO analogues. The platelet decreases were apparently mild to moderate in mice and rats, but were pronounced in some of the monkeys of the chronic toxicity study. Therefore, two monkeys had to be prematurely sacrificed, because their platelet counts dropped to even lower levels when inotersen administration was resumed following temporary treatment interruption. Platelet reductions with sporadic deterioration resulting in severe thrombocytopenia were similarly evident among members of a large set of 102 systemically administered 2'-MOE ASOs in non-human primates (Henry *et al*., 2017). In view of the variability of the effect across species and even between monkeys of the same dose group, it is assumed that inotersen could augment pre-existing immunoglobulins that interact with platelets to increase platelet clearance. In fact, the antibody profiles determined in a subset of monkeys with platelet declines suggest that inotersen induces various anti-platelet antibodies against several platelet-specific surface epitopes and a combination of these antibodies is apparently required to evoke the individual cases of severe thrombocytopenia. The clinical findings reinforced the observations that inotersen treatment is associated with reductions in platelet count, therefore specific recommendations for dose adjustments and platelet monitoring frequency have been included in section 4.2 of the approved SmPC.

Inotersen revealed no genotoxic potential in an ICH S2(R1) compliant standard battery of test (EMA/CHMP/ICH/126642/2008), which coincides with other phosphorothioate ASOs (Berman *et al.*, 2016; EMEA/CHMP/SWP/199726/2004). No risk for triplex formation was identified. Inotersen was also not carcinogenic in a short-term study in transgenic rasH2 mice. A 2 year carcinogenicity study in rats is currently ongoing and the Applicant proposed to submit the corresponding results by Q3 2018. In the absence of any signals observed in short term studies the CHMP found the proposal acceptable. The study is reflected as a category 3 study in the RMP and the requirement for the submission of the final study report is captured as well.

Inotersen did not impact on fertility, embryonic or pre- and postnatal development in mice and rabbits. However, only one dose level was investigated and dose-related effects, e.g. occurring with nearly 100 % reduction in TTR mRNA, could have been missed. Therefore, the CHMP considered that conducted animal studies do not allow reaching a definitive conclusion on reproductive toxicity of

inotersen. This conclusion is appropriately reflected in the approved SmPC section 4.6. In addition , TTR plays an important role in the transport of retinol, which is crucial for normal embryonic development. Except one dose group in mice, the pharmacological effects of inotersen were not captured in embryo-foetal development studies. As women of child-bearing potential will be receiving inotersen, adequate warnings for vitamin A supplementation and measures to prevent pregnancies in women of child-bearing potential are implemented in sections 4.4 and 4.6 of the SmPC and corresponding sections of the PL.

2.3.7. 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 Tegsedi in the treatment of stage 1 or Stage 2 polyneuropathy in adult patients with hereditary transthyretin amyloidosis (hATTR).
The CHMP furthermore concluded that Tegsedi was not expected to pose a risk to the environment.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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

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

- **Tabular overview of clinical studies**

Study Type and Number	**Study Description**	**PK, PD, or IM Assessments**
Phase 1 Study ISIS 420915-CS1	Phase 1 single and multiple ascending dose ranging study in healthy subjects	PK and PD
Phase 2/3 Study ISIS 420915-CS2	Phase 2/3 placebo-controlled study in patients with hATTR-PN	PK, PD and IM
Open Label Extension Study ISIS 420915-CS3	Phase 3 OLE for CS2 rollover patients	PK, PD and IM

2.4.2. Pharmacokinetics

The pharmacokinetics of inotersen were investigated in three clinical trials, ISIS 420915-CS1, ISIS 420915-CS2 and ISIS 420915-CS3. The bulk of pharmacokinetic data was generated in healthy

volunteers in the FIH-study (CS1) and in the pharmacokinetic subgroup in study ISIS 420915-CS2. In addition plasma trough levels of inotersen were measured in all patients in studies CS2 and CS3. The key pharmacokinetic parameters from study ISIS 420915-CS1 and the pharmacokinetic subset from study ISIS 420915-CS2 are presented in Table 1 below.

Table 1 Summary of Key Plasma Pharmacokinetic Parameters for Inotersen Following SC Administration(s)

Study/Day	Dose (mg)	N	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-24h} (µg*h/mL)	CL_{0-24h}/F (L/h)	$t_{1/2\lambda z}$ (day)
ISIS 420915-CS1							
Single Dose	50	3	0.416 (149)	4.00 (1.50-4.00)	3.98 (107)	12.5 (107)	NA
	100	3	2.26 (18.1)	1.50 (1.50-6.00)	21.1 (13.1)	4.74 (13.1)	NA
	200	3	4.98 (13.1)	4.00 (1.50-6.00)	50.6 (25.5)	3.95 (25.5)	NA
	400	3	6.83 (44.1)	3.97 (1.50-4.00)	82.4 (51.3)	4.86 (51.3)	NA
Multiple Dose Day 1	50	8	0.742 (36.1)	3.00 (1.50-8.00)	5.76 (22.5)	8.69 (22.5)	NA
	100	8	2.04 (34.4)	2.52 (2.00-4.00)	18.8 (29.9)	5.33 (29.9)	NA
	200	8	12.3 (16.7)	2.00 (1.50-2.00)	69.8 (46.1)	2.86 (46.1)	NA
	300	8	6.58 (43.6)	3.00 (3.00-8.00)	74.4 (41.4)	4.03 (41.4)	NA
	400	7	9.3 (63.3)	4.00 (2.00-6.00)	92.1 (62.1)	4.34 (62.1)	NA
Multiple Dose Day 22	50	8	1.06 (53.7)	3.00 (3.00-4.00)	6.43 (43.3)	7.78 (43.3)	21.5 (73.9)
	100	8	2.48 (53)	3.52 (1.50-4.00)	20.1 (32.7)	4.96 (32.7)	13.8 (25.6)
	200	7	6.27 (58.9)	4.00 (3.00-6.00)	56.2 (55.1)	3.56 (55.1)	17.5 (55)
	300	7	7.09 (42.3)	3.00 (1.50-4.00)	70.8 (31.5)	4.24 (31.5)	17.6 (29.1)
	400	6	7.74 (26)	3.98 (1.98-6.00)	86.6 (31.5)	4.62 (31.5)	16.7 (17.9)

ISIS 420915-CS2							
Day 1	300	10	10.1 (84.2)	3.18 (0.470, 8.03)	90.6 (48.5)	3.31 (48.5)	NA
Day 240	300	7	6.26 (38.8)	3 (2.00, 4.08)	74.3 (28.7)	4.04 (28.7)	NA
Day 449	300	8	7.83 (39.5)	3.77 (2.95, 6.00)	80.4 (68.8)	3.73 (68.8)	25.5 (45.1)[a]

Analytical methods

Inotersen quantification in plasma and urine

The bioanalytical methods employed for quantitation of inotersen levels included hybridization-based enzyme-linked immunosorbent assay (hybridization ELISA) methods for plasma and urine. All of the quantitative methods used were demonstrated to be specific and selective for detection of parent 20-mer oligonucleotide (inotersen). The hybridization ELISA method for human plasma used in Studies CS2 and CS3 includes a protein digestion procedure to eliminate potential interference of anti-drug antibodies (ADA) on the quantitation of inotersen.

The bioanalytical reports for the analytical methods used in each study as well as the method validation reports are provided by the Applicant.

Table 2 Summary of Bioanalytical Methods for Quantitation of Inotersen in Human Subjects

Ionis Report Number	**420915-MV04**	**420915-MV11**	**420915-MV13**	**420915-MV05**
Matrix:	Human K_2EDTA Plasma	Human K_2EDTA Plasma	Human K_2EDTA Plasma	Human Urine
Notes:	Original Assay	Including Proteinase K Treatment	Assay transfer from San Diego to New Jersey	Original Assay
Analyte:	Inotersen	Inotersen	Inotersen	Inotersen
Method of Detection:	Hybridization ELISA	Hybridization ELISA	Hybridization ELISA	Hybridization ELISA
Validation Samples:	1.00, 3.00, 60.0, 160, 200 and 20,000 ng/mL	1.00, 3.00, 60.0, 160, 200 and 20,000 ng/mL	1.00, 3.00, 60.0, 160, 200 and 20,000 ng/mL	1.00, 3.00, 50.0, 110, 150 and 15,000 ng/mL
Intra-Assay Accuracy (%Bias):	-37.6% to 8.5%[a]	-5.7% to 9.2%	-8.6% to 10.1%	-27.0% to 21.8%[a]
Intra-Assay Precision (%CV):	0.4% to 24.0%[a]	3.9% to 9.4%	5.9% to 16.4%	0.9% to 24.2%[a]

Ionis Report Number	**420915-MV04**	**420915-MV11**	**420915-MV13**	**420915-MV05**
Inter-Assay Accuracy (%Bias):	-18.5% to -4.7%	-7.8% to 4.1%	-6.4% to 16.0%	-12.3% to 6.7%
Inter-Assay Precision (%CV):	9.5% to 17.4%	4.2% to 8.1%	5.5% to 21.0%	10.2% to 13.6%
Quality Control Samples:	3.00 (LQC), 60.0 (MQC), 160 (HQC) ng/mL	3.00 (LQC), 60.0 (MQC), 160 (HQC) ng/mL	3.00 (LQC), 60.0 (MQC), 160 (HQC) ng/mL	3.00 (LQC), 50.0 (MQC), 110 (HQC) ng/mL
Intra-Assay Accuracy (%Bias):	-34.9% to 10.9%[a]	NC	NC	NC
Intra-Assay Precision (%CV):	0.4% to 24.0%[a]	NC	NC	NC
Inter-Assay Accuracy (%Bias):	-11.4% to -4.2%	-7.4% to 0.7%	-9.7% to 1.0%	-0.4 to 11.1%
Inter-Assay Precision (%CV):	10.8% to 16.7%	3.1% to 5.2%	4.5% to 8.4%	8.1% to 19.7%
LLOQ:	1.00 ng/mL	1.00 ng/mL	1.00 ng/mL	1.00 ng/mL
ULOQ:	200 ng/mL	200 ng/mL	200 ng/mL	150 ng/mL
Freeze/Thaw Stability:	8 Cycles	ND	ND	6 Cycles
Storage Stability in Matrix:	21 h 43 min at RT and 21 h 43 min at 5 °C	ND	ND	18 h 30 min at RT and 18 h 30 min at RT at 5 °C
Long-Term Stability in Matrix:	496 days at -70°C	ND	ND	450 days at -70°C

Range includes values not within target acceptance criteria (%Bias within ± 20% and %CV ≤ 20% [25% for LLOQ]) , as they were included in final statistics; however, overall validation acceptance criteria was met.
Abbreviations: CV = coefficient of variation; LTS = long term stability in frozen matrix at -70 °C ± 10 °C; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation; NC = not calculated; ND = not determined; RT = room temperature; LQC = low quality control; MQC = mid quality control; HQC = high quality control
Sources: Study Reports 420915-MV04; 420915-MV11; 420915-MV13; 420915-MV05

The methods are validated for linearity, intra- and inter-assay accuracy and precision, selectivity, specificity, dilution integrity, hook effect and stability. Incurred sample reanalysis were performed for plasma samples and the results met the current regulatory recommendations. However, in some cases the accuracy and precision exceeded the recommendations given in Guideline on Biochemical Validation. Despite not meeting the acceptance criteria for intra-assay accuracy and precision in some runs, the criteria were met when all runs were taken into account. This provided the reassurance that the overall study results were not affected. Matrix effects on haemolysed and hyperlipidaemic plasma

samples were not evaluated in the validation; nonetheless, hemolysis and lipemia are not expected to have an effect on the bioanalytical assay. This would be mainly due to the high specificity of the assay, patients with hATTR-PN are unlikely to have hyperlipidaemia and the minimal number of hemolyzed samples. It was also adequately justified why interference of co-medications with the determination of inotersen concentrations was not further examined. Due to the late appearance of ADAs, using the assay without the ADA migitation step within the Study CS1 is considered acceptable and the measured inotersen plasma concentrations can be considered valid.

Determination of anti-drug antibodies

A validated ELISA method was used to determine the presence and level of anti-inotersen antibodies in human plasma by a 3-tiered approach: (1) screen assay, which identifies initial positive or negative samples, (2) confirmation assay, which assesses specificity of the positive screen samples by free drug competition, and (3) titration assay, which estimates the level of antibody for the confirmed positive samples. Assessed validation parameters included intra-assay and inter-assay precision, hook effect, drug tolerance, matrix selectivity, effect of haemolysis on matrix selectivity, specificity, stability, precision of titers, relative assay sensitivity, and established the screening cut point factor and confirmatory cut point. Inter-assay precision of 23.6 % was observed at LPC2 level; however, according to the Guideline on Biochemical Validation precision should not exceed 20 %. The slightly higher inter-assay precision at the LPC2 level is expected to have minimal impact on the study data because it was mainly driven by 2 samples that had higher OD than the remaining samples tested. Study samples having higher OD than expected, would be classified as screen positive and further evaluated in confirmation assay. This finding is not expected to have any impact on the overall study results.

Hyperlipidaemic plasma samples were not evaluated in method development or validation of the ADA assay; nonetheless, hyperlipidaemia is not anticipated for the patient population for the current submission (hATTR-PN).. The applicant justified based on the high specificity of the assay and the lack of signals in placebo treated patients, why the possible interference of co-medications on the determination of ADAs was not investigated. The Applicant was requested to show long-term stability of positive control under the storage conditions (-70 °C). . The applicant provided literature data which supported the stability of antibodies at temperatures $\leq$ 20 $^{\circ}$ C for 2 years or longer. Stability of the study samples has not been evaluated. Since the analytes of interest in both positive controls and study samples are mostly IgG, the stability of the positive control is expected to represent the stability of study samples. The neutralizing capacity of antibodies present in positive samples has not been tested. The applicant highlighted that anti-inotersen antibodies bind to inotersen in circulation and do not make it to the intracellular site of activity of inotersen. Therefore it is expected that they have no effect on inotersen pharmacological activity. This is further supported by the fact that neither the non-clinical data, nor the human PD and efficacy data indicate a loss of drug effect with the appearance of antibodies.

Bioanalytical assay for characterisation of inotersen metabolites

Inotersen metabolites in plasma and urine samples were identified and profiled in an exploratory manner using ion-pair HPLC-ES/MS in selected samples collected from Study ISIS 420915-CS1. Chromatographic separation of inotersen and its chain-shortened metabolites was followed by on-line mass measurements using a single-quadrupole mass spectrometer.

Determination of diflunisal concentration in human plasma

Diflunisal concentration in human EDTA K_3 plasma was determined using a validated HPLC/MS/MS method. Diflunisal was extracted from human EDTA K_3 plasma using an automated protein

precipitation procedure. Performance of the analytical method has been adequately demonstrated. The data on high and low concentrations of diflunisal demonstrate satisfactory stability of the analyte in human plasma for up to 665 days after storage at -20 °C.

Absorption

After subcutaneous injection inotersen is rapidly absorbed into the plasma. Peak plasma concentrations are typically reached within a few hours within both healthy volunteers and patients and plasma exposure increases dose-proportionally within the dose range from 100 mg to 400 mg with less than proportional increases in lower doses. In the multiple dose part of study CS1 similar median Cmax, Tmax and AUC were observed independent of treatment day. These results were comparable with data from hATTR-patients in study CS2, suggesting time-invariant PK.

Distribution

After reaching Cmax, plasma inotersen concentrations decline in a multi-phasic manner with an initial distribution half-life in hours and a terminal elimination half-life of 2 to 4 weeks in healthy subjects and approximately 1 month in patients with hATTR-PN. Plasma concentration decreases more than 90% compared to Cmax within 24 hours after subcutaneous injection. In in-vitro plasma protein binding studies in plasma from humans and cynomolgus monkeys inotersen showed high plasma protein binding of more than 94% independent of plasma concentration. Based on the population PK analysis the apparent volume of distribution in patients with hATTR was estimated to be 293 L, which is consistent with the drug being extensively distributed to tissue. Based on non-clinical drug-distribution studies and similarity of PK between monkeys and humans the main targets of distribution in humans are assumed to be kidney and liver.

Metabolism

Metabolites of inotersen were found in human plasma and urine and were produced by ubiquitous endonucleases and exonucleases and consisted of chain-shortened 6-mer to 12-mer oligonucleotide fragments. Intact inotersen is the most abundant detectable oligonucleotide in human plasma and comprises more than 74% of all oligonucleotides. Each detectable chain-shortened oligonucleotide comprised less than 7% of the total detectable amount.

Excretion

Due to being highly bound to plasma protein glomerular filtration of inotersen is minimal. Consistent with fast distribution to tissue and low renal excretion less than 1% of the administered dose can be found in urine within the first 24 hours. Chain-shortened oligonucleotides collected over 24 hours account for 13.5% of the administered dose. This is consistent with urine being the major excretion pathway for chain-shortened metabolites of inotersen.

Elimination

The elimination of inotersen appears to be a combination of metabolism through endo- and exonucleases and excretion of the metabolites through urine. This is the expected route of elimination for this chemical class of antisense oligonucleotides.

Dose proportionality and time dependencies

Inotersen showed dose proportionality within the clinically relevant dose range of 100 mg to 400 mg, however AUC and C_{max} increased greater than dose proportionally from 50 mg to 100 mg. Consistent with this, plasma clearance was independent from dose between 100 mg and 400 mg but by comparison 2-fold higher for the 50 mg dose. The half-life of inotersen in healthy volunteers was dose independent over the whole investigated dose range. No major differences in AUC and C_{max} were observed over the treatment duration, which is consistent with no plasma accumulation of inotersen and time-invariant pharmacokinetics.

Special populations

So far the pharmacokinetics of inotersen in special populations has not been investigated in a dedicated study. Due to the small sample size and high variability of data conclusions from the population pharmacokinetics remain limited and influences of several factors remain a possibility. Clinical significance however appears unlikely and overall the data support a flat dosing regimen without adjustments for special populations.

While age has not been identified as a significant covariate in the population model based on analysis between patients of ≤ 65 years of age and ≥ 65 years data about the amount of patients included in the age groups ≥ 65 years (65-75years, 75-85 years, above 85 years) was presented during the evaluation procedure (see Table 3). PK parameters of inotersen in three different age subgroups have been also presented. No major difference was observed in PK parameters of inotersen between the age groups although clearance was slightly lower and AUC higher in patients over 75 years of age.

Table 3

	Age <65 (Older subjects number /total number)	**Age 65-74 (Older subjects number /total number)**	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
PK Trials	85	53	13	0

Population PK analysis and intra- and inter-individual variability

Population PK analysis was performed to characterize the PK of inotersen in healthy subjects and in patients with hATTR. Pooled plasma concentration time data and other demographic data from studies CS1, CS2, and CS3 were used to identify the intrinsic and extrinsic factors associated with the inter-individual variability in the PK of inotersen.

Overall the developed population PK-model seems to adequately describe the observed data. The influence of several covariates on the PK parameters however could not be definitely assessed due to high variability and limited amount of data.

Data collected after the onset of immunogenicity were excluded from the population PK analysis. 452 records, or 12.5% of the plasma concentrations, which occurred after the formation of anti-drug antibodies were excluded from the analysis. The antibodies have no effect on inotersen pharmacological activity but trough concentrations of inotersen were elevated. Leaving these elevated trough/ADA samples and immunogenicity out of the final model has been justified. The lack of incorporation of Cl/F from the peripheral compartment has also been adequately explained.

Pharmacokinetics in target population

The effect of disease status on PK seems to be negligible. While the bulk of pharmacokinetic data were created in healthy volunteers they seem to also adequately describe PK in patients.

Pharmacokinetic interaction studies

The potential of inotersen for cytochrome P450 inhibition and induction as well as the potential to act as a substrate or inhibitor of major human drug transporters were investigated in in-vitro studies. The results showed the lack of potential for inotersen to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 as well as the lack of potential for inotersen to induce CYP1A2, CYP2B6, or CYP3A4. Inotersen is also not a substrate or inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, BSEP, OCT1, OCT2, OAT1, or OAT3. These are the expected results for the chemical class of antisense oligonucleotides. Consequently no clinical drug-drug-interaction studies were performed and the in-vitro studies are considered sufficient evidence that no interactions are to be expected at clinically relevant concentrations.

Commonly used concomitant medications in studies CS2 and CS3 included diuretics (30.5%), antithrombotic (36.4%), and non-NSAID analgesics (83.4%) in patients with hATTR-PN. As requested the Applicant has provided lists of specific diuretics, antithrombotics and non-NSAID analgesics and their frequencies taken during the studies. Several different agents have been taken during the studies but the amount of patients taking these different agents was small (<5 patients).

Additionally transthyretin and cytochrome P450s share common transcriptional regulatory pathways (such as Hepatic nuclear factors). Transthyretin (TTR) is a liver produced plasma protein, which functions as a carrier protein for thyroxine and vitamin A. There is no evidence that TTR regulates the expression of genes at the transcriptional level. Although TTR and cytochrome P450s are regulated by common transcriptional regulatory pathways such as the hepatic nuclear factors (HNFs), TTR itself does not have any reported role in regulating the expression of HNFs or cytochrome P450s (Buxbaum 2009; Lau 2017). Therefore, it is not expected that changes in TTR levels after administration of inotersen would impact CYP levels or any co-medications that are metabolically influenced by P450s.

Effects of anti-drug antibodies (ADAs) on PK

In study CS2 34 out of 112 patients (30.4%) developed anti-drug antibodies following chronic treatment with inotersen. Inotersen concentration-time profiles were similar between ADA-positive and ADA-negative subjects on all examined days showing minimal effect on C_{max} and AUC. Beginning at later time points plasma C_{trough} levels were substantially higher for ADA-positive subjects. Similar observations were made in study CS3 for subjects that developed ADAs after switching to inotersen from placebo. ADAs were not associated with loss of efficacy and did not have any major effects on the clinical safety of inotersen.

Although the presence of ADA does prevent an accurate estimation of drug accumulation over time, TTR level (PD) over time can indirectly reflect drug accumulation in the hepatocytes. Similar TTR reduction between ADA positive and ADA negative subjects suggested inotersen accumulation was not affected by the presence of ADA. According to clinical studies no major differences in Cmax, AUC0-24, Clss//F were noticed between ADA positive and ADA negative patients at week 1, week 35 and week 65 in a subgroup in study CS2 but the amount of subjects was small in both groups (n=4 and n=6, respectively). However, plasma trough and post-treatment inotersen concentrations and ADA

data, as well as TTR levels, are being monitored in the open label-extension study CS3, with planned treatment up to 5 years, and the results will be reported once available.

2.4.3. Pharmacodynamics

Mechanism of action

The Applicant has conducted three clinical studies and investigated the pharmacodynamics of inotersen primarily in the first-in-human study ISIS 420915-CS1 with additional, supportive data generated in the Phase 2/3 study, ISIS 420915-CS2, and the open-label extension (OLE) study for patients rolled over from study CS2, ISIS 420915-CS3.

The primary pharmacodynamic biomarker has been the change of TTR protein in plasma compared to baseline, with RBP4 and retinol levels acting as additional markers. Reduction of TTR protein is considered the relevant effect of inotersen to produce clinical benefit for patients. Reduction of mutated TTR can also be achieved by orthotopic liver transplantation; however, wild-type TTR protein continues to be produced by the donor liver and can deposit in the pre-existing amyloid deposits in the tissues after transplantation. Total reduction of both, mutated and wild-type TTR protein, represents a novel therapeutic approach for treatment of hereditary transthyretin amyloidosis (hATTR). It is however not known what levels of TTR reduction are required to generate a clinical benefit for patients.

Primary and Secondary pharmacology

Primary pharmacology

In study ISIS 420915-CS1 a clear dose and time dependent pharmacodynamic effect was established. Levels of TTR, RBP4 and retinol all decreased in a similar fashion in the multiple dose cohorts as predicted by the non-clinical studies. No steady-state for inotersen tissue concentration was achieved. During the four week treatment duration TTR suppression levels in the 300 mg and 400 mg cohorts were very similar. Since only a minimal additional clinical benefit could be assumed for the 400 mg dose compared with the 300 mg dose, while at the same time the incidence rate for adverse events might increase, the sponsor chose the 300 mg dose for the further clinical development program in patients.

Plasma concentrations of TTR, RBP4 and retinol were slowly rising after the final dose; however they still remained significantly reduced at the end of the observation period (study day 92). This prolonged pharmacodynamic effect is best explained by slow elimination of inotersen from the tissue and congruent with the long elimination half-life of 2 to 4 weeks in healthy volunteers.

In study ISIS 430915-CS2 patients with hATTR were treated with 300 mg of inotersen weekly for 65 weeks. TTR levels decreased until week 13 and then remained at a constant level of suppression of about 70% compared to baseline with more than 80% of patients reaching a TTR suppression of more than 60% by that time. A similar time dependent effect was found for RBP4. Maximum levels of TTR suppression seem to be reached at about the time inotersen concentrations in the liver reach steady state. The 13 week mark seems to be in line with the long elimination half-life.

Further supportive data was generated in study ISIS 430915-CS3, the open-label extension to study CS2. Patients either continued treatment at the same dosing schedule they had in study CS2 or were switched to 300 mg inotersen weekly from placebo. In the inotersen-inotersen group, TTR suppression

levels remained comparable over the whole duration of the extension study, showing that the pharmacodynamic effect can be maintained over extended periods of time.

Patients in the placebo-inotersen group reached TTR suppression levels of over 70% by week 13. This is of note because it shows that similar pharmacodynamics effects can be achieved with and without the loading doses during week one of treatment. From a pharmacodynamic point of view, a once weekly treatment regimen without additional administrations of inotersen on days 3 and 5 of week 1 is therefore supported.

Overall, the pharmacodynamic endpoints investigated throughout the clinical development programme are consistent with the non-clinical data and show a pronounced and sustainable reduction of TTR protein over a treatment period of up to 3 years.

Secondary pharmacology

No studies were conducted to specifically assess secondary pharmacology. Inotersen is expected to be highly specific for transthyretin mRNA. No unexpected off-target effects were observed in the nonclinical toxicity studies. The adverse events in healthy volunteers and patients do not appear to be related to specific pharmacodynamic effects.

Pharmacodynamic interactions

Inotersen acts in a highly specific way by first binding to and subsequently degrading transthyretin mRNA which then leads to decreasing TTR levels. No other gene products should be affected. There are also no approved drugs that are known to have a TTR lowering effect. Therefore any pharmacodynamics interaction between inotersen and co-administered drugs appears unlikely.

Genetic differences in PD response

More than 130 known mutations for TTR are described in the scientific literature. Inotersen was designed to avoid hybridization to any known TTR mutation site. Therefore both mutated and wild-type TTR mRNA should be affected in the same manner and mutated TTR should be reduced independent of the specific mutation.

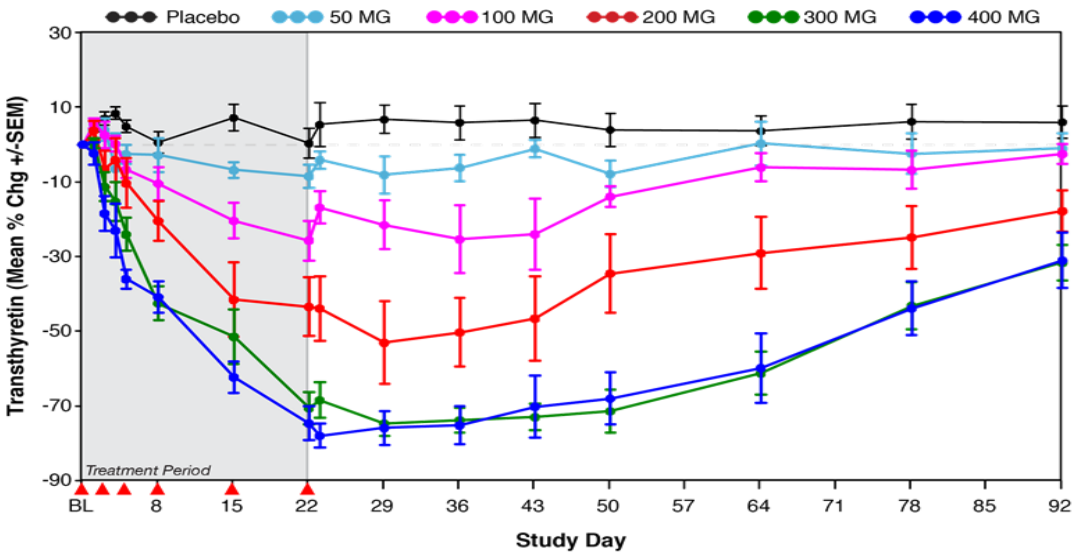
In study ISIS 420915-CS2 patients were stratified by their mutation status (V30M TTR mutation vs non-V30M TTR mutation). TTR and RBP4 showed similar suppression levels compared to baseline for subjects with V30M and non-V30M mutations. Based on the mechanism of action no difference was expected. These results are also supported by the overall efficacy data.

Dose-response relationship

Study ISIS 420915-CS1 investigated the exposure-response relationship of multiple doses on inotersen and levels of TTR, RBP4 and retinol. A sigmoid inhibitory exposure-response relationship was observed with the higher dose levels (300 mg and 400 mg) reaching plasma trough concentrations in the flat part of the curve. It also has to be noted that, due to the long elimination half-life of inotersen (2 to 4 weeks in healthy volunteers) tissue steady state levels were not reached during the observation period. In studies ISIS 420915-CS2 and ISIS 420915-CS3 highest levels of TTR suppression were reached by week 13 and remained stable during continued treatment.

Study ISIS 420915-CS2 compared individual trough plasma levels of inotersen given at a dose of 300 mg once weekly with TTR levels on days 85 and 449. A flat exposure-response relationship was found on both days with consistent levels of about 70% reduction found over the entire exposure range. The data show that a single dose of 300 mg inotersen given once weekly achieves similar pharmacodynamic effects in patients with hATTR independent of body weight.

Figure 2 Mean TTR Reduction by Dose: Study CS1



Source: CTD Section 5.3.4.1 CS1 CSR Figure 8; Ackermann EJ, Guo S, Benson MD, et al. 2016

Abbreviations: BL=baseline; Chg=change; SEM=standard error of the mean

Effect of anti-drug antibodies

A significant proportion of subjects under long-term treatment showed development of late onset (median onset of 202.5 days in CS2 and 327 days in CS3) anti-inotersen antibodies, with 30.4% in CS2 and 39.5% in CS3 being positive post baseline. Inotersen plasma trough concentrations were generally higher in ADA-positive subject. Study ISIS 420915- CS2 however showed similar levels of TTR suppression for ADA-positive and negative subjects. It can therefore be assumed that anti-inotersen antibodies are drug binding antibodies, but not neutralizing antibodies, since pharmacodynamics responses were not affected.

2.4.4. Discussion on clinical pharmacology

The results from the three clinical studies conducted with inotersen allow for an adequate assessment of its pharmacokinetic characteristics. Adsorption, distribution, metabolism and excretion are well described and conform to expectations for the chemical class of antisense oligonucleotides. The pharmacokinetic properties are adequately described in the SmPC. Inotersen showed dose proportional and time invariant PK.

The analytical methods have overall been well described and all points for clarification have been adequately addressed by the Applicant.

The pharmacokinetic data is, to certain extent, limited because only few non-white patients were recruited in the studies. It should also be noted that the pharmacokinetic subgroup in study ISIS 420915-CS2 did not include any female patients. Due to the small sample size and a high variability of data the influence of several covariates on PK could therefore not be definitely ascertained. The CHMP found to be sufficient the provided justification for excluding data from ADA-positive patients from the population PK model.

Since the pharmacokinetics of inotersen has not been investigated in a dedicated study in special populations influences of several factors remain a possibility. Data in patients over 65 years has not

been analysed for potential effects of different age ranges. Clinical significant influences in special populations however appear unlikely and the limits of the population PK-model do not preclude a flat dosing in all patients. Therefore the CHMP considers that no special dosing recommendations in elderly are required. The limitations of the PK-model are adequately reflected in the SmPC section 5.2.

In vitro studies with inotersen showed a general lack of interaction potential with cytochromes and major drug transporters. These results are expected for antisense oligonucleotides since the metabolic pathways for small molecules and ASOs are generally different and non-overlapping. The lack of human DDI-studies is therefore justified. A more detailed discussion of concomitant medications used in the clinical studies was provided during the evaluation procedure. No definite conclusions on the lack of interactions between inotersen and the other agents can be drawn based on the conducted clinical studies as the data is sparse. Possible effects of inotersen on CYP levels not by direct interaction but through common transcriptional regulatory pathways shared by transthyretin and cytochrome P450s can most likely be ruled out, since there is no evidence that TTR regulates the expression of genes at the transcriptional level.

The pharmacodynamic effects of inotersen have also been well characterised. The proposed mechanism of action is plausible based on the mechanism of action of ASOs which well established and highly specific. Therefore no pharmacodynamic interactions are to be expected. Non-clinical data corroborates the primary pharmacodynamics.

Inotersen showed the predicted time and dose dependent suppression of TTR, RBP4 and retinol with all biomarkers responding in a similar pattern. Suppression followed a sigmoid dose-response curve. Individual trough plasma concentrations at the dose of 300 mg SC once weekly vary greatly but are generally in the flat part of the exposure-response curve. Therefore, at the clinically relevant dose of 300 mg a flat concentration-response curve has been established.

It has to be noted that doses of inotersen lower than 300 mg SC weekly have not been clinically investigated and therefore the clinical significance of lower suppression levels of TTR cannot be evaluated.

Inotersen was designed to bind to a part of TTR mRNA without any known mutations. Therefore, efficacy of inotersen is expected to be independent of mutation status. This is further supported by TTR and RBP4 suppression levels which showed similar values compared to baseline for subjects with V30M and non-V30M mutations.

A significant portion of patients developed anti-inotersen antibodies. While C_{max} and AUC appeared unaffected C_{trough} levels showed significant increases in ADA-positive patients. Pharmacodynamic effects however were not significantly affected by the presence of antibodies. Therefore, anti-inotersen antibodies appear to be drug binding but not drug neutralising. In the absence of pharmacodynamic or efficacy signals pointing towards a neutralising effect the CHMP considered acceptable that the neutralizing capacity of antibodies present in positive samples was not tested.

2.4.5. Conclusions on clinical pharmacology

Overall the pharmacokinetics and pharmacodynamics of inotersen have been thoroughly investigated and well described. The results generally reflect the expected behaviour of a substance belonging to the chemical class of antisense oligonucleotides. The analytical methods have overall been well described. The influence of certain factors, including ADAs, on drug exposure cannot be definitely ascertained based on the presented population PK-model results. However possible influences appear to be minor and are considered by the CHMP as clinically not significant.

Inotersen has shown predictable and consistent pharmacodynamic effects (reduction of TTR, RBP4 and retinol) over treatment durations up to three years. Since the magnitude of TTR reduction required to optimally attenuate progression of disease is not known a possible clinical benefit of lower doses of inotersen remains unexplored.

2.5. Clinical efficacy

The clinical development of inotersen consists of a Phase 1 first-in-human study in healthy volunteers (ISIS 420915-CS1; herein referred to as CS1), a Phase 2/3 pivotal study in patients with hATTR (CS2), and a Phase 3 Open Label Extension (OLE) study (CS3). CS1 provided pharmacodynamic (PD) evidence and a dose-response relationship for the actions of inotersen. The pivotal double-blind Phase 2/3 study recruited Stage 1 and Stage 2 subjects with hATTR-PN with a Neuropathy Impairment Score (NIS) ≥10 and ≤130. This study investigated the change from baseline in Modified Neuropathy Impairment Score + 7 (mNIS+7) composite score and in the Norfolk Quality of Life - Diabetic Neuropathy (QoL-DN) questionnaire total score at week 65/66. The Supportive study CS3 is an open label extension for an additional 260 weeks (5 years), which recruited patients who had satisfactorily completed CS2.

2.5.1. Dose response study

CS1 was a Phase 1, double-blinded, placebo-controlled, dose-escalation study conducted at a single center. The study consisted of 4 single-dose (randomized to 3 active:1 placebo) and 5 multiple-dose (randomized to 8 active:2 placebo) treatment cohorts. Subjects in the single-dose treatment cohorts received a single SC dose of study drug on Day 1: Cohort A (50 mg), Cohort B (100 mg), Cohort C (200 mg), and Cohort D (400 mg). Subjects in the multiple-dose treatment cohorts received 3 SC doses of study drug on alternate days (Days 1, 3, and 5) during Week 1 followed by once weekly SC administration during Weeks 2 to 4 (Days 8, 15, and 22) for a total of 6 doses: Cohort AA (50 mg), Cohort BB (100 mg), Cohort CC (200 mg), Cohort DD (400 mg), and Cohort EE (300 mg).

A maximum of 86 subjects were planned; 65 subjects were randomized and analyzed: 16 subjects in the single-dose treatment cohorts and 49 subjects in the multiple-dose treatment cohorts.

In CS1, dose- and concentration-dependent reductions in TTR levels were observed after both single and multiple doses, based on evaluations in the 50 to 400 mg dose range. The exposure-response relationship between plasma trough concentrations and serum TTR was nonlinear and was best described with an inhibitory sigmoid Imax model. Based on modelling and simulation of the Phase 1 data, a clinical dose of 300 mg per week was selected as the Phase 3 dose to achieve TTR reduction of ≥ 70% in majority of patients (66%) and over 50% TTR reduction in approximately 90% of patients.

In CS1, the 300 mg dose level showed a satisfactory safety profile and substantial PD effects after 6 doses (>70% mean reduction in plasma TTR levels). The PD effect observed with the 300 mg dose level was also similar to that observed with the 400 mg dose level, and therefore the 300 mg per week dose (with additional loading doses in the first week) was selected for the Phase 2/3 study. Preliminary PK/PD modelling, based on data from CS1 and extrapolation to steady-state, predicted mean total TTR (wild-type and mutant) steady-state reductions of ~80% with either a 300 mg/week or 400 mg/week regimen.

2.5.2. Main study(ies)

Both CS2 and CS3 provided substantial evidence of clinical efficacy of inotersen in subjects with hATTR. .

The primary, secondary, tertiary and exploratory efficacy endpoints used in the main studies CS2 and CS3 are summarised in Table 4 below:

Table 4 Summary of Studies Contributing to Inotersen Efficacy Profile

Study ID	**Pivotal Study: CS2 (ISIS 420915-CS2)**	**Open-Label Extension Study: CS3 (ISIS 420915-CS3)**
Phase	Phase 2/3	Phase 3
Critical design features	Multicenter, double-blind, placebo-controlled	Multicenter, OLE
Study population	Stage 1 and Stage 2 subjects with hATTR-PN with an NIS ≥10 and ≤130 at CS2 Baseline	
Number of subjects planned	135, approximately 50% Stage 1 and 50% Stage 2 subjects	Eligible subjects who had satisfactorily completed CS2[a]
Number of subjects randomized/enrolled and dosed	Randomized: 173; Dosed: 172[b] (65.7% Stage 1 hATTR-PN; 34.3% Stage 2 hATTR)	Enrolled and Dosed: 114 received inotersen (at interim analysis)
Treatment regimen	300 mg inotersen or placebo, SC injection Week 1: (Days 1, 3, and 5) Weeks 2 to 65: once-weekly (for a total of 67 doses)	300 mg inotersen once weekly SC injection[c]
Sites (locations)[d]	Argentina, Brazil, France, Germany, Italy, New Zealand, Portugal, Spain, UK, and US	Argentina, Brazil, France, Germany, Italy, Portugal, Spain, UK, and US
Primary efficacy endpoints	Changes from Baseline at Week 66 in: Modified Neuropathy Impairment Score +7 Norfolk QoL-DN Total Score	Efficacy measures were a secondary objective in CS3. In general, the efficacy assessments in CS3 were consistent with those identified as primary and secondary endpoints in CS2 (with the exclusion of the NIS+7 and modified +7 from CS3 and the designation of PND as an efficacy measure in CS3, rather than as an exploratory measure, as in CS2). Changes from the CS2 Baseline *and* CS3 Baseline were evaluated.
Secondary efficacy measures	BMI; mBMI; Components of mNIS+7 (NIS and modified +7 composite scores); Norfolk QoL-DN questionnaire symptoms domain score (Stage 1 subjects only) and physical functioning/large fiber neuropathy domain score (Stage 2 subjects only); GLS by ECHO; NIS +7	
Tertiary efficacy measures	SF-36 questionnaire; Individual components of NIS; Individual components of modified +7; Individual domain scores of Norfolk QoL-DN +7 (total score and individual components)	
Exploratory measures	ECHO parameters (except GLS); NT-proBNP Polyneuropathy Disability score NSC (total score and individual domain scores)	SF-36 questionnaire ECHO parameters (except GLS) NT-proBNP
Pharmacodynamic measures[d]	Changes from Baseline in TTR and retinol binding protein 4 levels Proportion of subjects with at least 60% reduction in TTR	
Study status	Treatment period complete; Post-treatment period ongoing for subjects not enrolled in OLE	Ongoing
Data cut-off	28-Mar-2017	28-Feb-2017

a. Exceptions were allowed with approval from the Sponsor. One subject was discontinued from CS2 due to Sponsor decision as a result of unblinding but was allowed to enroll in CS3.

b. One subject in the inotersen group was randomized in error and did not initiate treatment with study drug

c. Subjects who had a dose reduction or schedule change in CS2 were permitted to continue with the adjusted dose level or schedule in CS3.
d. The PD measures of TTR and retinol binding protein 4 levels were identified as secondary objectives in the CS2 SAP. The proportion of subjects with at least 60% reduction in TTR was identified as an exploratory measure.
Abbreviations: BMI=body mass index; CSR=clinical study report; ECHO=echocardiogram; GLS=global longitudinal strain; mBMI=modified body mass index; mNIS+7=modified Neuropathy Impairment Score +7; NIS=Neuropathy Impairment Score; Norfolk QoL-DN=Norfolk Quality of Life-Diabetic Neuropathy; NSC=Neuropathy Symptoms and Change; NT-proBNP=N-terminal prohormone of brain natriuretic peptide; SAP=Statistical Analysis Plan; SF-36=Short Form 36 Health Survey; UK=United Kingdom; US=United States

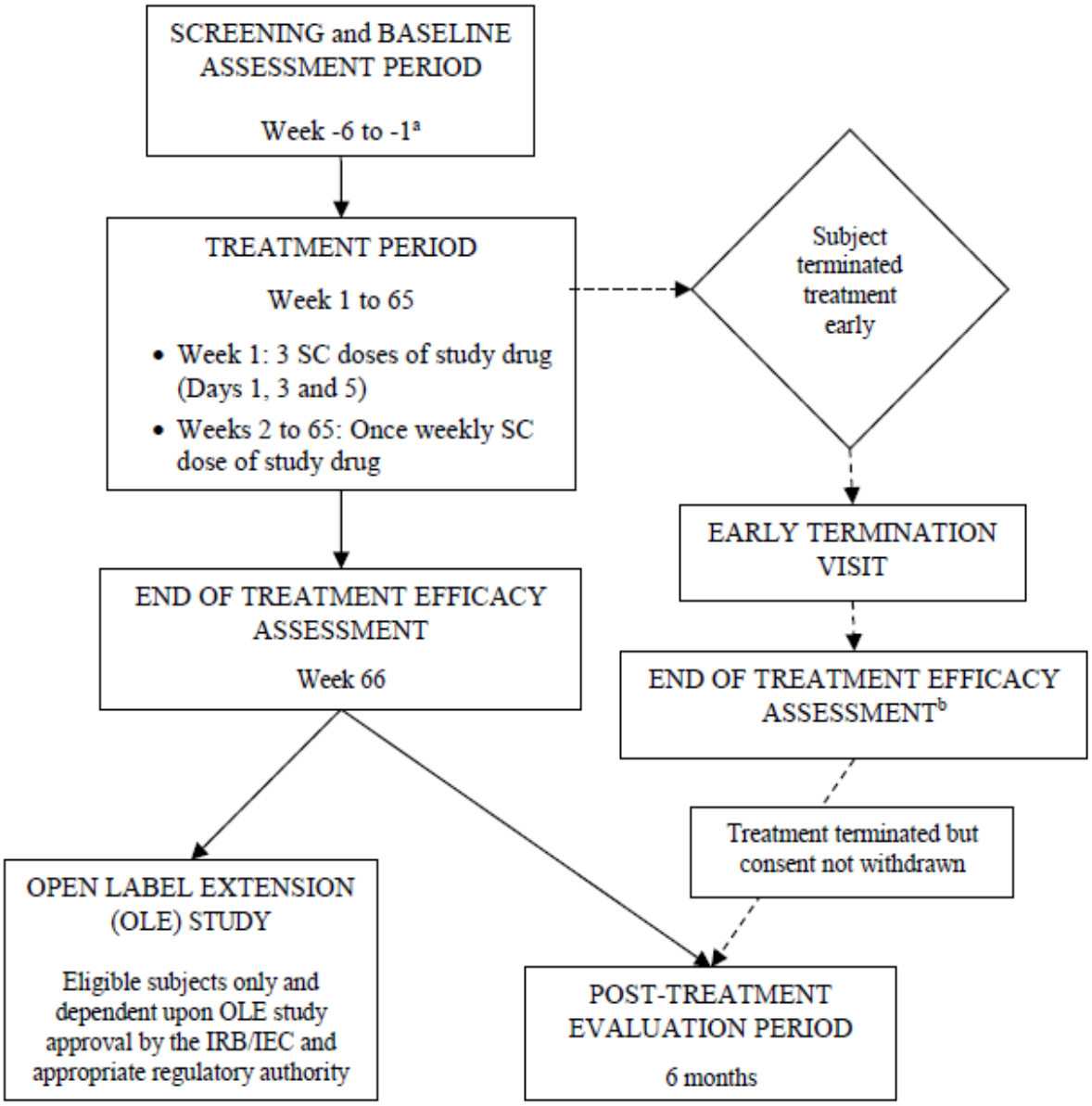
Pivotal Study - Study CS2

Title: A Phase 2/3 Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of ISIS 420915 in Patients with Familial Amyloid Polyneuropathy (NEURO-TTR Study)

Methods

CS2 was a Phase 2/3 multicenter, double-blind, randomized, stratified, placebo-controlled study of inotersen in Stage 1 and Stage 2 subjects with hATTR-PN with a Neuropathy Impairment Score (NIS) ≥10 and ≤130. Approximately 135 subjects were planned to be randomized 2:1 to 300 mg inotersen or placebo, 173 were finally randomised and 172 received a dose. Approximately 50% Stage 1 and 50% Stage 2 subjects were planned to be enrolled in the study.

Figure 3 Study Design and Treatment Schema.



a) Exceptions to the 6-week period to perform Screening evaluations and Baseline assessments were allowed for the TTR genotyping and amyloid biopsy tests. These tests were allowed up to 10 weeks prior to Study Day 1 and were only conducted if appropriate documentation was not already available. In addition, ERG and ophthalmology examinations were allowed up to 1 week after Study Day 1, if needed for scheduling purposes.
b) Subjects who terminated treatment early were to complete the early termination visit and EOT efficacy assessments within 14 days from the last dose of study drug. These subjects then entered the post-treatment evaluation period.

Abbreviations: EOT=end of treatment; ERG=electroretinography; IEC=Independent Ethics Committee; IRB=Institutional Review Board; OLE=open-label extension; SC=subcutaneous)

In addition, a small number of subjects (approximately 20) at selected sites were to be enrolled in a PK subgroup to undergo additional sampling for PK, electrocardiogram (ECG), complement, coagulation, inflammatory, and haematology assessments. Subjects who met additional ECHO inclusion criteria also had the option to participate in an ECHO subgroup to receive additional transthoracic ECHO assessments during the Treatment Period.

Study Participants

To be eligible for study participation, potential subjects were required to satisfy all of the eligibility criteria within 6 weeks of Study Day 1 or at the time point specified in the individual inclusion or exclusion criterion.

Inclusion Criteria

1. Subjects with Stage 1 or Stage 2 hATTR-PN and all of the following:

    a. NIS score ≥ 10 and ≤ 130

    b. Documented TTR variant by genotyping

    c. Documented amyloid deposit by biopsy

    d. In Germany, Portugal, and Argentina only, Stage 1 subjects were also required to meet at least 1 of the following criteria: 1) failed tafamidis treatment, 2) intolerant to tafamidis treatment, or 3) not eligible for tafamidis treatment.

2. Willing to take vitamin A supplements

3. Aged 18 to 82 years at the time of informed consent

4. Satisfied the following:

    a. Females: Nonpregnant and nonlactating; surgically sterile, postmenopausal, abstinent, or if engaged in sexual relations and of childbearing potential, the subject was required to use an acceptable contraceptive method from the time of signing the informed consent form until at least 3 months after the last dose of study drug.

    b. Males: Surgically sterile, abstinent, or if engaged in sexual relations and of child-bearing potential, the subject was required to utilize an acceptable contraceptive method during treatment and for 3 months after the last dose of study drug.

5. Must have given written informed consent (signed and dated) and any authorizations required by local law and agreed to comply with all study requirements protocol, or unwilling to cooperate fully with the investigator

Exclusion Criteria

1. Unwilling to comply with study procedures, including follow-up, as specified by this protocol, or unwilling to cooperate fully with the investigator

2. Screening laboratory results as described below, or any other clinically significant abnormalities in Screening laboratory values that rendered a subject unsuitable for inclusion:

    a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >1.9 times the upper limit of normal (ULN)

    b. Bilirubin ≥ 1.5xULN (subjects with bilirubin ≥ 1.5xULN may have been permitted following discussion with the medical monitor, if only indirect bilirubin was elevated, ALT/AST was not >ULN, and genetic testing confirmed Gilbert's disease)

    c. Platelets <125 x 10^9/L

    d. Positive (≥ trace) for protein on urine dipstick. In the event of a positive test, eligibility may have been confirmed by a quantitative total urine protein measurement of <1.0 g/24 hours

e. Positive (≥ trace) for blood on urine dipstick. In the event of a positive test, eligibility may have been confirmed with urine microscopy showing ≤ 5 red blood cells (RBCs) per high power field. If >5 RBCs per high power field and there was a clearly identifiable benign cause for the microscopic hematuria (e.g., chronic urinary tract infection secondary to neurogenic bladder), eligibility was to be determined by discussion with the medical monitor

f. Thyroid-stimulating hormone (TSH) values outside normal range (unless approved by the medical monitor)

3. Retinol level at Screening less than the lower limit of normal (LLN). For subjects with a TTR mutation at position 84 (e.g., Ile84Ser or Ile84Asn) and retinol <LLN, the exclusion criterion was signs or symptoms of vitamin A deficiency (such as evidence of vitamin A deficiency on electroretinography [ERG])

4. Uncontrolled hypertension (blood pressure >160/100 mmHg)

5. Positive test result for human immunodeficiency virus (HIV), hepatitis B or hepatitis C

6. Karnofsky performance status ≤ 50

7. Renal insufficiency as defined by estimated creatinine clearance calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula <60 mL/min/1.73 m2 at Screening. If the calculated creatinine clearance was thought to be artificially low, a 24-hour urine creatinine clearance was allowed with prior Sponsor approval

8. Presence of known type 1 or type 2 diabetes mellitus

9. Other causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease)

10. Treatment with another investigational drug, biological agent, or device within 3 months of Screening, or 5 half-lives of the study agent, whichever was longer

11. If previously treated with tafamidis, the subject must have discontinued treatment for 2 weeks prior to Study Day 1. If previously treated with diflunisal, the subject must have discontinued treatment for 3 days prior to Study Day 1

12. Previous treatment with any oligonucleotide or small interfering ribonucleic acid within 6 months of Screening. Subjects that were previously treated with oligonucleotides were to be approved by the medical monitor

13. Prior liver transplant or anticipated liver transplant within 1 year of Screening

14. New York Heart Association (NYHA) functional classification of ≥ 3

15. Acute coronary syndrome or major surgery within 3 months of Screening

16. Known primary amyloidosis

17. Known leptomeningeal amyloidosis

18. Anticipated survival <2 years

19. Active infection requiring systemic antiviral or antimicrobial therapy that was not completed prior to Study Day 1

20. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that was successfully treated. Subjects with a history of other malignancies that were

curatively treated may have also been eligible, but discussion with and approval by the medical monitor was required.

21. Any other conditions, which in the opinion of the investigator, made the subject unsuitable for inclusion, or could have interfered with the subject participating in or completing the study

22. Known monoclonal gammopathy of undetermined significance or multiple myeloma

Additional Entry Criteria for Subjects in the ECHO Subgroup

Subjects who participated in the ECHO substudy were also required to meet the following entry criteria in order to be included in this subgroup:

1. Left ventricular wall thickness of ≥ 13 mm on transthoracic ECHO at Baseline

2. No known history of persistent hypertension ≥ 150 mmHg within 12 months prior to Screening

3. Baseline ECHO was evaluable as ascertained by the central reader

Treatments

Study drug (inotersen or placebo) characteristics are listed in Table 5.

Table 5 Study Drug Characteristics

Product	**Strength**	**Volume/Formulation**	**Route**
Inotersen	200 mg/mL (189 mg/mL as parent acid)	1 mL or 1.5 mL solution per vial	SC 1.5 mL
Placebo	N/A	1 mL or 1.5 mL solution per vial	SC 1.5 mL

Abbreviations: N/A=not applicable; No.=number; SC=subcutaneous

Study drug was to be administered SC as a 300 mg dose (284 mg parent acid). A single 1.5 mL injection containing 300 mg inotersen was to be administered 3 times in the first week and then once weekly in Weeks 2 to 65.

In addition to study drug, all subjects were required to take daily oral supplemental doses of the recommended daily allowance (RDA) of vitamin A (approximately 3000 IU vitamin A or the closest approximate dose as available in the region in which the subject resides).

Objectives

- Primary objectives

The primary objective of this study was to evaluate the efficacy of inotersen as compared with placebo when administered for 65 weeks as measured by the change from Baseline in the modified neuropathy impairment score +7 (mNIS+7) and in the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire total score in subjects with hATTR-PN.

- Secondary objectives

The secondary objectives of the study were to evaluate the efficacy of inotersen as compared with placebo when administered for 65 weeks based on the change from Baseline in the following measures:

Norfolk QoL-DN questionnaire symptoms domain score in Stage 1 subjects and Norfolk QoL-DN questionnaire physical functioning/large fiber neuropathy domain score in Stage 2 subjects

Modified body mass index (mBMI) and body mass index (BMI) NIS and modified +7 Neuropathy impairment score +7 (NIS+7)

Global longitudinal strain (GLS) by echocardiogram (ECHO) in the ECHO subgroup and in the Cardiomyopathy-ECHO (CM-ECHO) Set

- Additional secondary objectives

To evaluate the pharmacodynamic (PD) effect of inotersen as compared with placebo based on the change from Baseline in TTR and retinol binding protein 4 (RBP4).

To evaluate the safety and tolerability of inotersen

To evaluate the plasma trough levels of inotersen in all subjects and to evaluate the plasma pharmacokinetic (PK) parameters of inotersen in a subset of subjects

- Tertiary objectives

Tertiary objectives of the study were to evaluate the change from Baseline as compared with placebo in the following measures:

Short form 36 health survey (SF-36) questionnaire

Individual components of NIS, modified +7, and +7

+7

Individual domain scores of the Norfolk QoL-DN questionnaire

- Exploratory objectives

Exploratory objectives of the study were to evaluate the change from Baseline as compared with placebo, in the following exploratory measures:

ECHO parameters (except GLS) in the ECHO subgroup and in the CM-ECHO Set

Plasma N-terminal prohormone of brain natriuretic peptide (NT-proBNP)

Polyneuropathy disability (PND) score

Neuropathy symptoms and change (NSC) score

Outcomes/endpoints

The primary efficacy endpoints were the change from Baseline to Week 66 in the Modified Neuropathy Impairment Score +7 (mNIS+7) composite score and in the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire total score. The mNIS+7 consists of two composite scores: the NIS composite score (maximum of 244 points) and the modified +7 composite score (maximum of

102.32 points) and has a maximum of 346.32 points. The mNIS+7 assessment procedure included an evaluation of each component, as well as additional components needed for the NIS+7 total score.

Sample size

The planned sample size for CS2 was revised (Protocol Amendment 7, dated 16-Nov-2015) from 195 subjects to 135 subjects based on published results from the placebo-controlled Phase 3 diflunisal trial and a retrospective, multi-national natural history study in 283 patients with hATTR-PN [Berk, 2013; Adams, 2015a], as well as uncontrolled data for another TTR mRNA targeted therapeutic oligonucleotide [Adams, 2015b]

The study was designed with 135 patients to detect a 9.6-point difference in the mean change from Baseline in mNIS+7 score between the 2 groups and a 10.7-point difference in the change from Baseline in the Norfolk QoL total score between the 2 groups.

Randomisation

Subjects were randomized after all Screening and Baseline assessments were completed and after the investigator verified that subjects were eligible per the study entry criteria. No subject began treatment prior to randomization and assignment of a unique subject identification number.

Using an Interactive Voice/Web-Response System (IXRS), eligible subjects were randomized 2:1 to receive inotersen or placebo, respectively. There were 2 separate and independent randomizations: one for subjects in the PK subgroup and one for remaining subjects who were not in the PK subgroup. Within each randomization, subjects were stratified for:

Previous treatment with tafamidis or diflunisal vs no known previous treatment

Stage 1 vs Stage 2 disease

V30M TTR mutation vs non-V30M TTR mutation

Blinding (masking)

Sponsor personnel or their designees who were involved in the conduct of the study, monitors, study center personnel, and subjects were blinded throughout the study until all subjects completed the treatment period and the EOT efficacy assessments and the database was locked. In addition, the Sponsor, clinical research organization (CRO) personnel involved in the regular conduct of the study, investigators, study center personnel, and the subjects did not have access to any post-Baseline PK or PD data (e.g. TTR, RBP4) that may have resulted in unblinding of treatment assignments.

If a subject experienced a serious adverse event (SAE) and/or if knowledge of the treatment assignment would have impacted the clinical management of the subject, the investigator only had the ability to unblind the treatment assignment for that subject using the IXRS. Every reasonable attempt was to be made in order to complete the EOT efficacy assessment or early termination visit procedures prior to subject unblinding, as knowledge of the treatment arm had the potential to influence assessment of the subject.

Statistical methods

The primary efficacy analyses were the comparison of change from Baseline to Week 66 in modified Neuropathy Impairment Score +7 (mNIS+7) Composite Score and in the Norfolk Quality of Life Diabetic Neuropathy (Norfolk QoL-DN) questionnaire total score between the inotersen 300 mg group and the placebo group. To account for multiplicity between both endpoints a sequential testing approach was pre-defined and mNIS+7 was to be tested first and the QoL endpoint second.

Both endpoints were analysed using a Mixed Effects Model with Repeated Measures (MMRM) based on the Full Analysis Set (FAS) that included all enrolled subjects according to randomized allocation who received at least 1 injection of inotersen and who have a baseline and at least 1 post-Baseline efficacy assessment for mNIS+7 or Norfolk QoL questionnaire. The MMRM method included fixed factors for treatment, time, treatment-by-time interaction, and the 3 stratification factors. Covariates for baseline and baseline-by-time interaction were also included and an unstructured covariance matrix was used for repeated measures.

Results

Recruitment

Two hundred seventy-eight subjects were screened for entry into the study. A total of 173 subjects were randomized into the study and 172 subjects received study treatment. One subject in the inotersen group was randomized in error and did not initiate study drug.

Conduct of the study

Most randomized subjects (80.3%) completed study treatment according to the protocol. The proportion of subjects who discontinued study treatment early was higher in the inotersen group (23.0%) compared with the placebo group (13.3%) due primarily to AEs. In the inotersen group, over one-third of the AEs that led to permanent discontinuation of study treatment were associated with thrombocytopenia (4 inotersen subjects) or glomerulonephritis (2 inotersen subjects), which are known to be associated with inotersen treatment.

The majority of subjects who completed treatment (133 of 139 subjects) entered the CS3 OLE study at the end of the treatment period. Subjects who did not continue into the OLE study, either because they elected not to after completion of treatment in CS2 or because they discontinued CS2 treatment early, were followed for safety and efficacy in the 6-month post-treatment evaluation period. At the time of data cut-off for the submitted CSR (28 March 2017), a total of 3 subjects were still ongoing in post-treatment follow-up.

There were no subjects unblinded for safety reasons by the investigator during the study.

The majority of subjects had at least 1 protocol deviation during the study (Table 11). The most frequently reported major protocol deviations involved study procedures that were not performed according to the protocol or study drug errors (primarily missed doses). The incidence of major protocol deviations was similar between treatment groups.

Table 6 Subjects With Protocol Deviations (Randomized Subjects)

	Placebo (N=60)	Inotersen 300 mg (N=113)	Total (N=173)
Any protocol deviations, n (%)	60 (100)	111 (98.2)	171 (98.8)
Any major protocol deviation, n (%)	49 (81.7)	90 (79.6)	139 (80.3)
Drug error	18 (30.0)	29 (25.7)	47 (27.2)
Eligibility criteria	1 (1.7)	4 (3.5)	5 (2.9)
Improper informed consent procedures	4 (6.7)	9 (8.0)	13 (7.5)
Missed visit	0	9 (8.0)	9 (5.2)
Restricted concomitant meds	3 (5.0)	0	3 (1.7)
Study procedure	40 (66.7)	76 (67.3)	116 (67.1)
Visit out of window	8 (13.3)	14 (12.4)	22 (12.7)
Other	4 (6.7)	7 (6.2)	11 (6.4)
Any minor protocol deviation, n (%)	60 (100)	108 (95.6)	168 (97.1)
Drug error	11 (18.3)	20 (17.7)	31 (17.9)
Eligibility criteria	1 (1.7)	0	1 (0.6)
Improper informed consent procedures	20 (33.3)	41 (36.3)	61 (35.3)
Missed visit	4 (6.7)	9 (8.0)	13 (7.5)
Study procedure	55 (91.7)	100 (88.5)	155 (89.6)
Visit out of window	31 (51.7)	69 (61.1)	100 (57.8)
Other	6 (10.0)	7 (6.2)	13 (7.5)

Baseline data

Demographic characteristics were well balanced between the treatment groups (Table 7).

Subjects were predominantly White (91.9%) and 68.6% of the subjects were male. The mean age of subjects was 59.2 years. The majority of subjects were enrolled at sites in North America (47.7%) and Europe (34.9%), with the remainder (17.4%) enrolled in South America/Australasia.

Regional enrolment was well balanced between the treatment groups.

Based on data entered in the electronic case report form, approximately two-thirds of treated subjects (67.4%) had Stage 1 hATTR-PN at Baseline and 32.6% of subjects were Stage 2. V30M TTR mutations were observed in 51.7% of subjects, and 57.6% of subjects received prior treatment with tafamidis or diflunisal. In the CM-ECHO Set, subjects were older (mean age: 62.7 years vs 59.2 years), a higher proportion were from North America (North America 58.3% vs 47.7%, South America 10.2% vs 17.4%) and a lower proportion had V30M TTR mutations (41.7% vs 52.3%) compared with the SS.

A total of 27 different TTR mutations were observed in treated subjects. The most common TTR mutation was V30M, and the incidence was similar between treatment groups (50.0% inotersen, 55.0% placebo).

Table 7 Demographic Characteristics (Safety Set)

	Placebo (N=60)	Inotersen 300 mg (N=112)	Total (N=172)
Age (years)			
Mean (SD)	59.5 (14.05)	59.0 (12.53)	59.2 (13.04)

Median	63.0	62.0	62.5
Minimum, Maximum	28, 81	27, 78	27, 81
Age group (years)			
≤18	0	0	0
19 to 64	34 (56.7)	64 (57.1)	98 (57.0)
≥65	26 (43.3)	48 (42.9)	74 (43.0)
Sex, n (%)			
Male	41 (68.3)	77 (68.8)	118 (68.6)
Female	19 (31.7)	35 (31.3)	54 (31.4)
Ethnicity, n (%)			
Hispanic or Latino	7 (11.7)	17 (15.2)	24 (14.0)
Not Hispanic or Latino	53 (88.3)	95 (84.8)	148 (86.0)
Race, n (%)			
American Indian or Alaskan Native	0	0	0
Asian	3 (5.0)	1 (0.9)	4 (2.3)
Black	1 (1.7)	3 (2.7)	4 (2.3)
Native Hawaiian/Other Pacific Islander	0	0	0
White	53 (88.3)	105 (93.8)	158 (91.9)
White and Grayish-Brown	1 (1.7)	0	1 (0.6)
Other	2 (3.3)	3 (2.7)	5 (2.9)
Weight (kg)			
Mean (SD)	71.07	70.59 (17.032)	70.76
Median	69.93	70.10	69.95
Minimum, Maximum	38.2, 126.0	37.0, 140.4	37.0, 140.4
Region, n (%)			
Europe	23 (38.3)	37 (33.0)	60 (34.9)
North America	26 (43.3)	56 (50.0)	82 (47.7)
South America/Australasia	11 (18.3)	19 (17.0)	30 (17.4)
Randomization stratum by IXRS, n (%)			
Previous treatment with tafamidis or diflunisal			
Yes	33 (55.0)	61 (54.5)	94 (54.7)
No	27 (45.0)	51 (45.5)	78 (45.3)
Disease stage			
Stage 1	39 (65.0)	74 (66.1)	113 (65.7)
Stage 2	21 (35.0)	38 (33.9)	59 (34.3)
V30M TTR mutation			
Yes	32 (53.3)	58 (51.8)	90 (52.3)
No	28 (46.7)	54 (48.2)	82 (47.7)
Randomization stratum by CRF, n (%)			
Previous treatment with tafamidis or diflunisal			
Yes	36 (60.0)	63 (56.3)	99 (57.6)
No	24 (40.0)	49 (43.8)	73 (42.4)
Disease stage			
Stage 1	42 (70.0)	74 (66.1)	116 (67.4)
Stage 2	18 (30.0)	38 (33.9)	56 (32.6)
V30M TTR mutation			
Yes	33 (55.0)	56 (50.0)	89 (51.7)
No	27 (45.0)	56 (50.0)	83 (48.3)

Note: Randomization strata were determined by IXRS and CRF data. Per the SAP, if the randomization strata recorded from IXRS was different from the actual data recorded in the CRF, the randomization strata recorded from IXRS was used in the analysis of efficacy.

Due to the randomisation scheme 2:1 more patients were recruited in the inotersen group in CS2 study. The percentages of the demographic characteristics for each subgroup were similar in the placebo and in the inotersen group. A slightly higher percentage of patients with Stage 2 disease (Stage 2 to Stage 1 ratio 34/66) were included in the inotersen group compared to the placebo (Stage 2 to Stage 1 ratio 30/70).

Baseline hATTR-PN disease characteristics were consistent with study entry criteria and the disease severity of enrolled subjects. The mean duration of hATTR-PN disease from the time of symptom onset was 63.9 months. Approximately 40% of subjects had a concomitant diagnosis of hATTR-CM at study entry, with a mean duration of disease from the time of symptom onset of 41.1 months. Baseline mNIS+7 composite scores and Norfolk QoL-DN total scores ranged from 11.2 to 174.7 and from -2.0 to 127.0, respectively.

Numbers analysed

A total of 172 of the 173 randomized subjects received at least 1 dose of study drug and were included in the SS (, which was the population used for the analyses of all safety measures. The majority of randomized subjects were included in the FAS (95.4%), which was the primary population for analysis of efficacy and PD outcomes. The proportion of subjects in the inotersen group (93.8%) included in the FAS was slightly lower than the placebo group (98.3%).

A lower proportion of subjects in the inotersen group were included in the PPS compared with the placebo group (Table 8). The primary reason that excluded subjects from the PPS in both treatment groups was <80% of prescribed doses of study drug received.

A total of 66 subjects (38.2%) participated in the ECHO substudy, and the proportion of subjects included in the ECHO subgroup was similar between treatment groups. Approximately 62% of subjects either had a diagnosis of hATTR-CM at study entry or were eligible to participate in the ECHO substudy (whether consented or not) and comprised the CM-ECHO Set. A higher proportion of subjects in the inotersen group (66.4%) were included in the CM-ECHO Set compared with the placebo group (55.0%).

A total of 10 subjects in the inotersen group (8.8%) were included in the PK subgroup, which was used for all PK analyses.

Table 8 Analysis Populations (Randomized Subjects)

	Placebo (N=60) n (%)	**Inotersen 300 mg (N=113) n (%)**	**Total (N=173) n (%)**
Number of subjects:			
Randomized	60 (100)	113 (100)	173 (100)
Dosed	60 (100)	112 (99.1)	172 (99.4)a
In the Safety Set (SS)	60 (100)	112 (99.1)	172 (99.4)
In the Full Analysis Set (FAS)	59 (98.3)	106 (93.8)	165 (95.4)
In the Per-Protocol Set (PPS)	52 (86.7)	83 (73.5)	135 (78.0)
In the PK Subgroup	8 (13.3)	10 (8.8)	18 (10.4)
In the PK Set	0	111 (98.2)	111 (64.2)
In the PK Subgroup (PK Set)	0	10 (8.8)	10 (5.8)

In the ECHO Subgroup	22 (36.7)	44 (38.9)	66 (38.2)
In the CM-ECHO Set	33 (55.0)	75 (66.4)	108 (62.4)
In the TTR Subgroup	18 (30.0)	37 (32.7)	55 (31.8)

Outcomes and estimation

Results of primary analysis

The primary endpoints demonstrated statistically significant differences from baseline to week 66. The differences were large with -19.73 (95% CI: -26.43, -13.03; p=0.00000004) for the mNIS+7 Score (maximum score 346) and -11.68 (95% CI: -18.29, -5.06; p<0.0006) for the Norfolk QoL-DN (maximum score 156) using the pre-specified MMRM analysis (Figures 4 and 5 below). It is noted that for mNIS+7 the difference at week 35 showed statistically significant results with a difference of -8.69 (95% CI: -13.49, -3.90; p=0.0005). For Norfolk QoL-DN the difference of -6.14 (95% CI: -11.77, -0.52; p=0.032) was statistically significant at week 35 (MMRM analysis). However, these evaluations were based on the pre-specified MMRM analysis that is not considered of primary importance (see section on statistical methods above).

Figure 4 On-Treatment LSM Change From Baseline in mNIS+7 Composite Score using MMRM analysis (Full Analysis Set).

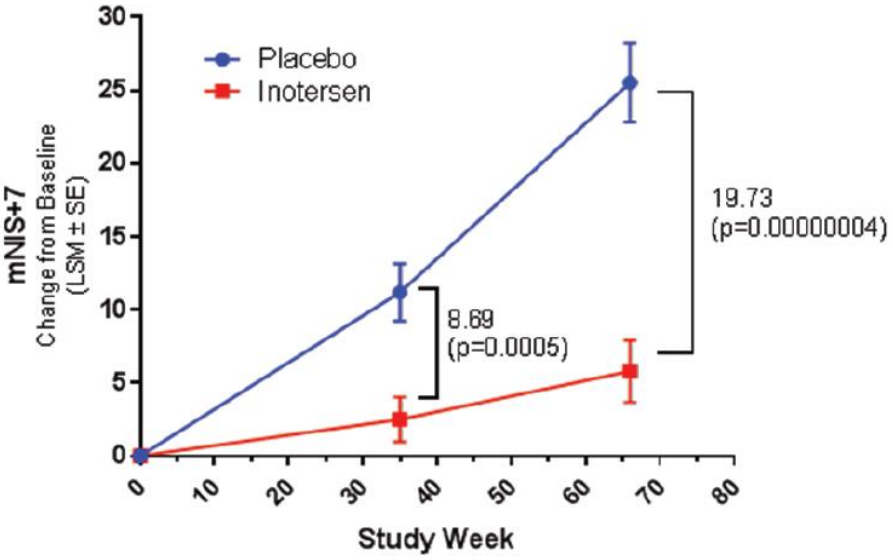
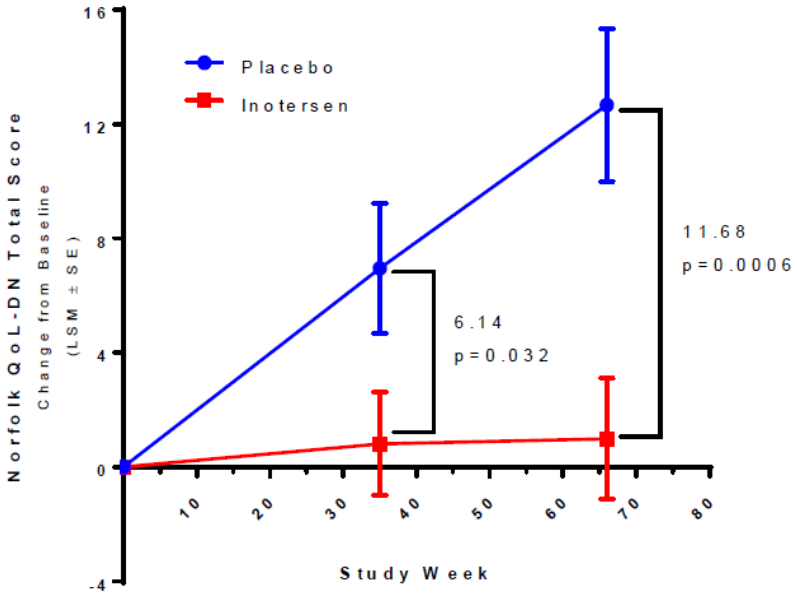
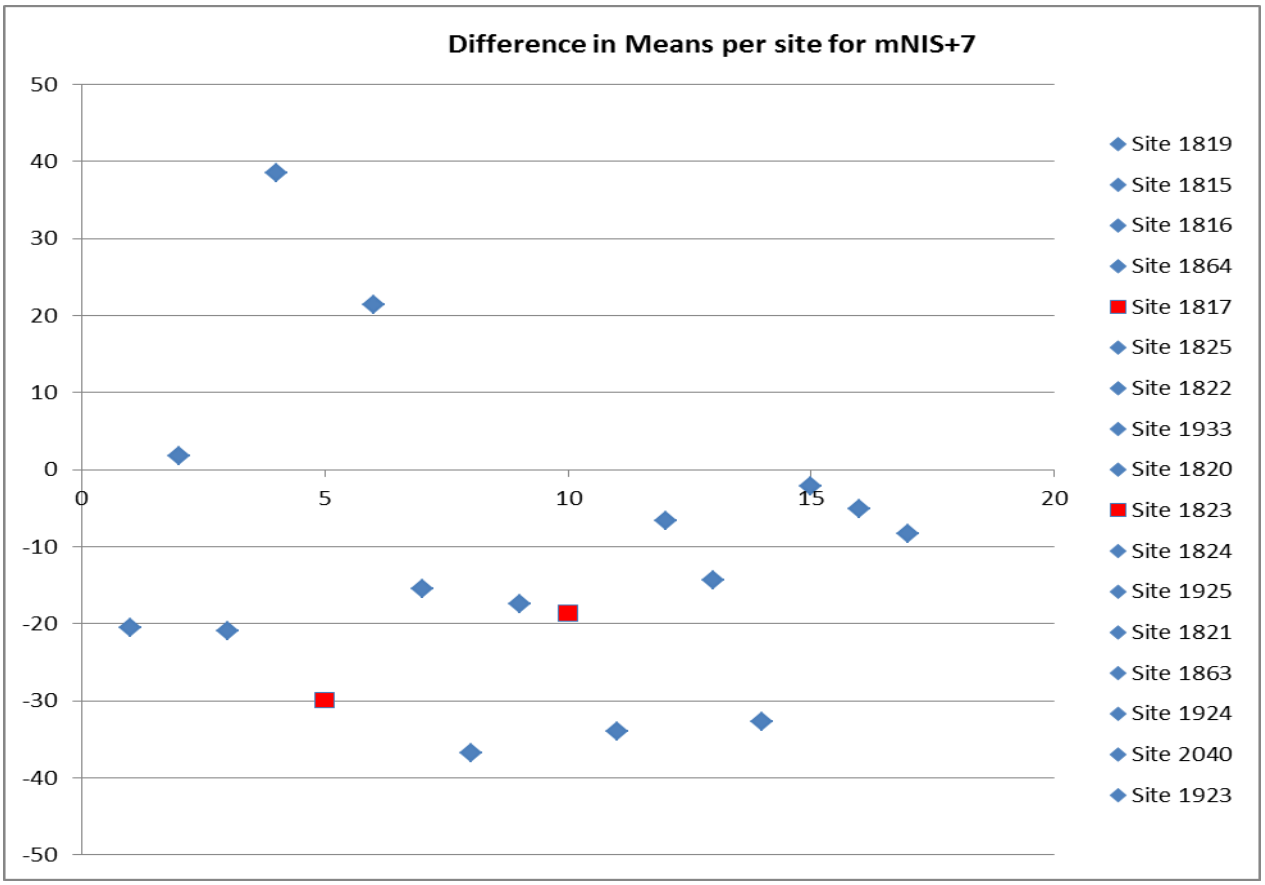


Figure 5 On-Treatment LS Mean Change From Baseline in Norfolk QoL-DN Total Score using MMRM analysis (Full Analysis Set)



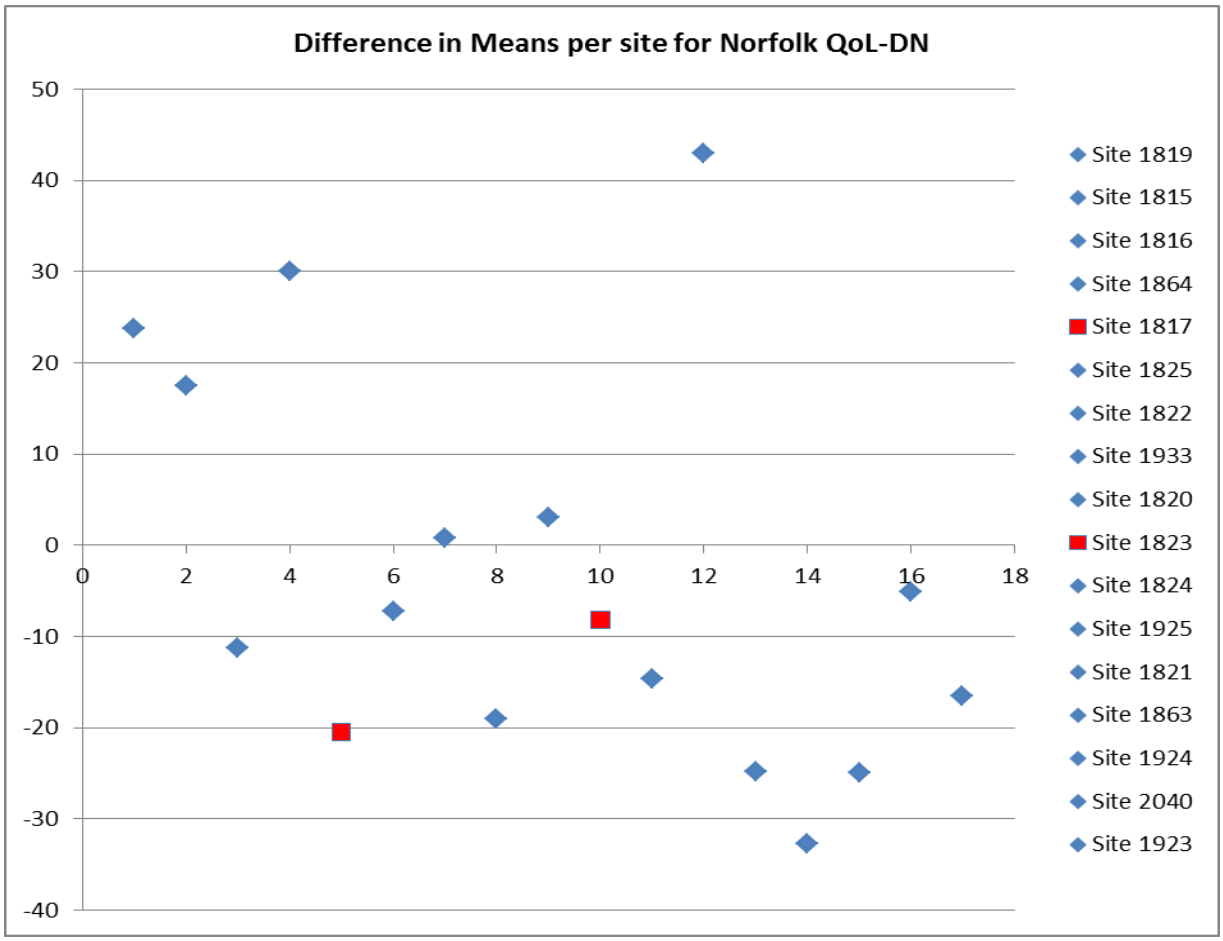
In order to clarify whether any sites could have had a major influence on the results, an analysis was performed by the CHMP with the primary efficacy results pooled from all sites, all European sites except Site 1817, all USA sites except Site 1823, and all South American/New Zealand sites except Site 1863 and presented by the Applicant in a Table. From this analysis the centres that recruited more patients (1817 in Europe and 1823 in USA) did not dominate the results and no specific trends in individual sites or in regions could be detected. There were not one or two sites that played a major role in the differences in the means for the endpoints favouring inotersen versus placebo and defining the outcome of the study, as it can be seen from the graphs below.

Figure A. Difference in means per site for mNIS+7



Note: The difference in means for mNIS+7 for site 1817 is -29.93 and for site 1823 is -18.64 (marked with red squares)

Figure B. Difference in means per site for Norfolk QoL-DN



Note: The difference in means for mNIS+7 for site 1817 is -20.50 and for site 1823 is -8.18 (marked with red squares)

It is also noted that a number of predefined sensitivity analyses have been performed and these were confirmatory of the results obtained with the primary analysis of the primary endpoints (please see below), including sensitivity analysis 6, which is considered of highest relevance (see discussion on statistics below) and results show, that also this analysis yielded significant results for both primary endpoint at week 66 with point estimates of -14.89 (95% CI: -22.55; -7.22, p<0.001) and -8.56 (95% CI: 15.42; -1.71, p=0.015) for mNIS+7 and Norfolk QoL questionnaire, respectively. Considering sensitivity analysis 6, the results for week 35 are -7.57 (95% CI: -12.3 to 2.284; p-value=0.002) and -4.69 (95% CI: -10.56 to 1.18; p-value=0.116) for mNIS+7 and Norfolk QoL questionnaire, respectively.

Sensitivity analyses

Results for all 13 sensitivity analyses of the change from Baseline in the mNIS+7 composite score conducted in CS2 were consistent with the primary analysis (Table 9). Statistical significance was maintained at Week 35 and Week 66 for all pre-specified sensitivity analyses, including all missing data sensitivity analyses.

Table 9 Sensitivity Analyses for Primary Endpoints (CS2 Full Analysis Set)

	Difference [Inotersen 300 mg– Placebo] (p-value)
	Week 66
Primary Analysis	
mNIS+7	-19.73 (<0.001)
Norfolk	-11.68 (<0.001)
Sensitivity Analysis	
Sensitivity Analysis 1 (Non-parametric analysis)	
mNIS+7	-18.81[a] (<0.001)
Norfolk	-12.00 (<0.001)
Sensitivity Analysis 2 (Conservative assessment level imputation)	
mNIS+7	-19.60 (<0.001)
Norfolk	-11.61 (<0.001)
Sensitivity Analysis 3 (Excluding assessments done at early termination visits)	
mNIS+7	-20.04 (<0.001)
Norfolk	-11.64 (<0.001)
Sensitivity Analysis 4 (Multiple imputation assuming missing at random)[b]	
mNIS+7	-19.43 (<0.001)
Norfolk	-10.91 (0.002)
Sensitivity Analysis 5 (Multiple imputation assuming copy increments from reference)[b]	
mNIS+7	-15.74 (<0.001)
Norfolk	-9.05 (0.010)
Sensitivity Analysis 6 (Multiple imputation assuming jump to reference)[b]	
mNIS+7	**-14.89 (<0.001)**
Norfolk	**-8.56 (0.015)**
Sensitivity Analysis 7 (Data at withdrawal visit included)[b]	
mNIS+7	-19.19 (<0.001)
Norfolk	-11.43 (<0.001)
Sensitivity Analysis 8 (Per Protocol Set)	
mNIS+7	-18.80 (<0.001)
Norfolk	-10.82 (0.002)
Sensitivity Analysis 9 (Adjustment for pooled site)	
mNIS+7	-19.91 (<0.001)
Norfolk[a]	-11.22 (0.002)
Sensitivity Analysis 11 (HRDB and nerve conductions scored using points and NIS-sensation excluded)	
mNIS+7	-16.32 (<0.001)
Sensitivity Analysis 12 (Excluding HRDB)	
mNIS+7	-19.57 (<0.001)
Sensitivity Analysis 13 (Modified mNIS+7 Baseline definition)	
mNIS+7	-19.60 (<0.001)

Note: Stratification factors used in the sensitivity analyses of change from Baseline in mNIS+7 composite score were based on strata determined by IXRS at randomization.

a. Hodges-Lehmann estimate of difference inotersen minus placebo.

b. Sensitivity analysis was performed using the SS. All other sensitivity analyses were performed using the FAS unless otherwise noted.

A sufficient number of predefined sensitivity analyses have been performed supporting the primary efficacy results, which were analysed using MMRM. As mentioned above, this MMRM analysis is not supported. Instead sensitivity analysis 6 is considered of highest relevance and should be considered as primary instead of the MMRM analysis.

Of note, the amount of missing assessment level data was small (i.e. <4% of subjects in each treatment group had a missing sub-component at any visit), with the exception of the heart rate to deep breathing (HRDB) component. HRDB cannot be assessed in subjects with active pacing or with

atrial fibrillation, both of which were common in the study. Therefore, Sensitivity Analysis 12 was included to examine the change in mNIS+7 excluding HRDB data from analysis. Statistical significance was maintained in this analysis of change from Baseline in mNIS+7 composite score without this component score included.

The LSM changes from Baseline in mNIS+7 composite score using the PPS were similar to the primary analysis using the FAS. The difference in LSMs between treatment groups in the PPS was -7.54 (95% CI: -12.38, -2.70; p=0.003) and -18.80 (95% CI: -25.66, -11.94; p<0.001) at Week 35 and Week 66, respectively.

A responder analysis was conducted in CS2 to examine the difference in response between the 2 treatment groups over a range of thresholds (0 to 30 points). A responder was defined as a subject who was in the FAS set and who had a change from Baseline that was less than or equal to the threshold value. Subjects in the FAS who discontinued from the study prematurely or had missing data were considered non-responders.

In the CS2 responder analysis (sensitivity analysis 10), the response rate in the inotersen group was consistently higher than in the placebo group over all thresholds evaluated (0- to 30-point increase), with an approximate 2-fold difference observed between the inotersen and placebo groups at each threshold. Statistical significance in favour of inotersen treatment was demonstrated at all thresholds beyond a 0-point change (i.e., ≥2-point change). Using the smallest statistically significant threshold, a ≤2-point increase in mNIS+7, the response rate in the inotersen group (37.7%) was 2 times higher than that observed in the placebo group (18.6%). Importantly, although not statistically significant at ≤0 points, almost one-third of inotersen-treated subjects showed either improvement or no progression in neuropathy, as measured by mNIS+7.

For a more complete responder analysis in subjects with <0 Change or ≤2 points increase from Baseline to Week 66 in mNIS+7 Composite Score and/or Norfolk QoL-DN Total Score (CS2 Full Analysis Set, N=165), the followingTable 10**: Summary of Subjects with ≤0 Change or ≤2 points increase from Baseline to Week 66 in mNIS+7 Composite Score and/or Norfolk QoL-DN Total Score (CS2 Full Analysis Set, N=165)** Table 10 was compiled by the CHMP based on data provided by the Applicant in the summary of clinical overview and study report.

Table 10: Summary of Subjects with ≤0 Change or ≤2 points increase from Baseline to Week 66 in mNIS+7 Composite Score and/or Norfolk QoL-DN Total Score (CS2 Full Analysis Set, N=165)

	Placebo (N=59)	**Inotersen 300 mg (N=106)**	**Total (N=165)**
mNIS+7 Composite Score (observed cases only)			
≤0 change from Baseline to Week 66, n (%)	10/52 (**19.2**)	31/85 (**36.5**)	41/137 (29.9)
p-value (Chi-square test)		**0.032**	
mNIS+7 Composite Score (missing data = non-response)			
≤0 change from Baseline to Week 66, n (%)	10/59 (**16.9**)	31/106 (**29.2**)	41/165 (24.8)
p-value (Chi-square test)		0.081	
mNIS+7 Composite Score (missing data = non-response)			
≤2 points increase from Baseline to Week 66, n (%)	11/59 (**18.6**)	40/106 (**37.7**)	51/165 (30.9)
p-value (Chi-square test)		**0.011**	
Norfolk QoL-DN Total Score (observed cases only)			
≤0 change from Baseline to Week 66, n (%)	14/52 (**26.9**)	42/84 (**50.0**)	56/136 (41.2)
p-value (Chi-square test)		**0.008**	
Norfolk QoL-DN Total Score (missing data = non-response)			
≤0 change from Baseline to Week 66, n (%)	14/59 (**23.7**)	42/106 (**39.6**)	56/165 (33.9)
p-value (Chi-square test)*		**0.039**	
mNIS+7 Composite Score and Norfolk QoL-DN Total Score (observed cases only)			
≤0 change from Baseline to Week 66, n (%)	5/52 (**9.6**)	18/84 (**21.4**)	23/136 (16.9)
p-value (Chi-square test)		0.074	
mNIS+7 Composite Score and Norfolk QoL-DN Total Score (missing data = non-response)			
≤0 change from Baseline to Week 66, n (%)	5/59 (**8.5**)	18/106 (**17.0**)	23/165 (13.9)
p-value (Chi-square test)*		0.131	

**p values have been calculated by the CHMP*

Sensitivity analysis 6 is considered as the analysis of highest importance (instead of the MMRM which uses a hypothetical strategy to address the effect had no patient discontinued treatment), since this analysis accounts for the unfavourable effect of treatment discontinuation, does not exclude patients from analysis and uses a reasonable assumption for missing data handling in lack of data collected after treatment discontinuation needed to reliably address treatment policy strategy. A responder analysis based on the safety set instead of the FAS would have been preferred, but counting the patients not included in the FAS as non-responders is not expected to relevantly change results and conclusions.

Secondary, Tertiary and Exploratory Efficacy Results

The CS2 study incorporated multiple secondary, tertiary, and exploratory endpoints to evaluate further the efficacy of inotersen compared to placebo.

Table 11 Summary of On-Treatment Secondary, Tertiary and Exploratory Efficacy Endpoints, Study CS2 (CS2 Full Analysis Set)

Parameter	Placebo Change from Baseline n LSM (SE) 95% CI	Inotersen Change from Baseline n LSM (SE) 95% CI	Inotersen-placebo Change from Baseline LSM 95% CI p-value
Secondary endpoints			
Norfolk QoL-DN Symptoms Domain Score Stage 1	33 1.11 (0.778) -0.43, 2.66	55 -1.42 (0.608) -2.63, -0.21	-2.53 -4.49, -0.57 0.012
Norfolk QoL-DN Physical Functioning/Large Fiber Domain Score Stage 2	19 9.04 (2.481) 4.04, 14.03	29 0.78 (2.021) -3.28, 4.85	-8.25 -14.71, -1.80 0.013
Body Mass Index	49 -0.80 (0.204) -1.21, -0.40	82 -0.30 (0.159) -0.61, 0.02	0.50 0.00, 1.01 0.051
NIS Composite Score	52 18.65 (1.762) 15.16, 22.13	85 5.40 (1.403) 2.62, 8.17	-13.25 -17.65, -8.85 <0.001
NIS+7 Composite Score	52 20.39 (1.815) 16.80, 239.98	85 5.90 (1.444) 3.04 8.75	-14.50 -19.03, -9.96 <0.001
Modified +7 Composite Score	52 6.95 (1.540) 3.91, 10.00	85 0.46 (1.221) -1.95, 2.87	-6.49 -10.32, -2.66 0.001
Tertiary endpoints			
SF-36 Physical Component Summary Score[a]	51 -3.65 (1.011) -5.65, -1.65	84 -0.05 (0.802) -1.64, 1.53	3.59 1.07, 6.12 0.006
SF-36 Mental Component Summary Score[a]	51 -1.35 (1.121)	84 1.07 (0.888)	2.42

Parameter	Placebo Change from Baseline	Inotersen Change from Baseline	Inotersen-placebo Change from Baseline
	n LSM (SE) 95% CI	n LSM (SE) 95% CI	LSM 95% CI p-value
	-3.57, 0.87	-0.68, 2.83	-0.37, 5.22 0.088
SF-36 Mental Health Domain Score[a]	51 -2.48 (2.079) -6.60, 1.63	84 2.59 (1.645) -0.67, 5.84	5.07 -0.11, 10.25 0.055
Individual Components of NIS and Modified +7	See Clinical Overview		
Individual Domains of Norfolk QoL-DN	See Clinical Overview		
Exploratory endpoints			
NSC Total Score[a]	52 8.10 (1.121) 5.89, 10.32	85 1.77 (0.891) 0.01, 3.53	-6.33 -9.12, -3.55 <0.001
PND Score[a] Week 65, n Improved, n (%) Not changed, n (%) Worsened, n (%)	 52 2 (3.8) 37 (71.2) 13 (25.0)	 86 9 (10.5) 56 (65.1) 21 (24.4)	 Not applicable

Source: Module 2.7.3, Section 3.2

Note: mBMI was also designated as secondary endpoint but is not shown here. Interpretation of mBMI results were confounded by observed changes in albumin levels that differed slightly between groups. NIS+7, +7, and individual components of +7 were also designated as secondary or tertiary endpoints but are not shown here. NIS+7, +7 and the nerve conduction component of +7 were statistically significant at Week 66. The vibration of the big toe component of +7 was not statistically significant. These endpoints were included in the study for completeness as they were used in previous hATTR-PN studies.

a. *Analysis based on data collected up to 52 days after last dose of study drug.*

Table 12: Secondary, Tertiary, and Exploratory Endpoint Subgroup Analysis applying Sensitivity Analysis 6

Week 65/66 Analysis, Change From Baseline	**Parameter**	**Sensitivity Analysis 6 (Jump to Reference)**			
		LSM diff	**95% CI Low**	**95% CI High**	**p-value**
mNIS+7	Composite Score	-14.89	-22,55	-7,22	<0.001
m+7	Composite Score	-4,8	-8,82	-0,78	0,02
m+7	HRDB	-0,04	-0,24	0,16	0,7041
m+7	Nerve Conduction Score	-0,41	-0,83	0	0,051
m+7	Touch-Pressure Sensory Score	-1,83	-4,02	0,36	0,1009
m+7	Heat-Pain Sensory Score	-2,63	-4,54	-0,72	0,0069
+7	Composite Score	-0,74	-1,62	0,14	0,099
+7	HRDB	-0,04	-0,24	0,16	0,7041
+7	Nerve Conduction Score	-0,5	-1,02	0,02	0,0602
+7	Vibration Detection Threshold Score	0,08	-0,16	0,32	0,5236
NIS	Composite	-9,54	-14,55	-4,52	<0.001
NIS	Cranial Nerves Score	NA	NA	NA	NA
NIS	Muscle Weakness Score	-7,06	-10,11	-4,02	<0.0001
NIS	Reflexes Score	-0,68	-1,67	0,31	0,1771
NIS	Sensation Score	-2,23	-3,43	-1,04	0,0002
NIS+7	Composite Score	-10.65	-15,79	-5,52	<0.001
Norfolk	Total	-8,56	-15,42	-1,71	0,015
Norfolk	Symptoms Domain Score -Stage 1	-1,75	-3,46	-0,04	0,045
Norfolk	Physical Functioning/Large Fiber Neuropathy Domain - Stage 2	-6,34	-12,07	-0,62	0,03
Norfolk	Physical Functioning /Large Fiber Neuropathy Score	-5,08	-8,33	-1,83	0,0022

Norfolk	Symptoms Score	-1,97	-3,45	-0,49	0,0091
Norfolk	Activities of Daily Living Score	-1,71	-2,82	-0,60	0,0025
Norfolk	Small Fiber Neuropathy Score	-0,07	-1,02	0,87	0,8781
Norfolk	Autonomic Neuropathy Score	-0,56	-1,24	0,12	0,1075
mBMI	Modified Body Mass Index	6,96	-23,05	36,97	0,649
BMI	Body Mass Index	0,33	-0,12	0,78	0,155
NSC	Total Score	-4,72	-7,3	-2,13	0,0004
NSC	Muscle Weakness	-2,45	-3,72	-1,18	0,0002
NSC	Sensory (hypo/loss of sensation)	-0,01	-0,46	0,44	0,959
NSC	Sensory (paresthesia, hyper sensation)	-1,25	-2,2	-0,31	0,0094
NSC	Autonomic (GI/urinary incontinence)	-0,53	-1,01	-0,04	0,0338
NSC	Autonomic (other than GI/urinary incontinence)	-0,65	-1,3	0,01	0,0521
SF-36	Physical Component Summary Score	2,72	0,54	4,91	0,0147
SF-36	Mental Component Summary Score	2,25	-0,38	4,89	0,094
SF-36	Mental Health Domain Score	3,77	-0,87	8,4	0,1114
TTR	Change from Baseline	-0,11	-0,13	-0,09	<0.0001
Retinol Binding Protein 4	Change from Baseline	-16279.6	-19289.12	-13270.1	<0.0001
NT-proBNP	log-transformed	0,07	-0,1	0,25	0,415

The analysis of change from Baseline in NIS composite score in CS2 showed a statistically significant difference in favour of inotersen treatment at Week 35 and Week 66 (-13.25, p<0.001), consistent with the mNIS+7 primary endpoint results . Statistical significance was also observed for the PPS . The analysis of change from Baseline in NIS+7 composite score showed a statistically significant difference in favour of inotersen treatment compared with placebo at CS2 Week 35 and Week 66 (-14.50, p<0.001), consistent with the mNIS+7 primary endpoint results .

With respect to BMI and mBMI the results cannot be considered conclusive since the Inotersen-placebo change from Baseline was small (0.5) and not statistically significant. Only a trend in favour of inotersen was observed and this could not be confirmed with the results from mBMI.

The secondary and tertiary efficacy endpoint analyses were mostly supportive of the positive efficacy outcome for the inotersen group. However, the CHMP considered that the evaluation of secondary endpoints should be repeated using sensitivity analysis 6 which was considered of highest relevance in this setting. The Applicant presented the requested analysis and has highlighted the differences in statistical significance compared to the MMRM analysis. Overall results for secondary, tertiary and exploratory endpoints using sensitivity analysis 6 were mostly consistent with results of the MMRM analysis, although the effect sizes were, as expected, overall smaller. It is worth mentioning that, when sensitivity analysis 6 was applied, changes in the nerve conduction score in m+7, the composite score and the nerve conduction score in +7, the reflexes score in NIS and the autonomic (GI/urinary incontinence and other) in NSC were not statistically significant and had p-values greater than 0.05, due to a reduced effect estimate compared to the MMRM analysis.

Ancillary analyses

Subgroup analysis

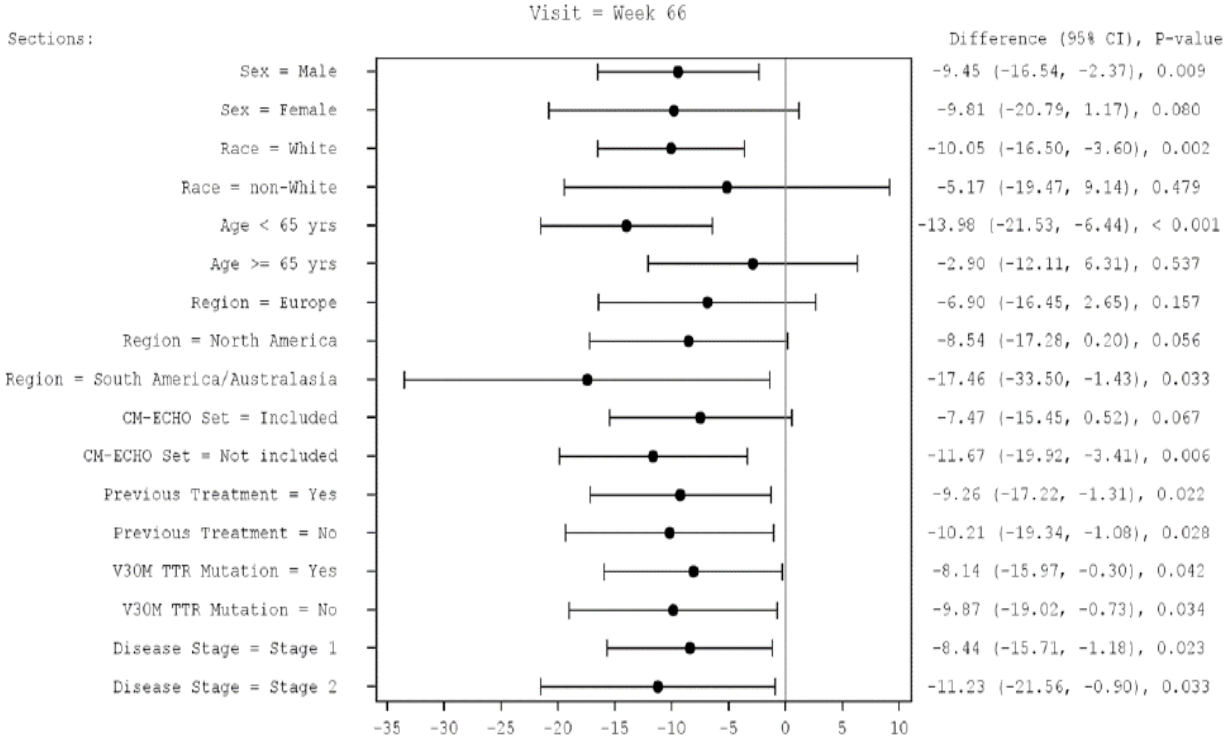
Changes from Baseline in mNIS+7 composite score and Norfolk QoL-DN total score were examined in 17 subgroups in CS2, including the stratification factors, presence or absence of cardiomyopathy, region, and selected demographics. A statistically significant benefit for inotersen treatment compared to placebo treatment was observed in all 17 subgroups based on mNIS+7 and in 11 subgroups based on Norfolk QoL-DN at Week 66. The 8 subgroups representing the stratification factors or presence/absence of cardiomyopathy were statistically significant for both mNIS+7 and Norfolk QoL-DN at Week 66 in all cases but one, which showed a trend (Norfolk QoL-DN: previous treatment with tafamidis or diflunisal, p=0.052).

With respect to the subgroup analysis, it is noted that a very small number of non-white patients were included in the CS2 study 6 in the placebo group and 6 in the inotersen 300mg group and values were obtained at week 66 for 5 patients in the placebo group and 3 in the inotersen group.

In the case of female and male population the numbers of patients were more balanced and the differences in LSMs between treatment groups were similar. No statistically significant treatment-by-sex interactions were observed.

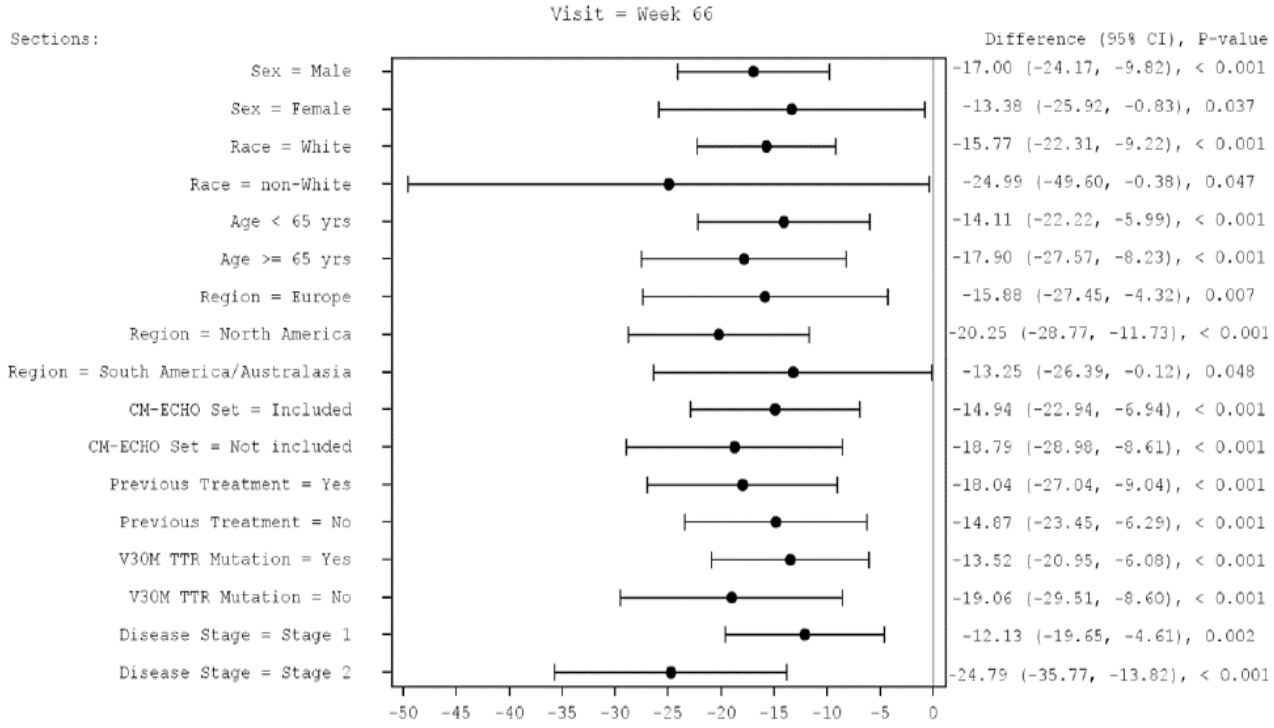
However, subgroups were also analysed using an MMRM analysis which is not considered to address a treatment effect of relevance in this setting. Instead, sensitivity analysis 6 is considered of highest importance and the Applicant has repeated subgroup evaluation using sensitivity analysis 6. All relevant changes compared to the MMRM analysis have been highlighted, but overall results using sensitivity analysis 6 are similar compared to the primary MMRM analysis, although the effect sizes are, as expected, overall smaller.

Figure 6 Forest Plots of Subgroup Analysis for Norfolk QOL-DN Total Score With Multiple Imputation Data Assuming Jump to Reference (On-Study) - CS2 Safety Set



Source: /project41/isis237007/stats/interim/prog/figures/f_mnis_mi_sub.sas 14MAR2018 21:52
Difference in LS means, confidence intervals, and p-values are from an ANCOVA model with change from baseline as the dependent variable, baseline, treatment, 3 strata factors and treatment by subgroup factors as covariate.

Figure 7 Forest Plots of Subgroup Analysis for mNIS+7 With Multiple Imputation Data Assuming Jump to Reference (On-Study) - CS2 Safety Set



USER ID HUANGTH: /project41/isis237007/stats/interim/prog/figures/f_mnis_mi_sub.sas 14MAR2018 21:52
Note: Difference in LS means, confidence intervals, and p-values are from an ANCOVA model with change from baseline as the dependent variable, baseline, treatment, 3 strata factors and treatment by subgroup factors as covariate.

From the Forest plots (Figures 6 and 7) consistency can be observed for mNIS+7 and Norfolk QoL-DN in the subgroups. In the case of mNIS+7 the effects were significant in all subgroups, whilst there were non-significant effects in some subgroups for the Norfolk QoL-DN such as in non-white patients (due to the very small number of non-white patients) and in patients 65 years or older. However, this was also observed for the MMRM analysis.

It is noted that the effect seems to be larger for stage 2 than for stage 1 patients (especially for the mNIS+7).

Summary of main study

The following table (Table 18) summarises the efficacy results from the main study CS2 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 13 Summary of efficacy for trial CS2

Title: A Phase 2/3 Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of ISIS 420915 in Subjects with Familial Amyloid Polyneuropathy	
Study identifier	Pivotal Study: CS2 (ISIS 420915-CS2) EudraCT number: 2012-001831-30

Design	A Phase 2/3 multicenter, double-blind, randomized, stratified, placebo controlled study of inotersen in Stage 1 and Stage 2 subjects with hATTR-PN with a Neuropathy Impairment Score (NIS) ≥10 and ≤130.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	65 week (15 months) 6 weeks (screening and baseline assessment)[a] 260 weeks in study CS3	
Hypothesis	Superiority to placebo		
Treatments groups [a total of 172 of the 173 randomized subjects received at least 1 dose of study drug]	Inotersen		300 mg inotersen sodium (equivalent to 284 mg of parent acid), 1.5 mL SC injection Week 1: Days 1, 3 and 5 Weeks 2 to 65: once-weekly n= 113 (randomized), 112 dosed and were included in the Safety Set
	Placebo		SC injection Week 1: Days 1, 3 and 5 Weeks 2 to 65: once-weekly 60 randomised, 60 dosed
Endpoints and definitions	Two primary endpoints	mNIS+7 and Norfolk QoL-DN	Change from Baseline to Week 66 in the modified Neuropathy impairment score +7 (mNIS+7) score and in the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire total score.
	Secondary endpoints	-Norfolk QoL-DN questionnaire symptoms domain score (Stage 1 subjects only) and the Norfolk -QoL-DN questionnaire physical functioning/large fiber neuropathy domain score (Stage 2 subjects only) (Week 66) -Modified Body Mass Index (mBMI) (Week 65) -BMI (Week 65) -NIS (Week 66) -modified +7 (Week 66) -NIS+7 (Week 66) -Global longitudinal strain (GLS) by echocardiogram (ECHO) in the ECHO Subgroup and in the CM-ECHO Set (Week 65)	▪ Change from Baseline to Week 66 in the Norfolk QoL-DN questionnaire symptoms domain score (Stage 1 subjects only) and the Norfolk QoL-DN questionnaire physical functioning/large fiber neuropathy domain score (Stage 2 subjects only) ▪ Change from Baseline to Week 65 in the mBMI ▪ Change from Baseline to Week 65 in the BMI ▪ Change from Baseline to Week 66 in the NIS ▪ Change from Baseline to Week 66 in the modified +7 ▪ Change from Baseline to Week 66 in the NIS+7 ▪ Change in GLS by ECHO from Baseline to Week 65 in the ECHO subgroup and in the CM-ECHO Set
	Secondary Pharmacodynamic endpoints		▪ Change and percent change from Baseline to Week 65 in TTR level ▪ Change and percent change from Baseline to Week 65 in RBP4 level.

<table>
<tr><td></td><td>Tertiary Endpoints</td><td></td><td>▪ SF-36 questionnaire Physical Component Summary score, Mental Component Summary score, and Mental Health Domain score. The SF-36 scoring methods are described in the Module 5.3.5.1, CS2 CSR, Appendix 16.1.9, Section 3.2.1.2.
▪ Individual components of the NIS score (cranial nerves, muscle weakness, reflexes, and sensory)
▪ Individual components of the modified +7 score (HRDB, nerve conduction, heat-pain sensory (i.e., heat as pain), and touch-pressure sensory)
▪ Individual components of the +7 score (nerve conduction and vibration detection threshold)
▪ +7 score
▪ Individual domain scores of the Norfolk QoL-DN (physical functioning/large fiber neuropathy, symptoms, activities of daily living, small fiber neuropathy, and autonomic neuropathy)</td></tr>
<tr><td>Database lock</td><td colspan="3">Data Cut-off Date: 28-MAR-2017 (First Subject Enrolled: 15-MAR-2013)</td></tr>
<tr><td colspan="4"><u>Results and Analysis</u></td></tr>
<tr><td>Analysis description</td><td colspan="3">Primary Analysis</td></tr>
<tr><td>Analysis population and time point description</td><td colspan="3">Full Analysis Set (FAS): Inotersen:106, PL: 59,
Safety Set (SS): INO:112, PL: 60,
Per-Protocol Set (PPS): INO: 83, PL: 52
<table>
<tr><td></td><td>PL (n)</td><td>INO (n)</td><td>PL (%)</td><td>INO (%)</td></tr>
<tr><td>Randomised</td><td>60</td><td>113</td><td>100.0</td><td>100.0</td></tr>
<tr><td>Dosed</td><td>60</td><td>112</td><td>100.0</td><td>99.1</td></tr>
<tr><td>Safety set (SS)</td><td>60</td><td>112</td><td>100.0</td><td>99.1</td></tr>
<tr><td>Full Analysis Set (FAS)</td><td>59</td><td>106</td><td>98.3</td><td>93.8</td></tr>
<tr><td>Per Protocol Set (PPS)</td><td>52</td><td>83</td><td>86.7</td><td>73.5</td></tr>
<tr><td>PK Subgroup</td><td>8</td><td>10</td><td>13.3</td><td>8.8</td></tr>
<tr><td>PK Set</td><td>0</td><td>111</td><td>0.0</td><td>98.2</td></tr>
<tr><td>PK Subgroup (PK Set)</td><td>0</td><td>10</td><td>0.0</td><td>8.8</td></tr>
<tr><td>ECHO Subgroup</td><td>22</td><td>44</td><td>36.7</td><td>38.9</td></tr>
<tr><td>CM-ECHO Set</td><td>33</td><td>75</td><td>55.0</td><td>66.4</td></tr>
<tr><td>TTR Subgroup</td><td>18</td><td>37</td><td>30.0</td><td>32.7</td></tr>
</table></td></tr>
<tr><td>Descriptive statistics and estimate variability</td><td>Treatment group</td><td>Placebo</td><td>Inotersen 300mg</td></tr>
<tr><td></td><td>Number of subjects (Baseline)</td><td>N=59</td><td>N=106</td></tr>
<tr><td></td><td>Number of subjects (Week 66)</td><td>N=52</td><td>N=85</td></tr>
<tr><td></td><td>On-Treatment mNIS+7 Composite Score (CS2 Full Analysis Set) Change from Baseline</td><td>Week 66
Mean value
23.89</td><td>Week 66
Mean value
4.16</td></tr>
</table>

	SD	24.190	15.672
	Number of subjects	N=52	N=85
	Statistical analysis of change from Baseline LSM	**Week 66 25.53**	**Week 66 5.80 Difference in LSM -19.73 p-value=0.00000004**
	SE	2.690	2.127
Notes	The LSM changes from Baseline in mNIS+7 composite score using the PPS were similar to the primary analysis using the FAS. The difference in LSMs between treatment groups in the PPS was -7.54 (95% CI: -12.38, -2.70; p=0.003) and -18.80 (95% CI: -25.66, 11.94; p<0.001) at Week 35 and Week 66, respectively Sensitivity analysis 6: Difference [Inotersen 300 mg– Placebo] at week 66 was -14.89 (<0.001)		
Descriptive statistics and estimate variability	Treatment group	Placebo	Inotersen 300mg
	Number of subjects (Baseline)	N=58	N=105
	Number of subjects	N=52	N=84
	On-Treatment **Norfolk QoL-DN Total Score** (CS2 Full Analysis Set) Change from Baseline	Week 66 Mean value **10.77**	Week 66 Mean value **-0.08**
	SD	21.134	18.967
	Number of subjects	N=52	N=85
	Statistical analysis of change from Baseline LSM	**Week 66 12.67**	**Week 66 0.99 Difference in LSM -11.68, p-value=0.0006**
	SE	2.666	2.117
Notes	The LSM changes from Baseline in Norfolk QoL-DN total score using the PPS were similar to the primary analysis using the FAS. The difference in LSMs between treatment groups in the PPS was -6.15 (95% CI: -11.88, -0.41; p=0.036) and -10.82 (95% CI: 17.65, -3.99; p=0.002) at Week 35 and Week 66, respectively. Sensitivity analysis 6: Difference [Inotersen 300 mg– Placebo] at week 66 was -8.56 (0.015).		

Clinical studies in special populations

Elderly patients

Subgroup analyses were performed based on data collected in the pivotal study CS-2 and CS-3, but not a study dedicated in older subjects. The disposition of the elderly patients in the two trials based on age brackets is presented below:

	Age <65 (Older subjects number /total number) Placebo, Inotersen	**Age ≥65 (Older subjects number /total number) Placebo, Inotersen**	**Age 85+ (Older subjects number /total number)**
Controlled Trials CS2 (safety set) CS2 Study Report	98 out of 173 Placebo:34 Inotersen: 64	74 out of 173 Placebo: 26 Inotersen: 48	0
Open Label Extension CS3 study Age group at CS3 screening (yrs)	57 out of 114 Placebo:19 Inotersen: 38	57 out of 114 Placebo: 21 Inotersen: 36	0

A subgroup analysis by age in two groups <65 years and ≥65 years was performed. Efficacy was investigated in the study CS2 and safety was evaluated in study CS3. The oldest subjects who participated in the pivotal CS2 trial were a 78 year old white male who received inotersen and an 81 year old white female who received placebo. The values at baseline were comparable for patients ≥65 years without noticeable differences (mean value of mNIS+7 Composite Score was 80.19 for placebo and 80.85 for inotersen 300mg and mean value of Norfolk QoL-DN was 49.55 for placebo and 47.19 for inotersen 300mg).

In study CS2, subjects aged <65 years and ≥65 years showed a statistically significant difference in favour of inotersen treatment compared with placebo at Week 35 and Week 66, based on changes from Baseline in mNIS+7 composite score. The differences in LSMs between inotersen and placebo were statistically significant at Week 66 in both age groups. Subjects ≥65 years of age also showed a statistically significant difference in favour of inotersen treatment at Week 35. A statistically significant treatment-by-age interaction at the 10% significance level was observed at Week 35 (p=0.064), but not at Week 66.

Changes from Baseline in Norfolk QoL-DN total score showed a statistically significant difference in favour of inotersen in subjects <65 years of age at Week 66 and trends in favour of inotersen treatment were observed at Week 35 (MMRM analysis). Statistical significance was not achieved at either time point in subjects ≥65 years of age, but there was a trend in favour of inotersen treatment at both time points. The differences in LSMs were similar for the 2 age groups at Week 35. At Week 66, a statistically significant treatment-by-subgroup interaction was observed at the 10% significance level (p=0.069).

These analyses were repeated using sensitivity analysis 6, which is considered of highest relevance. In the case of mNIS+7 the effects were significant in all subgroups and in patients 65 years or older. However, it was noted that the effect on Norfolk QoL-DN is considerably smaller and non-significant (but still in favour of inotersen) for older patients compared to that for patients lower than 65 years of age (using either MMRM or sensitivity analysis 6).

CM-ECHO set

In the CS2 CM-ECHO Set, some Baseline disease characteristics suggested that subjects in the inotersen treatment group had slightly more severe cardiomyopathy at study entry compared with subjects in the placebo group. A higher proportion of subjects in the inotersen group (66.4%) were included in the CM ECHO Set compared with subjects in the placebo group (55.0%) in study CS2. Approximately 45% of subjects in CS3 had a concomitant diagnosis of hATTR-CM at CS2 entry, with a mean duration from diagnosis of 36.4 months at the time of CS3 entry.

Table 14 Number of Subjects Included in Each Analysis

Cohort	Placebo at Baseline	Placebo at Week 35	Placebo at Week 66	Inotersen Baseline	Inotersen Week 35	Inotersen Week 66
mNIS+7						
random	60	—	—	112	—	—
FAS	59	55	52	106	95	85
CM-ECHO	32	32	31	70	63	59
Non CM-ECHO	27	23	21	36	32	26
sum(CM-ECHO + non-CM-ECHO)	59	55	52	106	95	85
Norfolk						
FAS	58	57	52	105	94	84
CM-ECHO	32	32	31	70	63	59
Non CM-ECHO	26	25	21	35	31	25
sum(CM-ECHO + non-CM-ECHO)	58	57	52	105	94	84

Source: Module 5.3.5.1, CS2 CSR Table 12, Table 18, Table 23, and Table 28

Subjects in the inotersen group had a slightly longer duration from onset of hATTR-CM symptoms and a higher mean Baseline NT-proBNP concentration at study entry compared with the placebo group. The mean duration of hATTR-PN disease from the time of diagnosis and onset of symptoms was also longer in subjects in the inotersen group (35.0 months and 63.4 months, respectively) compared with subjects in the placebo group (23.3 months and 54.0 months, respectively) in the CM-ECHO Set.

The LSM changes from Baseline in mNIS+7 showed a statistically significant difference in favour of inotersen treatment compared with placebo at Week 35 and Week 66 in the CM-ECHO Set and the non-CM-ECHO Set (Table 19). The differences in LSMs between treatment groups were similar in the CM-ECHO Set and the non-CM-ECHO Set at both time points.

Table 15 On-Treatment mNIS+7 Composite Score by CM-ECHO Set (CS2 Full Analysis Set)

	Placebo (N=59)	Inotersen 300 mg (N=106)
Included in CM-ECHO Set[a]		
Absolute value		
Baseline, n	**32**	**70**
Mean (SD)	80.33 (38.304)	83.16 (36.419)
Change from Baseline		
Week 35, n	32	63
Mean (SD)	10.66 (14.963)	2.81 (15.561)
Week 66, n	31	59
Mean (SD)	24.79 (24.110)	7.19 (16.571)
Statistical analysis of change from Baseline[b], total n	56	95
Week 35, n[a]	32	63
LSM (SE)	10.99 (2.544)	3.73 (1.892)
95% CI	5.96, 16.02	-0.01, 7.47
Difference in LSM (SE)		-7.26 (3.128)
95% CI / p-value		-13.44, -1.07 / p=0.022
Week 66, n[a]	**31**	**59**
LSM (SE)	25.88 (3.456)	8.71 (2.556)
95% CI	19.04, 32.72	3.66, 13.77
Difference in LSM (SE)		**-17.17 (4.268)**
95% CI / p-value		**-25.62, -8.73 / p<0.001**
Treatment-by-subgroup interaction p-values		
Week 35		0.422
Week 66		0.252
Not included in CM-ECHO Seta		
Statistical analysis of change from Baselineb, total n	56	95
Week 35		
Na	23	32
LSM (SE)	11.44 (3.120)	0.15 (2.618)
95% CI	5.28, 17.61	-5.03, 5.32
Difference in LSM (SE)		-11.30 (3.911)
95% CI		-19.03, -3.57
p-value		0.004
Week 66		
Na	21	26
LSM (SE)	24.78 (4.217)	-0.40 (3.716)
95% CI	16.45, 33.12	-7.75, 6.95
Difference in LSM (SE)		**-25.18 (5.497)**
95% CI		**-36.06, -14.31**
p-value		**<0.001**
Treatment-by-subgroup interaction p-values		
Week 35		0.422
Week 66		0.252

Source: Module 5.3.5.1, CS2 CSR, Table 2.33
Note: Analysis based on data collected up to 52 days after the last dose of study drug.

a. Based on randomization strata by IXRS.

b. *Based on an MMRM with fixed categorical effects for treatment, time, each of the 3 randomization stratification factors (based on IXRS strata), treatment-by-time interaction, treatment-by-subgroup interaction, and treatment-by-time-by-subgroup interaction, and fixed covariates for the Baseline value of the endpoint and the Baseline-by-time interaction.*

Based on Norfolk QoL-DN total score, subjects who received inotersen in the CM-ECHO Set appeared to progress faster than subjects who received inotersen in the non-CM-ECHO Set (Table 20). A statistically significant treatment-by-subgroup interaction was observed at Week 35 (p=0.089), but not at Week 66.

Table 16 On-Treatment Norfolk QoL-DN Total Score by CM-ECHO Set (CS2 Full Analysis Set)

	Placebo (N=59)	Inotersen 300 mg (N=106)
CM-ECHO Set[a]		
Absolute value		
Baseline , n	**32**	**70**
Mean (SD)	54.21 (28.200)	53.12 (26.904)
Change from Baseline		
Week 35, n	32	63
Mean (SD)	3.63 (18.955)	0.88 (18.527)
Week 66, n	**31**	**59**
Mean (SD)	10.19 (22.670)	1.22 (21.373)
Statistical analysis of change from Baseline[b], total n	57	94
Week 35, n[a]	32	63
LSM (SE)	5.21 (3.007)	2.83 (2.204)
95% CI	-0.73, 11.15	-1.52, 7.19
Difference in LSM (SE)		-2.38 (3.667)
95% CI / p-value		-9.63, 4.87 / p=0.518
Week 66, n[a]	**31**	**59**
LSM (SE)	11.93 (3.482)	2.88 (2.561)
95% CI	5.04, 18.82	-2.18, 7.94
Difference in LSM (SE)		**-9.05 (4.266)**
95% CI / p-value		**-17.49, -0.61 / p=0.036**
Treatment-by-subgroup interaction p-values		
Week 35		0.089
Week 66		0.298
Not included in CM-ECHO Seta		
Statistical analysis of change from Baselineb, total n	57	94
Week 35		
na	25	31
LSM (SE)	9.26 (3.523)	-3.14 (3.138)
95% CI	2.29, 16.22	-9.34, 3.06
Difference in LSM (SE)		**-12.40 (4.540)**
95% CI		**-21.37, -3.42**
p-value		**0.007**
Week 66		
na	21	25
LSM (SE)	13.58 (4.204)	-2.77 (3.844)
95% CI	5.27, 21.89	-10.37, 4.83
Difference in LSM (SE)		-16.35 (5.530)
95% CI		-27.28, -5.41
p-value		0.004
Treatment-by-subgroup interaction p-values		
Week 35		0.089
Week 66		0.298

Source: Module 5.3.5.1, CS2 CSR, Table 2.41
Note: Analysis based on data collected up to 52 days after the last dose of study drug.

a. Based on randomization strata by IXRS.

b. *Based on an MMRM with fixed categorical effects for treatment, time, each of the 3 randomization stratification factors (based on IXRS strata), treatment-by-time interaction, treatment-by-subgroup interaction, and treatment-by-time-by-subgroup interaction, and fixed covariates for the Baseline value of the endpoint and the Baseline-by-time interaction.*

In CS2, mean GLS values were abnormal at Baseline in both groups in the CM-ECHO Set, as compared with established reference ranges [Smiseth, 2016]. No statistically significant improvements in GLS were observed with inotersen compared with placebo in the CM-ECHO Set or the ECHO Subgroup (Table 20). Based on ranges utilized in other cardiovascular conditions [Smiseth, 2016], no clinically significant worsening of GLS was observed in either treatment group. Consequently, it was difficult to evaluate a potential benefit of inotersen treatment over the time frame of CS2.

Table 17 On-Treatment Global Longitudinal Strain (%) (CS2 CM-ECHO Set and ECHO Subgroup)

	CM-ECHO Set		ECHO Subgroup	
	Placebo (N=33)	Inotersen 300 mg (N=75)	Placebo (N=22)	Inotersen 300 mg (N=44)
Absolute value (%)				
Baseline				
N	30	68	20	41
Mean (SD)	-14.63 (3.614)	-14.44 (3.995)	-14.21 (3.564)	-14.61 (4.073)
Median	-14.25	-13.90	-14.05	-14.10
Minimum, Maximum	-23.1, -8.5	-24.0, -6.7	-23.1, -8.5	-24.0, -7.6
Change from Baseline (%)[a]				
Week 65				
N	25	50	16	30
Mean (SD)	0.46 (2.702)	0.69 (3.134)	1.05 (2.745)	0.25 (3.163)
Median	-0.10	1.25	0.80	1.00
Minimum, Maximum	-4.5, 6.3	-6.1, 7.7	-4.5, 6.3	-6.1, 5.0
Statistical analysis of change from Baseline				
Total n included in statistical analysis			17	36
Week 65				
N	25	50	16	30
LSM (SE)	0.94 (0.588)	1.14 (0.497)	1.61 (0.747)	0.72 (0.577)
95% CI	-0.23, 2.11	0.15, 2.13	0.10, 3.11	-0.44, 1.88
Difference in LSM		0.20		-0.89
95% CI		-1.17, 1.56		-2.67, 0.90
p-value		0.771		0.322

Source: Module 5.3.5.1, CS2 CSR, Table 5.02 and Table 5.03
Note: Analysis based on data collected up to 52 days after the last dose of study drug.
a. Positive changes from Baseline indicate a worsening in GLS.

Mean GLS values were also abnormal at CS3 Baseline in both groups in the CM-ECHO Set, as compared with established ranges [Yingchoncharoen, 2013; Smiseth, 2016]. However, the changes in GLS observed in both groups (inotersen-inotersen and placebo-inotersen) over time were small and variable.

Taken together, the duration of the studies was likely too short to evaluate potential benefits of inotersen on GLS.

No statistically significant differences were observed between the inotersen group and the placebo group in the CS2 CM-ECHO Set in selected parameters of left ventricular (LV) size and function including interventricular septum (IVS) thickness, posterior wall thickness, LV ejection fraction, LV mass, LV mass index, left atrial strain, or E/Em lateral ratio. The changes in key ECHO measurements related to cardiac wall thickness (LV mass, intraventricular septum thickness, posterior wall thickness) showed an overall decrease at Week 65 that was numerically greater in the inotersen group compared with the placebo group.

Further analyses of cardiac wall thickness parameters (LV mass, IVS thickness, and posterior wall thickness) were conducted in a subgroup of subjects with severe cardiomyopathy, defined as a Baseline IVS thickness $\geq$ 1.5 cm. The placebo subjects showed an increase in cardiac wall thickness parameters while the inotersen subjects showed a reduction in wall thickness. The differences in the

LSM changes from Baseline between the inotersen and placebo groups were statistically significant for all 3 parameters (Table 20). The decrease in cardiac thickness and mass suggest regression of cardiac amyloid.

Table 18 Comparison of Change from Baseline to Week 65 in LV Mass, Posterior Wall Thickness, and Interventricular Septum Thickness (CS2 Randomized Set, Subjects with Baseline IVS Thickness of At Least 1.5 cm [N=64])

Parameter	Placebo (n=19) Change from Baseline n[a] LSM (SE) 95% CI	Inotersen (n=45) Change from Baseline n[a] LSM (SE) 95% CI	Placebo-Inotersen Change from Baseline LSM 95% CI p-value
LV mass (g)[b]	16 11.721 (12.648) -13.787, 37.229	35 -18.639 (8.765) -36.315, -0.963	-30.359 -57.424, -3.295 0.0288
Interventricular septum thickness (cm)[b]	16 0.086 (0.070) -0.056, 0.227	35 -0.102 (0.049) -0.200, -0.003	-0.187 -0.337, -0.038 0.0150
Posterior wall thickness (cm)[b]	16 0.063 (0.071) -0.080, 0.207	35 -0.095 (0.050) -0.195, 0.005	-0.158 -0.311, -0.006 0.0425

Source: Module 2.7.3, Table 29

a. Number of patients with non-missing data on Baseline covariates and change from Baseline at time point.

b. Based on an analysis of covariance (ANCOVA) with fixed categorical effects for treatment, gender and each of the 3 randomization stratification factors, and fixed continuous covariates for the Baseline value and age.

Abbreviation: LV=left ventricular; n=number of subjects; SE=standard error

Statistically significant differences in the ratio of geometric means for NT-proBNP were observed between the inotersen and placebo groups in the FAS at Week 13 and Week 35, but not at Week 65.

Table 19 On-Treatment NT-proBNP Results (Full Analysis Set) (extract from Table 56 Module 5.3.5.1, CS2 CSR, Section 11.9)

	Placebo (N=59)	Inotersen 300 mg (N=106)

Statistical analysis of log(post-Baseline value)-log(Baseline)		
Total n included in statistical analysis	59	100
Week 13		
n	59	98
Ratio of geometric means (post-Baseline/Baseline, back-transformed)	0.9 (1.06)	1.1 (1.05)
95% CI (back-transformed)	0.8, 1.0	1.0, 1.2
Ratio of geometric means (inotersen/placebo, back-transformed)[a]		1.2
95% CI (back-transformed)		1.0, 1.4
p-value		0.012
Week 35		
n	57	92
Ratio of geometric means (post-Baseline/Baseline, back-transformed)	1.1 (1.08)	1.4 (1.06)
95% CI (back-transformed)	1.0, 1.3	1.2, 1.5
Ratio of geometric means (inotersen/placebo, back-transformed)[a]		1.2
95% CI (back-transformed)		1.0, 1.5
p-value		0.038
Week 65		
n	52	82
Ratio of geometric means (post-Baseline/Baseline, back-transformed)	1.2 (1.09)	1.4 (1.07)
95% CI (back-transformed)	1.0, 1.5	1.2, 1.6
Ratio of geometric means (inotersen/placebo, back-transformed)[a]		1.1
95% CI (back-transformed)		0.9, 1.4
p-value		0.340

Source: Table 2.63, Table 2.66

Note: Analysis based on data collected up to 52 days after last dose of study drug. Higher values of NT-proBNP indicate worsening. A ratio of <1 represents a decrease in NT-proBNP.

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

The Applicant has performed a comparison and analyses of results across studies, however the results are presented separately for CS2 Full Analysis Set, CS3 Full Analysis Set, CS2 Safety Set and CS3 Safety Set and not a pooled analysis.

Supportive study

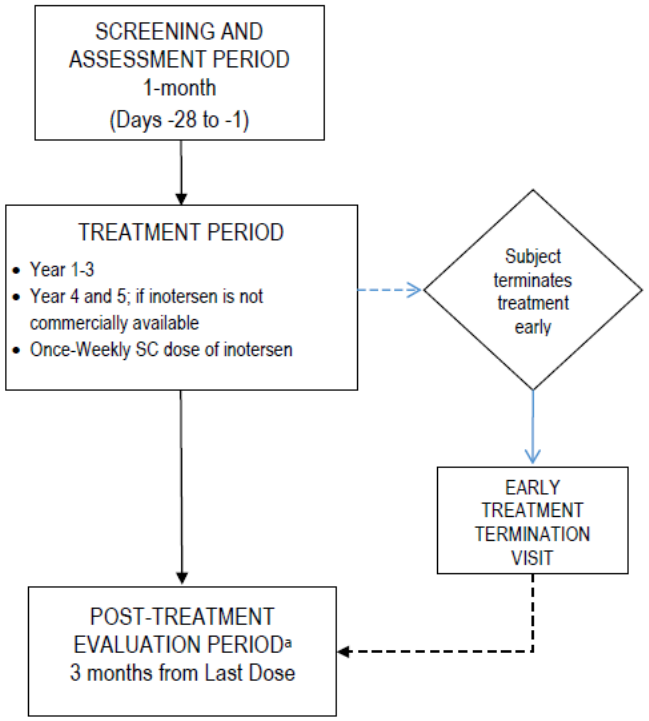
Supportive study CS3

The Supportive study CS3 was an open label extension multicentre study for an additional 260 weeks (5 years, currently ongoing), which recruited patients who had satisfactorily completed CS2.

Eligible subjects who had satisfactorily completed CS2 receive 300 mg inotersen once weekly for up to 260 weeks (5 years) in the OLE. Subjects who had a dose reduction or schedule change in the parent study are permitted to continue with the adjusted dose level or schedule in the OLE. Under special

circumstances, subjects who participated in CS2 but did not complete the full treatment period were allowed to participate in this study with approval from the Sponsor. All subjects receive supplemental doses of the recommended dietary allowance of vitamin A. In CS3, the efficacy of extended dosing with inotersen was evaluated as a secondary objective, and the majority of efficacy assessments identified for CS2 were also evaluated in CS3.

Figure 8 CS3 Design and Treatment Schema.



a. If a subject discontinues treatment in this study, but is continuing to receive treatment with inotersen via another mechanism (i.e., commercially available or expanded access), the entry of the subject into the 3-month post-treatment evaluation period may be omitted.
Abbreviation: SC=subcutaneous

There was no randomization in this OLE study. All subjects received inotersen. After completion of treatment, subjects enter the 3-month post-treatment evaluation period that consists of clinic and non-clinic visits for safety monitoring.

Treatment with 300 mg inotersen once weekly for up to 260 weeks (5 years) was continued for patients who participated in ISIS 420915-CS2.

Consistent with the pivotal study, CS3 is evaluating efficacy of inotersen by measuring mNIS+7 and the Norfolk QoL-DN total score, but in CS3, they are assessed as secondary endpoints. The efficacy endpoints in CS3 included changes from CS2 Baseline and CS3 Baseline at Week 78, Week 156, and at the end of each subsequent treatment year. For some endpoints, other time points were also summarized.

The efficacy data from CS3 are supportive of those from CS2 and should be considered in the context of the following important limitations:

- The interim analysis data are summarized only and do not include the primary statistical analysis; the mixed effects model with repeated measures (MMRM) analyses will be completed at the end of the study.

- The open-label design, in contrast to placebo-controlled, has the potential to bias subjective endpoints.

- The study is ongoing, with an enrollment period of more than 2.5 years. Therefore, efficacy data were not complete at the time of the interim analysis, and subject numbers were limited at many time points, particularly beyond Week 78.

In addition, an investigator-initiated, open-label study to assess long-term safety and tolerability of inotersen 300 mg subcutaneous injection once weekly for 24 months enrolled approximately 20 patients with ATTR-CM [Benson, 2017]. The investigator-initiated study does not contribute with data supporting the efficacy claims.

The results obtained with the open label extension study corroborated the results obtained with CS2 study and efficacy was maintained throughout the whole duration of the study. However, the effect size for the inotersen-inotersen group was not as large as in the case of placebo-inotersen group. According to the Applicant, changes from CS2 Baseline in mNIS+7 composite score and Norfolk QoL-DN total score showed less progression in the inotersen-inotersen group compared with the placebo-inotersen group at CS3 Weeks 26, 52, and 78, indicating continued benefit with earlier inotersen treatment. This argument however, is a postulation that would require further confirmation with the continuation of the CS3 study.

2.5.3. Discussion on clinical efficacy

The clinical development programme of inotersen is comprised of the Phase 1 first-in-human study in healthy subjects (CS1), the Phase 2/3 pivotal study in hATTR-PN patients (CS2), and the Phase 3 open label extension study (CS3).

Design and conduct of clinical studies

A dose-finding study (CS1) investigating doses of from 50 mg to 400 mg found a maximum effect in TTR reduction with the 300 mg and 400 mg doses. Only minimal differences in the effect of inotersen were detected between the 300 mg and the 400 mg doses and the effect did not increase further with the 400 mg dose. Therefore, the decision to take the dose of 300 mg forward into phase 2/3 is acceptable.

The single pivotal Phase 2/3 study to support the marketing authorisation application was a multicenter, randomized, double-blind, placebo-controlled study of 15 months' duration. The protocol was discussed with Food and Drug Administration (FDA) and the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP). The applicant followed the scientific advice given.

The sample size calculation and the randomization procedure are acceptable. In general, the procedures to maintain the blind in the personnel involved in the trial conduct were also appropriate. However, the role of the external partner of the Applicant was initially unclear. The Applicant clarified that individuals that had access to unblinded safety, efficacy and PD data were R&D leads and Safety and Pharmacovigilance lead of the Partner Company. The individuals were not part of the team

responsible for the conduct of the study and the firewall was maintained between these individuals, others at GSK, and the study team.

The pivotal study CS2 recruited subjects with stage 1 or 2 hATTR-PN with a Neuropathy Impairment Score (NIS) ≥10 and ≤130. This study investigated the change from baseline in Modified Neuropathy Impairment Score+7 (mNIS+7) and in Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) at week 65/66 as co primary endpoints. The choice of end-points has been discussed during a Scientific Advice procedure in 2012 (Procedure No.: EMEA/H/SA/2286/1/2012/III). The Applicant has followed the recommendations of CHMP which, amongst others, stated that for the intended population, Neuropathy Impairment Score-lower limb (NIS-LL) is not appropriate. The Applicant has discussed the validity of Norfolk QoL-DN. This was not the case with modified NIS+7 score and at the CHMP's request the Applicant presented relevant arguments. The existence of recent publications on the performance of modified NIS+7 and its use in clinical studies supporting its validity has been noted by the CHMP.

Efficacy data and additional analyses

The baseline disease characteristics showed that the subjects in the inotersen group, on average, had more advanced autonomic neuropathy, sensorimotor neuropathy, and cardiomyopathy before starting treatment. However, (i) a relatively large effect size was observed between inotersen 300 mg and placebo groups with regard to change from baseline in the primary endpoints, mNIS+7 and Norfolk QoL-DN, (ii) other baseline characteristics were well balanced between treatment groups (i.e. random imbalances are covered by the statistical test), and (iii) change from baseline is analysed for all endpoints and evaluation includes a baseline covariate.

Both co-primary endpoints chosen, the changes from Baseline in mNIS+7 composite score and Norfolk QoL-DN total score, showed statistically significant benefit for inotersen compared with placebo at Week 66, as well as, at the earlier time point assessed, Week 35. Statistically, the primary endpoints were analysed in a hierarchical manner in order to protect against familywise type I error rate. The effect size observed in the two primary endpoints is considered large and clinically relevant, since the primary endpoints are considered to reflect the clinical condition of the patients and able to detect disease progression. The primary efficacy endpoint data were analyzed primarily using a Mixed Effects Model with Repeated Measures (MMRM).

Thirteen sensitivity analyses were performed and confirmed the robustness of the efficacy results and the clinically relevant effect size for the sensitivity analyses were consistent with the primary analysis, and statistical significance was maintained with all analysis at both Week 35 and Week 66.

However, the primary MMRM analysis is not considered of primary importance due to the following deficiencies: (1) an imbalanced number of patients was excluded from the FAS (1 and 6 patients were excluded for placebo and inotersen, respectively). (2) An imbalanced number of patients in the FAS was excluded from the MMRM analysis (3 (4) placebo and 11 (12) inotersen patients were excluded from analysis of mNIS+7 (Norfolk QoL DN)). (3) A hypothetical strategy estimand is used which addresses the effect had no patient discontinued treatment. This may yield an overoptimistic effect which is less relevant from a clinical efficacy perspective, since AEs are the main reason for treatment discontinuation (TD). A treatment policy strategy is considered of highest relevance reflecting an overall expected treatment effect irrespective of early treatment discontinuation. Of the 13 sensitivity analyses, No 6 is considered to be the most appropriate because in case data after TD (which is needed to reliably address a treatment policy strategy) is missing using placebo-based multiple imputation and hence addressing the treatment effect if treatment had no effect after TD is a

reasonable assumption to handle missing data. Sensitivity analysis 6 accounts for the unfavourable effect of treatment discontinuation and does not exclude patients from analysis. Therefore the CHMP considered that the results of this analysis are the most suitable to be included in the product information as they reflect the most clinically relevant estimand. Due to an increasing treatment effect over time and due to using placebo-based imputation, this estimand depends on the time point of analysis.

Dose interruptions frequently occurred especially in the inotersen arm. However, subjects were mostly followed-up despite dose interruptions. Most subjects (49 of 59 inotersen and 20 of 23 placebo) with dose interruptions were completely followed up until the end of the study and hence had non-missing week 66 data. All data despite dose interruptions were included in the MMRM and all sensitivity analyses. Results of all sensitivity analyses – in particular sensitivity analysis 6 that addresses the treatment effect if treatment has no effect after treatment discontinuation (i.e. a kind of 'worst case' assumption for dose interruption effect) - are consistent with the MMRM analysis for week 66.

For Norfolk QoL-DN, results for all 9 sensitivity analyses were also consistent with the primary analysis, and statistical significance was maintained across all analyses at Week 66. It is acknowledged that a number of sensitivity analyses were performed without identifying discrepancies and these analyses can be considered as confirmatory of the primary analysis of the primary efficacy endpoints.

There was also a responder analysis (sensitivity analysis 10) that showed consistency of benefit of inotersen over multiple thresholds. Clinically relevant progression of disease was improved or arrested in 36.5% of subjects treated with inotersen as evidenced by improvement (negative change) or no worsening in the mNIS+7 composite score ($p<0.032$) and 50% of subjects treated with inotersen had improvement (negative change) or no worsening based on the Norfolk QoL-DN total score ($p<0.008$) (observed cases). Based on the FAS and calculated by the CHMP, for mNIS+7 clinically relevant progression of disease was improved or arrested in 29.2% and 16.9% of subjects treated with inotersen and placebo, respectively ($p=0.081$). For Norfolk QoL-DN total score 39.6% (inotersen) and 23.7% (placebo) showed improvement or no worsening ($p=0.039$).

Subgroup analyses revealed that there were either statistically significant differences or trends in favor of inotersen treatment in the various subgroups, supportive of the positive efficacy outcome. Analyses in non-White subjects were limited due to small numbers. However, subgroup analyses were based on the MMRM analysis and they were repeated using sensitivity analysis 6. Overall, results for subgroup analysis using sensitivity analysis 6 were similar compared to the primary MMRM analysis, although the effect sizes are, as expected, overall smaller. From the Forest plots, consistency can be observed for mNIS+7 and Norfolk QoL-DN in the subgroups, in favour of inotersen. In the case of mNIS+7 the effects were statistically significant in all subgroups, whilst there were non-significant effects in some subgroups for the Norfolk QoL-DN such as in females, non-white patients (due to the very small number of non-white patients), patients 65 years or older and the regions Europe and North America. However, this was also observed for the MMRM analysis.

It is noted that the effect seems to be larger for stage 2 than for stage 1 patients (especially for the mNIS+7).

The results presented for the open label extension study CS3 corroborated the results for CS2 study and efficacy was maintained throughout the whole duration of the study. Whereas, the effect size was smaller for the inotersen-inotersen group compared to the placebo-inotersen group in CS3, it was larger in the inotersen-inotersen group when considering the whole study duration of CS2+CS3, indicating that benefits may be larger when starting treatment earlier in the disease. However, no

definitive conclusion can be drawn at this point in time and this is expected to be further investigated and confirmed by the final results of CS3.

The Applicant has provided the requested evaluation of the treatment effect estimates per study site.

The Applicant stated that the two sites that recruited many patients are centres of excellence: one in Europe, site 1817 and one in the USA, site 1823. The Applicant performed different analyses with results from all sites separately, from all sites pooled, from Europe only without site 1817, from USA only without site 1823, and from South America/New Zealand without site 1863.

Based on the presented analyses, the CHMP concluded that there was no specific site influencing to a greater extent the overall study results.

CM-ECHO set

Approximately 40% of subjects had a concomitant diagnosis of hATTR-CM at study entry, with a mean duration from diagnosis of 23.7 months. 45 (40.2%) subjects in Inotersen group and 22 (36.7%) subjects in the placebo group were diagnosed with hATTR-CM. The baseline characteristics did not show significant differences between the placebo and inotersen groups.

It noted that a significant improvement was not consistently observed in GLS or ECHO parameters, which are associated with an improvement in cardiomyopathy. According to the Applicant the lack of statistical significance is not surprising, given the relatively short treatment period and that no clinically significant worsening was observed in the placebo group for any of the ECHO parameters included in the exploratory analysis in CS2 study. Prognosis and progression of disease in patients with hereditary transthyretin amyloidosis cardiomyopathy is better than in patients with primary amyloidosis with a median survival of 24 to 66 months (as reviewed by Mankad AK and Shah KB, Curr Cardiol Rep 2017; 19: 97). Furthermore, only patients in earlier stages of cardiac disease were included (NYHA I and II). The CHMP considered that the outcome measurement time points of 35 and 62 weeks may indeed be too short to detect relevant effects on cardiac parameters in these patients. In addition, the results in study CS3 are difficult to interpret due to the low number of patients and the switch from placebo to inotersen.

In a subgroup of the ECHO-CM patients with more advanced cardiac disease, the effects of inotersen appeared to be stronger but these very limited data and post-hoc analyses need to be interpreted with great caution.

In addition, CHMP considered that ECHO parameters are not considered sufficient to demonstrate clinically relevant efficacy in patients with cardiomyopathy. Either a beneficial effect on morbidity/ mortality is expected or in case of an orphan disease, at least a beneficial effect on functional parameters e.g. 6 Minute Walk Test or VO2 max during exercise testing in the absence of a detrimental effect on morbidity/mortality in the relevant population should be demonstrated. These parameters were not investigated in CS2 and CS3.

Advanced disease (stage 3) patients

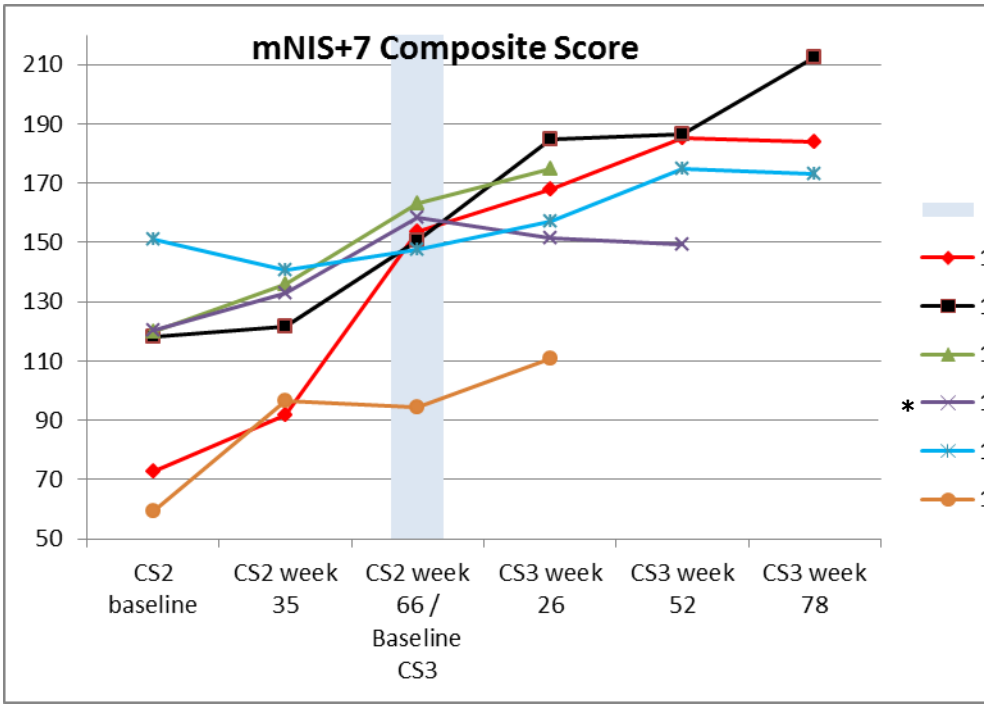
The disease hATTR-PN is classified into 3 stages such that i) Stage 1 patients do not require assistance with ambulation, ii) Stage 2 patients do require assistance with ambulation, and iii) Stage 3 patients are bound to wheelchair. Approximately two-thirds of treated subjects (67.4 %, n = 116) had Stage 1 hATTR-PN at Baseline and only 32.6 % (n = 56) of subjects were classified as having Stage 2.

Patients with stage 3 hATTR polyneuropathy were not enrolled in the pivotal study CS2 but the Applicant identified a small number of six (6) patients on placebo treatment who progressed to stage 3 during the CS2 study. These patients switched to treatment with inotersen at the beginning of the

extension study CS3. The applicant calculated annualised rates of the disease progression in these patients which suggested some positive effect of inotersen for these patients in the CS3 study. Of However, 4 out of these 6 patients discontinued treatment prematurely and the variability of the measured endpoints does not allow annualisation of the observed short-term effects. In addition, this was a post-hoc analyses including only a few patients of the placebo group, thus rendering the analysis subject to bias.

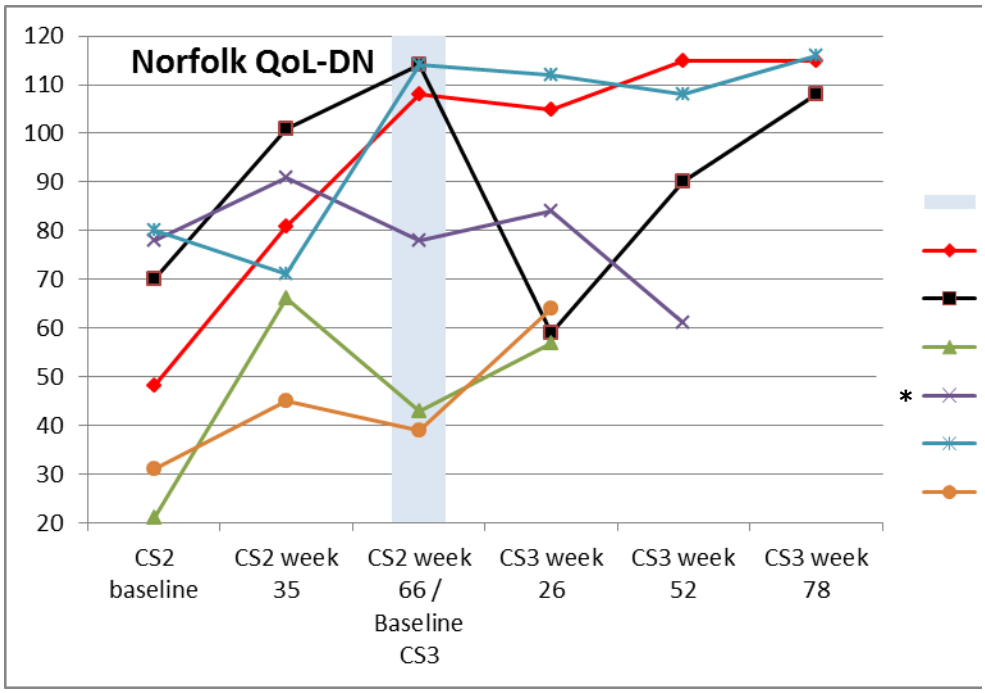
In order to be able to evaluate the outcome more clearly, the assessors prepared visual representations of mNIS+7 Composite Score (absolute values) and Norfolk QOL-DN Total Score for the six patients who progressed to Stage 3 disease, based on the data presented by the Applicant (Figures C and D). The y-axes in both graphs represent the endpoints' scores and a higher number translates into worse condition. The shaded column in the graphs represents the last measurement at week 66 for study CS2, when the patients were still on placebo and the first measurement at baseline of study CS3 when the patients started receiving inotersen treatment. The graphs indicate that at least in one of the primary endpoints a slower progression of the disease condition could be observed for 4 of these patients at stage 3 who received inotersen treatment, compared to being at stage 2 and receiving placebo.

Figure C **Graphical representation of the efficacy results for mNIS+7 Composite Score for the six patients who progressed to Stage 3 disease (y-axis represents the mNIS+7 Composite Score)**



**For this patient the last measurement was performed at week 41 and not week 52*

Figure D **Graphical representation of the efficacy results for Norfolk QOL-DN Total Score for the six patients who progressed to Stage 3 disease (y-axis represents the Norfolk QoL-DN Total score)**



**For this patient the last measurement was performed at week 41 and not week 52*

The Applicant also presented safety data of the six patients who were on placebo treatment in CS2 and progressed to Disease Stage 3. For 4 of these patients thrombocytopenia was recorded as a TEAE and for 1 patient decreased platelet count was observed, while these patients were on treatment with inotersen. With respect to renal function, the eGFR values remained at baseline levels in 5 patients and slightly improved in one patient. Although the AEs are in line with the inotersen safety profile in the overall population – it cannot be ruled out that inotersen could also have a more unfavourable safety profile in subjects with a more advanced disease stage. Not only the limited data hamper any firm conclusions but also the fact that most of the patients had various dose pauses during CS3 and 4 of 6 patients did not complete treatment (discontinued, withdrew voluntarily or terminated treatment early).

From the mechanism of action and biological plausibility, it would be expected that treatment with inotersen delays disease progression in all subjects regardless of disease stage. However, evidence of beneficial effects of starting inotersen treatment in patients at an advanced stage (i.e. stage 3) is unconvincing and efficacy demonstrated for earlier disease stages cannot simply be extrapolated to stage 3. Also, comparing the mean annualized rate of progression in CS3 with the one in CS2 may be biased towards concluding that progression is slower under inotersen as a result of selection of six placebo patients from CS2 that likely showed a stronger worsening of disease compared to other placebo patients not progressing to stage 3. Furthermore, it remains unknown whether the benefit/risk balance continues to be favourable in patients progressing to stage 3 of the disease, while being treated with inotersen. Based on the very limited data of subjects progressing to stage 3, the expected slowing of the disease progression cannot be confirmed.

Initially, the Applicant applied for a broad indication for the "treatment of adult patients with hereditary transthyretin amyloidosis". Due to the limitations of the submitted clinical data in patients with stage 3 polyneuropathy and in patients with cardiomyopathy, the CHMP requested a restriction of the indication to "treatment of stage 1 or stage 2 polyneuropathy in adult patients with hereditary transthyretin amyloidosis".

The arguments were as follows:

As per the inclusion/exclusion criteria, no patients with Stage 3 disease were included in the pivotal study CS2. Therefore, the study population is not reflected in the indication initially proposed by the applicant. Extrapolation of efficacy from earlier disease stages to stage 3 is not possible, the data on the 6 patient that progressed to stage 3 on placebo and were later treated with inotersen are too limited, and the analyses provided by the applicant are likely flawed. Thus, evidence is insufficient to allow a conclusion of efficacy of inotersen in patients with stage 3 disease.

A subgroup of 45 patients (40%) in the inotersen group were diagnosed with Cardiomyopathy hATTR-CM. Only patients with less advanced cardiac disease were included (NYHA I-II, mean NT-proBNP levels were only moderately increased). Although some positive effects of inotersen on ECHO parameters and biomarkers were observed, the clinical relevance of the findings cannot be judged in the absence of clinical functional tests such as the 6MWT or VO2max.

The Applicant has finally agreed to restrict the indication to "treatment of stage 1 or stage 2 polyneuropathy in adult patients with hereditary transthyretin amyloidosis (hATTR)".

2.5.4. Conclusions on the clinical efficacy

A clear statistically significant difference in the change from baseline in the two primary efficacy endpoints mNIS+7 and Norfolk QoL-DN was observed between placebo and inotersen 300 mg group in patients with stage 1 and stage 2 hereditary transthyretin amyloidosis polyneuropathy (hATTR-PN), which is supported by a substantial number of predefined sensitivity analyses. Also, sensitivity analysis 6 which is considered of highest relevance instead of the pre-specified primary MMRM analysis yielded clearly significant results. The clinical relevance of the results was further supported by responder analysis (sensitivity analysis 10).

Convincing effects on CM have not been shown. Therefore the Applicant has proposed a revised therapeutic indication with focus on polyneuropathy patients in accordance with the population studied.

Efficacy and safety of inotersen has not been systematically studied in patients with stage 3 disease and extrapolation of data from patients with Stage 1 and Stage 2 hATTR-PN is considered difficult. Six patients from the placebo group that progressed to stage 3 and were treated with inotersen in the open label study but these data are insufficient to conclude on relevant efficacy. In the light of the safety concerns, especially thrombocytopenia and bleeding risk, the CHMP considered that efficacy needs to be more robustly shown in stage 3 patients. Moreover, four of the six patients who progressed to stage 3 did not complete treatment due to thrombocytopenia and other TEAEs. Given the uncertainties related to the effect of inotersen in stage 3 hATTR-PN patients the applicant agreed, during the procedure, not to pursue any longer an indication in this population.

Based on the totality of presented evidence, the CHMP considered that efficacy of inotersen was demonstrated for treatment of stage 1 or Stage 2 polyneuropathy in adult patients with hereditary transthyretin amyloidosis (hATTR).

2.6. Clinical safety

The clinical safety of inotersen is based on a completed pivotal phase 2/3 study (ISIS 420915-CS2; in this report, final on-study data are presented with cut-off date: 28th March 2017) and interim data from a currently ongoing open-label study (ISIS 420915-CS3; original data cut-off date = 28th February 2017; updated cut-off date 15th September 2017). Subjects in CS2 were either randomised to placebo or inotersen 300 mg per week, while all subjects entering CS3 received inotersen, irrespective of treatment in CS2. The main body of the safety data base is generally considered adequately sized for a rare disease like hereditary transthyretin amyloidosis (hATTR).

The **CS2 safety set** informs on approximately 15-months safety data of inotersen compared to placebo, whereas the **CS3 grouping** reports on clinical safety for an additional 5-year period. Two further groupings of safety data are presented in the dossier: the **longitudinal safety set** refers to the safety profile of inotersen over the combined treatment periods in studies CS2 and CS3 (with reference to the CS2 baseline) in subjects, who received inotersen in both studies. The **integrated inotersen safety set** additionally includes subjects, who were randomised to placebo in the CS2 study and references results relative to the CS3 baseline. Results from a phase 1 single – and multiple dose study (CS1) are considered to be supportive in nature, while not pooled with the pivotal and the open-label experience due to different study designs. In addition, the safety profile of inotersen is assumed to be different in healthy volunteers and patients with transthyretin amyloidosis (ATTR). In this assessment, the focus was set on results from the placebo-controlled study CS2, which forms the basis of information reflected in the SmPC.

Subjects, who met additional ECHO inclusion criteria in CS2 formed the CM-ECHO subset and received additional transthoracic ECHO assessments during the treatment period in CS2. Safety parameters were intensively recorded in studies CS2 and CS3 (AESIs and OAEIs). In addition, laboratory parameters cover the most important safety issues with inotersen taking into account the signals from the preclinical data and the mechanistic features of the drug.

There is an ongoing inotersen open - label study referenced in the dossier (Benson 2017) including both, hereditary and wild-type ATTR-CM patients. This investigator-initiated study is referred to as not contributing to this application. Upon CHMP's request, few available safety data (serious adverse events) were provided by the applicant. This data did not reveal any new safety concerns.

Patient exposure

Placebo-controlled safety data of inotersen derive from a total of 113 subjects with hATTR of which 112 subjects received at least one dose of inotersen (300 mg once weekly). 85 of 112 (75.9%) of inotersen-treated subjects in CS2 continued inotersen treatment in CS3. Similarly, 81.7% of subjects on placebo during CS2 received inotersen in CS3 (n=49). The number of subjects currently contributing to the open-label long-term experience of inotersen is 134 (as per the CS3 interim data cut-off 15th September 2017 provided at Day 106). The new interim CS3 data extends the cumulative CS3 safety data by 6.5 months. 112 (69.6%) and 69 (42.9%) patients had at least 1 year and 2 years of inotersen exposure. The longest inotersen treatment exposure is currently for 4.36 years in 2 subjects. 27 of 134 subjects (20.1%) discontinued treatment for any reason in CS3 so far. Long-term safety information is currently limited as CS3 is ongoing and final results are not awaited in the course of this procedure. Hence, any conclusion on long-term outcomes of safety concerns is preliminary.

Discontinuation rates in CS2 were almost twice as high for subjects on inotersen compared to placebo – treated subjects (23% vs. 13.3%), with the main reasons being adverse events (AEs) or

serious adverse events (SAEs) (14.2% vs. 1.7%). SAEs leading to discontinuations were determined the following adverse events of special interest (AESIs): thrombocytopenia in four patients and glomerulonephritis in two patients. Discontinuations due to disease progression were more pronounced in subjects on placebo compared to inotersen (5% vs. 1.8%). Subjects on inotersen-inotersen during CS3 were reported to discontinue more frequently due to TEAEs compared to those on placebo-inotersen (9.4% vs. 4.1%), which might account for certain (S)AEs worsening over time. The reason for a higher percentage of subjects from the placebo-inotersen group that discontinued due to investigators judgement compared to those on inotersen-inotersen (7.5% vs. 1.4%) was not related to a specific risk.

Subjects not continuing treatment in the open-label study CS3 were captured in the CS2 post-treatment evaluation period (6 months duration) for further efficacy and safety assessment. Withdrawal from study due to an AE/SAE during this post-treatment evaluation period in 7% of subjects, who received inotersen in CS2, occurred as a consequence of the discontinuation from inotersen treatment due to an AE/SAE.

A lower percentage of subjects on inotersen in CS2 received between 61 and 70 of the 67 planned doses compared to those on placebo (68.8% vs. 85%) probably due to the higher rate of discontinuations in the inotersen group.

Dose holds were reported more frequently in inotersen-treated subjects compared to placebo-treated subjects in CS2 (52.7% vs. 38.3%). In particular, those differences were highest for holds due to platelet stopping rule ($<75 \times 10^9$ /L; inotersen 11% vs. placebo 0%) and renally related issues (investigator decision; 13.4% vs. 0%), in line with the known class effects of AONs. In total, 63.4% of subjects (85 out of 134) had dose pauses during CS3. Increases in dose pauses during CS3 in each treatment group and for all reasons are considered a consequence of the increased exposure to inotersen during CS3.

Baseline demographics of subjects in CS2 are in accordance with the target population, i.e. a mean age of 59.2 years, and slightly more male than female patients. Stratification was conducted for hATTR-PN disease stage (slightly more subjects with stage 1 than stage 2[67.4% vs. 32.6%], existence of the main mutation associated with hATTR, i.e. Val30Met (V30M; balanced in study patients), and previous treatment with tafamidis or diflunisal (yes [57.6%] or no). Compared to the CS2 safety set, patients included and evaluated in the CM-ECHO set were slightly older and less had V30M mutations. Of interest, more subjects on inotersen qualified for inclusion in this subset compared to placebo (66.4% vs. 55%), contributing to the assumption that subjects on inotersen were more diseased than those on placebo.

Supplemental medications given to all subjects in both studies were vitamin A products (at recommended daily allowance) in order to compensate a mechanistically – induced hypovitaminosis A and eye drops for dilatation (ERG examination).

Adverse events

Analyses were provided by system organ class (SOC), time to occurrence of adverse events, severity, and adverse event causality.

The incidence of adverse events of subjects from CS2 was ~ 100% for inotersen and placebo.

TEAEs were frequently found to be related to treatment and leading to a substantially higher number of discontinuations or withdrawals in subjects on inotersen compared to placebo in CS2. Adverse

events of special interest (AESIs) and SAEs were also higher in subjects on inotersen. TEAEs resulting in dose reduction and fatal TEAEs have been reported with inotersen only.

Dose reductions subsequently leading to TEAEs were more frequently observed with longer inotersen exposure. A substantial number of dose interruptions/delays was reported for subjects on inotersen in CS2 (24.1%) and even more pronounced in subjects, who were switched from placebo during CS2 to inotersen during CS3 (46.9%). Irrespective of the reason, more than 70% of subjects on inotersen had at least one dose hold over the course of studies CS2 and CS3 combined, hampering an accurate clinical safety interpretation over time.

Differences in TEAE incidences between inotersen and placebo could be seen on the level of the following SOCs (differences of ≥ 10%) for *Blood and Lymphatic System Disorders* (6.7% placebo vs 27.7% inotersen), *General Disorders and Administration Site Conditions* (55% placebo vs 85.7% inotersen), *Investigations* (30% placebo vs 52.7% inotersen), *Metabolism and Nutrition Disorders* (13.3% placebo vs 24.1% inotersen), and *Eye Disorders* (38.3% placebo vs 27.7% inotersen).

SOC differences in TEAE occurrence between inotersen and placebo in the *Blood and Lymphatic Disorders SOC* derived from a higher frequency in TEAEs of *anaemia and thrombocytopenia* (described within adverse events of special interest). Anaemia (more pronounced in the inotersen group) might be caused by baseline imbalances in autonomic neuropathy (associated with impaired erythropoietin response in hATTR). This association is further supported by the fact that more inotersen than placebo subjects had haemoglobin and haematocrit values below LLN at baseline.

TEAEs from the *Cardiac Disorders SOC* were balanced across treatment groups; however, the number of events was higher for inotersen than placebo, possibly as a consequence of more severe cardiomyopathy for inotersen-treated subjects at baseline. Medical history/conditions in line with cardiomyopathy (subjects were included in the CM-ECHO subset) preceded severe and/or serious TEAEs of atrial fibrillation and congestive heart failure. However, also vascular adverse events like arterial hypotension and hypertension were observed more frequently in the inotersen group and more severe CKD renal impairment stages (i.e. stages 4 and 5) have only been reached by (single) subjects on inotersen. eGFR decreases from baseline to post-baseline of more than 25% or 50% were clearly attributed to inotersen treatment (see below). Such AEs in the different organ classes may be related to each other (e.g. sinustachycardia due to hypotension, oedema due to renal failure in patients with underlying cardiomyopathy, decreased renal function due to hypotension etc.). Upon CHMP's request, the Applicant has identified 15 patients that had concomitant deterioration in parameters of renal function and AEs related to cardiac and/or vascular disorders. 10 of these patients were on inotersen, 5 on placebo (representing 8.9 and 8.3% of the patients). The data did not indicate that renal AEs were a reason for cardiac events and vice versa in the study. The incidence of TEAEs did not increase with continuous treatment.

There were several safety aspects that can be rated tolerability issues rather than significant safety problems, including TEAEs from the Ear and Labyrinth disorders SOC and Gastrointestinal Disorders SOC, the latter being a consequence of the underlying polyneuropathy. Inotersen as an antisense oligonucleotide was also found to be related to a higher number of TEAEs from the General Disorders and Administration Site Conditions SOC including injection site related events and constitutional symptoms. Even though the effect of inotersen on disease progression might lead to less weight loss in the target population, TEAEs in the Metabolism and Nutrition Disorders SOC (decreased appetite reported in 9.8% of subjects on inotersen) are likely to occur as a consequence of gastrointestinal events. Notably, two SAEs in this SOC led to death (cachexia) in subjects on inotersen.

A higher frequency of TEAEs in the Investigations SOC for inotersen was mainly driven by TEAEs of platelet count decreased.

Orthostatic hypotension (incidence: 6.3% inotersen and 0% placebo) within the Vascular Disorders SOC is a known manifestation of hATTR-PN that is thought to be in line with inotersen-treated subjects having more advanced cardiac disease than those in the placebo group.

The most commonly reported on-study TEAEs by preferred terms in the inotersen group affecting more than 10% of subjects with a difference in incidence of more than 5% with placebo were *injection site erythema (31.3% vs. 0%), nausea, fatigue, headache, injection site pain (20.5% vs. 6.7%), peripheral edema, pyrexia (19.6% vs. 8.3%), chills (19.9% vs. 3.3%), myalgia, vomiting (15.2% vs. 5%), thrombocytopenia (13.4% vs. 1.7%), anaemia (13.4% vs. 3.3%), injection site pruritus (11.6% vs. 0%), and platelet count decreased (10.7% vs. 0%)*.

Similar findings derive from CS3. However, a slightly lower occurrence of overall TEAEs between treatment groups (placebo-inotersen 93.9% and inotersen-inotersen 89.4%) was noted. This finding might account for the fact that a majority of TEAEs tended to occur with initial inotersen treatment and diminish over time. This is for most TEAEs supported by higher frequencies in the placebo-inotersen group during CS3 (in a majority of events in line with incidences from the inotersen group in CS2) compared to inotersen-inotersen. Differences found in the two treatment groups more pronounced in subjects who received placebo during CS2 are thought to be either a consequence of the progression of the underlying disease (e.g. urinary tract infection, myalgia, fall, and TEAEs from the GI disorders SOC) or a higher susceptibility to first-time treatment with inotersen (e.g. injection side rash, injection side erythema, vomiting, injection side pain, thrombocytopenia, diarrhoea, chills).

Analysis of TEAE occurrence by time of first administration of inotersen revealed that most of the TEAEs had their highest incidence within the first 6 months after treatment initiation with inotersen, followed by a reduced reporting rate 6 to 12 months after start of treatment. The re-increase in reporting in months 12 to 24 can best be explained by transitioning subjects from placebo to inotersen in CS3, i.e. TEAEs in subjects from the placebo-inotersen group. TEAEs that nearly exclusively occurred during the initial 6 months are *pyrexia, injection site reactions (erythema, pain), and headache*. Low reporting rates after 24 months of treatment initiation are thought to be a result of the low number of subjects included and hamper any firm conclusion on real long-term effects of inotersen.

Severity of TEAEs was mild or moderate in approximately two third of subjects in both studies. 28% of subjects on inotersen and 22% on placebo had at least one severe TEAE during CS2. Differences were mainly based on a higher incidence of severe cardiac disorder events (probably based on pre-existing cardiomyopathy) and GI events with inotersen. In CS3, the incidence of severe TEAEs was double as high in subjects with continuous inotersen treatment compared to those who previously received placebo. The most commonly reported severe TEAEs with inotersen were cardiac failure congestive, cardiac failure, cachexia, and renal impairment.

Causality assessment for TEAEs with inotersen and placebo revealed that most of the TEAEs observed in the clinical studies CS2 and CS3 were drug-related (for inotersen but not for placebo; 77.7% vs. 38.3%). Most of the TEAEs related to inotersen treatment were of tolerability issues, e.g. injection site reactions, chills, pyrexia, headache, and fatigue. Except for thrombocytopenia (related to drug for 11% of inotersen and 1.7% for placebo subjects), drug-related TEAEs were not TEAEs of special interest. Long-term inotersen was not obviously associated with a different pattern in drug-related TEAEs.

Inclusion of adverse drug reactions into the SmPC is based on a frequency at least twice as high in inotersen treated patients compared to placebo and biological plausibility.

Upon CHMP's request the Applicant has provided a comprehensive list of all adverse events and their frequencies that occurred during studies CS2 and CS3. Generally the events lack a biological plausibility or can be better explained by the underlying disease or concomitant treatment. None of these TEAEs require inclusion in the SmPC. The safety data that derived from analysis of the longitudinal and the inotersen integrated safety set mirrored the data from CS2 and CS3, while a majority of the events were those from CS2.

Overall, interpretation of safety data is hampered by the underlying disease itself presenting with various symptoms overlapping with adverse events from inotersen. In addition, few long-term safety data are included in the dossier and safety profile might change when longer-term data will become available.

Adverse events of special interest (AESI)

Ocular adverse events potentially related to vitamin A deficiency

Inotersen is capable to induce a vitamin A deficiency state due to inhibition of TTR that is – together with retinol binding protein 4 - responsible for the transport of retinol to respective tissues. All subjects received supplemental vitamin A throughout the studies.

Vitamin A deficiency – related adverse events were not detected in excess in studies CS2 or CS3 based on an overall similar occurrence of TEAEs in the respective treatment groups. A lower proportion of subjects on inotersen vs. placebo (27.7% vs. 38.3%) had TEAEs in the Eye Disorders SOC while on study. No subjects met the protocol-defined stopping rules for ocular effects.

Electroretinogram assessments performed do support the TEAE evaluation regarding changes from baseline to evaluation endpoint. Slight imbalances between treatment groups in abnormalities from baseline to endpoint are not considered to be clinically significant.

Laboratory values for vitamin A but not for retinyl palmitate were reduced in almost every subject receiving inotersen during CS2 and CS3. Retinyl palmitate measurements support the hypothesis that sufficient amounts of vitamin A are probably transported through chylomicrons when RBP4 levels are low. Since all patients received 3000 IU vitamin A during studies CS2 and CS3 the retinyl palmitate levels in chylomicrons without substitution are unknown. Therefore it remains unexplored if inotersen could be used safely without supplementation. However, data from the placebo arm in study CS2 show no increased incidence of AEs that could be attributed to vitamin A and the supplemented dose is well within the range that is considered safe based on the scientific literature. Therefore, while vitamin A supplementation is based on hypothetical risks it does not seem to pose a risk to patients.

In regard to an anticipated risk for hypovitaminosis A in pregnancy opposed to the risk probably deriving from an oversupply of vitamin A in pregnancy, concrete recommendations are provided to prescribers in SmPC section 4.4. The Applicant has proposed distinguished instructions to compensate for potential vitamin A deficiency for non-pregnant patients as well as planned and unintentional pregnancies. Overall this approach is welcomed.

Adequate levels of vitamin A are required for normal embryo-foetal development. While high concentrations of vitamin A may have teratogenic effects, low levels could also be harmful to the foetus. Due to the long half-life of inotersen a significant deficit of vitamin A can develop even after discontinuing the drug if supplementation is also discontinued. On the other hand the risk of teratogenic effects is highest in the early pregnancy. The "WHO Guideline on Vitamin A supplementation in pregnant women" describes a risk for single doses of a vitamin A supplement greater than 25 000 IU, particularly between day 15 and day 60 following conception. This is much higher than the dose given during inotersen treatment and it cannot be excluded that the risks of

developing a vitamin A deficit during early pregnancy might be greater than the risks associated with continuing supplementation.

Based on the available scientific data it is therefore not justified to give a strict recommendation to discontinue supplementation of vitamin A during the first trimester of pregnancy of an unplanned pregnancy.

Thrombocytopenia

Thrombocytopenia has been occasionally noted following antisense oligonucleotide treatment in preclinical test species but appears to be compound-specific rather than a common oligonucleotide class effect (Frazier et al. 2015). Other findings attributed thrombocytopenia to the class of ASOs caused by the backbone of antisense oligonucleotides and not by a specific nucleotide sequence. Chi et al. (2017) reviewed the risk of ASO-induced thrombocytopenia and came to the conclusion that two different mechanisms might be involved: a) a mild, transient and dose-dependent thrombocytopenia that is not necessarily involved in bleeding episodes; and b) a marked thrombocytopenia, emerging after repeated exposure to AONs and resulting in multiple hemorrhages in monkeys. The latter was also found in clinical studies with other ASOs, e.g. drisapersen. The pathogenesis of mild and severe thrombocytopenia is unclear but seems different.

TEAEs of thrombocytopenia including platelet count decreases were reported with a frequency of 24.1% in subjects on inotersen (versus 1.7% on placebo). Continuous treatment with inotersen in CS2 and CS3 did obviously not lead to an increased reporting rate of TEAEs up to the new cut-off date (incidence decreased in CS3 to 14.1%). The occurrence of thrombocytopenia TEAEs in subjects who received placebo in CS2 and were then switched to inotersen, was similar to those for subjects on inotersen in CS2 (26.5% for placebo-inotersen – treated subjects). Discontinuations due to thrombocytopenia events have been reported for four subjects in CS2 (including one death due to intracranial haemorrhage in a subject subsequent to Grade 4 thrombocytopenia) and two subjects in CS3 (assumes to a total of 5.4% of subjects in the longitudinal safety set with data cut off 28th February 2017). Most of the events were rated mild to moderate in severity. Three subjects from CS2 and one subject from CS3 presented with severe/serious AEs. However, classification of severity is strongly doubted on since Grade 3 and Grade 4 TEAEs (according to CTCAE criteria) related to platelet count values of 25 to 50 x 10^9/L and <25 x 10^9/L should at least be considered "severe" in nature.

Thrombocytopenia events **Grade 4** according to CTCAE criteria were confirmed in 3 of 112 subjects (2.7%) on inotersen in CS2 . In one of the subjects grade 4 thrombocytopenia could not be confirmed on repeated testing but was mentioned for completeness in the table for Grade 3 to 5 events of platelet counts in the ISS. Grade 3 to Grade 4 thrombocytopenia in these subjects was observed within the first six months of treatment with inotersen (i.e. Week 8 – Week 20).

In CS3 (updated at Day 106), clinical narrative descriptions revealed five additional subjects with decreased platelet counts in line with **Grade 3** (four subjects) **or Grade 4** thrombocytopenia (one subject). These patients were all on inotersen during CS2 and (except one subject) also had events of thrombocytopenia in CS2. However, platelet count decreases to values suspect of Grade 3 and Grade 4 thrombocytopenia were first detected in CS3 (between Week 77 and 151 of inotersen treatment). The Applicant informed on an additional serious and severe event of thrombocytopenia Grade 3 after the DLP of CS3. This patient had occasional steep drops in platelet counts after approximately 22 months of inotersen treatment that recovered without interruption. However, this patient presented with further platelet decrease of 80% within ~8 weeks some months later that culminated into hospitalisation and treatment with corticosteroids. Except for a single occurrence of positive anti-PF4 IgM, no further antiplatelet antibodies were reported for this subject.

In the aforementioned subjects with Grade 3/ 4 thrombocytopenia, TEAEs related to haemorrhages have been additionally found in the narratives and included for example menorrhagia, haematomas, gingival bleeding, bruising and an intracranial haemorrhage as the worst presentation of bleeding events leading to death in this subject (severe thrombocytopenia with a nadir of $<10 \times 10^9$/L was concomitantly reported with the SAE of intracranial haemorrhage the day prior to death). The subject had platelet count decreased at several occasions (approximately during three months starting within the first weeks of treatment). Platelet count monitoring rules were changed as a consequence of this event (from every 2-3 weeks monitoring to weekly controls). In subjects with Grade3/4 thrombocytopenia events in study CS3, ecchymosis, haematoma, haematuria periorbital haemorrhage, and scleral haemorrhage were reported in the narratives. In one subject (CS3), narrative description could not fully rule out possible punctate left frontal lobe corona radiata intraparenchymal haemorrhage. This subject also had a bleeding from an injury on his foot that did not stop spontaneously. Corticosteroids were commenced in this subject at a platelet cut-off $>25 \times 10^9$/L.

Mean decreases in platelet counts followed a gradual pattern over the first 6 months of treatment with inotersen in CS2. However, mean (%) changes from baseline in CS2 should be interpreted with caution since the number of subjects included in the calculation by week was highly variable. Within three and six months of treatment, mean platelet counts in the inotersen group decreased by 20% and 24% from baseline. At Week 65 (EOT) mean platelet counts decreased by 26% from baseline confirming that the most relevant mean decrease in platelet counts takes place in the first 6 months of treatment. Evaluation of mean platelet count decreases in CS3 is clearly impeded by the low number of subjects. However, it appears that no further significant mean reductions in platelets occurred. Of interest, an initial rise in platelet values from baseline was observed in studies CS2 and CS3 upon initiation of inotersen treatment, i.e. at Week 2/3. The cause for this finding was explained to be activation of the innate immune system that translated into increase in high sensitivity (hs)CRP with the first doses of inotersen and followed by reactive thrombocytosis. This information is adequately reflected in the SmPC.

Differences between placebo and inotersen safety profiles are further supported by the reported **platelet abnormalities**:

More than half of the subjects on inotersen had any value below the LLN for platelets (i.e. $<140 \times 10^9$/L). Decreases by more than 30% and 50% from baseline have been reported in 70.5% and 18% of subjects. Platelet counts below the LLN were more frequently reported in subjects continuing on inotersen during CS3 compared to those previously treated with placebo.

Duration of thrombocytopenia was 28 weeks during CS2 and 41 weeks in CS3 in subjects on inotersen. Patients meeting the stopping rule for platelet counts set in the protocol for CS2 (i.e. platelet count $< 75\times10^9$/L, affecting approximately 10% of inotersen-treated subjects in CS2 and CS3) needed to pause any further doses until platelet count had recovered to $>100\times10^9$/L. The overall duration of interruptions was longer in CS3 (8 weeks vs. 4.5 weeks in CS2). When the data from CS2 and CS3 are combined, the full dose of inotersen was successfully reinitiated in 11 subjects, but in 8 subjects a reduced dose (150 mg) was required to maintain platelet counts above 75×10^9/L. Platelets, although not systematically evaluated, tended to recover following treatment interruption or dose reduction; however, thrombocytopenic states recurred after treatment resumption accommodated by a reduction in doses while no systematical dose reduction has been presented. Although the concern that reduction in dose along with long dosing pauses due to thrombocytopenia negatively affects efficacy could be refuted, it remains unclear whether frequent treatment interruptions and rechallenges might even trigger immunological responses and enhance even steeper decreases in platelets. The formation of ADAs in subjects after at least one dose pause is of the same magnitude as in subjects tested positive

without dose pauses and therefore occurring over the whole duration of the study. This might be in support of a triggered response after dose pauses.

At least severe thrombocytopenia seems to be immunologically mediated while other causes, e.g. bone marrow dysfunction, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura, thrombotic microangiopathy, sepsis, platelet activation, and systemic complement activation could not be confirmed.

All subjects with IgG positivity had experienced platelet declines compared to none with IgM positivity. All subjects with confirmed thrombocytopenia Grade 4 were reported to be positive for post-baseline treatment-emergent anti-platelet IgG antibodies but not all subjects with IgG anti-platelet antibodies developed Grade 3 or 4 thrombocytopenia. Treatment-emergent anti-PF4 IgA was additionally present in one of these subjects and anti-PF4 IgM in two of them. In 4 of 8 subjects with Grade 3 (subjects, who were re-challenged with inotersen) or Grade 4 thrombocytopenia, positive test results for antibodies directed against platelet surface glycoproteins were detected in addition to antiplatelet IgG antibodies. The combination of anti-platelet IgG plus positivity for GP IIb/IIIa (and even anti-PF4 antibodies) may be contributory to the cases of Grade 4 thrombocytopenia. This assumption can be extended to cases of Grade 3 thrombocytopenia as well. These patients might be at risk for even further and steeper decreases in platelet counts. Further evidence supportive of immunologically related thrombocytopenia derives from the response to corticoid therapy and non-adequate response to platelet transfusion (rapid elimination from the body).

Analyses of platelet count decreases over different time intervals were used to establish **platelet monitoring rules**. However, platelet decreases in a number of subjects did not follow theoretical calculations and presented unpredictable platelet declines from any baseline or previous value over short time periods. The following baseline and post-baseline monitoring options as well as stopping rules were discussed during the procedure:

- The recommendation not to initiate treatment with inotersen in subjects with baseline platelet counts < 100 x 10^9/L is not in line with the exclusion criteria set in study CS2 (i.e. patients with platelets < 125 x 10^9/L are excluded). However, argumentation brought by the Applicant based on analyses of inotersen-treated subjects presented with the original clinical safety summary for whom platelet counts are not expected to drop from >100×10^9/L to <75 x 10^9/L within a 2-week period is accepted taking into account that patients with a platelet count of 100 x 10^9/L or less will have weekly platelet monitoring.
- Although the proposed 2-week monitoring frequency in subjects with baseline platelet counts of > 100 x 10^9/L is not in line with once-weekly measurement in clinical studies, the risk is manageable: a majority of subjects with decreases from >100 x 10^9/L to <75 x 10^9/L between two platelet count measures had at least one value of less than 100 x 10^9/L in the past that would have set them at weekly monitoring anyway.
- The Applicant's proposed recommendation of a twice weekly monitoring (until three successive values above 75) in subjects with platelet counts between 25 and 75 x 10^9/L (section 4.2) is considered adequate. Additional recommendations have been included in the SmPC for subjects with platelet counts of <50x10^9/L in whom more frequent monitoring should be considered if additional risk factors for bleeding are present.
- Experience with reduction in dose frequency derives from a total of four subjects only (all of them had Grade 3 thrombocytopenia and had recovered to platelet counts of more than 75 x 10^9/L). These subjects could only be managed on reduced dose frequency by additional dose interruptions after re-challenge with inotersen.

- A single change in the treatment schedule (i.e. extension of the dosing interval from once weekly to every 2 weeks) is recommended in SmPC section 4.2 in contrast to protocol requirements of up to 2 dose adjustments. This approach is conservative and agreed by the CHMP.

- Re-initiation of inotersen is proposed to be in line with the study protocols, i.e. not earlier than after recovery of platelets to > 100 x 10^9/L). For this scenario, section 4.2 proposes to re-initiate dosing at reduced frequency of every 2 weeks.

- Re-initiation of inotersen in 16 recovered subjects with previous platelet decreases <75 x 10^9/L led anew to thrombocytopenic states in all but one of these subjects and subsequently to further treatment interruptions. Clarifications provided by the Applicant could not sufficiently rule out that immunological responses are triggered by dose interruptions. In a majority of subjects, antiplatelet antibody testing was not adequately performed. In addition, when adjusted for treatment duration, a relatively higher number of subjects with at least one dose pause developed ADAs compared to subjects without dose pauses.

- Results from baseline anti-platelet antibody testing are not considered to be predictive for severe decreases in platelet counts under treatment. The overall clinical significance of post-baseline antiplatelet antibodies remains uncertain. Although significantly more subjects on inotersen were reported to have had positive testing results upon direct and indirect testing of antiplatelet antibodies (including epitopes), two testing laboratories (one of which was a specialised reference laboratory) revealed variable results, questioning the validity of the testing systems at present. Furthermore, antiplatelet antibody testing was not performed at the platelet nadirs of the reviewed Grade 3 thrombocytopenic subjects in CS3. It should, however, be noted that all subjects with Grade 3 thrombocytopenia in CS3 continue inotersen, despite numerous dose interruptions. The excess of anti-PF4 IgM in inotersen-treated subjects in CS2 and CS3 (CS3: 20% vs. 0% treatment-emergent anti-PF4 IgM in subjects on placebo-inotersen vs. inotersen-inotersen) has been hypothesised to derive from the loading dose regimen in CS2 by intensively stimulating an innate immune system response during the first weeks of treatment. Emerging anti-PF4 IgMs are not necessarily thought to be of pathogenic value and were not found to emerge at the time of platelet nadirs in CS2 and CS3.

- However, post-baseline anti-platelet antibodies including antibodies directed against platelet surface glycoproteins are in sum considered key to the steepness of platelet decreases in subjects treated with inotersen. Although, for patients with Grade 3 thrombocytopenia antiplatelet antibody testing results could not verify steeper decreases in platelet counts based on the provided data and the implementation of various treatment interruptions upon continued inotersen treatment in the respective subjects, the current platelet count testing measures (including additional wording for platelet counts of <50 x 10^9/L in SmPC in regard to monitoring frequency considerations, corticosteroid treatment and subject-based risk factors) and dosing recommendations address the potential risk for severe bleedings.

- A recommendation has been added to monitor platelet counts every 2 weeks for 8 weeks following discontinuation of inotersen treatment.

- Currently, there are no data to support different platelet cut-off values for subjects treated with inotersen and antithrombotic agents. A footnote to Table 1 in the SmPC has been included to indicate anticoagulant and antiplatelet medication as risk factors for bleedings. Section 4.4 of the SmPC includes a warning on the use of antithrombotic and antiplatelet medication with

inotersen and that is considered sufficient by the CHMP for managing the risk of bleedings in conjunction with the use of antithrombotic and antiplatelet medication.

Renal impairment

20.5% of subjects treated with inotersen and 10% of placebo-treated subjects reported TEAEs of renal impairment in CS2, while TEAEs in the Renal and Urinary Disorders SOC were similar in inotersen- and placebo-treated subjects (25% vs. 26.7%). Severe and serious renal impairment TEAEs were more pronounced in inotersen-treated subjects compared to placebo (4.5% vs. 1.7% and 5.4% vs. 0%) including PTs of *glomerulonephritis, tubulointerstitial nephritis, acute kidney injury, renal failure and renal impairment*. Of interest, albuminuria and proteinuria were not characteristically pronounced in subjects on inotersen compared to placebo. Urinalysis revealed baseline imbalances at the expense of inotersen, e.g. for protein and protein/creatinine ratio. Subjects on inotersen had mean values of P/C ratio >ULN throughout the study. In the updated CS3 safety set, renal impairment TEAEs were found in a similar frequency in the placebo-inotersen and inotersen-inotersen group (12.2% vs. 10.6%). Urinalysis parameters fluctuated in both treatment groups. Supported by the analysis of the longitudinal safety set, TEAEs of renal impairment were found more pronounced within the first year of inotersen treatment.

A total of 13 subjects experienced 17 **severe or serious TEAEs** in the Renal and Urinary Disorders SOC in CS2 and CS3 (16 events in CS2). One of these subjects was on placebo. Risk factors were present in these subjects at baseline and renal impairment was also in line with the underlying hATTR. Half of the events were rated possibly related to inotersen or placebo. Two SAEs (*glomerulonephritis*) led to discontinuation from treatment. In addition, two subjects, who died during study CS2 (*one with cardiac failure congestive and one with cachexia*) were reported to have had a worsening of renal function prior to death (see section 'Serious adverse events and deaths').

Four cases of **glomerulonephritis** were reported (three happened in CS2 and were rated possibly related to inotersen; one "chronic glomerulonephritis" occurred during CS3 in a subject on inotersen-inotersen). Common for these cases was elevated protein and/or albumin at baseline, presentation of significant proteinuria at the time of the events together with a decrease in eGFR in two of the subjects. Two subjects presented with reduced complement C3. The subject reported in CS3 (updated safety set) did neither present renal function loss nor significant proteinuria. Therefore, glomerulonephritis diagnosis is disputable. Renal biopsy reports were presented for the three subjects in CS2 and in two of the subjects two distinct processes have been found i.e. the underlying disease leading to amyloid deposits in the glomeruli and additional immunological contribution of inotersen evident by glomerular deposits for complement factors and IgG. In the third subject, pauci-immune glomerulonephritis was diagnosed lacking concomitant amyloid disposition. This subject also presented with anti-drug antibodies. None of the subjects fully recovered despite immunosuppressive therapy and one subject required permanent haemodialysis due to end stage renal failure.

Renal function assessed by serum and urine parameters was variable over time for subjects on placebo and inotersen. Mean eGFR was found to be decreased in subjects on inotersen from Week 10 post-baseline but remained stable on these reduced values >80 mL/min/1.73 m^2. Subgroup analysis of subjects (on placebo and inotersen) in CS2 with disease stage 1 and 2 revealed that mean baseline eGFR values were quite similar in subjects on placebo and inotersen up to Week 10, from where separation between placebo and inotersen could be recognised in subjects with disease stage 2 but not with disease stage 1. In other words, subjects with hATTR disease stage 2, suffering from longer disease and more pronounced renal amyloidosis might present a patient group at risk for renal function

decline while treated with inotersen. There is currently no information on patients with stage 3 disease. However, even worse effects could be expected in those patients. Possible mechanistic explanation behind the observed decline in eGFR has been discussed, although contributing non-clinical data are not available. The Applicant hypothesised changes in renal tubular creatinine secretion as a consequence of greater tubular dysfunction in the inotersen arm due to renal amyloid deposits and probably additional accumulation of inotersen in the proximal tubular epithelium, which might consequently affect eGFR. Twice as many subjects on inotersen compared to placebo had shifts from Grade 1 (≥90 mL/min/1.73 m^2) to Grade 2 (≥60 mL/min/1.73 m^2 to <90 mL/min/1.73 m^2) during CS2. A higher number (double as high) of subjects on inotersen-inotersen in CS3 experienced shifts from Grade 1 or 2 to Grade 3 or 4 compared to subjects on placebo-inotersen. A more pronounced effect of inotersen on kidney function with longer treatment duration cannot be excluded.

Mean serum creatinine and BUN increased over the course of CS2 in inotersen-treated subjects to a variable extend compared to placebo.

Renal parameter abnormalities:

CKD renal impairment stages 4 and 5 have only been reached by (single) subjects on inotersen. eGFR decreases from baseline to post-baseline of more than 25% or 50% were clearly attributed to inotersen, the latter reduction affected a total of 8 subjects on inotersen (and none on placebo) in accordance with the presentations of severe and/or serious TEAEs of renal impairment. Serum creatinine increases of >44.2µmol/L (0.5mg/dL) from baseline affected 12 subjects on inotersen (10.7% vs. 1 subject on placebo). The Applicant demonstrated overlap of these subjects, i.e. the 8 subjects with 50% and more decrease in eGFR had at the same time a serum creatinine increase >44.2µmol/L. It appears that subjects who presented with reduced eGFR at baseline progressively declined early during treatment, while decreases of eGFR in subjects with near normal baseline values were found steeper and starting within the first 6 months of inotersen initiation (four subjects). As per the presentation in the responses some of the patients with renal function decline had pre-existing medical conditions and some had alternative explanation for these findings. Nevertheless, it seems that older subjects and those with disease stage 2 are more susceptible than others for those changes. Numerous dose interruptions were deemed necessary in subjects with renal function impairment hampering clear differentiation of conditions in line with the underlying disease or renal toxicity elicited by inotersen. However, inotersen seems to affect renal function indicated by the aforementioned eGFR decreases for which mechanistical explanation is only hypothetical (see above).

Proteinuria was measured by analysing A/C and P/C ratios. A/C ratios >5x ULN post-baseline were evenly distributed between subjects on inotersen and placebo. P/C ratio (including higher molecular weight non-albumin proteins, which may be tubular as well as glomerular in origin) >5x ULN was found higher in subjects on inotersen compared to placebo. Since baseline mean ratios for both, A/C and P/C were higher in subjects assigned to inotersen than to placebo and for both treatment groups higher than ULN, and moreover, mean as well as individual ratios fluctuated over time, it is again difficult to assess a clear contribution of inotersen to this finding. Results are, however, in line with TEAEs of proteinuria (more subjects on inotersen vs. placebo) and albuminuria (similar between treatment groups) in CS2. In addition, there were marked differences in serum creatinine increases of >0.5 mg/dL from baseline in subjects on inotersen over placebo (10.7% vs. 1.7%), which is considered an indicator of acute renal failure. Divergences in serum creatinine increases of >0.5 mg/dL from baseline and in urine protein between treatment groups seem again to be related to more diseased subjects that were included in the inotersen arm, in some of which, however, inotersen might have contributed to more pronounced proteinuria.

Results from CS3 are not significant regarding renal parameter abnormalities.

Findings on renal function decline and glomerulonephritis detection implemented in the SmPC:

A contraindication for subjects with a urine protein to creatinine ratio (UPCR) ≥ 113 mg/mmol (1 g/g) has been included. Similarly, subjects with an eGFR of 45 mL/min/1.73 m2and less (in line with CKD Stage 3b and higher) should not receive inotersen based on a) the number of subjects with such baseline values in whom inotersen was permanently discontinued (4 of 5 subjects, even though not due to renal issues), b) the remaining uncertainty that the decline in renal function in some patients might have been triggered by inotersen especially in those patients with an anticipated increased risk for renal deterioration, i.e. those with pre-existing renal function impairment (older subjects and those with advanced disease stage (stage 2) might have an increased susceptibility).

Monitoring algorithm for early glomerulonephritis detection was evaluated and proposed to be based on urinary P/C ratio (significant proteinuria was common for all of the three glomerulonephritis cases) and on eGFR.

Wording in regard to renal function monitoring in section 4.4 of the SmPC includes:

- General renal monitoring (UPCR and eGFR) every 3 months or more frequently, as clinically indicated, based on history of chronic kidney disease and/or renal amyloidosis. Since the mean decline in eGFR of 5 to 10% from baseline reported in the clinical studies is not considered of significance in regard to the underlying disease with considerable fluid shifts and general susceptibility to urinary infections and dehydration, the proposed monitoring interval is deemed acceptable by the CHMP.
- Monitoring algorithm of every 4 weeks for detection of acute glomerulonephritis is based on patients at risk *"(…) Patients with UPCR more than or equal to twice the upper limit of normal, or eGFR < 60 ml/min, which is confirmed on repeat testing (…)"*. In the CHMP's view this is acceptable recommendation since it provides a more conservative monitoring strategy compared to study protocol requirements.

Notwithstanding the Applicant's rationale that each of the three subjects with glomerulonephritis would have been detected with this algorithm, uncertainty remains in regard to a worsening of renal function (progressive decline in eGFR and increase in serum creatinine) with inotersen in addition to the presentation of glomerulonephritis. Therefore, and in line with adverse drug reactions from the Renal and urinary disorders SOC listed in section 4.8 of the SmPC, the warning subheading "Glomerulonephritis" in section 4.4 includes "renal function decline".

A mandatory dose pause is required for patients with UPCR ≥2g/g, followed by glomerulonephritis diagnosis. In case of an eGFR decrease of >30%, dosing is proposed to be withheld while examining the cause. Inotersen treatment can be resumed after exclusion of glomerulonephritis diagnosis but not before the renal function has improved. The above warnings and precautions are properly reflected in SmPC section 4.4.

Renal damage may be triggered by concomitant nephrotoxic medication. Hence, cautious wording has been proposed in regard to nephrotoxic drugs and other drugs that might impair renal function with reference in sections 4.4 and 4.5 of the SmPC.

In conclusion, the complexity of findings in at least three (a fourth case is not confirmed) subjects with glomerulonephritis hampers a clear differentiation of presentations of renal findings but strongly argues for coexistence of renal amyloid deposits that derive from the underlying disease and concomitant drug-induced alteration of the immune system particularly related to complement activation and/or immune complex deposition. Glomerulonephritis is hence a designated identified risk

and reflected in the RMP. The extent to which inotersen worsens pre-existing renal impairment, especially in older subjects and those with advanced disease (stage 2) remains uncertain. Although experience on patients with disease stage 3 is currently limited and does not point to a worsening of renal function with inotersen treatment, the outcome in a larger safety set is unknown.

Other adverse events of interest (OAEIs)

Coagulation abnormalities were not reported in the clinical studies while several events might be suspect of a broader coagulation in inotersen-treated subjects, i.e. embolic stroke, pulmonary embolism, deep vein thrombosis (in CS2 and CS3), thrombosis, venous thrombosis and Mesenteric artery occlusion (***thrombotic events***). It may be hypothesised that thrombotic and embolic events are caused by inflammatory lesions. TEAEs were balanced between placebo and inotersen in CS2. Only one additional subject in CS3 was noted. Upon request, the Applicant summarised available laboratory parameters which could indicate inflammatory and immunological processes. However, no evidence of a contribution of inotersen derived from these limited data. Therefore, occurrence of thrombotic and embolic events is probably attributed to the underlying immobility and cardiac failure in this patient population.

Hepatic abnormality TEAEs were more frequent for inotersen-treated subjects (12.5%) compared to placebo (6.7%). Liver transaminase increases, i.e. alanine aminotransferase increased [ALT] and aspartate aminotransferase increased [AST] (3.3% placebo vs. 2.7% inotersen and 3.3% placebo vs. 4.5% inotersen) were the liver abnormalities most frequently mentioned in both treatment groups.

Mean baseline values of ALT (more specific for liver) and AST (also expressed in other tissues) were found similar in subjects on placebo and inotersen at baseline in CS2: 24.2 U/L vs. 23.4 U/L and 26.6 U/L in each group. No increase from baseline was found in subjects on placebo, while highest mean ALT (mean AST) in inotersen-treated subjects was 32 U/L (31 U/L) at Week 18. In CS3, mean baseline ALT and AST were higher for subjects continuously treated with inotersen as compared to subjects previously treated with placebo. No significant changes were noted in either group in CS3. Total bilirubin mean baseline and post-baseline values were unremarkable.

No SAEs were reported. One subject presented with Gilbert's disease with a single increase in ALT $\geq$ 3 x ULN and elevation in total bilirubin $\geq$ 2 x ULN. Several stopping rules have been implemented in the protocol but were not met during the studies.

Hepatobiliary laboratory abnormalities as defined by confirmed cut-offs of ALT >3x ULN, 5x ULN, 8x ULN, and 10x ULN in CS2 solely occurred in subjects on inotersen.

Of the six subjects with ALT >3x ULN, one had a diagnosis of Gilbert's disease, that is thought to be causal for the increases in transaminases. In four of the subjects, increases occurred at single occasions during the study and resolved in a short period of time while inotersen was continued. One subject was reported with gradual increases in ALT, AST, and ALP during inotersen treatment in CS2 and CS3 lacking alternative causes and considered possibly related to inotersen.

Laboratory value abnormalities were less frequent reported in CS3.

In conclusion, a significant risk regarding liver abnormalities could not be deduced from available clinical data. GGT and GLDH measures would have been more specific parameters examining the risk of substance-specific liver toxicity over time, but are not included in the panel for liver toxicity evaluation. Hepatic toxicity is not reflected in the RMP, while use in patients with hepatic impairment is listed as missing information. Annual liver monitoring is recommended in the SmPC. Liver function is known to be affected by AONs in preclinical studies (Henry et al. 2007), and increases in mean ALT and AST have been reported with inotersen treatment but not with placebo together with single

subjects presenting with transaminases above defined cut-off values. The Applicant presented a discussion on on-treatment liver enzyme measurements and stopping rule implementation in the SmPC. The CHMP considered that no stopping rules are needed. Since the highest mean ALT and AST were detected in Week 18 of CS2, transaminase testing is proposed to be conducted at baseline, after 4 months of treatment followed by yearly monitoring thereafter. More data is expected in a post marketing setting in order to characterize the possible risks related to hepatic abnormalities (hepatotoxicity)/hepatic impairment and this is one of the objectives of registry study proposed as a post-authorisation measure.

Although commonly seen with other s.c. antisense oligonucleotide treatment and thought to be an inflammatory response to the injected drug, **injection site reactions** with inotersen were found rather a tolerability than a safety issue and affected 51% of subjects vs. 12% of subjects on placebo. The most common PTs of ISRs in CS2 were injection site erythema, pain, and pruritus. More severe presentations of ISR events (e.g. atrophy, induration, or haemorrhages) occurred in single subjects only. The total absence of injection site erythema in patients receiving placebo raised a question on the effectiveness of blinding in study CS2. However, post-hoc analyses confirmed that the magnitude of inotersen's mean effect and difference from the placebo group remains approximately at the same level. Of note, a bias is nearly excluded since subjects did not know the injection site reaction status of other subjects. The frequency of ISR events reported in CS3 (in subjects on continuous inotersen treatment) was found lower compared to CS2 and thus longer inotersen treatment is not related to more frequent or severe presentations. This is also supported by analysis of local cutaneous reactions. Injections site reactions and adequate measures to alleviate them are included in the SmPC.

More subjects on inotersen revealed non-specific events of **flu-like symptoms** like pyrexia and influenza-like illness that are known AEs for the class of AONs (Frazier et al. 2015) compared to placebo (7.1% vs. 1.7% and 9.8% vs. 0%). Symptoms emerged early during treatment and diminished thereafter. Both events are listed in the SmPC section 4.8 (pyrexia "very common", influenza like illness "common").

CNS-related AEs might appear as a consequence of a loss of TTR – associated neuroprotection or as a consequence of the underlying autonomic neuropathy in hATTR. An imbalance of CNS TEAEs could be found in CS2 deriving from a higher frequency of headache, events related to paraesthesia, and syncope/presyncope. Headache is a known AE related to antisense treatment. The occurrence of syncope/loss of consciousness can alternatively be explained by the underlying disease and past medical history of the subjects (especially those with cardiomyopathy).

Haemorrhage TEAEs were reported with similar incidence in subjects on placebo and inotersen (33.3% vs. 35.7%), while some differences are seen in the reporting of haematomas/ subdermal bleeds favouring placebo (20.5% and 11.7%). Analysis of haemorrhage TEAEs by platelet categories prior to the event in CS2 could not specifically attribute events to low platelet counts compared to normal range platelets. No significant difference was reported in the incidence of bleeding events in subjects with and without antiplatelet or anticoagulation drugs and no difference in severity of events with or without such agents was noted. Although most of the bleeding events were rated mild to moderate, one single severe, serious and finally lethal bleeding TEAE of intracranial haemorrhage occurred in strong relation to Grade 4 thrombocytopenia (while this patient did not receive anticoagulation). Upon update of CS3 subjects after DLP, one SAE of haemorrhage intracranial was reported in a placebo-inotersen treated subject after a fall, considered unrelated to the drug. Platelet counts in this subject were reduced but not below $100x10^9$/L. The subject had unstable anticoagulation parameters at baseline and during treatment with inotersen in CS3 while receiving anticoagulation treatment. Up to the data cut-off of 28th February 2017, bleeding events were balanced in subjects

with normal range platelets and those with platelets <LLN in CS3. Slightly more subjects with anticoagulation treatment in CS3 (irrespective of the treatment assignment in CS2) had a bleeding event compared to those without concomitant drugs (33% vs. 24%).

TEAEs potentially related to **complement activation** were evaluated using an unspecific MedDRA query of hypersensitivity. No significant difference was noted between treatments. Complement factors were not routinely measured in CS2. The reports of complement C3 values below LLN were found to increase during CS2. 55% of inotersen-treated subjects had any post-baseline C3 value below LLN compared to placebo (21%). Mean complement C3 deceased by 33% from baseline to Week 65 in CS2 (however, based on a low number of subjects with any measures).

Other complement split product measures are of limited value due to the imbalance and overall low numbers of subjects. Complement split product measurement (C5a and Bb) was conducted in study CS1. Upon request, the Applicant presented all complement split product data for studies CS1 and CS2. Interpretation of data is hampered given the fact that otherwise healthy volunteers in CS1 were reported to have had complement factors C5a and Bb >ULN at baseline as well as at several time points during the study in the placebo multiple dose cohort, in the inotersen single dose and in the multiple dose cohorts. No specific pattern could be detected and complement factors were often measured at a single time point only, probably in line with biological variation or secondary to acute phase response. Assessment of complement factors in study CS2 is clearly hampered by the irregularity of measurements applied and the overall low number of subjects. Since abnormalities in complement parameters were also reported at baseline for subjects in either treatment group, the only conclusion that can be drawn from these data is that there might be relevant complement activation in subjects with hATTR. The contribution of inotersen remains unknown at this time.

15% of thyroxine is transported via TTR protein, while 65% of thyroxine is transported by thyroxine blinding globulin and 20% by albumin and retinol. **Hypothyroid states** might therefore theoretically occur with inotersen treatment, but relevance is not clear at this time. A gradual increase in subjects with thyroxine values <LLN from baseline to Week 65 for inotersen vs. placebo was noted (19.8% vs. 8.5%). At the same time, thyrotropin values above ULN were not remarkably different between inotersen and placebo. Three subjects were affected by hypothyroidism TEAEs in study CS2 compared to one subject of placebo. During CS3, no increased reporting rate of TEAEs was noted. The overall risk for post - baseline low thyroxine levels is comparable for males and females. Overall differences between males and females are unlikely to be of clinical relevance.

Serious adverse event/deaths/other significant events

Ten **deaths** were reported during the clinical program up to the most recent data cut-off of 15th September 2017 in subjects on inotersen (inotersen in CS2 or inotersen-inotersen in CS3) and none in the placebo or placebo-inotersen group. Five of the deaths occurred during the placebo-controlled CS2 and another five in the uncontrolled single-arm extension study CS3.

While being aware of the 1:2 randomisation to placebo: inotersen, respectively, and differences in exposure, there is still an imbalance of death cases with 5 deaths occurring in the inotersen group vs. none in the placebo group in study CS2.

The causes of death were: cachexia in two subjects (not related to inotersen), intestinal perforation (not related to inotersen), cardiac failure congestive in two subjects (one in CS2 and another in CS3; not related to inotersen), intracranial haemorrhage (possibly related to inotersen), cardiac failure (not related to inotersen), cardiac failure acute/bacteriaemia/septic shock (CS3), neuropathy peripheral (CS3), and endocarditis (CS3). One of the five deaths reported during CS2 is considered related to

inotersen (i.e. a subject with an intracranial haemorrhage as a consequence of severe thrombocytopenia reported with a nadir of $<10 \times 10^9$/L the day prior to death). A decreasing platelet count was reported over several study visits (approximately during three months starting within the first weeks of treatment). Two of the five deaths in CS2 were due to cachexia, which is a common cause for death in subjects with hATTR (e.g. Hund et al. 2012). The narratives of the two subjects revealed sufficient justification for a lack of inotersen contribution to these deaths. Another common death cause in subjects with hATTR is cardiac failure (congestive), which was also the cause for another death in CS2. Two of these subjects (*one with cardiac failure congestive and another with cachexia*) had a worsening of renal function prior to death and therefore, relation to inotersen treatment against the background of the striking imbalance in death cases was requested to be re-assessed. Inotersen could finally not be rated as a contributor to these deaths.

Last but not least, the cause for death was intestinal perforation during surgery for sigmoid volvulus in another subject; sigmoid volvulus is thought to be a consequence of disease progression in the subject.

Five subjects died during the open-label experience. These patients seemed to have had a complicated disease progression during the study lacking evidence of inotersen involvement. Three of them had the Thy60Ala mutation, known to preferably induce cardiomyopathy (Ando et al. 2013; patient information on hereditary ATTR Thr60Ala amyloidosis from NHS Foundation Trust) but also polyneuropathy. The causes of death in these three subjects are in line with the findings in subjects with Thr60Ala mutation, i.e. congestive heart failure (in a subject with significant amyloid heart infiltration during CS2), cardiac failure acute/bacteraemia and septic shock, and neuropathy peripheral. The first two subjects had stage 2 disease and NYHA stage II at baseline. All of them had cardiomyopathy and orthostatic hypotension, which is a common feature in hATTR. Two of them also displayed a loss in renal function most obviously in line with cardiac impairment.

Another subject died during CS3 under liver transplantation surgery from a myocardial lesion (rupture in the right ventricle) reported as cardiac failure, which is considered a chance event.

Last but not least, death due to endocarditis subsequent to a systemic infection (which is considered causal) was reported two months after the last inotersen dose in study CS3 in a subject with various preceding (S)AEs. Death in this subject occurred 10 years after the first symptoms of hATTR, which is in line with life expectancy in subjects with the Val30Met mutation. The patient had disease stage 2 at baseline and was diagnosed with hATTR-CM five years prior to death.

In conclusion, the imbalance in deaths is not considered to be caused by inotersen based on the compilation of data provided by the Applicant, except for the case of intracranial haemorrhage due to Grade 4 thrombocytopenia. The open-label experience does not raise additional concerns since death causes are found similar to those in CS2. Currently, there seems to be no evidence for non-specific effects related to systemic ASO treatment. Overall, patients in the inotersen group had more advanced disease compared to patients on placebo and this circumstance may have, at least in part, contributed to the observed excess mortality on inotersen in CS2.

Although the data provided by the Applicant did not fully address the requested discussion on excess mortality, i.e. it has not been taken into account that active treatment would have been expected to delay disease progression, it is unlikely that additional helpful insights can be gained from this discussion.

SAEs were more common with inotersen compared to placebo (32.1% vs. 21.7%) and found to be in accordance with AESI /OESI but also included safety issues related to the underlying disease (e.g. infections, cardiac disorders, gastrointestinal disorders, nervous system disorders, metabolism and

nutrition disorders). Seven renally - related SAEs were reported in six subjects on inotersen and included three events of glomerulonephritis, one events of acute kidney injury, and one event each of renal failure, renal impairment and tubulointerstitial nephritis. Thrombocytopenia was reported as SAE in 2 (1.8%) subjects treated with inotersen in CS2 and in one subject in CS3.

Treatment-related SAEs in CS2 further condensed SAEs to AESIs or OESIs. Some PTs are mentioned under different SOCs while probably belonging to the same presentation, i.e. thrombotic/embolic events of embolic stroke, deep vein thrombosis, pulmonary embolism as a possible consequence of inflammatory lesions caused by inotersen.

Laboratory findings

Main changes in laboratory parameters have been presented in respective sections of AESIs or OAEIs.

In addition, **serum albumin** was found to be parallel translated to lower levels for subjects on inotersen relative to placebo, probably due to baseline inconsistencies between treatment groups (subjects with <LLN: inotersen 28%, placebo 10%). Patients with FAP show up with significantly decreased serum albumin levels as the disease progresses (Kugimiya et al. 2011) in line with a loss of antioxidative function of albumin to effectively suppress TTR amyloid formation. The incidence of serum albumin below the LLN increased to almost 50% of subjects in the inotersen-inotersen group during CS3, while the placebo-inotersen group achieved levels of inotersen in CS2.

C-reactive protein (measured as hsCRP) is known to increase during treatment with antisense oligonucleotides. Baseline mean hsCRP was already above the ULN in both groups in CS2, which might be a consequence of inflammatory processes of the underlying disease. Acute post-dose elevations in mean hsCRP levels were observed after the first exposure to inotersen (~20x of mean baseline value) and decreased from Week 5 on to placebo levels. During CS3, post-baseline abnormality frequencies were similar in both treatment groups and similar to results from the placebo group in CS2 (each around 50%).

Immunological alterations are depicted by elevated IgG and IgM more pronounced in subjects on inotersen versus placebo.

Other findings

Vital signs recorded in CS2 were not significantly different between inotersen and placebo. A significant effect of inotersen on systolic and diastolic blood pressure (reduction) was noted within the first 15 days of exposure in study CS2. This effect is attributed to the loading scheme that has been applied for the first week of treatment (a total of three doses) and further supported by a number of TEAEs consistent with hypotension within the first 15 days of inotersen. For the remainder of the studies, no significant differences were noted for either treatment group. Omitting the loading dose is not thought to compromise long-term efficacy (supported by popPK modelling data; see also section 2.4.3 Pharmacodynamics).

Weight decreased was generally more pronounced in subjects on placebo in line with disease progression in this group. However, the "protective" effect of inotersen seems to diminish with ongoing treatment in CS3.

QTcF: Inotersen did not interfere with Ikr- and hERG- currents up to the highest concentration of 300 µM and did not significantly prolong the QTc-interval or affect other cardiovascular (arterial blood pressure, heart rate, ECG) parameters in preclinical studies (see NC AR). Hence, no thorough QT/QTc study was performed and only ECG data from the clinical development program are available. 14 (4)

subjects on inotersen had a shift to >500 msec and/or QTcF of >60 msec in CS2. It could be clarified that 15 of 18 subjects had a pacer placement, for which QTc stopping rules can be disregarded. In the remaining 3 subjects, alternative explanations for reaching stopping criteria were mentioned.

In regard to C-SSRS evaluation, results in CS2 are clearly in favour of inotersen compared to placebo.

Safety in special populations

Gender: the only PT that was found notably more pronounced in female compared to male subjects in all treatment groups was urinary tract infection. There was no consistent preponderance of one SOC or PT over another in CS2 and CS3 that would change the assessment of overall safety concerns.

Age effects in the inotersen group in CS2 (disadvantaging subjects ≥ 65 y) were more pronounced for the cardiac disorders SOC, Musculoskeletal and Connective Tissue Disorders SOC, chills, and platelet count decreased. These effects were not seen in the placebo group. Results from CS3 data and the longitudinal safety set contributed to the observations for the cardiac disorders SOC in CS2. The extent to which inotersen increases the risk for additional or worsening of pre-existing cardiac disorders over time especially in subjects >65 years of age, is not clear. An age effect was observed for platelet count decreased and discussed in relation to the identified risk for thrombocytopenia. In addition, Arnold et al. 2015 pointed towards an increased risk for severe bleedings not only predicted by platelet counts of 10 – 20 x 10^9/L, but also age. As a result, a warning in regard to age over 60 being a risk factor for bleedings has been implemented in SmPC section 4.4 and mentioned in 4.2 in conjunction with the monitoring and treatment recommendations for platelet count. The finding is in line with the results in special population by age. Tabulated adverse events (see table below) in various age groups were provided by the applicant upon request and were found in line with the anticipated age effects already noted: i.e. a nearly 3-times higher incidence of TEAEs from the Cardiac disorders SOC in subjects aged 65 and older compared to those aged <65. In addition, also based on limited data, renal investigations were more pronounced in older subjects, finding which is considered biologically plausible.

Category - Subcategory	Age at ISIS 420915 Baseline: Age <65 (N=83) Subject (%)	Age at ISIS 420915 Baseline: Age 65-74 (N=53) Subject (%)	Age at ISIS 420915 Baseline: Age 75-84 (N=16) Subject (%)	Age at ISIS 420915 Baseline: Age 85+ (N=0) Subject (%)
Total AEs	83 (100.0%)	52 (98.1%)	16 (100.0%)	0
Total Serious AEs	27 (32.5%)	25 (47.2%)	7 (43.8%)	0
- Fatal	3 (3.6%)	4 (7.5%)	0	0
- Hospitalization/prolong existing hospitalization	21 (25.3%)	22 (41.5%)	7 (43.8%)	0
- Life-threatening	2 (2.4%)	1 (1.9%)	0	0
- Disability/incapacity	3 (3.6%)	0	1 (6.3%)	0
- Other (medically significant)	16 (19.3%)	13 (24.5%)	5 (31.3%)	0

AE leading to drop-out	10 (12.0%)	10 (18.9%)	4 (25.0%)	0
Psychiatric disorders	19 (22.9%)	12 (22.6%)	3 (18.8%)	0
Nervous system disorders	53 (63.9%)	34 (64.2%)	11 (68.8%)	0
Accidents and injuries	42 (50.6%)	27 (50.9%)	12 (75.0%)	0
Vascular disorders	24 (28.9%)	19 (35.8%)	2 (12.5%)	0
Cerebrovascular disorders	4 (4.8%)	1 (1.9%)	0	0
- Aphasia	0	1 (1.9%)	0	0
- Embolic Stroke	1 (1.2%)	0	0	0
- Haemorrhage intracranial	1 (1.2%)	0	0	0
- Transient ischaemic attack	1 (1.2%)	0	0	0
- Speech disorder	1 (1.2%)	0	0	0
Infections and infestations	61 (73.5%)	42 (79.2%)	10 (62.5%)	0
Anticholinergic syndrome	0	0	0	0
Quality of life decreased	0	0	0	0
Sum of the following	32 (38.6%)	27 (50.9%)	9 (56.3%)	0
- Orthostatic hypotension	9 (10.8%)	5 (9.4%)	1 (6.3%)	0
- Falls	12 (14.5%)	16 (30.2%)	5 (31.3%)	0
- Black outs	0	0	0	0
- Syncope	10 (12.0%)	6 (11.3%)	1 (6.3%)	0
- Dizziness	12 (14.5%)	9 (17.0%)	4 (25.0%)	0
- Ataxia	0	0	0	0
- Foot fractures	2 (2.4%)	0	1 (6.3%)	0
- Rib fracture	1 (1.2%)	0	1 (6.3%)	0
- Hand fracture	0	1 (1.9%)	0	0
- Ankle fracture	0	1 (1.9%)	1 (6.3%)	0
- Lower limb fracture	1 (1.2%)	0	0	0
- Hip fracture	0	0	2 (12.5%)	0
- Pelvic fracture	0	0	1 (6.3%)	0
- Wrist fracture	0	1 (1.9%)	0	0
Other AE appearing more frequently (>=10%) in older patients[1]				

Cardiac disorders				
- Cardiac failure congestive	0	6 (11.3%)	2 (12.5%)	0
Eye disorders				
- Conjunctival haemorrhage	2 (2.4%)	1 (1.9%)	2 (12.5%)	0
Gastrointestinal disorders				
- Constipation	11 (13.3%)	8 (15.1%)	5 (31.3%)	0
- Dysphagia	2 (2.4%)	2 (3.8%)	2 (12.5%)	0
General disorders and administration site conditions				
- Chest pain	1 (1.2%)	1 (1.9%)	2 (12.5%)	0
- Chills	12 (14.5%)	15 (28.3%)	4 (25.0%)	0
- Fatigue	18 (21.7%)	23 (43.4%)	7 (43.8%)	0
- Gait disturbance	5 (6.0%)	4 (7.5%)	3 (18.8%)	0
- Injection site pain	12 (14.5%)	13 (24.5%)	4 (25.0%)	0
- Oedema	2 (2.4%)	1 (1.9%)	2 (12.5%)	0
- Oedema peripheral	18 (21.7%)	16 (30.2%)	6 (37.5%)	0
Injury, poisoning and procedural complications				
- Contusion	5 (6.0%)	5 (9.4%)	3 (18.8%)	0
- Fall	12 (14.5%)	16 (30.2%)	5 (31.3%)	0
- Hip fracture	0	0	2 (12.5%)	0
- Laceration	1 (1.2%)	1 (1.9%)	3 (18.8%)	0
Investigations				
- Albumin urine present	1 (1.2%)	0	2 (12.5%)	0
- Blood creatinine increased	0	3 (5.7%)	3 (18.8%)	0
- Blood urea increased	1 (1.2%)	3 (5.7%)	3 (18.8%)	0
- Blood uric acid increased	1 (1.2%)	0	2 (12.5%)	0
- Glomerular filtration rate decreased	4 (4.8%)	3 (5.7%)	4 (25.0%)	0
- N-terminal prohormone brain natriuretic peptide increased	1 (1.2%)	0	4 (25.0%)	0

- Weight decreased	6 (7.2%)	3 (5.7%)	3 (18.8%)	0
Metabolism and nutrition disorders				
- Decreased appetite	5 (6.0%)	6 (11.3%)	3 (18.8%)	0
- Dehydration	2 (2.4%)	1 (1.9%)	2 (12.5%)	0
Musculoskeletal and connective tissue disorders				
- Muscular weakness	8 (9.6%)	7 (13.2%)	4 (25.0%)	0
- Musculoskeletal pain	2 (2.4%)	5 (9.4%)	3 (18.8%)	0
- Myalgia	12 (14.5%)	13 (24.5%)	4 (25.0%)	0
Nervous system disorders				
- Balance disorder	1 (1.2%)	3 (5.7%)	2 (12.5%)	0
- Dizziness	11 (13.3%)	9 (17.0%)	4 (25.0%)	0
Respiratory, thoracic and mediastinal disorders				
- Dyspnoea	7 (8.4%)	10 (18.9%)	1 (6.3%)	0
- Dyspnoea exertional	1 (1.2%)	2 (3.8%)	2 (12.5%)	0

Note 1: Safety analysis based on data collected from the first dose of ISIS 420915 until the patient's last contact date within CS2 or CS3. CS2 data from 'Placebo (CS2) and ISIS 420915 300mg (CS3)' group is not summarized.
Note 2: For each treatment, a patient is counted only once within each category or subcategory.
Note 3: Adverse events were coded using MedDRA version 19.1.
[1] AE appearing more frequently (>=10%) in older patients were included in this table by system organ class and preferred term. For each treatment, a patient is counted only once within each system organ class or preferred term.

No additional safety concerns arose for subjects with hATTR-CM compared to those with hATTR-PN.

Mean eGFR in inotersen-treated subjects was notably lower in subjects on inotersen with the V30M TTR mutation compared to inotersen-treated subjects with other mutations. In contrast, subjects with the V30M TRR mutation who were on placebo in CS2 had higher eGFR compared to subjects with other mutations. If – as hypothesised by the Applicant – subjects with V30M mutation are affected by a higher incidence of renal amyloidosis, eGFR would need to be similarly reduced in placebo- and inotersen-treated V30M subjects. It is therefore assumed that the type of mutation is not causative for the differences in eGFR in inotersen- and placebo-treated subjects.

The influence of **extrinsic factors** on the safety of inotersen was determined.

Several **regional imbalances** were found. However, the European subgroup did not display a higher number of TEAEs in any subgroup as compared to the other regions. In CS3, a strikingly higher number of subjects from North America had TEAEs from the cardiac disorders SOC compared to European patients. It is not clear, if this is due to regional differences in disease mutations or overall regional incidence of cardiac – related events. Notable differences in the occurrence of **TEAEs by previous treatment** (diflunisal or tafamidis) were found for the renal and urinary disorders SOC: more subjects on inotersen than placebo had TEAEs while not receiving prior treatment with these substances. More subjects with prior diflunisal or tafamidis treatment reported eye disorders TEAEs

with inotersen. In CS3, eye disorders TEAEs were also more pronounced in subjects previously treated with diflunisal or tafamidis while in the placebo-inotersen group (i.e. during the first exposure with inotersen), the relevance of this finding being unclear.

Immunological events

Anti-drug antibodies were detected in 30% of inotersen- treated patients in CS2. Testing was performed on study Day 1, Day 28, Day 85, Day 197, Day 323 and Day 449 (Week 65). In the 6-months Post Treatment Evaluation Period of 6 months (for patients who did not enter the open-label extension study CS3), immunogenicity assessments were performed at Day 491 (Week 71), Day 631 (Week 91) and upon Early Termination. These time points are considered relevant. Titers were persistent in most of the subjects tested positive for ADAs. ADAs were first reported at week 13 (Day 85) but median onset was not before 202 days, and median time to reach peak titer was after another 100 days of onset. Median peak antibody titers were rather low (300) but there were single patients with high ADA titers of 102 000 (three female patients). For the first two patients, the peak titer was reached before the last sampling time point and no potential immunogenicity-related AEs were reported. The last patient permanently discontinued study drug at Day 114 due to Hypersensitivity. A MedDRA "hypersensitivity" SMQ has been provided as summary of AEs potentially related to complement activation. There was no apparent association between low ADA titer vs. high ADA titer (lower 50-percentile vs. upper 50-percentile) with respect to TEAEs potentially related to complement activation, using the MedDRA "hypersensitivity" SMQ. In general, hypersensitivity PTs were slightly more frequent in the ADA positive subjects, particularly those related to local reactions of the injection site. Data related to complement factor C3 are limited. There was one inotersen-treated IM positive patient with decreased C3 reported as a TEAE. Overall the data available do not suggest a relationship between immunogenicity and C3 status.

Median titers increased up to Day 197 (Week 29; median titer 1600) during treatment and decreased thereafter by Day 449 (Week 65; median titer 200). Increased ADA titers were observed during the Post - Treatment Evaluation Period in single patients. The Applicant was requested to provide all data currently available on post-treatment immunogenicity, and to discuss critically whether this finding could be attributed to long-term stimulation of antibody production or to poor drug tolerance of the assay with ADAs becoming detectable only after drug levels have reached zero. However, no new data related to post-treatment immunogenicity is available. Drug tolerance of the ADA assay is acceptable, and no further conclusions can currently be drawn before more data from study CS3 becomes available.

TEAEs were analysed for subjects with and without ADA antibodies and generally found similar, including AESIs of thrombocytopenia (the sum of thrombocytopenia and platelet count decreased) and renal impairment (23.5% vs. 24.7% and 17.6% vs. 22.1%). Ocular AEs potentially related to vitamin A deficiency and flu-like symptoms were reported more frequently in ADA positive subjects. The clinical implications are not clear. Immunogenicity status in CS2 did not negatively affect severity or discontinuations due to TEAEs.

Almost **50%** of patients continuously treated with inotersen in **CS3** were positive for anti-drug antibodies compared to 25% of subjects, who received inotersen as early as in CS3 (placebo-inotersen) and titers were mostly persistent. The latter is in line with inotersen immunogenicity status in CS2. The longer subjects are exposed to inotersen (inotersen-inotersen group), the more ADA formation is to be expected. The first positive ADA assessment was reported at an earlier time point for subjects from the inotersen-inotersen group (week 13) as compared to those from the placebo-inotersen group (Week 26). The latter onset is not in line with the results for inotersen in CS2, where ADAs were found as early as by Week 13. The difference between studies CS2 and CS3 is probably not a true difference in ADA onset but rather a consequence of the limited sample size of placebo-

inotersen subjects in CS3. In addition, the loading dose regimen might have contributed to some of the subjects from CS2 having an early onset of ADA formation. Of note, all positive IM samples observed at Week 7 during CS3 are from subjects who received inotersen treatments for 15 months in CS2, while the earliest onset for placebo-inotersen – treated subjects in CS3 was at Week 26. In Study CS3, inotersen-inotersen treated subjects received inotersen treatment 15 months longer than placebo-inotersen treated subjects, leading to longer median onset of ADA formation. While placebo-inotersen subjects showed higher median peak titer, inotersen-inotersen subjects have a much wider range of different titers over all. With the overall small sample size of placebo-inotersen subjects and high variability of titers, differences between groups are difficult to interpret but overall the groups are probably comparable. In addition, the immunogenicity observed may be associated with slight re-increase in mean and median TTR levels between Weeks 52 and 117 in the IM-positive inotersen-inotersen group as well as hypersensitivity-type TEAEs including local cutaneous reactions at the injection site (LCRIS). Thorough analysis and discussion of efficacy and safety of inotersen based on immunogenicity status, taking into account all data available up to Day 91 of this procedure, revealed no significant differences in IM positive and IM negative subjects regarding efficacy. With regard to safety by immunogenicity status, there were no clinically relevant differences in the overall incidence, severity or seriousness of the AEs between the groups, while injection site – related events, including LCRIS, as well as flu-like symptoms occurred more frequently in the ADA-positive subjects. These symptoms may be a marker for subjects who are more likely to develop ADAs, as generally symptoms occurred early in treatment (e.g. ISRs were frequently reported within the first 6 months) and did not lead to treatment discontinuation, while ADAs were reported rather late (onset between Days 200 and 250 based on Studies C2 and C3). Immunogenicity and symptoms potentially related to hypersensitivity in ADA-positive patients, in particular reactions at the injection site, will be monitored as part of routine pharmacovigilance. SmPC Section 4.8 reflects that more reactions at the administration site were reported in subjects with anti-drug antibodies.
Some imbalances in the occurrence of TEAEs and affected SOCs as well as for the AESIs and OAEIs were noted in CS3, especially in the placebo-inotersen group, while no such imbalances were observed in the inotersen group in CS2. However, the number of ADA-positive subjects in the placebo-inotersen group is rather small hampering any firm conclusion on potential differences. Platelet decreases of $\geqslant$30% and $\geqslant$50% from baseline in CS3 were more frequently experienced by ADA positive subjects in both treatment groups. In line with what it is expected from AON treatment, i.e. induction of inflammatory processes, injection site reactions (and subsequently LCRIS) as well as potential complement activation were more frequently reported in ADA-positive subjects in both treatment groups. Long-term safety consequences remain unknown.

Safety related to drug-drug interactions and other interactions

No formal drug-drug interaction studies have been conducted by the Applicant. The CHMP considers that such studies are not required.

Discontinuation due to adverse events

Discontinuations from study drug due to TEAEs mainly occurred in CS2 and were reported in a higher number of subjects on inotersen versus placebo (14.3% vs. 3.3%). No specific TEAEs were involved in these numbers. The only TEAEs mentioned in n=2 subjects were *thrombocytopenia and cachexia* with the latter being fatal in both subjects. Cachexia is a known complication of the underlying disease. However, no subject on placebo was affected. Two subjects had TEAEs leading to discontinuation from the renal and urinary disorders SOC (acute renal injury/ glomerulonephritis).

In CS3 up to the updated data cut-off 15th September 2017, 10 subjects (7.5%) discontinued the study due to a TEAEs with 8 of them (9.4%) assigned to the inotersen-inotersen group (4.1% placebo-inotersen). Most discontinuations in the inotersen-inotersen group occurred due to TEAEs from the Cardiac disorder SOC (4 patients), Infections and infestations SOC (2 patients), and the Nervous System Disorders SOC (2 patients). Other AEs leading to discontinuation of treatment in CS2 and CS3 occurred without specific pattern.

Discontinuations due to AEs of thrombocytopenia and platelet count decreased in the longitudinal safety set were the main reasons for stopping inotersen treatment (4.5%).

Post marketing experience

N/A

2.6.1. Discussion on clinical safety

hATTR (presenting as hATTR-PN and/or hATTR-CM) is a rare disease with certain geographic clusters described in Portugal, Japan, and Sweden with approximately 10 – 15,000 afflicted patients worldwide (Hawkins et al. 2015). For this reason, the size of the presented safety database for inotersen is generally considered adequate at the time of marketing authorisation.

The main safety set to inform the SmPC derives from study CS2.

112 subjects received at least one dose of inotersen (300 mg once weekly) and 60 subjects were treated with placebo (2:1) in CS2. Up to the latest data cut-off date for interim analysis (15th September 2017), a total of 134 subjects from CS2 were exposed to inotersen during CS3 (49 subjects previously treated with placebo and 85 subjects previously treated with inotersen). 112 (69.6%) and 69 (42.9%) patients had at least 1 year and 2 years of inotersen exposure based on the total inotersen treated set.

Differences between treatment groups were noted in the overall exposure that derive from **discontinuations and dose pauses** with inotersen that were to a high extent due to subjects reaching stopping rules or TEAEs. Study completion rate for CS2 was higher in subjects on placebo (87% vs. 77%). Discontinuation rates in CS2 were almost twice as high for subjects on inotersen compared to placebo – treated subjects (23% vs. 13.3%), most of all due to AEs or SAEs (14.2% inotersen vs. 1.7% placebo) including pre-defined AESIs.

Overall, general observations in exposure and (S)AEs in CS3 are in support of more pronounced effects on tolerability early in treatment with inotersen, while it cannot be excluded from the available data that safety issues also translate into long-term effects.

Demographics of subjects in CS2 were found in accordance with the disease to be treated. Compared to the CS2 safety set, the patients included and evaluated in the CM-ECHO set (with cardiomyopathy) were slightly older and fewer had V30M mutations as per stratification. More subjects on inotersen qualified for inclusion in this subset compared to placebo (66.4% vs. 55%), since they were more affected by autonomic neuropathy and cardiomyopathy.

Nearly all subjects in both treatment groups in CS2 reported **TEAEs** while 91% of subjects in CS3 did so. Disproportionality in the number of events in CS2 between treatment groups might be a consequence of more subjects on inotersen having had more frequent occurrence of one and the same event. A majority of TEAEs were AESIs in the inotersen group but not in the placebo group in CS2.

Common adverse events in study CS2 on PT level were either in line with AON – specific safety issues (e.g. injection site reactions, constitutional symptoms, thrombocytopenia or platelet count decreased, as well as gastrointestinal symptoms) or in line with ongoing underlying disease and increased autonomic neuropathy or cardiac involvement (e.g. oedema, anaemia, cachexia, orthostatic hypotension). Progressing autonomic neuropathy was attributed to inotersen-treated subjects rather than placebo-treated subjects due to baseline imbalances. Most of the events had their peak incidence within the first 6 months of treatment, were reported less frequently 6 to 12 months after treatment initiation and again more frequently in months 12 to 24, likely as a consequence of transitioning subjects from placebo to inotersen in CS3.

Currently, no impact of inotersen on an overlap of TEAEs in different organ system (cardiac, vascular, and renal system) could be identified when compared with placebo. Nevertheless, it cannot be excluded that treatment with inotersen was associated with cardiac AEs on the one hand and renal AEs on the other hand.

Types of TEAEs reported in CS3 were similar to CS2. The incidence in the placebo-inotersen group was well in line with incidences seen in the inotersen arm during CS2 due to a higher susceptibility to inotersen given for the first time or in line with disease progression (e.g. TEAEs of falls and fractures in subjects treated with placebo during CS2). The overall frequency of TEAEs was lower for most SOCs. Data from the longitudinal safety set and the inotersen integrated set were found in overall accordance with data from the single studies, although long-term data are limited.

Severity of TEAEs was in a majority of subjects/events rated as mild or moderate for inotersen but severe AEs were more frequently reported with inotersen (28% vs. 22%), including events that were attributed to more diseased inotersen-subjects at baseline.

Most of the TEAEs observed in CS2 (and CS3) were drug-related (for inotersen but not for placebo; 77.7% vs. 38.3%).

An imbalance in **deaths** was found in the clinical program with 10 deaths reported in subjects on inotersen compared to none in subjects on placebo or placebo-inotersen. Five of these deaths occurred in the placebo-controlled study (CS2) and another five in the uncontrolled single-arm extension study CS3. Cause of death was cachexia in two subjects, cardiac failure congestive in two subjects, cardiac failure, intestinal perforation, cardiac failure acute/bacteriaemia/septic shock (CS3), neuropathy peripheral (CS3), endocarditis (CS3) and intracranial haemorrhage (CS2) in one subject each. The latter happened as a consequence of Grade 4 thrombocytopenia and is considered the only death related to inotersen while the other cases point towards progression or complication of the underlying disease. Baseline imbalances in disease severity favouring placebo may have, at least partly, contributed to the excess mortality observed on inotersen.

SAEs were found to be in accordance with AESI /OESI as defined by the Applicant (renal SAEs and thrombocytopenia), but also included safety issues related to the underlying disease in single subjects. Longer exposure to inotersen in CS3 does not seem to increase the incidence of SAEs.

The relevant safety concerns for inotersen deriving from the dossier pertain to the following **AESI**:

- **Ocular adverse events potentially related to vitamin A.** There is currently no evidence for an increased risk for Ocular adverse events potentially related to vitamin A as a consequence of inotersen's mode of action causing Vitamin A deficiency. Laboratory values for vitamin A but not for retinyl palmitate were reduced in almost every subject on inotersen during CS2 and CS3. While retinyl palmitate levels in chylomicrons without substitution are unknown, data from the placebo arm in study CS2 did not show an increased incidence of AEs that could be attributed to 3000 IU per day of vitamin

A. This is an indirect reassurance (also based on scientific literature) that the supplemented dose is safe. In regard to an anticipated risk for hypovitaminosis in pregnancy balanced against the risk of foetal harm deriving from an oversupply of vitamin A, section 4.4 of the SmPC recommends that for planned pregnancies vitamin A supplementation should be discontinued prior and during the pregnancy along with inotersen the treatment while in case of unplanned pregnancies no definitive recommendation can be given whether to continue or discontinue vitamin A supplementation during the first trimester.

Occurrence of severe thrombocytopenia. Thrombocytopenia was defined an AESI for inotersen. No consistent effect on platelets was seen in the Phase 1 study with multiple dosing. Only one TEAE was reported in a placebo-treated subject while affecting 27 of 112 subjects (24.1%) on inotersen in CS2. The occurrence of severe thrombocytopenia represents the most important safety concern related to inotersen treatment. One subject died due to intracranial haemorrhage as a consequence of Grade 4 thrombocytopenia with a platelet nadir of <10 x 109/L occurring within four weeks from a normal value (the normal value was based on estimation from a smear test). In CS3, a higher proportion of subjects in the placebo-inotersen group reported AESIs of thrombocytopenia than subjects in the inotersen-inotersen group during the on-study period (26.5% vs. 14.1%). Four subjects discontinued study due to thrombocytopenia (including the death case) in CS2 and 2 subjects in CS3 (5.4% of all subjects from the longitudinal safety set). Thrombocytopenia events Grade 4 according to CTCAE criteria affected 3 of 112 subjects (2.7%) on inotersen in CS2 (one additional subject remained unconfirmed for Grade 4 thrombocytopenia). Grade 3 or Grade 4 TEAEs in these subjects were observed within the first six months of treatment (i.e. Week 8 – Week 20). During CS3, clinical narrative descriptions revealed five additional subjects with decreased platelet counts in line with Grade 3 or Grade 4 thrombocytopenia all of which were reported between Week 77 and 151 of treatment). Some of the subjects with Grade 3 or 4 events had concomitant haemorrhages (e.g. menorrhagia, haematomas, gingival bleeding, bruising, ecchymosis, periorbital haemorrhage, scleral haemorrhage, haematuria, and intracranial haemorrhage). In one subject (CS3), narrative description could not fully rule out possible punctate left frontal lobe corona radiata intraparenchymal haemorrhage. A total of 8 of 112 patients from the longitudinal safety set (i.e. 7.1%) had Grade 3 or 4 thrombocytopenia. At least two different mechanisms seem to be involved, one of which leads to a gradual decline in platelets shortly after treatment initiation. Within three and six months of treatment, mean platelet counts in the inotersen group decreased by 20% and 24% from baseline and by 26% from baseline at week 65 (EOT). Further decrease in platelets is evident from CS3 data (e.g. CS3 Week 39: mean platelet counts reduced by 28.5% from CS2 baseline). The other potential mechanism leading to a sudden and severe decline in platelets is considered to be immunologically–mediated. Treatment-emergent antiplatelet antibodies (IgG and IgM) and anti-PF4 antibodies (IgG, IgM, and IgA) were seen in subjects receiving inotersen (~29% of inotersen-treated in CS2 and >53% of subjects in CS3 [where all subjects received inotersen]) and to a significantly lower extent in subjects receiving placebo (~6% in CS2). All subjects with severe thrombocytopenia (Grade 3/4) had confirmed anti-platelet antibody formation (in two subjects with Grade 4 thrombocytopenia antibodies against platelet surface glycoproteins were detected). Interpretation is hampered by the fact that not all subjects with anti-platelet antibodies experienced severe thrombocytopenia and that some subjects also had positive anti-platelet antibodies at baseline. Baseline monitoring is therefore not considered predictive for severe post-baseline platelet decreases and not further pursued as a risk minimisation measure. Nevertheless, there is evidence for a correlation of severe thrombocytopenia and the magnitude of antibody formation, i.e. a steeper decline in platelets is noted in subjects with more than one antiplatelet antibody species (the lowest platelet counts were found in subjects with antiplatelet IgG antibodies and concomitant formation of antibodies against platelet surface glycoproteins). To conclude, implementation of antiplatelet antibody testing in a routine setting is hampered by a lack of

sensitivity and specificity of the applied indirect testing systems and the need for specialised laboratories for direct testing.

Platelet abnormalities by CTCAE cut-offs showed consistent effects of inotersen on platelet counts.

Thrombocytopenic states were prolonged in subjects from the inotersen-inotersen group in CS3 compared to inotersen-treated subjects in CS2. Treatment interruption/dose pauses (in 10% of subjects with platelets <75 x 10^9/L in CS2 and in 13.4% of subjects in CS3), dose reduction or corticoid administration led to recovery of thrombocytes in a number of subjects but continuation with inotersen was subsequently associated with recurring thrombocytopenic states needing further interruptions. It appears that, based on comparable treatment duration, ADA development was increased in patients with treatment interruptions compared to those without interruption. Therefore, frequent dose pauses might trigger immunological reactions.

Additional analyses for baseline platelet counts to predict a certain pattern of platelet decreases over time to inform monitoring algorithm have to be interpreted with caution and various exceptions were noted from theoretical calculations (i.e. subjects presenting with unpredictable steep decreases in short time intervals irrespective of baseline or prior platelet counts). The risk minimisation measures that have evolved during this procedure and delineated in the SmPC are now considered sufficient to prevent severe thrombocytopenia events. An additional monitoring step has been included in the monitoring and treatment recommendations for platelet count contained in the SmPC for subjects with a platelet count of <50x10^9/L. For them, more frequent monitoring of platelets should be considered based on the existence of additional risk factors for bleeding (that might include 'age', anticoagulant or antiplatelet medication, or prior history of major bleeding events). The same platelet threshold and risk factors should be taken into account in order for the prescriber to decide on case by case basis on initiating the treatment with corticosteroids.

Analyses of haemorrhages (MedDRA query; OESI) in a low number of subjects revealed no obvious overall difference between inotersen and placebo. Similarly, analyses per platelet categories in inotersen-treated subjects with concomitant antiplatelet or anticoagulation drugs showed no increased risk for bleeding events.

- **Renal abnormalities** are a known class effect of antisense oligonucleotides due to their accumulation in the proximal tubules of the kidneys. The incidence of renal impairment was higher for subjects on inotersen vs. placebo during CS2 (20.5% vs. 10%) mainly including TEAEs of proteinuria, eGFR decreased, renal impairment, acute kidney injury, and renal failure. Severe and serious TEAEs were nearly exclusively reported for subjects on inotersen during CS2. At least two subjects who died during CS2 were reported to have experienced a loss in renal function prior to death, for which a contribution of inotersen could sufficiently be ruled out. Inotersen was found to be (possibly) related to four cases of glomerulonephritis (two of which led to discontinuation, amongst them one subject who turned to end stage renal disease; a new case of chronic glomerulonephritis was reported in CS3 in a subject on inotersen-inotersen). Glomerulonephritis is indicated as an identified risk in the RMP. All subjects with confirmed glomerulonephritis in CS2 had significant proteinuria prior to the onset of the events and two subjects had a significant decrease in eGFR. All of these cases did not emerge before approximately 3 months of treatment. The complexity of findings in the aforementioned subjects argues for coexistence of renal amyloid deposits that derive from the underlying disease in addition to concomitant drug-induced alteration of the immune system. Renal impairment TEAEs in CS3 were not found to increase over those in CS2. Chemistry (serum creatinine, BUN) and urinalysis data are in support of the clinical findings. The mechanism of renal function loss is unclear, while it seems possible that tubular dysfunction and tubular accumulation of inotersen is involved. Shifts to worse CKD stages were in support of these findings. Renal parameters abnormalities were found more pronounced in

subjects on inotersen compared to placebo. Numerous dose interruptions were necessary in subjects with renal function impairment. Routine monitoring is considered to capture renal impairment seen in the clinical program, while renal function decline also occurred in subjects without glomerulonephritis. Based on available data, for subjects with an eGFR of <45 ml/min/1.73m2 treatment with inotersen (section 4.3) is contraindicated and subjects with a decline in eGFR of >30% during treatment need to interrupt inotersen for further evaluation of the cause followed by resumption of dosing after improvement of their renal function. Monitoring interval of renal function is set to every 3 month in patients with eGFR >60 ml/min/1.73m2 and to every 4 weeks in patients with eGFR <60 ml/min/1.73m2.

Coagulation abnormalities as pre-defined by the Applicant (coagulation parameters, e.g. aPTT, INR, PT) did not constitute a safety issue in either study with similar incidences of respective TEAEs in subjects on inotersen and placebo. However, a broader view on coagulation abnormalities by including **thrombotic events** revealed SAEs of embolic stroke, pulmonary embolism, deep vein thrombosis (in CS2 and CS3), and Mesenteric artery occlusion that might be a consequence of inflammatory lesions caused by AON treatment. No increased incidence could be seen in subjects on inotersen vs. placebo.

Hepatic abnormalities (mainly based on liver transaminase increases of ALT and/or AST relative to baseline) were found double as high for inotersen compared to placebo. Laboratory parameters revealed slight increases in both, ALT and AST, from baseline to Week 18 up to 32 and 31 U/L for inotersen but not for placebo. The totality of liver function parameters did not raise serious concerns on liver toxicity. However, the observed changes up to Week 18 justify liver monitoring prior to inotersen treatment initiation and testing after 4 months followed by yearly measurement thereafter. This recommendation for monitoring of hepatic function is properly reflected in the SmPC.

Mild to moderate **injection site reactions** (not serious) were reported frequently in subjects on inotersen compared to placebo (51% vs. 12%) during CS2 and less during CS3. Frequent presentations of ISRs were (in decreasing order) injection site erythema, injection site pain and injection site pruritus and found to be ameliorated by injection site rotation.
Constitutional (pyrexia, flu-like symptoms) as well as CNS symptoms (headache, paraesthesia, syncope) were found higher in subjects on inotersen versus placebo but safety was not significantly influenced by these events.

Complement activation is discussed with antisense oligonucleotides (Chi et al. 2017). Although baseline C3 was reduced in some of the patients, post-baseline Complement C3 below LLN was more frequently reported in subjects on inotersen (55% vs. 21%). In CS1 (healthy volunteers), results from split product measurements are considered to be in line with normal biological variation and acute phase response to inotersen. Complement split product measurement was conducted infrequently in CS2 and CS3 and from the paucity of data, no specific pattern could be determined that could attribute specific TEAEs to these values.

Although inotersen's mechanism might theoretically lead to hypothyroid states (15% of thyroxine is transported by means of TTR), no increased rate in TEAEs based on **reduced thyroxine** was noted in the clinical program. Thyroxine post-baseline values below LLN were more frequently observed in subjects on inotersen compared to placebo. However, thyreotropin remained unchanged during CS2 while more subjects on inotersen-inotersen were affected by thyreotropin levels >ULN in CS3 compared to subjects on placebo-inotersen. The clinical relevance cannot be judged based on these data. The overall risk for post - baseline low thyroxine levels is comparable for males and females.

Several **laboratory findings** were identified: serum albumin in subjects on inotersen was parallel translated to lower levels relative to those on placebo. This might be a consequence of the more pronounced autonomic neuropathy in the inotersen group. However, the difference between inotersen-inotersen and placebo-inotersen during CS3 in post-baseline serum albumin <LLN was more than double at the expense of continuous inotersen treatment, which might also point towards an additional effect on pre-renal impairment by inotersen. Mean hsCRP was >ULN at baseline in both groups and acute post-dose elevations were observed after the first exposure to inotersen (~20x of mean baseline value) and decreased from Week 5 on to placebo levels.

Single presentations of inotersen-treated subjects with critically QTcF values (shifts to >500msec or increases from baseline >60msec) but these happened in a majority of subjects after pacer placement and are hence not meaningful.

There is no clear impact of the formation and persistence of **anti-drug antibodies in 30% of patients** treated with inotersen in study CS2 on TEAEs. A majority of ADAs emerged at later time points (median onset not before 202 days). Inotersen plasma trough concentrations were found to be higher in subjects tested ADA positive compared to ADA negative subjects (see PK part of the clinical AR) leading to the conclusion that subjects with ADAs have higher accumulation in tissue compared to ADA negative subjects. Hypersensitivity PTs related to local reactions of the injection site were slightly more frequent in the ADA positive subjects but no apparent association between low or high ADA titers to TEAEs potentially related to complement activation could be retrieved.

With continuous inotersen treatment in CS3, incidences of ADA positive results increased to 50% (25% of subjects in the placebo-inotersen group). First time points of ADA positivity were different in CS2 (earlier) and CS3 (later for subjects on placebo-inotersen) but considered either a consequence of the small sample size of placebo-inotersen subjects in CS3 or of the loading dose regimen employed in CS2.

No specific safety issues in subjects being ADA positive were noted except for platelet decreases of $\geq$ 30% and $\geq$ 50% from baseline in ADA-positive subjects as in ADA-negative subjects in both groups in CS3 supporting the hypothesis of an immunologically-mediated process. Long-term effects of anti-drug antibody formation probably resulting in activation of the complement system are unknown.

2.6.2. Conclusions on the clinical safety

The safety database at present seems adequately sized to allow the evaluation of clinical safety in patients receiving inotersen for the first year, i.e. based on safety data from completed CS2 study (65 Weeks). However, limitations in integrated safety evaluation pertain to a high amount of patients (more than 50%) having had one or more dose pauses during treatment. These interruptions might under-estimate severity and persistence of relevant TEAEs. Long-term exposure at this time refers to ongoing data collection in open-label study CS3 with approximately half of the study patients being treated with inotersen for 2 years, while the maximum exposure to inotersen to date is 4.36 years in two subjects The new interim safety data cut-off of 15th September 2017 added additional 24 months data on inotersen from 11 subjects. As an additional pharmacovigilance activity the Applicant agreed to set up a product registry in order to collect additional safety data meant to overcome limitations in the data presented and to adequately react in case of a worsening of the safety profile of inotersen. These data will comprise prospective assessment of platelet count decreases, acute renal failure including glomerulonephritis, ocular toxicities due to vitamin A deficiency, discontinuations during treatment including follow-up of patients after discontinuation, SAEs, dose reductions, and treatment

pauses.

Overall, the CHMP considers that the safety profile of inotersen is sufficiently documented and appropriate risk minimisation measures (requirements for initiating inotersen treatment, monitoring rules, and stopping criteria) are in place in order to manage the identified and potential risks related to inotersen treatment.

2.7. Risk Management Plan

Safety concerns

Important identified risks	• Thrombocytopenia including a serious bleeding episode • Glomerulonephritis
Important potential risks	• Ocular toxicity due to vitamin A deficiency
Missing information	• Use in pregnancy and lactation • Use in patients with hepatic impairment • Use in patients with renal impairment • Use in patients with prior or anticipated liver transplant • Use in patients with NYHA classification 3 and 4 heart failure • Extended long-term safety • Carcinogenicity in rats

Pharmacovigilance plan

Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 3 – Required additional pharmacovigilance activities				
Planned	A retrospective chart review will be conducted with the specific objective of evaluating adherence to and effectiveness of the proposed platelet monitoring schedule, the cut-off points proposed, dose adaptation, and initiation of corticosteroids on thrombocyte recovery	Effectiveness of the proposed platelet monitoring.	1. Protocol Submission	1. No later than 3 months after EC Decision

Planned	Evaluate and further characterize the events of thrombocytopenia when Tegsedi is prescribed in normal clinical practice. This will include an assessment of the effectiveness of the platelet monitoring / dose adjustment / treatment algorithm to manage the risk of severe thrombocytopenia. Evaluate and further characterize the events of glomerulonephritis when Tegsedi is prescribed in normal clinical practice. Acute renal failure and other potential renal toxicities would similarly be characterized. This will include an assessment of the effectiveness of the renal monitoring algorithm to manage the risk of glomerulonephritis. Data on retinal toxicity/eye disease related to vitamin A deficiency would be assessed and characterized. Discontinuations during treatment including follow-up of patients after discontinuation would be assessed and characterized.	A non-interventional, multinational, observational cohort study in the form of product registry in patients receiving Tegsedi for the treatment of hATTR with symptoms of polyneuropathy to prospectively assess platelet count decreases, acute renal failure including glomerulonephritis, ocular toxicities due to vitamin A deficiency, discontinuations during treatment including follow-up of patients after discontinuation, AEs, SAEs, dose reductions, corticosteroid therapy and treatment pauses compared to external data sources of similar patients not exposed to inotersen	1. Protocol Submission	1. No later than 3 months after EC Decision
Ongoing	To evaluate the safety and tolerability of extended dosing with ISIS 420915 in patients with Familial Amyloid Polyneuropathy.	Better characterize the risks of thrombocytopenia, glomerulonephritis, and the potential risk ocular toxicity due to vitamin A deficiency	1. Completed Enrollment 2. Last patient last visit 3. Final CSR	1. Q4 2017 2. Q1 2023 3. Q4 2023

Risk minimisation measures

Safety Concern	Risk Minimisation Measures	Pharmacovigilance activities
Important identified risks		

Safety Concern	Risk Minimisation Measures	Pharmacovigilance activities
Thrombocytopenia including a serious bleeding episode	Routine RMMs **SmPC** This risk minimisation measure is addressed in Sections 4.2, 4.3, 4.4, 4.5 and 4.8. Prescription only medicine.Additional RMMs **Patient Alert card**	Routine PV activities Adverse events of special interest (AESI): Platelet count reduction to < 25 x 10^9/L with or without bleeding events. Serious bleeding events associated with death, life-threatening nature, or hospitalization. Enhanced pharmacovigilance practices implemented during the collection, collation, assessment and reporting of thrombocytopenia-associated events. Intake of AE Reports: All thrombocytopenia-associated events actively followed up to achieve a complete dataset for each case: Targeted questionnaire for Post-marketing AE reports Expedited / Periodic Safety Reporting: Cases of thrombocytopenia meeting AESIs definition expedited to the regulatory authorities within 15 calendar days. Thrombocytopenia-associated events reviewed quarterly individually and cumulatively and aggregate safety data comprehensively analysed and discussed in PBRERs on an ongoing basis. Additional PV activities: A non-interventional, multinational, observational cohort study in the form of product registry in patients receiving Tegsedi for the treatment of hATTR with symptoms of polyneuropathy to prospectively assess platelet count decreases. A retrospective chart review to be conducted with the specific objective of evaluating adherence to and effectiveness of the proposed platelet monitoring schedule, the cut-off points proposed, dose adaptation, and initiation of corticosteroids on thrombocyte recovery

Safety Concern	Risk Minimisation Measures	Pharmacovigilance activities
Glomerulonephritis	Routine RMMs **SmPC** This risk minimisation measure is addressed in Sections 4.2, 4.3, 4.4, 4.5 and 4.8. Prescription only medicine Additional RMMs**Patient Alert card**	Routine PV activities. AESI: All events of acute glomerulonephritis Enhanced pharmacovigilance practices implemented during the collection, collation, assessment and reporting of glomerulonephritis - associated events. Intake of AE Reports: All glomerulonephritis-associated events actively followed up to achieve a complete dataset for each case. Targeted questionnaire for Post-marketing AE reports (spontaneous and organized data collection sources) implemented to solicit case details of all reports of glomerulonephritis or renal impairment <u>Expedited / Periodic Safety Reporting</u> ICSRs of acute glomerulonephritis - associated AESIs will be expedited to the regulatory authorities within 15 calendar days. Glomerulonephritis-associated events reviewed on a quarterly basis individually and cumulatively and aggregate safety data comprehensively analysed and discussed in PBRERs on an ongoing basis. Additional PV activities: a non-interventional, multinational, observational cohort study in the form of product registry in patients receiving Tegsedi for the treatment of hATTR with symptoms of polyneuropathy to prospectively assess acute glomerulonephritis
Important potential risk		

Safety Concern	Risk Minimisation Measures	Pharmacovigilance activities
Ocular toxicity due to vitamin A Deficiency	Routine RMMs **SmPC** This risk minimisation measure is addressed in Sections 4.4. Prescription only medicine Additional RMMs**Patient Alert card**	Routine PV activities AESI: All Serious ocular toxicity events Enhanced pharmacovigilance practices implemented during the collection, collation, assessment and reporting of ocular toxicity - associated events. Intake of AE Reports: All ocular toxicity -associated events actively followed up to achieve a complete dataset for each case: Targeted questionnaire for Post-marketing AE reports (spontaneous and organized data collection sources) implemented to solicit case details of all reports of ocular toxicity <u>Expedited / Periodic Safety Reporting</u> Ocular toxicity -associated events will be reviewed on a quarterly basis individually and cumulatively and aggregate safety data will be comprehensively analysed and discussed in PBRERs on an ongoing basis. Additional PV activities: A non-interventional, multinational, observational cohort study in the form of product registry in patients receiving Tegsedi for the treatment of hATTR with symptoms of polyneuropathy to fully characterize the potential risk of ocular toxicity.
Missing information		
Use in pregnancy and lactation	Routine RMMs **SmPC** Use in pregnancy and lactation is discussed in Section 4.6. Prescription only medicine.	Routine PV activities for the collection of all reports of pregnancy in patients taking Tegsedi during pregnancy. Review during routine signal detection programme. Aggregate safety data analysed and discussed in PSURs on an ongoing basis. Expedited reporting of major congenital anomalies. Routine pharmacovigilance activities to characterise the risk in nursing infants

Safety Concern	Risk Minimisation Measures	Pharmacovigilance activities
Use in patients with hepatic impairment	Routine RMMs **SmPC** Use in patients with hepatic impairment is discussed in Section 4.2 and 5.2. Prescription only medicine.	Routine PV activities
Use in patients with renal impairment	Routine RMMs **SmPC** Use in patients with renal impairment is discussed in Section 4.2, 4.3 and 5.2. Prescription only medicine.	Routine PV activities
Use in patients with prior or anticipated liver transplant	Routine RMMs **SmPC** Use in patients with prior or anticipated liver transplant is discussed in Section 4.2. Prescription only medicine.	Routine PV activities
Use in patients with NYHA classification 3 and 4 heart failure	Routine RMMs **SmPC** Use in patients with NYHA class 3 and 4 is discussed in Section 4.2. Prescription only medicine.	Routine PV activities
Extended long-term safety	None.	Routine PV activities Additional PV activities: A non-interventional, multinational, observational cohort study in the form of product registry in patients receiving Tegsedi for the treatment of hATTR with symptoms of polyneuropathy to fully characterize the missing information extended long term Tegsedi therapy.
Carcinogenicity in rats	None.	None.

Conclusion

The CHMP and PRAC considered that the risk management plan version 2.0 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils

the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the 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.

2.9. New Active Substance

The applicant compared the structure of inotersen 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 inotersen to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

2.10. Product information

2.10.1. User consultation

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

2.10.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Tegsedi (inotersen) 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 amyloidosis (hATTR) is a rare, progressive and fatal disease caused by mutations in the TTR gene. Single point mutations destabilize the tetrameric structure of the TTR protein, causing its dissociation into free misfolded monomers. These monomers subsequently

aggregate to insoluble, extracellular amyloid fibril deposits resulting in cellular degeneration and death. Accumulation of amyloid deposits in multiple organ systems, particularly the nervous system, gastrointestinal tract, kidney, and heart, is causing a range of disease manifestations including but not limited to progressive polyneuropathy, which includes sensorimotor neuropathy and autonomic neuropathy.

Amyloidosis is the general term used to refer to the extracellular tissue deposition of fibrils composed of low molecular weight subunits of a variety of proteins, many of which circulate as constituents of plasma. The condition of hereditary ATTR amyloidosis is classified as a rare genetic disease by ORPHANET.

Hereditary ATTR is a multisystemic disorder with diagnostic challenges [Gertz 2017 and Parmana, Adams, Obici et al., 2016]. It has been reported that Neuropathy of TTR-FAP is typically a slowly progressive polyneuropathy, walking difficulties requiring aid occur after a delay of 6 years (stage 2) and confinement to wheelchair (stage 3) at 10 years. Late-onset Val30MetTTR-FAP is more severe and progresses twice as fast as early-onset presentation. The mean delay to reach stage 2 is only 2.6 years, and to reach stage 3, 3.8 years. In other variants of TTR, 60 % of patients reach stage 3 in a delay of 4.6 years. Life expectancy is reduced to 7.3 years. Unusually rapid decline in neurologic deficits has been reported in Ala97Ser TTR-FAP patients [Adams D, Théaudin M, Cauquil C, et al., 2014]. The staging system used to classify severity of disease in subjects being considered for enrolment in the pivotal study of inotersen (ISIS 420915-CS2 [CS2]) was the following: Stage 1 does not require assistance with ambulation; Stage 2 requires assistance with ambulation; and Stage 3 is wheelchair bound. Subjects with Stage 1 and Stage 2 hATTR-PN and an NIS $\geq$ 10 and $\leq$ 130 have been recruited in CS2, whilst patients with disease stage 3 have not been included. However, more recent publications are considering the disease a spectrum.

While patients with hATTR may present predominantly with either polyneuropathy or cardiomyopathy manifestations, most patients with hATTR manifest signs and symptoms of both polyneuropathy and cardiomyopathy over the course of their disease and reference is made to one hereditary disease with a spectrum of clinical manifestations rather than attempt to classify the disease into two distinct syndromes [Swiecicki PL, Zhen DB, Mauermann ML et al. 2015].

Furthermore, in support of the approach of a spectrum of the disease, hATTR with polyneuropathy (hATTR-PN) has been reported to progress quickly following the onset of symptoms, with a rapid increase in Neuropathy Impairment Score [Hawkins P.N., Ando Y., Dispenzeri A. et al., 2015]. Several factors can affect the rate of neuropathy progression such as TTR genotype, age at symptom onset, and extent of neurologic impairment and in many patients neuropathy progression is relatively rapid [Adams D, Coelho T, Obici L, et al. 2015 and Berk J, Suhr O, Obici L, et al. 2013].

3.1.2. Available therapies and unmet medical need

Current treatments for hATTR-PN include orthotopic liver transplantation (OLT) or pharmacotherapy with Vyndaqel (tafamidis) or diflunisal. Orthotopic liver transplant (OLT) serves as an established option for patients with early-stage disease that allows suppression of the main source of variant TTR. However, large numbers of patients are not suitable transplant candidates (Sekijima, 2015,). The rate of success for this treatment can be from 0 to 100%, since this method cannot guarantee a cure for the underlying disease.

Tafamidis is a small molecule protein stabilizer of the mutant TTR tetramer [Adams, 2016]. Tafamidis is indicated in Europe "for the treatment of transthyretin amyloidosis in adult patients with Stage 1 symptomatic polyneuropathy to delay peripheral neurological impairment".

Diflunisal is a nonsteroidal anti-inflammatory drug (NSAID) which stabilizes TTR tetramers, thereby slowing the rate of amyloidogenesis. Known cardiovascular and renal side effects associated with the NSAID drug class may limit the use of diflusinal in an aging hATTR-PN patient population. Diflunisal is not approved for the treatment of hATTR-PN; however, off-label use has been reported in patients with Stage 1 and Stage 2 disease [Adams, 2016].

There is a clear unmet medical need, since there are no approved therapies in the EU for patients with Stage 2 and Stage 3 hATTR-PN. OLT, has notable limitations and later stage patients often are not candidates for OLT. In addition, patients have disease progression despite treatment with tafamidis or diflunisal. Approximately 55% of the patients in the inotersen pivotal study were treated previously with tafamidis or diflunisal.

Inotersen is a 20-nucleotide antisense oligonucleotide (ASO) drug targeted to human TTR messenger RNA (mRNA). ASOs bind to their target sequence by Watson-Crick base pairing in the same way that complementary nucleotides bind within a double stranded helix of DNA. Consequently, to maximize binding affinity, ASOs are designed to be 100% complementary to their target mRNA. Importantly, inotersen binds to a region of the TTR mRNA that is free of any reported mutation. The selective binding of inotersen to the TTR and messenger RNA (mRNA) causes the degradation of both mutant and wild type (normal) TTR mRNA. Degradation of TTR mRNA prevents the synthesis of TTR in the liver, resulting in significant reductions in the levels of mutated and wild type TTR protein secreted by the liver into the circulation.

3.1.3. Main clinical studies

The clinical pharmacology, efficacy and safety studies are summarised in Table 2 of this report.

Dose selection was based on the dose-exploration study (CS1) investigating doses from 50 mg to 400 mg. The maximum effect in TTR reduction was obtained with the 300 mg and 400 mg doses with minimal differences between the 300 mg and the 400 mg doses. Therefore, the 300 mg dose was taken forward into the phase 2/3 trial.

The pivotal trial CS2 was a Phase 2/3 multicenter, double-blind, randomized, stratified, placebo-controlled study of inotersen in subjects with Stage 1 and Stage 2 hATTR-PN with a Neuropathy Impairment Score (NIS) of ≥10 and ≤130. Approximately 135 subjects were planned to be randomized 2:1 to 300 mg inotersen or placebo. Approximately 50% Stage 1 and 50% Stage 2 subjects were planned to be enrolled in the study. No Stage 3 patients were enrolled. 173 patients were randomised and 172 patients received at least one dose of inotersen.

The hypothesis of the pivotal study was that decreasing the amount of liver-derived TTR protein circulating in the plasma by treatment with inotersen will result in a decrease in the formation of TTR amyloid fibril deposits, and thus slow or halt disease progression (as measured by the mNIS+7) and maintain or improve quality of life (as measured by the Norfolk QOL-DN).

The co-primary efficacy endpoints were the change from Baseline to Week 66 in the Modified Neuropathy Impairment Score +7 (mNIS+7) Composite Score and in the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire total score. The mNIS+7 consists of 2 composite scores: the NIS composite score and the modified +7 composite score, each of which in turn consists of 4 individual components, that measure deficits in the strength of muscles innervated by the cranial nerves, muscle strength, reflexes, and sensation of the big toe and index finger. The +7 composite score shares the autonomic nerve assessment with the modified +7 (i.e., HRDB) but only includes peripheral nerve assessment of the lower limb (nerve conduction tests) and sensory nerve assessment

(vibration detection threshold), which is replaced by quantitative sensation testing in the modified +7. The composite score as well as individual components were evaluated.

The choice of end-points is in line with the CHMP recommendations given in 2012 in a Scientific Advice procedure (Procedure No.: EMEA/H/SA/2286/1/2012/III).

3.2. Favourable effects

The baseline disease characteristics of the patients in the inotersen group were worse than those for the placebo group. However, (a) relatively large effect sizes were observed with regard to the change from baseline in mNIS+7 and Norfolk QoL-DN for inotersen 300 mg compared to placebo, (b) other baseline characteristics were well balanced between treatment groups (i.e. random imbalances are covered by the statistical test), and (c) change from baseline was analysed for all endpoints and evaluation included a baseline covariate. A subset of patients (n=45 (40%) in the inotersen group and 22 (36.7%) in the placebo group) were diagnosed with Cardiomyopathy (hATTR-CM) at baseline.

Changes from Baseline in mNIS+7 composite score showed a statistically significant difference in favour of inotersen compared with placebo at both Week 35 and Week 66. The difference in least squares means (LSMs) between treatment groups was -8.69 (95% confidence interval [CI]: -13.49, 3.90; p=0.0005) at Week 35 and -19.73 (95% CI: -26.43, -13.03; p=0.00000004) at Week 66, respectively. Changes from Baseline in Norfolk QoL-DN total score showed a statistically significant difference in favour of inotersen compared with placebo at both Week 35 and Week 66. The difference in LSMs between treatment groups was -6.14 (95% CI: -11.77, -0.52; p=0.032) at Week 35 and -11.68 (95% CI: -18.29, -5.06; p<0.0006) at Week 66, respectively.

The analyses were performed using a Mixed Effects Model with Repeated Measures (MMRM), which was not supported by CHMP for providing too optimistic results (for more detailed reasons see section on uncertainties of favourable effects below). A high number (thirteen, 13) of predefined sensitivity analyses were performed, all supporting the primary efficacy results. Using sensitivity analysis 6 (placebo-based multiple imputation),the analyses supported by CHMP, the results at week 66 were also statistically significant i.e. for mNIS+7 the difference Inotersen 300 mg– Placebo was -14.89 (95% CI: -22.55; -7.22, p<0.001) and for Norfolk QoL-DN the difference Inotersen 300 mg– Placebo was -8.56 (95% CI: 15.42; -1.71, p=0.015).

The results from the primary endpoints are supported by consistent effects in secondary, tertiary and exploratory endpoints (either statistically significant or trends in favour of inotersen). The clinical relevance is further supported by responder analysis (sensitivity analysis 10). It is notable that progression of disease was improved or arrested in 29.2%of patients treated with inotersen as evidenced by improvement (negative change) or zero worsening in the mNIS+7 (p<0.081), compared to 16.9% for the placebo group. In 39.6% of patients treated with inotersen there was improvement (negative change) or zero worsening in the Norfolk QoL-DN (p<0.039), compared to 23.7% for the placebo group (based on FAS and calculated by the CHMP). For mNIS+7, statistical significance in favour of inotersen treatment was demonstrated at all thresholds beyond a 0-point change (i.e., ≥2-point change) and response rates were approximately 2-fold higher for inotersen compared to placebo at each threshold tested supporting consistency of results.

Secondary and tertiary efficacy endpoint analyses as well as subgroup analyses were primarily performed using MMRM. In addition, sensitivity analysis 6, which is considered of highest relevance in this setting, was performed and the results compared to those of the MMRM analysis. For mNIS+7, all the subgroup analyses were statistically significant at Week 66, results which are consistent with the MMRM results. For Norfolk QoL-DN certain subgroups, such as females, non-whites, patients older than

65 years, and the regions Europe and North America, did not reach statistical significance, but in general results were consistent with MMRM analysis and showed numerically better results for inotersen. It was noted that the effect size seems to be larger for stage 2 than for stage 1 patients (especially for the mNIS+7).

The results obtained with the open label extension study CS3 corroborated the results obtained with the CS2 study and efficacy was maintained throughout the duration of CS3 (up to interim analysis). The effect sizes for the coprimary endpoints were smaller for the inotersen-inotersen group compared to the placebo-inotersen group during CS3. However, changes from CS2 Baseline in mNIS+7 composite score and Norfolk QoL-DN total score showed less progression in the inotersen-inotersen group compared with the placebo-inotersen group at CS3 weeks 26, 52, and 78, indicating that benefits may be larger when starting treatment earlier in the disease.

3.3. Uncertainties and limitations about favourable effects

The primary analysis should ideally be based on all randomized subjects. In this case, however, only patients with at least one dose and a baseline value and at least one post-baseline assessment for the mNIS+7 or the Norfolk QoL-DN questionnaire were included in the FAS, which is the primary analysis population. While it can be argued that excluding patients who were not dosed is acceptable since the decision is not being influenced by treatment, excluding patients without post-baseline values for both endpoints is not acceptable since this could bias the results.

The primary MMRM analysis has the following deficiencies: (1) an imbalanced number of patients (6 vs. 1 on inotersen vs. placebo) was excluded from the FAS. (2) An imbalanced number of patients in the FAS was excluded from the MMRM analysis. (3) A hypothetical estimand is addressed (effect had no patient discontinued treatment) that yields an overoptimistic effect and is less relevant, since AEs are the main reason for treatment discontinuation (TD). A treatment policy estimand is considered of highest relevance reflecting an overall expected treatment effect irrespective of early treatment discontinuation.

Of the 13 sensitivity analyses No 6 is considered to be the most appropriate, because in the absence of data after TD (which is needed to reliably estimate treatment policy) the use of a placebo-based multiple imputation addressing the treatment effect as if treatment has no effect after TD, is a reasonable assumption to handle missing data. This analysis accounts for the unfavourable effect of treatment discontinuation, does not exclude patients from analysis and is proposed to be included in the product information as it reflects the most appropriate estimand. Results of this analysis (sensitivity analysis 6) are also significant and relevant for both primary endpoint at week 66 with point estimates of -14.89 (95% CI: -22.55; -7.22) and -8.56 (95% CI: 15.42; -1.71) for mNIS+7 and Norfolk QoL questionnaire, respectively. CHMP considered that the results of this analysis are most appropriate to be presented in section 5.1 of the SmPC.

Subgroup analyses did not reach statistical significance in all of the subgroups but trends in favor of inotersen treatment were observed. However, there was no indication that inotersen would not work in any of the subgroups investigated.

Approximately 40% of subjects had a concomitant diagnosis of hATTR cardiomyopathy (CM) at study entry, with a mean duration from diagnosis of 23.7 months. These patients were included in the ECHO-CM subgroup that underwent evaluation for cardiac effects of inotersen.

No consistent statistically significant effects of inotersen were observed on selected cardiac parameters including left ventricular (LV) size and function including interventricular septum (IVS) thickness,

posterior wall thickness, LV ejection fraction, LV mass, LV mass index, left atrial strain, or E/Em lateral ratio. The changes in key ECHO measurements related to cardiac wall thickness (LV mass, intraventricular septum thickness, posterior wall thickness) showed an overall decrease at Week 65 that was numerically greater in the inotersen group compared with the placebo group.

Further analyses of cardiac wall thickness parameters (LV mass, IVS thickness, and posterior wall thickness) were conducted in a subgroup of subjects with more advanced cardiomyopathy, defined as a Baseline IVS thickness $\geq$ 1.5 cm. The placebo subjects showed an increase in cardiac wall thickness parameters while the inotersen subjects showed a reduction in wall thickness. The differences in the LSM changes from Baseline between the inotersen and placebo groups were statistically significant for all 3 parameters. The decrease in cardiac thickness and mass suggest regression of cardiac amyloid.

Therefore, in a subgroup of the ECHO-CM patients with more advanced cardiac disease, the effects of inotersen appeared to be stronger but these very limited data and post-hoc analyses need to be interpreted with great caution.

In addition, CHMP considered that ECHO parameters are not considered sufficient to demonstrate clinically relevant efficacy in patients with cardiomyopathy. At least a beneficial effect on functional parameters e.g. 6 Minute Walk Test or VO2 max during exercise testing should be demonstrated. These parameters were not investigated in CS2 or CS3. The CHMP therefore concluded that efficacy of inotersen in the treatment of cardiomyopathy associated with hATTR has not been sufficiently demonstrated.

Efficacy of inotersen has also not been demonstrated in patients with stage 3 hATTR polyneuropathy. Study CS2 only enrolled patients with stage 1 or stage 2 polyneuropathy and efficacy observed in these patients cannot be simply extrapolated to patients with stage 3 disease. Six patients, who were treated with placebo in the study CS2, progressed to disease stage 3 and received inotersen in the open label study CS3. An uncontrolled post-hoc analysis suggested that, in four of these six patients, the annualised rate of disease progression to be slower after initiation of inotersen treatment. However, selection of six placebo patients from CS2 that likely had a more pronounced disease progression than other placebo patients who didn't progress to stage 3 could have biased the results. In addition, 4 out of the 6 patients discontinued the treatment prematurely and the variability of the measured endpoints does not allow for extrapolation/annualization of perceived short-term effects.

3.4. Unfavourable effects

In long-term toxicity studies in mice, rats and monkeys, adverse events reported during inotersen treatment were mainly attributable to its 2'-MOE phosphorothioate backbone chemistry and to its extensive and persistent tissue distribution. These non-specific effects have been previously reported for other members of the pharmaceutical class of 2'-MOE ASOs. However, the decreases of platelets across animal species in association with increased anti-platelet antibodies, coincide with severe clinical TEAEs (see below). Severe thrombocytopenia was noted in two monkeys and resulted in premature sacrifice of these animals, because their platelet counts had dropped to even lower levels after inotersen administration was resumed following temporary interruption.

In line with non-clinical findings, inotersen triggered immunological reactions in clinical studies, which include, but are not necessarily limited to, haematological changes (thrombocytopenia) and renal abnormalities. 30 % of inotersen - treated patients were positive to anti-drug antibodies compared to no subject on placebo. Based on provided analyses, incidence or severity of TEAEs appeared not to be associated with ADA titers.

Thrombocytopenia TEAEs including TEAEs of platelet count decreases occurred in 24.1% vs. 1.7% of subjects on inotersen and placebo, respectively in CS2 and in 25/134 subjects (18.7%) in CS3. Platelet abnormalities below <140 x 10^9/L (LLN) occurred in 55.4% of inotersen-treated patients compared to 16.7% of placebo-treated patients in Study CS2. CTCAE Grade 3 or 4 thrombocytopenia emerged as a safety issue at different time points during inotersen treatment and concerned 8 of 112 patients from the longitudinal safety set (i.e. 7.1% as of data cut-off 28th February 2017), some of them accompanied by bleeding episodes. One subject died due to intracranial haemorrhage as a consequence of Grade 4 thrombocytopenia. Discontinuations due to thrombocytopenia events affected 5.4% of subjects from the longitudinal safety set. Dose pauses triggered by platelet counts <75 x 10^9/L affected 10% of inotersen-treated subjects in CS2 and 13.4% in CS3. Mean reduction in platelets from baseline to week 23 and week 65 was 24% and 26%, respectively. Treatment interruption due to low platelets lasted 4.5 weeks in CS2 and 8 weeks in CS3. Mean duration of thrombocytopenia was 28 weeks in CS2 and 41 weeks in CS3 (i.e. patients with confirmed thrombocytopenia remained thrombocytopenic with ongoing inotersen exposure). 29% (6%) of subjects on inotersen (placebo) developed anti-platelet antibodies during CS2. All subjects with severe thrombocytopenia (i.e. Grade 3 or 4) had confirmed anti-platelet antibody formation.

Inotersen-related effects on the kidneys are expected to occur due to its accumulation in this tissue and renal excretion, therefore being a target organ of toxicity. **Renal impairment** was reported by 20.5% of subjects treated with inotersen and 10% of placebo-treated subjects with the main presentations being proteinuria (6.3% vs. 3.3%) and eGFR decreased (5.4% vs. 3.3%). Glomerulonephritis (SAE) was reported in a total of 3 subjects (2.7%) in CS2, all of which were rated possibly related to inotersen and in one subject on placebo (1.7%), accompanied by significant proteinuria and loss of renal function (in two of the three subjects). Two subjects received immunosuppressive treatment, and two subjects discontinued due to glomerulonephritis TEAEs, amongst them one subject who turned to end-stage renal disease. None of the three subjects fully recovered from the event. Renal biopsy was conducted in all cases and revealed amyloid deposits in two subjects but also concurrent immune-complex deposits; in the third subject pauci-immune glomerulonephritis was diagnosed lacking concomitant amyloid disposition. Other severe and/or serious renal TEAEs reported in 7 subjects on inotersen and one on placebo during CS2 were renal impairment, tubulointerstitial nephritis, renal failure, acute kidney injury, and proteinuria (in the subject on placebo). Decline in renal function was in line with a ≥50% reduction in eGFR from baseline in 7.1% of patients on inotersen and in none on placebo, partly overlapping with serum creatinine increases of >44.2 µmol/L (0.5 mg/dL) from baseline in 10.7% of subjects on inotersen vs. 1.7% on placebo.

Ocular adverse events potentially related to Vitamin A deficiency were balanced in subjects on inotersen and placebo (20.5% vs. 20%). All subjects were supplemented with Recommended Daily Allowance (RDA) of 3000 IU of Vitamin A.

Inotersen administered subcutaneously caused **injection site (IS) reactions** in 50.9% of subjects (versus 11.7% on placebo), mainly IS erythema, IS pain, and IS pruritus. Injection site reactions are thought to be manageable by rotation of the injection site.

Remaining **other adverse events of interest**, like hepatic abnormalities and flu-like symptoms were reported in a higher number of subjects on inotersen vs. placebo, while CNS disorders, haemorrhages, adverse events potentially related to complement activation, reduced thyroxine, and thrombotic events were reported at similar incidences for both treatment arms.

With respect to **laboratory findings and vital signs**, inotersen had clear but transient elevating effect on C-reactive protein at treatment initiation, a known class effect of antisense oligonucleotides.

Abnormalities in IgG and IgM concentrations were also more pronounced with inotersen. Alterations in haematology values, such as anaemia, were observed more frequently in the inotersen –treated group compared to placebo, including three cases of eosinophilia in the CS2 study. Inotersen treatment was also associated with a lowered mean systolic and diastolic blood pressure in CS2 study during the initial loading period.

Deaths: Ten subjects died while being treated with inotersen compared to none on placebo (5 subjects in the placebo-controlled study CS2, 5 subjects in the uncontrolled open-label study CS3). The reasons for death did not follow a specific pattern and all except one of the deaths were considered not related to inotersen treatment: two cases of cachexia, two cases of cardiac failure congestive and one case for each of the following: intestinal perforation, cardiac failure, cardiac failure acute/bacteriaemia/septic shock, neuropathy peripheral, and endocarditis. One death due to intracranial haemorrhage as a consequence of Grade 4 thrombocytopenia was considered possibly related to inotersen treatment.

3.5. Uncertainties and limitations about unfavourable effects

General uncertainties on the unfavourable effects of inotersen result from the limited long-term safety evidence, based on data from uncontrolled experience, and the fact that several TEAEs may be either related to the (immunological) effects of the drug or to the progression of the disease and co-morbidities. The observed baseline imbalance in autonomic neuropathy in subjects on inotersen and placebo as well as various dose interruptions in the two studies additionally impede clear associations between drug and adverse effects.

An **imbalance in death cases** with no fatal events in subjects on placebo and 10 fatalities in subjects on inotersen (5 in the placebo-controlled study CS2 and 5 in the uncontrolled single-arm extension study CS3 up to the latest interim data cut-off of 15th September 2017) was observed. Detailed discussion of every single case did not suggest a causal relationship to inotersen treatment in nine of ten subjects (for intracranial haemorrhage, see unfavourable effect section). The difference in exposure (2:1 randomisation to inotersen vs. placebo, respectively) and the baseline imbalance in disease severity favouring placebo may have, at least partly, contributed to the observed excess mortality with inotersen in study CS2.

Although **Ocular adverse events potentially related to Vitamin A deficiency** were not found to be in excess in inotersen-treated subjects over placebo, 90.1% of subjects on inotersen but only 3.3% of subjects on placebo had at least one Vitamin A value <LLN in CS2. Retinyl palmitate measurements indicate a sufficient supply of vitamin A through chylomicron transportation when RBP4 levels are low. It remains, however, unexplored if inotersen could have been used safely without vitamin A supplementation since all patients received 3000 IU vitamin A during studies CS2 and CS3. No increase in TEAEs attributed to vitamin A was seen in subjects on placebo.

Adequate **Vitamin A levels** are especially crucial **in pregnancy**; however it remains unclear, if the proposed vitamin A supplementation recommendations in the SmPC ensure adequate Vitamin A levels for normal embryo-foetal development.

Severe thrombocytopenia is of concern with inotersen treatment and mechanistically thought to be different from a gradual mean decrease in platelets. No consistent effect on platelets was seen with multiple dosing in the Phase 1 study (CS1). Timing of severe platelet decline lacks a clear pattern and emerged between Week 8 and Week 20 in CS2 and between Week 77 and 151 in CS3 rendering any

prediction difficult. In addition, an immunological mechanism is suggested based on findings of a higher total antiplatelet antibody formation in subjects with Grade 3 and especially Grade 4 thrombocytopenia compared to those with Grade 1 or 2 thrombocytopenia. Antibodies involved typically more than one antiplatelet antibody species and antibodies against platelet surface glycoproteins seem to be responsible for the steepest decreases in platelets. A discussion of routine testing of antiplatelet antibodies was requested by CHMP in order to explore the possibility that antiplatelet antibody positivity might help identifying patients with platelet counts $<50 \times 10^9$/L in whom inotersen should permanently be discontinued or in whom inotersen could safely be resumed. Baseline and on-treatment antiplatelet antibody monitoring was not found helpful to inform acute clinical decision making given that sensitivity and specificity of direct and indirect testing systems are low for detection of immune thrombocytopenia (Heikal et al. 2013).

Subjects with Grade 3 thrombocytopenia will be in need for dose pauses according to the recommendations included in the SmPC (section 4.4). Experience from re-challenge with inotersen in a total of 4 subjects with Grade 3 thrombocytopenia in the clinical studies indicate that dose pauses do not safely prevent from further decreases in platelets. Based on the available data it could not be clarified, whether these dose interruptions additionally trigger antiplatelet antibody formation and/or overall immunogenicity (of note: the pattern of ADA formation points towards an increase in immunogenicity after dose pauses).

Renal impairment/ decline in renal function: The most common denominators in subjects presenting with a loss in renal function are disease stage and age, i.e. the mean decline in eGFR during CS2 seems to derive from subjects with stage 2 disease. No renal function disturbances were reported for six subjects progressing to stage 3 of the disease. It remains unknown whether these limited data translate into the absence of renal impairment in a representative number of subjects with advanced disease.

Risk minimisation measures for detection of glomerulonephritis have been discussed and include a stepwise approach for monitoring of UPCR and eGFR: monitoring is recommended to be undertaken every three months for all patients on inotersen while either UPCR ≥ 2x ULN or eGFR <60 ml/min/1.72m2 (both confirmed on repeat testing) should trigger monthly monitoring. Dose interruption is recommended after either an eGFR decrease of more than 30% or an UPCR ≥ 2g/g (226 mg/mmol). Based on the eGFR LLN value of 60 ml/min/1.73m2, a more than 30% reduction in eGFR reflects the contraindication threshold of 45 ml/min/1.73m2 that is mentioned in section 4.3 of the SmPC. The UPCR threshold of ≥ 2g/g (226 mg/mmol) has been chosen based on the fact that it is approximately equivalent to a 24-hour urine protein excretion of 2 g, which was the mandatory dose interruption rule in the CS2 and CS3 protocols. If glomerulonephritis is excluded as cause for the decline in renal function/proteinuria, re-challenge with inotersen can be considered as soon as renal function has improved, which is in line with section 4.3 of the SmPC.

A mechanistical hypothesis for renal function loss has been raised that probably involves impairment of tubular function as a consequence of amyloid deposits. This is, however, not confirmed by data but will be further addressed in the post-market setting (i.e. product specific registry).

Uncertainties arose regarding the occurrence of thrombotic and/or embolic events that were reported as SAEs (embolic stroke, pulmonary embolism, deep vein thrombosis, and Mesenteric artery occlusion) and were suspected to be due to immunologically mediated inflammation. This suspician could not be confirmed by clinical data and the events were rather attributable to the underlying immobility and cardiac failure in this patient population.

Liver function is known to be affected by AON treatment, and transaminases increases (mean ALT and AST) were noted with inotersen but not with placebo. Baseline and on-treatment liver enzyme measurement (after 4 months and yearly thereafter or more frequently as clinically indicated) were determined based on clinical CS2 data. Liver toxicity will be further characterized in the proposed product registry.

Complement activation was not thoroughly studied in the clinical program to further substantiate the findings on the immunogenic potential. However, a progressive increase in the number of subjects with complement factor C3 below LLN was observed in CS2. Further examination of complement split products could neither confirm nor exclude a contribution of complement activation.

The clinical significance of **thyroxine** levels below LLN in 19.8% of inotersen- vs. 8.5% of placebo-treated subjects is unclear but in line with theoretical considerations on the mode of action of inotersen.

Some adverse events, e.g. hypotension, hsCRP, and platelet count increases were more frequently or solely reported during the loading period in Study CS2 and did not occur or to a minor extent in CS3 study where no loading dose was administered. A loading dose is not part of the proposed posology.

TEAEs from the *Cardiac Disorders SOC* were balanced across treatment groups; however, the number of events was higher for inotersen compared to placebo, possibly as a consequence of more severe cardiomyopathy in inotersen-treated subjects at baseline. Also vascular adverse events like arterial hypotension and hypertension were observed more frequently in the inotersen group and more severe CKD renal impairment stages (i.e. stages 4 and 5) have only been reported in subjects on inotersen. Adverse events reported in the cardiac and renal organ classes may be more relevant in patients with advanced cardiac disease but such patients (with NYHA stage III and IV) were not included in the study.

Immunogenicity of inotersen has been discussed and currently there is no overall evidence of a significant increase in TEAEs related to immunogenicity.

3.6. Effects Table

Effects Table for Inotersen-Ionis for the treatment of adult patients with hereditary transthyretin amyloidosis (hATTR) to delay disease progression and improve quality of life (data cut-off: 28 March 2017).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
mNIS+7 Composite Score, difference from baseline at week 66	Modified Neuropathy Impairment Score +7	N/A	Inotersen 300mg (n=106) 25.53	Placebo Placebo (n=59) 5.80	Difference -19.73 (95% CI: -26.43;-13.03) analysed by MMRM, and -14.89 (95% CI: -22.55;-7.22), by sensitivity analysis 6	(1)
Norfolk QoL-DN Total Score, difference from baseline at week 66	Norfolk Quality of Life Diabetic Neuropathy questionnaire	N/A	Inotersen 300mg (n=106) 12.67	Placebo Placebo (n=59) 0.99	Difference -11.668 (95% CI: -18.29;-5.06) analysed by MMRM, sensitivity and -8.56 (95% CI:-15.42;-1.71), by sensitivity analysis 6	(1)
mNIS+7 Composite Score, sensitivity analyses		N/A	Inotersen 300mg (n=106)	Placebo (n=59)	All 12 sensitivity analyses were consistent with the primary analysis, and statistical significance was maintained. One additional analysis, responder evaluation supported the superiority of inotersen compared to placebo.	(1)
Norfolk QoL-DN Total Score, sensitivity analyses		N/A	Inotersen 300mg (n=106)	Placebo (n=59)	All 9 sensitivity analyses were also consistent with the primary analysis, and statistical significance was maintained	(1)
GLS in CM-ECHO set	On-Treatment Global Longitudinal Strain (%)	N/A	Inotersen 300mg (n=50) 1.14	Placebo (n=25) 0.94	Difference 0.2, not statistically significant p=0.771) analysed by MMRM	(1)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Unfavourable Effects						
Thrombocytopenia	Incidence of thrombocytopenia (including thrombocytopenia all grades and platelet count decreased)	%	24.1	1.7	Two processes: 1) early decreases in platelets with mean values in the normal range; 2) sudden onset (< 4 weeks) of steep decreases of platelets with unpredictable onset after treatment initiation; likely immunologically mediated; long-term incidences not known; fatal in 1 subject (intracranial haemorrhage)	(1)
	Incidence of Grade 3/4 thrombocytopenia	%	7.1	N/A	see above	(2)
Renal impairment	Incidence of renal impairment	%	20.5	10.0	Renal abnormalities mainly comprise proteinuria, eGFR decreased	(1)
	Incidence of glomerulonephritis	%	2.7	1.7	Renal biopsies in these 3 subjects in line with amyloid deposits as well as immune-complex deposits	(1)
Ocular adverse events potentially related to vitamin A deficiency	Incidence	%	20.5	20.0	Nearly all subjects on inotersen had any post-baseline value of Vitamin A <LLN despite Vitamin A supplementation	(1)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Hepatic abnormalities	Incidence of abnormal liver function	%	12.5	6.7	class effect of AON treatment; ALT >3x ULN, 5x ULN, 8x ULN, and 10x ULN in CS2 solely occurred in subjects on inotersen	(1)
Injection site reactions	Incidence of Injection site reactions	%	50.9	11.7	class effect of s.c. AONs; TEAEs reported were mainly IS erythema, IS pain, and IS pruritus; no severe TEAEs/SAEs, no discontinuations	(1)
Flu-like symptoms	Incidence of flu-like symptoms	%	16.1	1.7	class effect of AONs	(1)
CNS disorders	Incidence of CNS disorders	%	58.9	53.3	Due to a loss of transthyretin – associated neuroprotection or as a consequence of the underlying autonomic neuropathy in hATTR	(1)
Haemorrhages	Incidence of haemorrhages	%	35.7	33.3	No increased incidence of haemorrhages in subjects with anticoagulation compared to no-coagulation treatment	(1)
Adverse Events Potentially Related to Complement Activation	Incidence of Adverse Events Potentially Related to Complement Activation	%	28.6	26.7	Complement C3 <LLN in 55% of subjects on inotersen vs. 21% on placebo	(1)
Reduced thyroxine	Incidence of TEAEs related to reduced thyroxine	%	2.7	1.7	gradual increase in subjects with thyroxine values <LLN from baseline to Week 65 for inotersen vs. placebo	(1)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Thrombotic events	Incidence of thrombotic events	%	3.6	3.3	thrombotic and embolic events with AONs might be a consequence of inflammatory lesions	(1)
Death	Number of deaths	No.	10	0	Imbalance in death cases not fully addressed up to day 106	(2)

Abbreviations: please see Table on Page 6

Notes: (1) Data from study CS2; (2) Data from evaluation of the longitudinal safety set.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Efficacy has been convincingly shown in one pivotal phase 2/3 trial conducted in 173 patients with Stage 1 or Stage 2 hereditary transthyretin amyloidosis (hATTR) polyneuropathy.

Statistically significant and clinically relevant results were shown in both co-primary endpoints. Norfolk QoL-DN has been previously validated and the mNIS+7 has been considered appropriate to reflect the clinical condition and its progress (e.g. assessment of the nerve conduction in the upper limb). Two very similar versions of the mNIS+7 have been tested in Phase 3 studies in patients with hATTR-PN and found to discriminate the progression of disease in subjects with hATTR-PN and to differentiate between progression in those treated with active drug and those treated with placebo. While the subscales have been validated in other studies in patients with hATTR-PN the performance of mNIS+7 has also been assessed and showed face validity and test-retest reproducibility. A good correlation between mNIS+7 and the validated Norfolk QoL-DN has been demonstrated for the overall population and for all stratification subgroups. As a result, the choice of endpoints is considered acceptable.

The clinical relevance is further supported by the results of a responder analysis (sensitivity analysis 10).

The 13 sensitivity analyses of the primary endpoints together with the analyses of secondary, tertiary and exploratory endpoints provided supportive evidence for the robustness of the efficacy data in favour of inotersen. Out of the 13 sensitivity analyses, sensitivity analysis 6 (placebo based imputation) is considered by the CHMP to be of highest clinical relevance and consequently is reflected in the SmPC.

It is usually expected that the scientific results are replicated in a second clinical trial. However, in this rare and ultimately fatal disease with a clear unmet medical need, a marketing application supported by data generated in a single pivotal trial is considered by CHMP acceptable. The submitted results are considered compelling with respect to data quality, statistical significance, clinical relevance and internal and external validity.

However, in the CHMP view the submitted data are insufficient to demonstrate efficacy of inotersen in the treatment of cardiomyopathy manifestations associated with hATTR. In addition, the study population was limited to stage 1 and 2 patients with hATTR-PN and extrapolation of efficacy data to stage 3 patients is considered challenging. Very limited data on 6 patients who progressed to stage 3

during treatment with placebo in study CS2 may be indicative of slowing of disease progression but 4 of these 6 patients discontinued treatment early and the annualised progression rate presented by the Applicant is considered to be flawed. Therefore and taking the safety profile of inotersen into account, initiation of inotersen treatment in stage 3 patients is not endorsed. It is noted that no data are yet available for patients who, while being treated with inotersen, progressed to stage 3 of the disease.

Inotersen obviously elicits immunological and pro-inflammatory effects in line with other drugs from the class of antisense oligonucleotides (e.g. Frazier et al. 2014), which are thought to translate into the main safety findings i.e. severe presentations of thrombocytopenia (including fatal bleeding in one subject) and renal abnormalities (including reports of four cases of glomerulonephritis (GN) and decline in renal function without GN). Immunological processes are thought to be involved in severe thrombocytopenia based on some investigational data for platelet count reductions. However, only theoretical considerations are available for the observed gradual decline in renal function, which could be related to inotersen treatment and/or the underlying disease.

Two risk minimisation options for clinical management of patients at risk experiencing severe thrombocytopenia have been thoroughly discussed during this procedure, i.e. platelet count monitoring and/or antiplatelet antibody monitoring in subjects with a platelet count threshold of $<50 \times 10^9$/L. Antiplatelet antibody measurement was, however, not considered feasible to further minimise the anticipated risk of bleeding given its rather complicated implementation into a routine setting.

In order to overcome the uncertainty of an increased risk of platelet count drops in subjects with Grade 3 thrombocytopenia, a tightened gradual monitoring scheme was introduced in section 4.2 of the SmPC that is considered to be sufficiently tight to capture sudden and steep drops on one hand, and present the lowest possible extra burden for the patient on the other hand. Low pre-specified platelet cut-off values trigger more frequent monitoring, reduction in dose frequency or even dose interruption (until platelet count recovery is confirmed): the originally proposed monitoring of platelets: $>100 \times 10^9$/L every 14 days (weekly dosing), ≥ 75 to $<100 \times 10^9$/L weekly (dosing every 2 weeks), $<75 \times 10^9$/L twice weekly (dose interruption), and $<25 \times 10^9$/L daily (discontinuation of inotersen and corticosteroid initiation) has been amended to include an additional monitoring step for subjects with platelet counts $< 50 \times 10^9$/L. This step includes consideration of more frequent than twice weekly platelet monitoring and individual corticosteroid treatment if additional bleeding risk factors are present. These risk factors are further specified in a footnote of table 1 in SmPC section 4.2 and include age >60 years (patients considered to be more susceptible to thrombocytopenia-related bleedings based on publically available literature (Cortalezzo et al. 1991); reference which is also included in section 4.4), patients receiving anticoagulant or antiplatelet medication, and patients with prior history of major bleeding events.

The anticipated uncertainties in regard to the monitoring and dose adjustment algorithm in the overall management of severe thrombocytopenia will be further elucidated as part of additional pharmacovigilance activities (i.e. product specific registry).

Renal function parameters and reported TEAEs of renal impairment need to be interpreted against the background of the underlying disease including recurrent infections, fluid shifts and cardiomyopathy leading to variability in renal function over the course of the studies while an effect of inotersen on the kidney function over time (beyond those evaluated in the clinical studies) cannot be excluded from current data.

Monitoring algorithm of proteinuria (UPCR) and renal function (eGFR) as well as their cut-offs for dose pauses sufficiently account for detection of glomerulonephritis during inotersen treatment.

Two contraindications related to renal impairment have been implemented during this procedure: subjects with pre-treatment UPCR ≥1g/g (113 mg/mmol) should not receive inotersen and this is according to the clinical study protocol exclusion criterion. In addition, renal amyloidosis in those subjects is considered advanced and the CHMP considers that in this population the B/R of inotersen treatment has not been established.

While subjects with an eGFR of < 45 ml/min/1.73m2 were excluded from inotersen treatment as per the original study protocol, a higher eGFR of < 60 ml/min/1.73m2 was included in later versions of CS2 study protocol. Additional data were presented that could reasonably rule out a worsening of renal function in subjects with a pre-treatment eGFR of < 60ml/min/1.73m2, hence supporting a contraindication threshold of < 45 ml/min/1.73m2. Lower eGFR CKD stages have not been studied, except for five patients. In four of them, inotersen was permanently discontinued, even though not due to decline in renal function.

Both contraindications were considered appropriate by CHMP in order not to deny access to inotersen treatment to too many subjects on one hand and to sufficiently prevent a further decline of renal function in patients with pre-existing advanced (amyloidosis – related) renal function impairment (i.e. those with advanced disease stage and older subjects) on the other hand. .

The uncertainties identified in the clinical program will further be addressed as part of post-marketing activities (i.e. a registry that collects specific information [through a structured questionnaire] on subjects who report episodes consistent with acute renal failure and results of any nephrologist consultation and specific tests, including reports of renal biopsies).

The **imbalance in death cases** remains unexplained, although the difference in exposure and an imbalance in disease severity at baseline favouring placebo may have contributed to the observed increased mortality in patients treated with inotersen. However, the overall data point towards the progression of the disease as a plausible explanation rather than towards non-specific effects related to the systemic ASO administration.

3.7.2. Balance of benefits and risks

Hereditary ATTR is a progressive and life-threatening disease where a high medical need has been identified due to very limited treatment options, esp. in patients beyond stage 1 disease.

Statistically significant, robust and clinically relevant beneficial effects of inotersen have been shown in patients with stage 1 and 2 hereditary transthyretin amyloidosis polyneuropathy. The benefits were measured by employing two validated and clinically relevant primary endpoints. The secondary, tertiary and exploratory endpoints and a number of sensitivity analyses supported the positive outcome.

The safety profile of inotersen, especially with regard to immune-mediated thrombocytopenia and associated bleeding risk and glomerulonephritis, is of concern. Mechanistic confirmation of inotersen's contribution to immunological alterations and pro-inflammatory effects on the main safety findings is insufficient up to the cut-off date of this procedure. However, the safety risks are considered to be manageable by implementing stringent risk-minimisation measures which are expected to be both effective and feasible in clinical practice. These include specific monitoring, dose reduction as well as stopping rules. These risk minimisation measures are thoroughly described in the respective sections of the SmPC. Post-marketing measures will be in place to further collect data on the main identified safety concerns in a real-world treatment setting.

Thus, in patients with stage 1 or stage 2, the benefits of treatment with inotersen are considered to outweigh the risks.

On the other hand, efficacy of inotersen has not been sufficiently shown on cardiac manifestations of hATTR and in patients with advanced (stage 3) polyneuropathy. Considering the substantial uncertainty regarding efficacy and the safety concerns with inotersen, the B/R balance of inotersen treatment in patients with only cardiac manifestation of hATTR and in patients with advanced (stage 3) polyneuropathy is considered unfavourable.

The applicant has agreed to restrict the indication accordingly.

3.8. Conclusions

The overall B/R ratio of Tegsedi in the treatment of stage 1 or Stage 2 polyneuropathy in adult patients with hereditary transthyretin amyloidosis (hATTR) is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP, by consensus, is of the opinion that Tegsedi is not similar to Vyndaqel, as defined in 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 Tegsedi is favourable in the following indication:

Treatment of stage 1 or Stage 2 polyneuropathy in adult patients with hereditary transthyretin amyloidosis (hATTR) 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 TEGSEDI 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 TEGSEDI is marketed, all patients who are expected to been administered the products are provided with a patient alert card (wallet size), aiming at preventing and/or minimising the important identified risks of thrombocytopenia, glomerulonephritis, and the important potential risk of ocular toxicity due to vitamin A deficiency, and reminding patients:

- To carry the card with them at all times during the treatment and up to 8 weeks following treatment discontinuation;
- The list of signs and symptoms of thrombocytopenia, glomerulonephritis, and ocular toxicity due to vitamin A deficiency, highlighting that these might be severe or life-threatening, and advising patients to call immediately their doctor or attend the emergency room if such signs and symptoms appear;
- To undergo all blood or urine tests as arranged by their doctor;
- To have a list of all other medicines they are using for any visit to a Health Care Professionals (HCP);

In addition to a prompt to include the contact details of the patient's physician and a call for reporting, the patient card should also:

- Alert HCPs that the patient is taking TEGSEDI, its indication and the key safety concerns;
- Advise HCPs that, due to the risks of thrombocytopenia and glomerulonephritis, patients should have their platelet count monitored at least every 2 weeks, and urine to protein creatinine ratio and estimated glomerular filtration rate monitored at least every 3 months;
- Advise HCPs that if the platelet count falls below 25 x 10^9/L, Tegsedi treatment should be permanently discontinued and corticosteroid therapy is recommended;

- Advise HCPs that if glomerulonephritis is confirmed, Tegsedi treatment should be permanently discontinued and early initiation of immunosuppressive therapy should be considered.

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 inotersen is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

4.1. Conclusions

The overall B/R of Tegsedi is positive.

REFERENCES

Adams D, Suhr OB, Hund E, et al. First European consensus for diagnosis, management, and treatment of transthyretin familial amyloid polyneuropathy. *Curr Opin Neurol.*2016;29:S14-S26.

Berman CL, Barros SA, Galloway SM, et al. OSWG recommendations for genotoxicity testing of novel oligonucleotide-based therapeutics. Nucleic Acid Therap 2016; 26: 73-85.

Castańo A, Drachman BM, Judge D, et al. Natural history and therapy of TTR-cardiac amyloidosis: emerging disease-modifying therapies from organ transplantation to stabilizer and silencer drugs. *Heart Fail Rev*. 2015;20(2):163-178.

Coelho T, Ericzon B, Falk R, et al. A physician's guide to transthyretin amyloidosis. *Amyloidosis Foundation.* 2008;1-16.

Coelho et al., *"A Guide to Transthyretin Amyloidosis"*, Amyloidosis Foundation, 2016 Edition

Coutinho P, Martins da Silva A, Lima JL, et al. Forty years of experience with type 1 amyloid neuropathy. Review of 483 cases. In: Glenner GG, Pinho e Costa P, Falcao de Freitas A, editors. *Amyloid and Amyloidosis*. Amsterdam, The Netherlands;1980.

Geary RS. Antisense oligonucleotide pharmacokinetics and metabolism. *Expert Opin Drug Metab Toxicol*. 2009;5:381-391.

Gertz, MA. Hereditary ATTR amyloidosis: Burden of illness and diagnostic challenges. *Am J Manag Care*. 2017;23:S107-S112.

Henry SP, Jagels MA, Hugli TE, et al. Mechanism of alternative complement pathway dysregulation by a phosphorothioate oligonucleotide in monkey and human serum. Nucleic Acid Ther 2014; 24(5): 326-335.

Henry SP, Narayanan P, Shen L, et al. Assessment of the effects of 2'-methoxyethyl antisense oligonucleotides on platelet count in cynomolgus nonhuman primates. Nucleic Acid Therap 2017; 27: 197-208.

Herlenius G, Wilczek HE, Larsson M, et al. Ten years of international experience with liver transplantation for familial amyloidotic polyneuropathy: results from the Familial Amyloidotic Polyneuropathy World Transplant Registry. Familial Amyloidotic Polyneuropathy World Transplant Registry. Transplantation. 2004;77(1):64-71.

Liepnieks JJ, Benson MD. Progression of cardiac amyloid deposition in hereditary transthyretin amyloidosis patients after liver transplantation. *Amyloid.* 2007;14(4):277- 282.

Liepnieks JJ, Zhang LQ, Benson MD. Progression of transthyretin amyloid neuropathy after liver transplantation. *Neurology.* 2010;75(4):324-327.

Olejniczak K, Gunzek P, Bass R. Preclinical test strategies. Drug Information Journal 2001; (35): 321-336.

Plante-Bordeneuve V, Said G. Familial amyloid polyneuropathy. *Lancet Neurol.* 2011;10:1086-1097.

Rabinovich-Guilatt L, et al. Impact of dosing regimen of custirsen, an antisense oligonucleotide, on safety, tolerability and cardiac repolarization in healthy subjects. Br J Clin Pharmacol. 2015.

Quintas A, Vas DC, Cardoso I, et al. Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants. *J Biol Chem.* 2001;276(29):27207-27213.

Sekijima Y, Yoshida K, Tokuda T, et al. Familial transthyretin amyloidosis. *Gene Reviews* 2009; [Internet], Pagon RA, et al. editors. Seattle, WA.

Stangou AJ, Hawkins PN. Liver transplantation in transthyretin-related familial amyloid polyneuropathy. *Curr Opin Neurol.* 2004;17(5):615-620.

Stewart M, Loftus J, Lenderking W, et al. Evaluating the quality of life and burden of illness in an ultra-rare disease in the U.S.: transthyretin familial amyloid polyneuropathy (TTR-FAP) patients & caregivers. Abstract presented at: the IXth International Symposium on Familial Amyloidotic Polyneuropathy (ISFAP); November 10-13, 2013; Rio de Janeiro, Brazil. Abstract E16.

Suhr O, Danielsson A, Rydh A, et al. Impact of gastrointestinal dysfunction on survival after liver transplantation for familial amyloidotic polyneuropathy. *Dig Dis Sci.*1996;41(10):1909-1914.

Shen L, Frazer-Abel A, Reynolds PR, et al. Mechanistic understanding for the greater sensitivity of monkeys to antisense oligonucleotide-mediated complement activation compared with humans. J Pharmacol Exp Ther 2014; 351(3): 709-717.

Sipe JD, Benson MD, Buxbaum JN, et al. Amyloid fibril proteins and amyloidosis: chemical identification and clinical classification International Society of Amyloidosis 2016 Nomenclature Guidelines. Amyloid. 2016;23(4):209-213.

Yazaki M, Tokuda T, Nakamura A, et al. Cardiac amyloid in patient with familial amyloid polyneuropathy consists of abundant wild-type transthyretin. *Biochem Biophys Res Commun.* 2000;274;702-706.

Yazaki M, Mitsuhashi S, Tokuda T, et al. Progressive wild-type transthyretin deposition after liver transplantation preferentially occurs onto myocardium in FAP patients. *Am J Transplant.* 2007;7:235-242.

Yu RZ, Kim TW, Hong A, et al. Cross-species pharmacokinetic comparison from mouse to man of a second-generation antisense oligonucleotide, ISIS 301012, targeting human apolipoprotein B-100. *Drug Metab Dispo*. 2007;35:460-468.