28 February 2019
EMA/180717/2019
Committee for Medicinal Products for Human Use (CHMP)

# Assessment report

## WAYLIVRA

International non-proprietary name: volanesorsen

Procedure No. EMEA/H/C/004538/0000

## Note

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

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# List of abbreviations

| | |
|---|---|
| 2'-MOE | (2'-O-[2-methoxyethyl]) |
| ADA | Anti-drug antibodies |
| ALT | Alanine transferase |
| AR | Assessment report |
| ASO | Antisense oligonucleotide |
| AUC | Area under curve |
| BE | Bio-equivalence |
| CPP | Critical process parameter |
| DDI | Drug-drug interaction |
| ELISA | Enzyme-linked immunosorbent assay |
| FCS | Familial chylomicronemia syndrome |
| FPL | Familial partial lipodystrophy |
| GCP | Good clinical practice |
| GFR | Globular filtration rate |
| HDL | High-density lipoproteins |
| HPLC-UV | High-performance liquid chromatography-ultraviolet (HPLC-UV) |
| HTG | Hypertriglyceridemia |
| ICH | International council for harmonisation |
| IP-HPLC-ES/MS | Ion-pair high performance liquid chromatography electrospray/mass spectrometry |
| IM | Immunogenicity |
| IV | Intravenous |
| LBM | Lean body mass |
| LDL-C | Low-density lipoprotein cholesterol |
| LOOI | List of outstanding issues |
| LPL | Lipoprotein lipase |
| LRP-1 | Low-density lipoprotein receptor-related protein 1 |
| MAA | Marketing authorisation application |
| mRNA | Messenger ribonucleic acid |
| MRT | Mean residence time |
| QWBA | Qualitative and quantitative whole-body autoradiography |
| PD | Pharmacodynamics |
| PDA | Photodiode array |
| PDE | Permitted daily exposure |
| PK | Pharmacokinetics |
| PP | Process parameter |
| SC | Subcutaneous |
| SHTG | Severe high triglycerides |
| TG | Triglycerides |
| TRL | Triglyceride rich lipoproteins |
| VLDL-C | Very low-density lipoprotein cholesterol |
| Vss | Steady-state volume |

# 1. Background information on the procedure

## *1.1. Submission of the dossier*

The applicant Akcea Therapeutics Ireland Ltd. submitted on 26 July 2017 an application for marketing authorisation to the European Medicines Agency (EMA) for WAYLIVRA, 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 June 2016.

WAYLIVRA, was designated as an orphan medicinal product EU/3/14/1249 on 12 March 2014 in the following condition: Treatment of familial chylomicronemia syndrome.

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

The applicant applied for the following indication: Waylivra is indicated as an adjunct to diet for the treatment of patients with familial chylomicronemia syndrome (FCS).

**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).

## *Information on Paediatric requirements*

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0031/2017 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0031/2017 was not yet completed as some measures were deferred.

## *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.

### Conditional marketing authorisation

The applicant requested consideration of its application for a Conditional marketing authorisation in accordance with Article 14-a of the above mentioned Regulation.

## New active Substance status

The applicant requested the active substance volanesorsen 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.

### *Scientific Advice*

The applicant received Scientific Advice from the CHMP on 22 May 2014 (EMEA/H/SA/2741/1/2014/II). The Scientific Advice pertained to clinical aspects of the dossier.

## *1.2. Steps taken for the assessment of the product*

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

Rapporteur: Johann Lodewijk Hillege Co-Rapporteur: Bart Van der Schueren

| | |
|---|---|
| The application was received by the EMA on | 26 July 2017 |
| The procedure started on | 17 August 2017 |
| The Rapporteur's first Assessment Report was circulated to all CHMP members on | 3 November 2017 |
| The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on | 31 October 2017 |
| The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on | 17 November 2017 |
| The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on | 14 December 2017 |
| The applicant submitted the responses to the CHMP consolidated List of Questions on | 23 February 2018 |
| The following GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the assessment of the product: | |
| – A routine GCP inspection at one investigator site in the UK between 22–25 January 2018, one investigator site in the Netherlands between 29 January-1 February 2018 and one sponsor site in the United States between 26 February 2018-2 March 2018. The outcome of the inspection carried out was issued on | 26 April 2018 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on | 06 April 2018 |
| The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on | 12 April 2018 |

| | |
|---|---|
| The CHMP agreed on a 1st list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on | 26 April 2018 |
| The applicant submitted the responses to the CHMP List of Outstanding Issues on | 29 May 2018 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on | 14 June 2018 |
| Ad Hoc Expert group was convened to address questions raised by the CHMP on<br>The CHMP considered the views of the Ad Hoc Expert group as presented in the minutes of this meeting. | 19 June 2018 |
| The CHMP agreed on a 2nd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on | 28 June 2018 |
| The applicant submitted the responses to the CHMP List of Outstanding Issues on | 21 August 2018 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on | 06 September 2018 |
| The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on | 19 September 2018 |
| The CHMP agreed on a 3rd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on | 20 September 2018 |
| The applicant submitted the responses to the CHMP List of Outstanding Issues on | 16 October 2018 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on | 01 November 2018 |
| The CHMP agreed on a 4th list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on | 15 November 2018 |
| The applicant submitted the responses to the CHMP List of Outstanding Issues on | 31 January 2019 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on | 14 February 2019 |
| 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 WAYLIVRA on | 28 February 2019 |

# 2. Scientific discussion

## *2.1. Problem statement*

Waylivra is indicated as an adjunct to diet for the treatment of adult patients with genetically confirmed familial chylomicronemia syndrome (FCS) and at high risk for pancreatitis, in whom response to diet and triglyceride lowering therapy has been inadequate.

### 2.1.1. Epidemiology

Familial chylomicronemia syndrome (FCS) is a rare disorder of lipid metabolism characterized by extremely high serum triglycerides (TG) (> 750 mg/dL, 8.5 mmol/L), which are carried primarily in chylomicrons (dietary lipids) (Brahm and Hegele 2015), and affects an estimated 3000-5000 patients globally. Chylomicrons are large (~ 1 micron in diameter) lipoprotein particles that, if elevated, can result in several clinical manifestations.

Chylomicronemia causes reduced blood flow in the microcirculation and has been identified to affect several organ systems including the central nervous system, the musculoskeletal system, and the gastrointestinal system, resulting in cognitive impairment, muscle and joint pain, and abdominal pain, respectively. The most serious effects are on the pancreatic microcirculation leading to severe abdominal pain and pancreatitis (Valdivielso et al. 2014).

The inflammatory changes caused by excessive chylomicronemia induce acute pancreatitis, which can be fatal or lead to pancreatic damage, resulting in permanent exocrine or endocrine insufficiency (Symersky et al. 2006).

### 2.1.2. Biologic features

The mechanism of this disease includes functional deficiency of lipoprotein lipase (LPL), an essential enzyme in hydrolysis of plasma TGs, a prerequisite for effective chylomicron clearance. Patients with FCS have inherited recessive defects that limit or impair functionality of LPL, resulting in chylomicronemia. These include mutations in the LPL gene encoding for LPL, or mutations in genes that code for other proteins necessary for proper LPL function, including APOC2, LMF1, APOA5 and GPIHBP1 (Brahm and Hegele 2015, Ahmad et al. 2017, Stroes et al. 2017). FCS patients who do not have one of the aforementioned identified genetic mutations continue to be identified through clinical presentation. With further research on such patients, it is likely that new genetic mutations impairing LPL function will be identified.

### 2.1.3. Clinical presentation, diagnosis

Patients with FCS have a number of severe and potentially life-threatening complications associated with the disease. Complications associated with the disease are believed to result from TG levels being above a critical threshold; > 500 mg/dL (5.6 mmol/L) is a well-established threshold for increased pancreatitis risk and > 750 mg/dL is widely thought to represent a threshold above which there is chylomicron accumulation which increases pancreatitis risk. In addition, patients with FCS frequently experience other manifestations that have impact on their daily lives including cognitive, emotional (i.e., psychosocial), and physical impairments that include abdominal pain, steatorrhea, bloating, asthenia, fatigue, anxiety and depression, fear and worry, “brain fog”, lack of concentration, and impairment of memory, all occurring frequently and moderate to very severe in magnitude. All these complications diminish quality of life, impact on employment choices, ability to secure and maintain

employment, and days lost from work due to FCS. Most frequently reported symptoms across these domains include abdominal pain, steatorrhea, bloating, asthenia, and fatigue.

Acute pancreatitis presents the most significant risk in patients with FCS, with potential mortality and other significant complications (Davidson 2017). Approximately 65-80% of patients with FCS will experience at least one episode of acute pancreatitis, with the majority experiencing recurrent episodes (Gaudet et al. 2016). Long-term complications as a result of acute pancreatitis may include chronic pancreatitis, pancreatogenic (Type 3c) diabetes and endocrine and exocrine pancreatic insufficiencies, with their attendant complications and hepatosplenomegaly (Symersky et al. 2006).

### 2.1.4. Management

Patients with FCS are on strict dietary fat restriction. Traditional lipid-lowering medications used to treat hypertriglyceridemia (HTG), such as fibrates, statins and fish oils, niacin (not registered any more in EU) and off label lomitapide are minimally effective in patients with FCS because their effectiveness depends, at least in part, on a functional LPL enzyme, notably deficient in a large proportion of these individuals (Brahm and Hegele 2015, Stroes et al. 2017). The previously approved gene therapy (Glybera) was restricted to those FCS patients with LPL deficiency, suffering from severe or multiple pancreatitis attacks and detectable levels of LPL protein with a genetic confirmed testing. However, this product has been withdrawn from use in the European Union since October 2017 following the marketing authorisation holder's decision not to apply for a renewal for commercial reasons.

***About the product***

Volanesorsen (also known as volanesorsen sodium, ISIS 304801, ISIS-ApoCIII$_{Rx}$) is a 2′-*O*-2-methoxyethyl (2′-MOE) antisense oligonucleotide (ASO) inhibitor of the molecular target apoC-III, a key regulator of TG metabolism.

Antisense technology interrupts the protein production process by degrading the target messenger ribonucleic acid (mRNA) and thus reducing the translation of the specific protein. Antisense inhibitors are designed to be sequence specific with a high binding affinity to their unique target mRNA. Volanesorsen is designed to bind to a specific segment within the 3′-untranslated region of the human apoC-III mRNA and promote a reduction in apoC-III protein.

FCS is primarily a consequence of impaired triglyceride rich lipoprotein (TRL) clearance, manifested by the severe elevation in plasma TG and TG-rich lipoproteins such as very low-density lipoproteins (VLDL) and/or chylomicrons. Levels of the apoC-III protein, which reside on the surface of apoB containing lipoproteins and high density lipoproteins, are elevated in patients with FCS and the majority of apoC-III is associated with TG-rich lipoproteins (TLRs) in these patients. Volanesorsen therapy causes suppression of the ApoC-III protein, which leads to reductions in plasma TG in FCS patients.

The claimed indication for Waylivra was as an adjunct to diet for the treatment of patients with familial chylomicronemia syndrome (FCS).

The indication approved by the CHMP was as an adjunct to diet in adult patients with genetically confirmed familial chylomicronemia syndrome (FCS) and at high risk for pancreatitis, in whom response to diet and triglyceride lowering therapy has been inadequate.

The recommended dose regimen of Waylivra is a starting dose of 285 mg once weekly for 3 months. Following 3 months, dose frequency should be reduced to 285 mg every 2 weeks.

## *Type of Application and aspects on development*

The applicant sought advice on the clinical development of this product from the CHMP, regarding the patient population definition to be included in the pivotal study, and the selection of appropriate endpoints in support of the claimed indication.

## *2.2. Quality aspects*

### *Introduction*

The finished product is presented as solution for injection containing 285 mg of volanesorsen (as volanesorsen sodium) as active substance in 1.5 ml solution.

Other ingredients are: sodium hydroxide and hydrocloric acid.

The product is available as a single-dose, type I glass pre-filled syringe with a siliconised chlorobutyl rubber stopper and staked needle with shield, filled to deliver 1.5 mL of solution, as described in section 6.5 of the SmPC.

## 2.2.1. Active substance

### *General information*

The active substance, volanesorsen sodium, is a single stranded synthetic oligonucleotide with an antisense mechanism of action.

The chemical name of volanesorsen is

DNA, d(P-thio)([2'-O-(2-methoxyethyl)]rA-[2'-O-(2-methoxyethyl)]rG-[2'-O-(2-methoxyethyl)]m5rC-[2'-O-(2-methoxyethyl)]m5rU-[2'-O-(2-methoxyethyl)]m5rU-m5C-T-T-G-Tm5C-m5C-A-G-m5C-[2'-O-(2-methoxyethyl)]m5rU-[2'-O-(2-methoxyethyl)]m5rU-[2'-O-(2-methoxyethyl)]m5rU corresponding to the molecular formula $C_{230}H_{301}N_{63}O_{125}P_{19}S_{19}Na_{19}$.

The sodium salt has a relative molecular mass of 7582.7 Da g/mol and the following structure (see **Figure 1**).

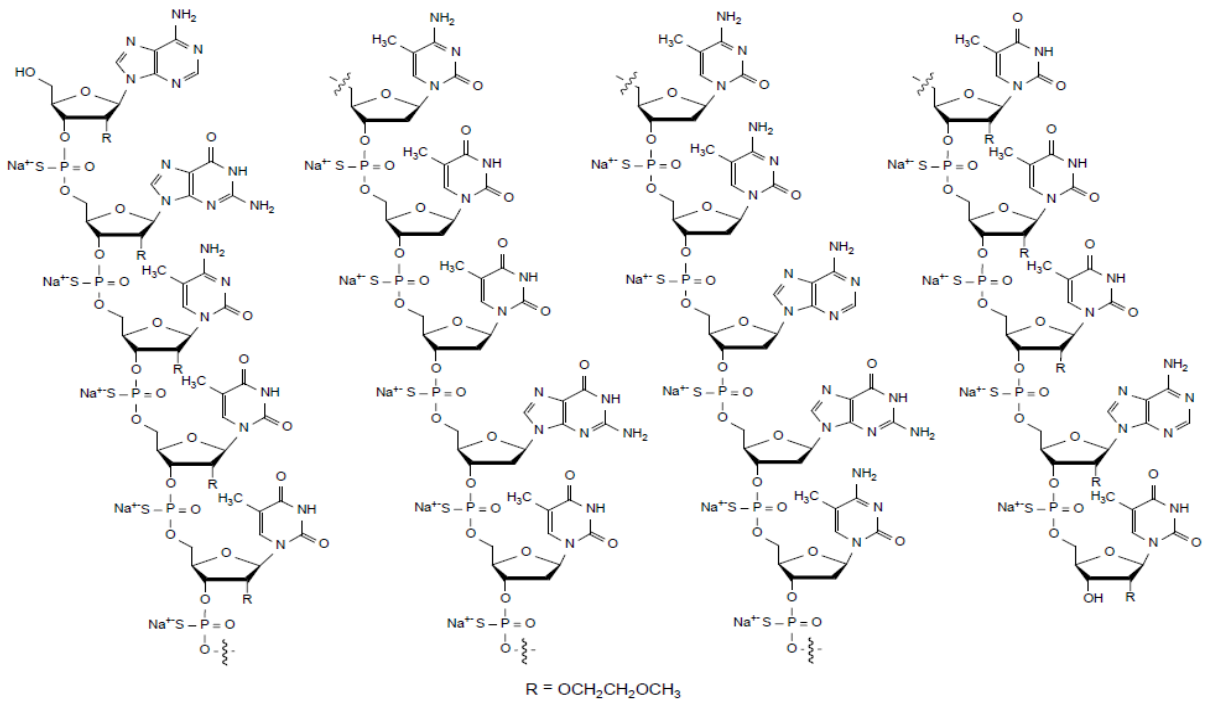



**Figure 1: Structure of volanesorsen**

The sequence is: 5’ AGMeCMeUMeUMeCTTGTMeCMeCAGMeCMeUMeUMeUAMeU-3′ . The underlined residues are 2’ -O-(2 methoxyethyl) nucleosides, all other residues are 2′ deoxynucleosides.

Volanesorsen is the nonadecasodium salt of a 20-base residue (20-mer) phosphorothioate oligonucleotide. Each of the 19 internucleotide linkages is a 3’-O to 5’-O phosphorothioate diester. The five sugar residues on the 3' and 5' ends are 2-O-(2-methoxyethyl)-D-ribose (MOE). The 10 sugar residues in the middle are 2-deoxy-D-ribose. All of the cytosine bases are methylated at the 5-position.

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 phosphorous atom is undefined and hence volanesorsen is a mixture of $2^{19}$ diastereoisomers (524,288), where no individual diastereoisomer contributes to approximately 0.0018% of the total.

Polymorphism has not been observed for volanesorsen. The active substance is a hygroscopic white to yellow solid, amorphous phosphorothioate oligonucleotide isolated in the sodium salt form, freely soluble in water and in aqueous sodium acetate buffer pH 3.

The general information provided for volanesorsen is acceptable.

The chemical structure of volanesorsen was elucidated by a combination of chemical and spectral analysis proton nuclear magnetic resonance ($^{1}$H NMR) spectroscopy, carbon nuclear magnetic resonance ($^{13}$C NMR) spectroscopy, phosphorus nuclear magnetic resonance ($^{31}$P NMR) spectroscopy, high resolution electrospray ionization time-of-flight mass spectrometry (ESI-TOF). Failure sequence analysis data, obtained on crude volanesorsen due to incomplete coupling in each synthesis cycle, using ion-pair high performance liquid chromatography-time-of-flight mass spectrometry (IP-HPLC-TOF-MS) confirmed volanesorsen has the claimed nucleotide sequence. The sequence is also assured via the automated chemical synthesis and associated process controls. The elemental analysis results are consistent with the molecular formula of volanesorsen. The measured masses and the theoretical masses for the fragments are in compliance. The characterisation data provide adequate evidence for

the primary structure of the active substance, with suitable assurance that this can be routinely monitored during commercial synthesis.

The following physicochemical characterisation tests are consistent with the expected properties: ultra violet (UV) spectrophotometry, hygroscopicity, X-ray powder diffraction (XRPD), thermogravimetric analysis (TGA), and pH of solution.

Carbon stereochemistry is defined and controlled by the use of chirally pure starting materials, and is not impacted by the manufacturing process of volanesorsen. The stereoselectivity experiments performed indicate that the ratio of absolute configurations at each internucleotide linkage varies within the sequence, but the ratios are reproducible, which implies that also the diastereomeric composition is reproducible and robust towards variations in synthesis conditions. The sequence of oligonucleotides and the mixture of activators, 4,5-dicyanoimidazole and N-methylimidazole, used during the synthesis of volanesorsen are fixed; therefore, the diastereoisomeric composition of volanesorsen is not expected to vary. The thermal melt temperature ($T_m$) test measures hybridisation temperature and is unique to oligonucleotides. $T_m$, coupled with UV spectroscopy, measures the affinity of an oligonucleotide to its complementary strand. It provides supportive evidence of the correct sequence and can serve as a useful control for the consistent structure of the molecule. $T_m$ results from ten volanesorsen batches are provided and the results are consistent.

***Manufacture, characterisation and process controls***

One manufacturer, Ionis Pharmaceutical Inc, USA, is responsible for the manufacture of the active substance. Volanesorsen is synthesised using well defined starting materials with acceptable specifications. The nucleoside phosphoramidites are the defined as starting materials and are adequately controlled.

There are no alternate processes or reprocessing steps in the commercial manufacture of volanesorsen. A typical batch of volanesorsen is defined as the quantity produced in a single freeze drying run. There are no defined in-process controls during the synthesis of the drug substance. In view of tight control of the intermediates of the process and the intrinsic in-process control of the equipment used for the synthesis and purification steps, this is accepted.
The specifications and control methods for intermediate products, starting materials and reagents are satisfactory. During the review, the limits for purity of the intermediates of the synthesis have been tightened, in line with batch data, and are now considered acceptable.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Volanesorsen may contain a number of product-related and process impurities. Product-related impurities, i.e., impurities that are oligonucleotides, can be divided into three main categories, starting material- and reagent-derived impurities, product-related impurities formed due to incomplete or side reactions, and product-related degradation products. Starting materials that are not reactive are separated from the support-bound product by solvent wash steps and consequently cannot be carried forward into the active substance. In contrast, reactive starting material impurities compete for incorporation during the coupling reaction and may lead to active substance oligonucleotide impurities that are not removed by subsequent processing steps. The latter are considered critical impurities and are, therefore, tightly controlled by starting material specifications. Product-related impurities can also be due to incomplete or side reactions that may occur during the manufacturing process. Potential product-related impurities, observed long-term and in stress studies, have adequately been discussed. Potential genotoxic impurities have been classified according ICH M7 and unknown potential mutagenic impurities evaluated adequately. In line with ICH M7, it was concluded that control of impurities during synthesis is not required.

The pharmaceutical development included elements of Quality by Design (QbD), in line with ICH Q8, Q9, Q10, and Q11 and it draws from the experience and prior knowledge of the manufacturer gained from a marketed oligonucleotide product. The CQA identified are identity, assay, purity, impurities, starting materials and reagent derived impurities, endotoxin and bioburden, elemental impurities. During the manufacturing process development a FMEA risk assessment was performed to identify the critical process parameters (CPPs) that impact one or more CQAs of the active substance. Design of experiments (detritylation volumes and delivery times for each synthesis cycle) and several laboratory studies (reduced phosphoramidite equivalents, sulfurisation volume and – time, capping volume and – time, phosphorus deprotection volume and – time, ammonium hydroxide quantity, ammonolysis temperature and -time, purification elution solutions and temperatures, detritylation kinetics (pH, time, and temperature) have been conducted on the CPPs. Based on these studies, proven acceptable ranges (PARs) have been defined for the investigated steps of the manufacturing process of the active substance. . The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs. The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. The basic manufacturing process and design and operating principles remained the same with the exception of scale and optimisation of some parameters. The changes made to the process throughout the course of development of volanesorsen resulted in reduction in the levels of some impurities and an improvement in overall purity between the first clinical lot and the process qualification (PQ) lots. Changes introduced have been presented in sufficient detail and have been justified.

Process qualification has been performed on three full scale production batches and the results indicate that the process is consistent and adequately controlled. The critical process parameters were consistent and amply within the PARs.

The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The active substance is packaged in a multi-component container closure system. The primary container closure system complies with the EC directive 2002/72/EC and EC 10/2011 as amended.

***Specification***

The active substance specification includes tests for appearance (visual), identification (sequencing by IP-HPLC-TOF-MS and $T_m$, most abundant mass by ion-pair high performance liquid chromatography-ultra violet mass spectrometry (IP-HPLC-UV-MS) and sodium counter-ion inductively coupled plasma-optical emission spectrometry (ICP-OES)), assay, purity, specified impurities, unspecified impurities, total degradation products, total impurities (all by IP-HPLC-UV-MS), residual solvents (gas chromatography (GC)), elemental impurities (inductively coupled plasma mass spectrometry (ICP-MS)), water (Karl-Fisher), bacterial endotoxins (Ph.Eur.) and microbial examination of nonsterile products (Ph.Eur.).

Justification for the applied tests and limits is presented based on the critical quality attributes (CQAs). Additional tests are included in the specification to ensure process consistency (appearance, water, residual solvents and $T_m$). Several other tests performed during development (i.e. sodium acetate, deamination, and small molecule impurities) are not included in the specification as the results of the tests confirm that none of them are CQAs of the active substance. The convention of defining the impurities in the specification as groups rather than individual components has been satisfactorily justified and it is acknowledged that oligonucleotides are excluded from the scope of the ICH guidance on impurities in drug substances (Q3A (R2)).

Volanesorsen active substance stability has been demonstrated at water contents of up to approximately 120%. Furthermore, studies conducted with oligonucleotides of similar chemistry to volanesorsen indicate that with a specified high water content, water activity values are below that required to support microbial growth. Additionally, the product compounding weights are adjusted to account for active substance water content measured at the time of compounding; hence, the latter does not impact finished product concentration. Based on these justifications, the specification limit for water content is considered acceptable.

A statistical approach has been adopted to take process variability into account in setting specification limits and the proposed impurity limits are supported by toxicology data. That approach is acceptable. The applicant is recommended to reassess the active substance specifications following the synthesis of additional batches.

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 deamindation impurity testing has been presented.

Batch analysis data (10 commercial scale batches, including the three qualification batches, manufactured by the proposed manufacturer) of the active substance are provided. The results are within the specifications and consistent from batch to batch.

***Stability***

Stability data from 3 full scale batches of active substance, from the proposed manufacturer, stored in the intended commercial package under long term conditions (- 20 ºC) and for up to 6 months under accelerated conditions (5ºC) according to the ICH guidelines were provided. Additional supporting data on large scale batches, from the proposed manufacturer, stored in the intended commercial package for up to 24 months under long term conditions (- 20 ºC), under accelerated conditions (5ºC) and for up to 3 months at 30 ºC, have been provided. The following parameters were tested: appearance, assay, purity, impurities, water content, and microbiological quality. The analytical methods used were the same as for release, a part from the deamindation method, which has been described, and were stability indicating. All tested parameters were within the specifications. At -20 °C, a slight decrease in assay is observed for some batches, yet as this trend is not observed at 5°C and 30°C, this is considered due to analytical variation.

Photostability testing following the ICH guideline Q1B was performed on three batches. Photostability studies indicate that while volanesorsen active substance is subject to a slow rate of photodegradation. Consequently, special precautions to protect volanesorsen from the effects of light during manufacturing, handling, and finished product formulation activities are not required. This is accepted. Results on stress conditions (thermal, acidic, basic, oxidative and photo) were also provide on two batches. Degradation mainly results in increase of full length phosphate diester, total abasic, early eluting impurities and late eluting impurities, and total degradation products.

The stability results justify the proposed retest period of 2 years stored at -20 °C in the proposed container.

## 2.2.2. Finished medicinal product

***Description of the product and Pharmaceutical development***

Volanesorsen sodium is a sterile solution for injection, containing no preservatives. The finished product is packaged as a single-dose with a 1.5 mL deliverable volume in a 2.25 mL glass prefilled syringe. The finished product composition is provided in **Table 1** below.

| Components | Quality Standards | Component Function | Nominal Quantity per 1.5 mL (mg) | Concentration (mg/mL) |
|---|---|---|---|---|
| Volanesorsen | Drug Substance Specification | Active Ingredient | 285 (as free acid)<br>300 (as sodium salt) | 190 (as free acid)<br>200 (as sodium salt) |
| Sodium hydroxide<br>(as 1 N solution in WFI[a]) | USP-NF, Ph.Eur., Ph.J. | pH adjustment | As needed | As needed |
| Hydrochloric acid<br>(as 1 N solution in WFI[a]) | USP-NF, Ph.Eur., Ph.J. | pH adjustment | As needed | As needed |
| Water for Injection | USP, Ph.Eur., Ph.J. | Vehicle | Q.S.[b] | Q.S.[b] |

a Water for Injection
b Quantity sufficient
Ph.Eur. European Pharmacopoeia
Ph.J. Japanese Pharmacopoeia
USP United States Pharmacopeia
USP-NF USP-National formulary

**Table 1**: Composition of volanesorsen finished product

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.

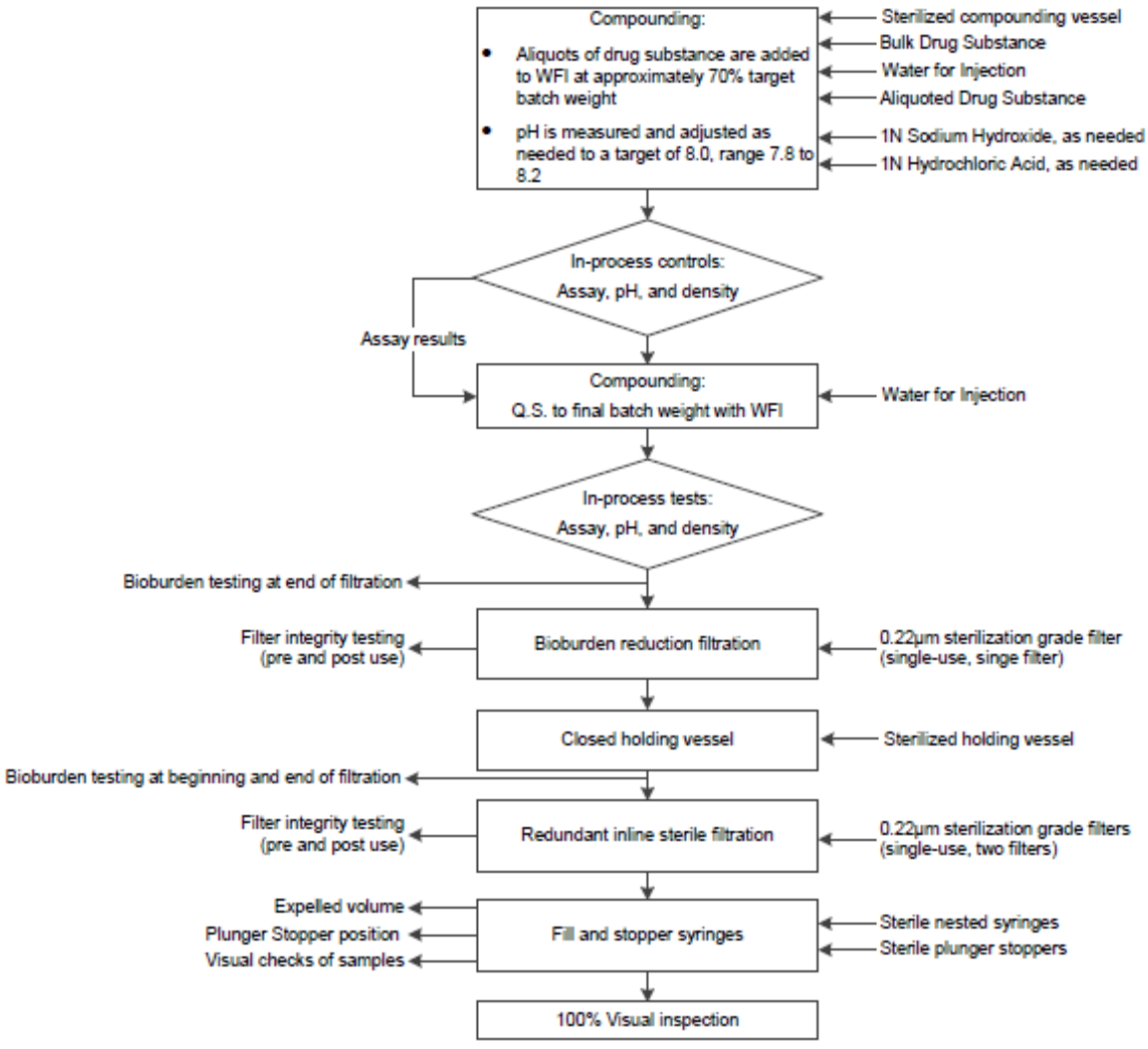
The pharmaceutical and formulation development section provided is generally satisfactory. The finished product formulation is simple and straightforward. The pH, volume and osmolality are acceptable for subcutaneous administration. The physico-chemical suitability of the chosen pH of the formulation is confirmed by the stability results. The finished product used for all phases of clinical development has the same composition as proposed, both in vial and the proposed prefilled syringe containers. The manufacturing process development has been described in detail. The proposed method of sterilisation has adequately been justified. Although tested at pH 7.5 and 8.5 and not at the target pH of 8.0, it is clear that a robust steam sterilisation process is not suitable for this finished product. The sterilisation process of the glass syringes with ethylene oxide according ISO 11135 is allowed as the drug product concerns an aqueous solution. Satisfactory additional details on the sterilisation process have been provided during the review. Sterilisation of the plunger stoppers by gamma irradiation is acceptable. Satisfactory details on the sterilisation process have been provided.

The primary packaging is a single-dose, Type I glass pre-filled syringe with a siliconised chlorobutyl rubber stopper and staked needle with shield, filled to deliver 1.5 ml of solution. The material complies with Ph.Eur. and EC requirements. The risk analysis on potential glass delamination confirms that the risk for glass delamination to occur over the shelf-life of the medicinal product is low. The results so far of the controlled extraction study, the simulated use extraction study, and elemental impurities testing of the registration stability batches indicate acceptable leachable profile for the finished product formulation in the container closure system. The results of the leachable study are still awaited for and

the applicant is recommended to provide them post approval with a variation and update the specification of the finished product as needed. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

***Manufacture of the product and process controls***

The manufacturing process consists of three main steps: compounding (compounding, dissolution and pH adjustment), filtration (bioburden reduction) and sterile filtration/filling. Conditions like temperature, duration of mixing times, volume of filter flush, bubble points are included in the description. The filters used have a 0.22 µm pore size. The syringes and stoppers are delivered ready to use.




**Scheme 1: Finished Product Manufacturing Process**

The critical steps are compounding (prior to and after final batch weight adjustment), bioburden reduction and filling. The in-process controls are adequate for this type of manufacturing process. The process is considered to be a non-standard manufacturing process as it involves an aseptic filling process. During the review, a major objection was raised on the absence of full-scale process validation data. In response, results of full-scale process validation of three batches have been

provided which addressed the major objection. The process qualification batches met all requirements outlined in the process qualification protocol and the results are consistent. Holding and processing times have been taken into account. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The validated batch size range targeted for commercial finished product manufacturing has been specified. Representative batch formulas for the targeted validated batch size range are provided.

***Product specification***

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (Ph.Eur.), degree of coloration (Ph.Eur.), clarity and degree of opalescence (Ph.Eur.), identification, assay, purity, degradation products (all by IP-HPLC-UV-MS), extractable volume (Ph.Eur.), uniformity of dosage units (Ph.Eur.), pH (Ph.Eur.), osmolality (USP), particulate matter (Ph.Eur.), bacterial endotoxins (Ph.Eur.), sterility (Ph.Eur.), container closure integrity (USP) and syringe performance (ISO 11040-8).

The finished product purity and individual degradation products limits are in line with the active substance specification and considered acceptable. The wider limit for total degradation products (NMT 5.5% for active substance and NMT 7.0% for finished product) is acceptable however it is noted that only limited batch data are available and that when further batch data are available it may be possible to tighten the limits. Therefore, the applicant is recommended to reassess the acceptance criteria for specified degradation products and total degradation products in finished product specification following the synthesis of additional batches. The data indicate volanesorsen finished product will not deaminate when stored at the proposed recommended storage condition and therefore the proposed specification does not include a test for deamination.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. The reference standards used for the finished product are the same as those used for the active substance.

Batch analysis results are provided for three registration batches (scale: 8 L, date: 2015) manufactured at the proposed commercial site of manufacture according the proposed process and packaged in the commercial packaging, confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

***Stability of the product***

Stability data from three small scale batches (8 L) of finished product stored under long term conditions (stored at 5±3°C with a 6 week transition to 30 °C / 75% RH) and under accelerated conditions (25 ºC / 60% RH) were provided. The batches of Waylivra are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing. Stability studies will also be initiated with the three validation batches (12 L, 30 L, and 30 L).

Samples were tested for appearance, degree of coloration, clarity and degree of opalescence, pH, assay, purity and impurity profile. Break-loose force, glide force, needle shield removal force, needle hold force, bacterial endotoxins, sterility and particulate matter are tested every year in the long term study. Container closure integrity and leachables are tested at the end of the accelerated and long-term study. The analytical procedures used are the same as used for release and are stability indicating. All results complied and the only trend observed is a minor increase in break loose force (syringe performance) that was well within the NMT 30 N specification limit.

In addition, three batches were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Assay, purity, degradation products, appearance, degree of coloration, clarity and degree of opalescence, particulate matter and pH were tested. For the unprotected product a decrease in full-length n (purity) and increases of degradation products full-length (P=O), total abasic, late eluting impurities, and T=total degradation products was observed, which was not observed for the dark control and the product stored in the carton box. In view of that, the product should be stored in the carton box to protect from light.

Three cycles of freeze-thaw storage were completed on two batches of the product; however, since the container closure integrity has not been verified in the frozen state over time or when exposed to freeze thawing, thus the product is not to be frozen. Intrinsic stability and degradation pathways and products of the finished product were evaluated by exposing samples to forced thermal degradation (80 °C, 2 weeks) and forced photodegradation (ICH Q1B, option 2; 6 × 106 lux hours CWL and 1073 watt hours/m2 UVA). Stressed and control samples were analyzed for assay, purity and degradation products by IP-HPLC-UV-MS and for deamination by IPHPLC-TOF-MS.

In line with ICH Q1E, extrapolation of 24 months long-term data to 30 months is allowed. The proposed shelf-life of 30 months when stored refrigerated at 2° to 8°C, in the original carton to protect from light, including 6 weeks transition not above 30°C, as stated in the SmPC (section 6.3) can be accepted.

***Adventitious agents***

In accordance with Directives 2001/83/EC and 2003/63/EC, starting materials, packaging materials with product contact, process materials, media fill components and cleaning detergents are compliant with chapter 5.2.8 "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" of Ph.Eur. and "Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products" (EMA/410/01/current version).

## 2.2.3. Discussion on chemical and pharmaceutical 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. During the review of the application, three recommendations have been given to the applicant in reference to tighten the criteria of the active substance and finished product once more experience with commercial manufactured is gained and to evaluate the results of the extractable and leachable studies and to implement any necessary control.

## 2.2.4. 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. Data have been presented to give reassurance on viral/TSE safety.

### 2.2.5. 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 some points for investigation.

## *2.3. Non-clinical aspects*

### 2.3.1. Introduction

A non-clinical program with volanesorsen was conducted using *in vitro* and *in vivo* studies to support chronic subcutaneous (SC) administration for the treatment of patients with FCS. The non-clinical testing strategy for volanesorsen followed a development pathway in line with existing regulatory guidance (ICH M3R2). The objectives of the non-clinical program were to evaluate the pharmacodynamic activity, pharmacokinetics (PK), and toxicology of volanesorsen.

### 2.3.2. Pharmacology

Volanesorsen was evaluated *in vitro* in human and monkey primary hepatocytes, human HepG2 hepatoma cells, APOC3 transgenic mouse primary hepatocytes and mice containing the human APOC3 transgene. The pharmacology of inhibiting apoC-III with species-specific ASOs was also examined in mice, rats, hamsters and monkeys.

#### *Primary pharmacodynamic studies*

**Identification of ISIS 304801 (Volanesorsen) and Species-Specific ApoC-III ASOs**

The APOC3 gene, which is conserved in eukaryotes, is ~500 base pairs in length, containing 3 Introns and 4 Exons. The human, rhesus monkey, and cynomolgus monkey genes are highly conserved with ~93% homology. To identify potential human candidates, ~350 second-generation 2′-O-methoxyethyl chimeric ASOs were screened against ~200 sites.

ISIS 304801 (volanesorsen) was identified as the optimal 2′-MOE antisense inhibitor of human apoC-III on the basis of its consistent and potent activity and maximal efficacy.

The binding site for ISIS 304801 lies within the 3′ untranslated region of the apoC-III mRNA transcript at position 489-508 bp, and based on in silico analysis, it is not predicted to target any other human gene. A bioinformatic analysis of the human transcripts in the NCBI Reference Sequence Database using the bowtie algorithm (Langmead et al. 2009) predicts ISIS 304801 is not homologous to any regions of the human transcriptome with either a single nucleotide mismatch, or with 16 or more consecutive nucleotide matches (data not shown).

Additional off-target analysis identified all 2-base mismatches and the potential effects of volanesorsen on these transcripts. There were 3 expressed putative potential off-targets with 2 mismatches identified: STIM2, RAC1 and RAC1P2. The sequence of the pseudogene RAC1P2 was determined to be 98% homologous to RAC1, and as real-time quantitative polymerase chain reaction (RT-qPCR) assays that distinguish RAC1 and RAC1P2 are not commercially available, one assay was used to measure both transcripts. At the highest concentration tested of 20 µM there was approximately 24% inhibition of RAC1, whereas STIM2, inhibition of 53% was reached at a concentration of 20 µM.

The half maximal inhibitory concentration ($IC_{50}$) of ISIS 304801 against apoC-III mRNA is > 200-fold lower than the $IC_{50}$ against the 3 expressed off-targets. At a concentration that produced near

maximal reduction of apoC-III mRNA (20 µM), no significant change was seen in the expressed putative off-targets.

While monkeys share the same sequence at this site, due to a lack of sequence homology in other species, different 2′-MOE ASOs (ISIS 440670, ISIS 440726, ISIS 353982, ISIS 353977, or ISIS 352952) were employed in studies targeting mouse, rat or hamster apoC-III, respectively (**Table 2**).

**Table 2.** Human, Mouse, Rat and Hamster ApoC-III ASO Sequences

| ISIS No | Length | Sequence | Chemistry | Species | Binding Site |
|---|---|---|---|---|---|
| 304801 | 20 | AGCTTCTTGTCCAGCTTTAT | 5-10-5 MOE gapmer | Human | 508 |
| 440670 | 20 | CAGCTTTATTAGGGACAGCA | 5-10-5 MOE gapmer | Mouse | 488 |
| 440726 | 20 | CCAGCTTTATTAGGGACAGC | 5-10-5 MOE gapmer | Mouse | 489 |
| 353982 | 20 | GAGAATATACTTTCCCCTTA | 5-10-5 MOE gapmer | Rat | 349 |
| 353977 | 20 | GAGCAACCTTCGGAGGCAGC | 5-10-5 MOE gapmer | Rat | 329 |
| 352952 | 20 | AGCCACCTCAGACTTTTGCA | 5-10-5 MOE gapmer | Hamster | 135 |

### *In Vitro* Pharmacodynamics

The *in vitro* pharmacological activity of volanesorsen was characterized in a human hepatoma cell line (HepG2) cells (study EX/3253:141), as well as in primary human hepatocytes (study EX/3270:139), cynomolgus monkeys (study EX/3270:146) and APOC3 transgenic (Tg) mice (study EX/3270:067). In these experiments, ISIS 304801 selectively reduced apoC-III mRNA in a concentration-dependent manner (data not shown). In human primary hepatocytes an IC50 of 2 nM was calculated.

### *In Vivo* Pharmacodynamics

The pharmacologic effects of 2'-MOE ASOs designed to inhibit apoC-III were examined in mice, rats, hamsters and non-human primate models. Because of the divergence between the mouse, hamster and human apoC-III sequences, species-specific ASOs were employed in their respective *in vivo* evaluations.

### *Rodent Models*

Inhibition of hepatic apoC-III expression by 2′-MOE ASOs consistently and significantly reduced hepatic mRNA and protein, with concomitant reductions in serum apoC-III, VLDL-C and TG levels in several models and species, including human APOC3 transgenic mice, mouse, rat and hamster (**Table 3**).

**Table 3**. Reduction of ApoC-III mRNA, TG, TPC and HDL-C in Various Rodent Models

| Model/Diet | EXPT# | SC Dose (mg/kg/wk) | Treatment (wks) | % Reduction ApoC3 mRNA | % Reduction TG | % Reduction TPC | HDL-C |
|---|---|---|---|---|---|---|---|
| *APOC3* transgenic mice | 3653:38 | 50 | 2 | -78% | -59% | ND | ↑56% |
| C57BL/6 (Chow) | 3309:271 | 12.5 | 6 | -66% | -30% | ND | ND |
| C57BL/6 (Western) | 3908:37 | 12.5 | 6 | -90% | -37% | -36% | ND |
| C57BL/6 (Western) | 3310:46 | 12.5 | 6 | -95% | -20% | ND | ND |

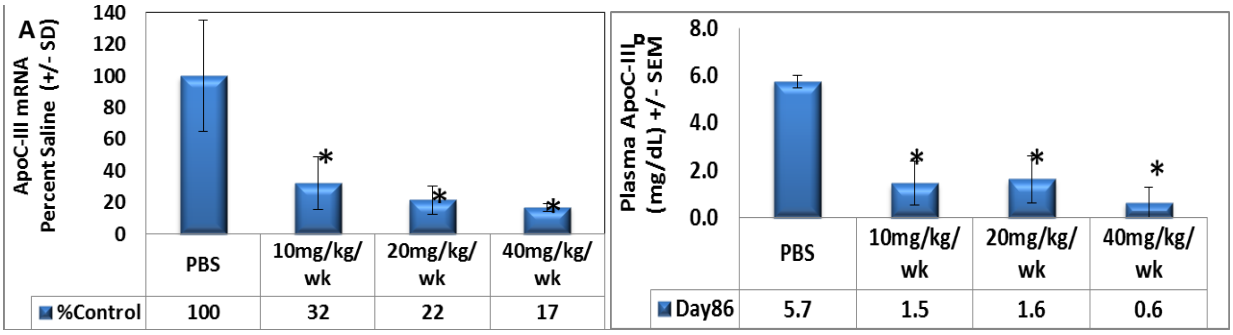
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ob/Ob* (Chow) | 3310:76 | 12.5 | 6 | -90% | -48% | ND | ND |
| *CETP* Tg *Ldlr-/-* (Western) | 3620:108 | 12.5 | 4 | -87% | -80% | -71% | ND |
| *CETP* Tg *Ldlr-/-* (HF/C) | 4312:19 | 12.5 | 18 | ND | -85% | -72% | ND |
| *CETP* Tg *Ldlr-/-* (HF/C) | 4312:21 | 12.5 | 6 | ND | -77% | -30% | ↑2.7 fold |
| Sprague Dawley Rat (Fructose) | 3310:08 | 25 | 6 | -84% | -86% | ND | ND |
| Hamster (Chow) | 2653:12 | 50 | 3 | -92% | -19% | ND | ND |

***Monkey model***

Because the binding site for volanesorsen is 100% homologous between rhesus monkey and human apoC-III mRNAs and lipid metabolism in nonhuman primates resembles that observed in humans, the effects of volanesorsen in rhesus monkeys that were made hypertriglyceridemic via administration of a high-fructose supplement were evaluated.

Volanesorsen significantly reduced rhesus monkey hepatic mRNA and plasma apoC-III levels in a concentration dependent manner, (**Figure 2**).

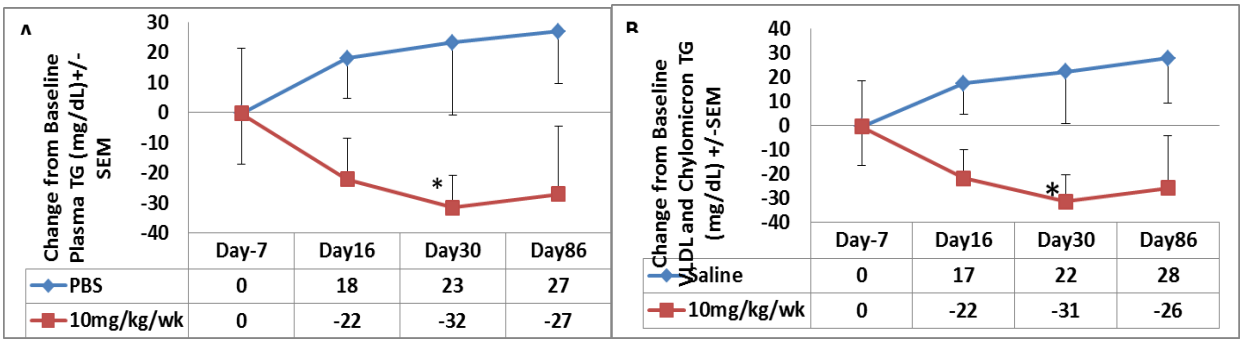
**Figure 2.** Reduction in Hepatic ApoC-III mRNA and Protein After 12-Week Administration of ISIS 304801 in HTG Rhesus Monkeys




*Monkeys (n = 5 per group) were treated twice weekly with a total dose of 10, 20, or 40 mg/kg/wk ISIS 304801 or PBS for 12 weeks. At study termination, hepatic apoC-III mRNA expression (A) was measured by qPCR and plasma apoC-III (B) was quantified on a clinical analyzer using a commercially available turbidometric assay (Kamiya Biomedical Co., Seattle, WA). Data are plotted as mean (± SD) for the qPCR and the mean (± SEM) for the plasma samples. *Significant difference from PBS cohort using one way ANOVA post hoc Tukey's multicomparison test ($p < 0.05$)*

Since the apoC-III reductions observed at the 10 mg/kg/wk were similar to results at the higher dose levels, further analyses were focused only on the 10 mg/kg/wk cohort. Reductions in TG, VLDL and chylomicron TG levels were also observed (**Figure 3**), whilst HDL-C levels increased with volanesorsen treatment (**Figure 4**).

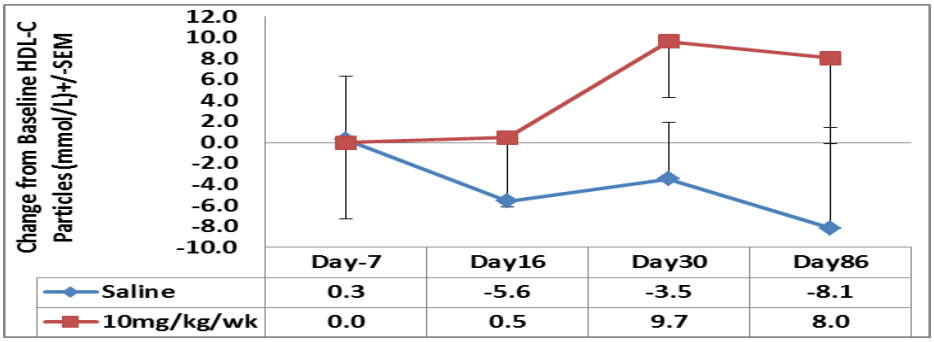
**Figure 3.** Plasma TG and VLDL and Chylomicron TG Reduction in the 10 mg/kg/wk ISIS 304801 Cohort vs. PBS Cohort in HTG Rhesus Monkeys




*Monkeys (n = 5 per group) were treated for a total weekly dose of 10 mg/kg/wk ISIS 304801 or PBS over 12 weeks and plasma total TG (A), and VLDL and chylomicron TG levels (B) levels were quantified using NMR analysis (Liposcience, Raleigh, NC). Data are plotted as mean (± SEM). *Significant difference from PBS cohort using one way ANOVA post hoc Tukey's multicomparison test (p < 0.05)*

After 10 weeks of treatment, postprandial TG clearance was increased by 38% and was similar to that observed in the C57BL/6 fat clearance study described above (EX/3309:271). Finally, volanesorsen did not significantly increase liver triglyceride levels in these animals (data not shown).

**Figure 4.** Change from Baseline in Plasma HDL-C Particles in the 10 mg/kg/wk ISIS 304801 Cohort vs. PBS Cohort in HTG Rhesus Monkeys




## *Secondary pharmacodynamic studies*

No secondary pharmacology studies were submitted.

## *Safety pharmacology programme*

### *Cardiovascular system*

#### *In vitro* hERG assay

Study ISIS 304801-IS01: There was no significant inhibition of the hERG current by ISIS 304801 at the concentrations tested (89.5 and 251 µM) and therefore an IC50 value could not be determined.

#### *In vivo* cardiovascular study

Study ISIS 304801-AS06: Eight naïve male cynomolgus monkeys were treated with ISIS 304801 via 1-hour intravenous (IV) infusion (12 mg/kg) or a SC administration (40 mg/kg). There were no test article-related changes observed in qualitative (Lead-II configuration) ECG assessment, quantitative

ECG parameters (QRS duration, PR, RR, QT, QTcB, and QTcF intervals), arterial BP (SBP, DBP and MAP), HR, BT or any clinical observations.

### *Central Nervous system*

Study ISIS 301804-AS03, Modified Irwin Study in Mice: Mice were administered vehicle control (phosphate buffered saline) or ISIS 304801 by SC injection at dose levels of 0, 50, 100, or 250 mg/kg. Detailed subjective observations of the mice were performed before dosing and at 30, 90, 150 and 300 min after administration of ISIS 304801 and again on Day 2.

No mortality or signs of clinical toxicity were observed during the study. No marked or statistically significant changes relative to the vehicle-treated control group were observed in locomotor activity or body temperature parameters.

### *Respiratory system*

Study ISIS 301804-AS04: mice were administered vehicle control (phosphate buffered saline) or ISIS 304801 by a single SC injection at dose levels of 0, 50, 100, and 250 mg/kg. Baclofen was used as a positive control and confirmed the validity of the assay.

A single animal receiving 250 mg/kg ISIS 304801 was found dead during the study. Upon gross examination, the lungs appeared congested and patchy. A decrease (35% relative to control) in respiratory rate was observed at the 250 mg/kg dose group by 180 min post-dose that fully reversed by 210 min. No changes in pulmonary function parameters were observed at doses ≤ 100 mg/kg.

## *Pharmacodynamic drug interactions*

No pharmacodynamic drug interaction studies were submitted.

## 2.3.3. Pharmacokinetics

The absorption, distribution, metabolism, and/or excretion (ADME) of volanesorsen were assessed in the mouse, rat, rabbit, and monkey. *In vitro* studies characterized the plasma protein binding across species, and evaluated the potential for interactions of volanesorsen with human cytochrome P450 (CYP) enzymes and transporters.

## *Absorption*

### *Mice*

Volanesorsen PK following SC administration in male and female CD-1mice was studied in a 6-week non-GLP pharmacokinetic study (study 304801-APK01) Results from this study are summarised in **Table 4.**

**Table 4.** Summary of Selected Volanesorsen Plasma Pharmacokinetic Parameters in Mice (study 304801-APK01)

| Dose Level (mg/kg) | Profile Day[a] | # of Doses | $C_{max}$ (µg/mL) | $AUC_{0-48h}$ (µg*h/mL) | $AUC_{0-168h}$ (µg*h/mL) | $MRT_{0-48h}$[b] (h) | $CL_{0-48h}/F$[c] (mL/h/kg) | $T_{max}$ (h) |
|---|---|---|---|---|---|---|---|---|
| 3 | 1 | 1 | 2.18 | 3.34 | NC | 3.27 | 897 | 0.5 |
| | 42 | 9 | 2.13 | 4.81 | 5.52 | 4.03 | 623 | 0.5 |

| Dose Level (mg/kg) | Profile Day[a] | # of Doses | $C_{max}$ (µg/mL) | $AUC_{0-48h}$ (µg*h/mL) | $AUC_{0-168h}$ (µg*h/mL) | $MRT_{0-48h}$[b] (h) | $CL_{0-48h}/F$[c] (mL/h/kg) | $T_{max}$ (h) |
|---|---|---|---|---|---|---|---|---|
| 30 | 1 | 1 | 34.6 | 66.8 | 68.9 | 2.79 | 449 | 0.5 |
| | 42 | 9 | 39.4 | 112 | 135 | 4.59 | 268 | 1 |
| 100 | 1 | 1 | 132 | 287 | 297 | 3.23 | 348 | 0.5 |
| | 42 | 9 | 142 | 473 | 533 | 4.48 | 211 | 1 |

[a] Day of study when profile samples were collected. Animals were dosed by SC administration on Days 1, 3, 5, 7 in mice and then once weekly thereafter

[b] MRT determined up to 48 hours post-dose only

[c] Post hoc analysis; $CL_{0-48h}/F$ = [Dose (mg/kg) * 1000]/ $AUC_{0-48h}$ (µg*h/mL); data not included in the report

Note: Plasma PK parameters were calculated based on pooled mean profiles obtained from sparse sampling in mice

NC = Not calculated

Volanesorsen PK in mice was further studied following once weekly SC administration for 13 weeks in as part of the 2-year carcinogenicity study (study 304801-AS15). Following SC administration, volanesorsen was absorbed rapidly into the systemic circulation with $T_{max}$ observed at 0.5 to 1 hours. After achieving $C_{max}$, the plasma concentrations then rapidly declined over the next 24 to 48 hours following SC administration. No large gender-difference in plasma TK was observed over the dose range studied at 6 to 40 mg/kg.

Plasma peak ($C_{max}$) and total ($AUC_{0-24h}$, $AUC_{0-48h}$, and, $AUC_{0-168h}$) exposures were dose-dependent and increased in a greater than dose-proportional manner following 13 weeks of SC administrations of volanesorsen. $MRT_{0-48h}$ was about 5 hours.

***Rats***

The PK of volanesorsen was studied in male Sprague-Dawley rats following a single SC dose of 5 mg/kg [$^3$H]volanesorsen (study 304801-APK02). Following a single SC injection of 5 mg/kg formulated [$^3$H]volanesorsen to rats, concentrations of the total radioactivity in blood and plasma decreased in a bi-exponential fashion with time. Blood and plasma clearance was initially rapid followed by a slow terminal elimination phase to the end of the study period. Radioactivity was mainly associated with the plasma and not the blood cell components, with mean whole blood to plasma ratios of approximately 0.53–0.83 from 0 to 48 hours after the 5 mg/kg dose.

Maximal blood and plasma levels were observed at 1 hour post-dose. The $C_{max}$ of radioactivity in whole blood was 5.1 µg equivalent/mL and concentrations declined thereafter such that they were below the limit of detection (0.024–0.031 µg/mL) at 672 hours and beyond. The $C_{max}$ in plasma was 6.96 µg equivalents/mL. Concentrations declined thereafter such that they were below the limit of detection (0.014–0.021 µg/mL) at 336 hours and beyond. The apparent $t_{1/2}$ of total radioactivity in plasma and blood was approximately 30.2 and 119 hours, respectively, which are underestimates due to several below quantification values (BLQ) in the elimination phase.

Volanesorsen PK was further studied following once weekly SC administration for 13 weeks in male and female rats as part of the 2-year carcinogenicity study (study 304801-AS16, **Table 5**.

**Table 5.** Summary of Volanesorsen Plasma Pharmacokinetic Parameters in Rats (Study 304801-AS16)

| Dose Level (mg/kg) | Profile Day[a] | # of Doses | $T_{max}$ (h) | $C_{max}$ (µg/mL) | $AUC_{0-24h}$ (µg*h/mL) | $AUC_{0-48h}$ (µg*h/mL) | $AUC_{0-168h}$ (µg*h/mL) | $MRT_{0-48h}$ (h) | $CL_{ss}/F$[b] (mL/h/kg) |
|---|---|---|---|---|---|---|---|---|---|
| 0.2 | 92 | 14 | 1 | 0.0362 | 0.234 | 0.295 | 0.426 | 12.2 | 469 |
| 1 | 92 | 14 | 0.5 | 0.771 | 2.97 | 3.39 | 4.48 | 8.16 | 223 |
| 5 | 92 | 14 | 1 | 12.1 | 43.1 | 48.0 | 71.2 | 7.03 | 70.2 |

A Day of study when profile samples were collected. Animals were dosed by SC administration on Days 1 and 8 and once weekly thereafter through Day 92
B $CL_{ss}/F$ represents $CL_{0\text{-}168h}/F$ calculated as [Dose (mg/kg) * 1000] / $AUC_{0\text{-}168h}$ (µg*h/mL)

Note: Plasma PK parameters were calculated by non-compartmental methods using the sparse sampling function

***Monkey***

Volanesorsen plasma PK following IV infusion and SC administration in monkeys was studied in a 13-week toxicology study (study 304801-AS02). Following IV administration the plasma PK profiles exhibited apparent multi-phasic disposition, with a rapid distribution phase (mean residence time [$MRT_{last}$] values of ~2 hours). Peak plasma concentrations occurred at the first time point taken following the end of the 1-hour IV infusion. Exposure was dose-dependent and dose-proportional for the 3 dose levels tested (4, 8 and 12 mg/kg).

Following SC administration, plasma concentrations peaked between 1 to 4 hours, and then decreased in an apparent multi-exponential fashion with time. A longer $MRT_{last}$ was observed (~6 to 9 hours) after SC administration compared to IV administration.

Dose-dependency of peak (Cmax) and total (AUC) plasma exposure measures in monkeys to volanesorsen after IV dosing on days 1, 3 and 5 followed by weekly SC dosing was demonstrated across the entire dose range tested and on all dose days examined. Mean $AUC_{0\text{-}48h}$ values after SC and IV dosing appeared similar, indicating near complete absorption with SC administration. Based on plasma exposure (mean $AUC_{0\text{-}48h}$), the estimated absolute bioavailability after 4, 8, and 12 mg/kg SC dosing ranged from 66–110%. The estimated mean apparent $t_{1/2}$ values (range: 13–36 days) appear generally comparable to estimated mean tissue half-life values (range: 13–19 days), consistent with expected equilibrium between plasma post-distribution concentrations and tissue concentrations.

Plasma trough concentrations showed a dose dependent trough (pre-dose) levels were observed after administration of the loading doses (Day 7), for all interim samples, and at the end of dosing (Day 91). In addition, an accumulation of plasma trough concentrations was found after multiple doses.

Volanesorsen plasma PK following SC administration in male and female monkeys were further studied in the 39-week toxicity study (study 304801-AS11). Results are summarised in **Table 6**.

**Table 6.** Summary of Plasma Pharmacokinetic Parameters in Monkeys Following Single and Multiple Subcutaneous Administrations of Volanesorsen (study 304801-AS11)

| Dose Level (mg/kg) | Profile Day | $C_{max}$ (µg/mL) | $AUC_{0\text{-}48h}$ (µg*h/mL) | $CL_{0\text{-}48h}/F^a$ (mL/h/kg) | $T_{max}$ (h) | $MRT_{0\text{-}48r}$ (h) | $t_{1/2}$ (day) |
|---|---|---|---|---|---|---|---|
| 3 | 1 | 9.78 ± 1.86 | 45.4 ± 6.26 | 67.3 ± 9.16 | 2 (1–2) | 5.86 ± 1.14 | NC |
| | 182 | 6.76 ± 1.53 | 60.4 ± 11.4 | 51.2 ± 10.6 | 2 (1–2) | 11.1 ± 1.06 | |
| | 273 | 4.03 ± 2.23 | 43 ± 17.8 | 78.6 ± 25.4 | 2 (1–4) | 12.1 ± 1.61 | |
| 6 | 1 | 26 ± 5.24 | 141 ± 32 | 44.5 ± 8.96 | 2 (1–4) | 5.13 ± 0.941 | NC |
| | 182 | 20.1 ± 3.25 | 275 ± 52 | 22.4 ± 3.60 | 3 (2–6) | 11.9 ± 1.97 | |
| | 273 | 17.8 ± 8.38 | 264 ± 160 | 32.6 ± 23.5 | 4 (2–4) | 12.7 ± 3.74 | |
| 12 | 1 | 48.2 ± 10.5 | 331 ± 76 | 38.0 ± 8.31 | 2 (1–6) | 6.13 ± 0.818 | 30.3 ± 4.90 |
| | 273 | 33 ± 13.9 | 434 ± 218 | 33.1 ± 13.4 | 4 (2–6) | 10.5 ± 2.39 | |

| Dose Level (mg/kg) | Profile Day | $C_{max}$ (µg/mL) | $AUC_{0-48h}$ (µg*h/mL) | $CL_{0-48h}/F^a$ (mL/h/kg) | $T_{max}$ (h) | $MRT_{0-48r}$ (h) | $t_{1/2}$ (day) |
|---|---|---|---|---|---|---|---|
| 20 | 1 | 73.2 ± 11.5 | 592 ± 126 | 35.1 ± 6.90 | 2 (1–4) | 6.69 ± 1.06 | 31.1 ± 10.9 |
| | 273 | 65.9 ± 20 | 1096 ± 423 | 21.1 ± 8.70 | 4 (2–10) | 11.9 ± 2.18 | |

SD = standard deviation; NC = not calculated; Note: $T_{max}$ values reported as median (range); all other values as mean ± SD

[a] $CL_{0-48h}/F$ = [Dose (mg/kg) / $AUC_{0-48h}$ (µg*h/mL)] x1000

Dose-dependent increases in mean trough (post-distribution phase) plasma concentrations were also observed in this study; with post-distribution plasma concentrations typically expected to be in equilibrium with the major tissues of distribution (i.e., reflecting accumulation and slow tissue elimination of volanesorsen). However, mean and median plasma trough values continued to increase after the loading dose phase of the study; with the mean increasing 4.4- to 15.4-fold (across the dose groups) by Day 147 compared to Day 14 (the start of the maintenance dosing phase) when the tissues would have been expected to have reached steady state

## *Distribution*

### *Mouse*

Kidney and liver were evaluated in mice after multiple SC doses of volanesorsen from 3 to 100 mg/kg for up to 6 weeks in the non-GLP study 304801-APK01. Higher volanesorsen mean tissue concentrations were observed in the liver than kidney at the 2 higher doses evaluated. Exposure generally increased less than dose-proportionally (~9- and 4- fold increase for liver and kidney, respectively), over the evaluated 33.3-fold dose range.

Kidney and liver were evaluated in mice after multiple SC doses of volanesorsen from 4 to 100 mg/kg for up to 13 weeks in study 304801-AS01. Similar volanesorsen mean tissue concentrations were observed in liver and kidney at the doses evaluated and generally increased but less than dose-proportionally (8.5- to 11.7-fold increase), over the evaluated 25-fold dose range. Consistent mean tissue concentrations were observed at 6 and 13 weeks (Days 44 and 93, respectively) indicating attainment of approximate steady-state conditions in tissues by 6 weeks with the employed dosing regimen. Estimated tissue elimination half-life values at the 100 mg/kg dose level were 21.7 and 25.8 days for kidney and liver, respectively.

Kidney and liver were further evaluated in the mouse carcinogenicity study after multiple SC doses of volanesorsen from 6 to 40 mg/kg for 13 weeks in toxicokinetic satellite animals in study 304801-AS15. Tissue exposure of volanesorsen was dose-dependent following 13 weeks of treatment; however, the increases in kidney and liver concentrations were less than dose-proportional with an observed 2.5- and 4.9-fold increase, respectively, over the 6.7-fold increase in dose. Liver concentrations were generally higher than kidney concentrations at equivalent dose levels. Although kidney concentrations in female mice were consistently higher (up to 2.5-fold) compared to males, no gender difference was observed in mouse liver concentrations.

The tissue concentrations of volanesorsen were also evaluated in main carcinogenicity study animals at the terminal necropsy intervals of the study (which varied by group due to survival). Tissue exposure of volanesorsen was also dose-dependent at the terminal necropsy following treatment with volanesorsen up to 2 years over a dose range of 6 to 40 mg/kg.

Liver and kidney were evaluated in mice following multiple SC doses of volanesorsen from 3 to 80 mg/kg in mice for up to 26 weeks in study 304801-AS10. In this study, mean kidney (but not liver) concentrations on Day 184 appeared consistently higher in female compared to male mice for all doses studied. Mean kidney concentrations increased either about or less than dose-proportionally (13.5-fold for males, 25.4-fold for females) over the evaluated 26.7-fold dose range. Mean liver concentrations also increased less than dose-proportionally (8-fold) over the evaluated 26.7-fold dose range. Slow clearance of volanesorsen from the evaluated tissues was observed during the 13-week recovery period, with concentrations of volanesorsen still measurable at the end of the recovery period (Day 273) in the high dose group (but not the 10 mg/kg dose group). Estimated mean tissue half-life values (based on 2 available mean time points only) at the 80 mg/kg dose level were 17.1 and 26.3 days for the kidney and liver, respectively.

***Rat***

Tissue distribution of radioactivity was studied in Sprague-Dawley rat following a single SC administration of 5 mg/kg or 25 mg/kg [$^{3}$H] volanesorsen in study 304801-APK02.

With respect to blood partition, radioactivity was mainly associated with the plasma and not the blood cell components, with mean whole blood-to-plasma ratios of approximately 0.53-0.83 from 0 to 48 hours after the 5 mg/kg dose.

The kidneys contained the highest levels of radioactivity at all time points evaluated, followed by the injection site, liver, mesenteric lymph nodes and bone marrow. The maximum levels of total radioactivity in kidney, injection site, liver, mesenteric lymph nodes, and bone marrow were 107, 64.4, 9.99, 7.95, and 7.08 µg equivalents/g, respectively. Tissues that contained moderate levels of radioactivity (tissues with maximum concentrations between 1 and 5 µg equivalents/g) were thyroid, spleen, bone, pancreas, walls of the gastrointestinal tract, adrenal glands, testes, skin, prostate and peri-renal fat. Very little radioactivity was associated with the brain and spinal cord with a mean maximum level of 0.060 and 0.194 µg equivalents/g, respectively.

For most tissues, maximum concentrations were observed at 24 or 48 hours after the 5 mg/kg dose. Mean concentrations of radioactivity in kidney and liver declined with a half-life of 23.0 and 13.6 days, respectively. Elimination of radioactivity from all tissues was slow with estimated terminal half-life values ranging from 10.5 to 77.9 days, reflecting the slow clearance of [$^{3}$H]volanesorsen-related radioactivity from tissues.

Tissue concentrations of radioactivity post-dose following administration at 25 mg/kg SC were comparable with the results obtained with the lower dose.

Kidney and liver distribution of volanesorsen in rats was evaluated following 0.2 to 5 mg/kg SC weekly administration of volanesorsen for 13 weeks during the 2-year carcinogenicity study (study 304801-AS16). The mean kidney and liver concentrations were BLQ at the 0.2 mg/kg dose and increased between the 1 to 5 mg/kg doses, in a greater than dose-proportional manner in this low dose range. The tissue levels were similar between male and female rats.

Tissue distribution of volanesorsen in rats following 3 to 80 mg/kg SC administration of volanesorsen for up to 26 weeks was investigated in study 304801-AS12. Mean liver and kidney concentrations indicated apparent gender-independent biodistributio and increased less than dose-proportionally (2.8-fold increase for kidney, 16.7-fold for liver) over the 26-fold dose range. Kidney contained higher concentrations than liver in rats at the 3 lower dose levels, while liver concentrations were similar to kidney concentrations in rats at the 80 mg/kg dose level, the latter likely due to saturation of uptake to the kidney. Mean kidney and liver concentrations of volanesorsen were typically similar at Day 93 and Day 184 or Day 121, indicating approximately steady-state was achieved in the tissues by 13 weeks.

***Monkey***

In monkeys the mean kidney cortex concentrations were higher than mean liver concentrations as measured in study 304801-AS02. Mean kidney and liver concentrations generally increased dose-dependently, but less than dose-proportionally for the highest dose (4.4- to 11.4- and 3.4- to 4.5 fold, respectively), over the evaluated 10-fold dose range. Slow clearance from tissue led to accumulation of volanesorsen following every-other-day and weekly administration. For kidney cortex and liver, after 4 loading doses of 8 mg/kg SC, the mean tissue concentrations at 48 hours after dosing (Day 9) were approximately 2.3- to 3.7-fold higher than that after a single dose (Day 3) consistent with expected tissue accumulation/ Reasonably consistent tissue concentrations were observed at 6 and 13 weeks (Days 44 and 93, respectively) for Groups 2 through 5, indicating attainment of approximate steady-state conditions in tissues by 6 weeks with the employed dosing regimen.

For all evaluated dose levels, volanesorsen concentrations 48 hours after the last dose (Day 93) were several hundred-to several thousand-fold higher in kidney cortex and liver than in plasma. Volanesorsen was cleared slowly from kidney cortex and liver after the 13-week administration of 4 to 40 mg/kg volanesorsen, with an estimated elimination half-life of 12.5 days in kidneys cortex and 18.9 days in liver.

Tissue distribution of volanesorsen in monkeys following 3 to 20 mg/kg administration of volanesorsen for up to 39 weeks (study 304801-AS11) was similar to that seen in the 13-week study 304801-AS02. Mean kidney cortex and liver concentrations increased dose-dependently. Following Day 275, the increase in exposure was slightly less than proportional to dose for liver (3.0-fold) and kidney cortex (4.2-fold) over the 6.6-fold dosing range. In the 2 groups where 6-month data from the interim necropsy was also obtained, the increase was also dose-proportional for both tissues. Based on the similarity of mean tissue concentrations between Days 184 and 275 across all dose groups, steady-state tissue concentrations appear to have been achieved by 6 months of treatment (Day 184) in this study. Typically, only moderate inter-animal variability in evaluated tissue concentrations was observed across dose levels and evaluated time points. Estimated apparent elimination half-life values for volanesorsen in kidney cortex and liver were 32 and 25 days, respectively.

***Protein Binding and Distribution in Blood Cells***

Volanesorsen was highly bound to plasma proteins over the evaluated volanesorsen plasma concentration range (5 and 150 µg/mL) in human, monkey, and mouse (**Table 7**).

**Table 7.** In Vitro Plasma Protein Binding (% Bound) of Volanesorsen in Whole Plasma of Human, Monkey and Mouse- study 304801 IS04

| Nominal Plasma Volanesorsen Concentration (µg/mL) | Extent of Plasma Protein Binding (%) | | |
|---|---|---|---|
| | Human | Monkey | Mouse |
| 5 | 99.24 ± 0.07 | 99.78 ± 0.04 | 98.12 ± 1.10 |
| 150 | 98.01 ± 0.14 | 99.48 ± 0.08 | 97.19 ± 0.47 |

% Bound values presented are mean ± standard deviation;

***Placental Transfer and Tissue Distribution in Reproductive Animals***

The effects of volanesorsen on fertility and embryo/fetal development were assessed in mice and rabbits following SC injection (study 304801-AS07 and -AS09, respectively). In mice, hepatic tissue concentrations of intact volanesorsen increased in a dose-dependent manner in paternal and maternal animals, and were 394 and 356 µg/g, respectively, at the highest dose tested (87.5 mg/kg/wk).

Relatively low concentrations of volanesorsen were measurable in placental tissue (13.5 µg/g), while no measurable levels were seen in fetal tissue (i.e., BLQ < 10 µg/g). Similarly in the rabbit (study 304801-AS09), dose-dependent and time-dependent exposure was seen in the maternal liver mean concentrations, with the highest concentration being observed at the highest dose on gestation day (GD) 20 (533 ± 131 µg/g). Quantifiable placental exposure was only observed in the highest dose group at GD20 (37.4 ± 12.7 µg/g), and no quantifiable exposure was seen in any evaluated fetal livers, indicating that volanesorsen is not readily transported to the embryo or fetus.

### *Metabolism*

The *in vivo* metabolite profile of volanesorsen was studied in mouse, rat, monkey, and humans using selected samples collected from a number of studies.

Plasma

Intact volanesorsen was the most abundant oligonucleotide in mouse, monkey, and human plasma. Volanesorsen accounted for >60% of the total oligonucleotides detected in plasma. Other drug-related moieties detected in plasma included a 19-mer oligonucleotide (N-1 from 3′-end only) and other metabolites, ranging from 12-mers down to 5-mers. Each individual metabolite detected was ≤1% of the total oligonucleotides detected in the plasma, except for the 3′-end deletion 5-mer and the 19-mer detected in the mouse samples collected 7 days post-dose, which accounted up to approximately 24% and 12% of the oligonucleotides detected, respectively. No detectable human-specific major or minor metabolites were present in human plasma.

Tissue

Relatively low volanesorsen metabolite levels (up to 20% of parent drug but typically < 10%) were measured in kidney and liver samples from mouse, rat, and monkey by a HPLC-PDA method: 12 and 100 mg/kg SC dosing for 6 and 13 weeks and at the end of recovery in mice, 80 mg/kg SC for 13 weeks and 17 weeks in rats, and 12 mg/kg SC for 6 weeks and 13 weeks and at the end of recovery in monkeys. These results are consistent with relatively slow but extensive formation of chain-shortened metabolites from the parent drug.

Urine

Metabolites excreted in urine from mice, monkeys, and humans were assessed by IP-HPLC-MS. In urine, metabolites ranging from 5-mer to 19-mer were identified. Compared to monkey and human urine, there was a greater amount of intact volanesorsen observed in mouse urine. In monkey and human urine, there was a great abundance of chain-shortened oligonucleotides observed together with the intact oligonucleotide.

*In vivo* metabolism of volanesorsen was also studied in rats following a single SC administration of 5 mg/kg or 25 mg/kg [$^{3}$H]volanesorsen ( Study 304801-APK02).

Plasma

Unchanged volanesorsen accounted for the majority of total plasma radioactivity (approximately 70%) in plasma at 2 hours. Trace amounts of metabolites were below detection by LC-MS and no radiolabelled metabolites were identified in plasma at either 2 hours or 24 hours. The minimal proportion of metabolites observed in plasma as compared to unchanged volanesorsen confirmed distribution rather than metabolism as the major contributing factor to the initial rapid plasma clearance.

The predominant component in the kidney and liver at 24 hours was unchanged volanesorsen, with low levels of 3′-deletion metabolites (6-mer or longer) (total of up to approximately 6% and 3% of dose in kidney and liver, respectively) also present.

Excreta

Urine collected from 0 to 6 hours and 6 to 24 hours contained significant amounts of radiolabelled metabolites of approximately 5 to 8 nucleotides in length. The major urinary components (6-mer and 7-mer, 3′-deletion) each accounted for 3 to 5% of dose while the more minor components (5-mer from 3′-deletion, 8-mer from 3′-deletion, and unidentifiable metabolite longer than 8-mer in length) each represented approximately 1% of dose. By comparing the proportions of metabolites excreted in urine, shorter oligonucleotides such as 6-mer and 7-mer from 3′-deletion are more readily excreted compared to unchanged volanesorsen likely due to reduced tissue and plasma protein binding.

Each of the metabolites identified in feces accounted for less than 0.3% of the administered dose over the 0- to 24-hour interval (5 and 25 mg/kg) among which 6-mer and 7-mer from 3′-deletion were the major components in feces.

Up to 18 radioactive components were present in plasma, excreta and tissues, of which 6- and 7-mer oligonucleotides following 3′-deletion were the major excreted metabolites.

Considering the small fraction of drug metabolized and excreted during the early collection interval, this confirms the primary mechanism of clearance from plasma as rapid uptake into tissue. Subsequent tissue clearance of the parent drug was slow, followed by elimination of metabolites in urine and feces. No sex difference was noted in the metabolism of volanesorsen following a 25 mg/kg dose, and there were no appreciable differences following a 5-fold increase in dose.

## *Pharmacokinetic drug interactions*

*In vitro* studies using primary human hepatocytes showed that volanesorsen up to 100 μM does not induce or inhibit major cytochrome P450 isoforms. Volanesorsen was also shown not to be a substrate or inhibitor of major drug transporters (see also Clinical Aspects of this Report). The applicant therefore conisdered that volanesorsen has a very low potential for involvement in CYP- or transporter-mediated drug-drug interactions and did not submit dedicated drug interaction studies.

## *Excretion*

Excretion and mass balance for volanesorsen were investigated in rats following a single SC administration of 5 mg/kg or 25 mg/kg [$^{3}$H]volanesorsen (study 304801-APK02).

After a single 5 mg/kg dose, excretion of [$^{3}$H]volanesorsen-related radioactivity in urine and faeces was slow and occurred mainly via urinary excretion, and to a lesser extent fecal elimination (i.e., only approximately 14% of administered dose was eliminated in the excreta within the first 24 hours). After the first 24 hours and up to 56 days post-dose, less than 2% of the administered dose was excreted daily. Less than 25% of dose was recovered in excreta by 168 hours post-dose, which increased to approximately 41% of dose by 28 days and up to 60% of dose by 56 days post-dose.

The mean overall recovery of radioactivity from excreta and tissues (including residual carcass) over the 56-day collection period was 77% of dose. The largest proportion of excreted radioactivity was recovered in urine (mean of 48% dose), 7.67% of dose in faeces and 1.98% of dose in cage washings, and 19.1% of the administered dose remained tissues/carcass, of which the kidneys and liver contained 5.81% and 1.20% of dose respectively, 1.22% of the dose remained associated with the injection site, and 6.83% of dose in the carcass (including fat, muscle, skin, bone and bone marrow). All other tissues each accounted for 0.53% or less of the dose.
After a single 25 mg/kg dose, the recovery of radioactivity from excreta over the first 24-hour period was 23.8% and 20.0% of dose in male and female rat, respectively, of which almost all was in the urine (only 0.97% and 0.48% of the dose, respectively in the faeces). The rate of urinary excretion was slightly higher for 25 mg/kg than that for the 5 mg/kg dose.

Transfer of volanesorsen into milk was evaluated in a pre- and post-natal development study in mice. The mean concentrations of volanesorsen in breast milk from lactating female mice increased in a dose-dependent manner. The mean volanesorsen concentration in milk was 0.6 µg/ml at the high dose of 87.5 mg/kg/wk while the mean volanesorsen concentration in maternal liver was 492 µg/g at the same dose level.

### *Immunogenicity studies*

The presence of anti-volanesorsen antibodies specific to volanesorsen was assessed using a standard ELISA type assay in chronic toxicology studies in mice and monkeys. None of the evaluated mice tested positive for anti-volanesorsen antibodies (study 304801-AS10).

The incidence of anti-volanesorsen antibody, time of onset, and antibody titer values in monkeys in study 304801-AS11 are summarized in **Table 8**.

**Table 8.** Incidence, Onset, and Titer of Anti-Volanesorsen Antibodies in Monkeys (study 304801-AS11, Amendment 1)

| Group | Dose Level (mg/kg) | IM (+) n (%) | IM Onset (Study Day) | Titer |
|---|---|---|---|---|
| 1 | 0 | 0 (0) | NA | NA |
| 2 | 3 | 7 (50) | 182 (182–273) | 200 (50–3200) |
| 3 | 6 | 10 (71) | 182 (70–182) | 1200 (100–25600) |
| 4 | 12 | 13 (72) | 182 (70–273) | 3200 (400–819200) |
| 5 | 20 | 13 (72) | 182 (70–273) | 1600 (100–205000) |

*IM (+) monkeys were defined as monkeys with at least 1 plasma sample confirmed as positive*
*IM onset and titer values are presented as median (range). Titer values include the Minimum Required Dilution of 50.*
*IM = immunogenicity n = number of IM (+) animals NA = not applicable*

The presence of anti-volanesorsen antibodies resulted in an increase in the median plasma trough concentrations of approximately 1.2- to 14-fold in the samples collected approximately 3 months or later after initiation of dosing in all dose groups. In general, the median trough plasma concentrations in IM-positive monkeys after 3 months of dosing were up to approximately 14-fold higher than the median trough plasma concentrations in the IM-negative monkeys, consistent with retention of antibody-bound volanesorsen in circulation (study 304801-AS11, amendment 1, Table 7). However, similar plasma $C_{max}$, $AUC_{0-48h}$, and half-life were observed between IM-negative and IM-positive animals though based on a limited sample size with relevant PK parameters. Similarly, the presence of antibodies to volanesorsen was not associated with consistent changes in tissue (liver and kidney) concentrations of volanesorsen.

## 2.3.4. Toxicology

### *Single dose toxicity*

No single dose toxicity studies were submitted which was considered acceptable as clinical administration could be supported by the submitted GLP repeated dose toxicity studies (see below).

### *Repeat dose toxicity*

Major findings from repeat dose toxicity studies are summarised in **Table 9**.

**Table 9.** Major findings from repeat dose toxicity studies with volanesorsen

| Study ID | Species/Sex/ Number/Group | Dose/Route | Duration | NOEL/ NOAEL(mg/ kg/d) | Major findings |
|---|---|---|---|---|---|
| 304801-AS01 (GLP) | Mouse 16 or 22/s/g | 0-4-12-40-100/SC | 13wks | ND | **Mortality**<br>=4: 1M moribund<br>=100: 1M moribund (dermatitis, scabbing at injection site)<br><br>**Clinical observations**<br>≥4: scabbing, loss of fur<br><br>**Haematology**<br>=100M:↑WBC, ↑Lymphocytes, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | ↑Neutrophils<br>≥40:↓PLT<br><br>**Serum chemistry**<br>=100:↑ALT (transient), ↑AST (transient),<br><br>**Pathology**<br>≥4: Minimal-mild liver inflammation<br>≥12: Basophilic granules in lymph nodes, Kupffer cells and proximal tubular epithelium, intracellular sinusoidal histiocytes in lymph nodes<br>≥40: Mild-moderate Kupffer cell hypertrophy, mild-moderate infiltrate in injection site, basophilic granules<br>=100: Minimal tubular vacuolation in kidney |
| 304801-AS10 (GLP) | Mouse: 18 or 12/s/g | 0-3-10-30-80 (volanesorsen), sc + 30 murine ASO, sc | 26 wks | 3 | **Mortality**<br>=80: 10 animals moribund due to (chronic) inflammation<br>**Clinical observations**<br>=80: scabbing (also at injection site, loss of fur<br>=80M: ↓body weight<br>**Haematology**<br>≥30:↑Monocytes, ↓RBC, ↓HB, ↓HCrit, ↑WBC, ↓PLT,<br>=80: ↑Neutrophils, ↑Lymphocytes, ↑Monocytes,<br>**Serum chemistry**<br>≥3: ↑IgM<br>≥30: ↑ALT,↑AST, ↓TG, ↑Chemokines/Cytokines, ↑IgG<br>=90: ↓Glucose,<br>**Gross pathology:**<br>≥30: ↑Liver weight, ↑Spleen weight<br>=80: ↑scabbing and alopecia at injection site<br>**Histopathology**<br>≥3: ↑basophilic granules in liver, Kupffer cell hypertrophy, mono-nuclear cell infiltrate in liver, sinusoidal hypertrophy in lymph nodes<br>≥10: Injection site reactions (basophilic granules, epidermal exudate @80, epidermal hyperplasia @80), chronic inflammation<br>≥30:Basophilic granules in kidney, mononuclear infiltrates in kidney, vacuolation proximal tubule, mononuclear infiltrates in salivary glands, histiocyte infiltrate in skin<br>=80: Heart w basophilic granules, degeneration of myofibre, histiocytic infiltrates<br><br>**Murine ASO (ISIS440670)**<br>**Serum Chemistry**<br>=30: ↓TG<br><br>**Histopathology**<br>=30: Kupffer cell hypertrophy, mononuclear cell infiltrates in liver, sinusoidal hypertrophy lymph nodes, histiocyre infiltrate in skin, basophilic granules in kidney proximal tubule<br>**Recovery**<br>=80:↑WBC (males), ↓RBC (males), ↓HB (males), ↓HCrit (males), ↑Neutrophils, ↑Lymphocytes, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | ↑Monocytes, Heart fibrosis and histiocytic infiltrate, chronic inflammation at injection sites , Kupffer cell hypertrophy and basophilic granules in liver, mononuclear cell infiltrates, basophilic granules and sinusoidal histiocyte hypertrophy in lymph nodes |
| 304801-AS14 (GLP) | Mouse: 6/s/g | 0-50-60 sc | 26 wks | ND | **Clinical observations**<br>≥50: ↑BW, ↑FC<br><br>**Haematology**<br>≥50: ↓RBC, ↓Hematocrit, ↓HB, ↓MCV, ↑Leukocytes, ↑Neutrophils, ↑Lymphocytes, ↑Monocytes<br><br>**Serum chemistry**<br>≥50:↑AST, ↑ALT, ↓Albumin, ↓TProt<br><br>**Gross pathology**<br>↑liver weight, ↑spleen weight<br><br>**Histopathology**<br>≥50: basophilic granules in heart valves (left AV, right AV) , Cytoplasmic alteration in liver, mononuclear cell infiltrate in liver<br>=60: basophilic granules in heart myocardium, myofiber degeneration, basophilic granules in heart valve, injection site, kidney and tubular cells, glomerulopathy, liver hypertrophy, karyomegaly and necrosis, increased cellularity in red pulp of spleen |
| 304801-AS12 (GLP) | Rat: 6/s/g | 0-3-10-40/20-80 sc | 26 wk | 3 | **Mortality**<br>=3: 1F (aspiration, non-treatment related), 1M 10mg/kg due to severe skin abrasion (inflammation/necrosis, non treatment related)<br>=20: 1M (treatment related)<br>=80: sacrificed on d121 due to treatment<br>**Clinical observations**<br>≥10: ↓body weight, ↓food consumption<br>≥20: Thin appearance<br>**Haematology**<br>≥20: ↑Leukocytes, ↑Lymphocytes, ↑Monocytes<br>**Serum chemistry**<br>≥20: ↑AST, ↑ALT, ↑Glob, ↓A/G<br>**Urinalysis**<br>≥20: ↑protein, ↑prot/crea,<br>**Gross Pathology**<br>≥3: ↓Thymus size<br>≥10:↑heart weight, ↑kidney weight, Liver weight, ↑Spleen weight, Kidney discoloration<br>≥20: Enlarged spleen<br>**Histopathology**<br>≥3: basophilic granules in kidney (incl mesangial cells and tubular cells), and Kupffer cells<br>≥20: basophilic granules in bone marrow, heart myocardium, heart valves and injection sites, mononuclear cell infiltrate in injection site and kidney, chronic progressive nephropathy, glomerulopathy, lymphoid hyperplasia in lymph nodes, lymphoid depletion in marginal zone |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | of spleen, increased extramedullary haematopoiesis in spleen, lymphoid depletion in thymus<br>≥80:, endocardial myxomatous change, centrilobular atrophy |
| 304801-AS13 (GLP) | Rat: 10/s/g | 0-40/20-80 | | ND | **Mortality**<br>5 animals in moribund condition, treatment related<br><br>**Clinical observations**<br>≥20:↓BW, ↓FC, pale appearance, injection sites swollen<br><br>**Haematology**<br>≥20:↓RBC,↓HB, ↓HCrit, ↓PLT<br><br>**Serum chemistry**<br>≥20:↑ALT, ↑AST, ↑A/G, ↑CK<br>=80: ↑BUN<br><br>**Urinalysis**<br>≥20: ↑Prot/Crea<br><br>**Gross Pathology**<br>≥20:↑Kidney weight, ↑Liver weight, ↑Spleen weight, ↑Thymus weight<br>≥20: Kidney discoloration, enlarged lymph nodes, enlarged spleen, small thymus, injection site discoloured<br><br>**Histopathology**<br>≥20:Basophilic granules proximal tubules and Kupffer cells, vacuolated histiocytes in kidney, Histiocytic and mononuclear infiltration in kidney and liver, tubular basophilia (kidney), eosinic globules (kidney), Kuppfer cell hypertrophy, extramedullary hematopoieiss of liver, liver atrophy, histiocytes hypertrophy lymphnode, histiocyte infiltration lymph node, lymphoid hyperplasia lmph node, histiocyte infilotration spleen, spleen lymphoid hyperplasia, spleen extramedullary haematopoiesis, thymus atrophy and histiocytic infiltration |
| 304801-AS11 (GLP) | Cynomolgus monkey: 7 or 9/s/g | 0-3-6-12-20 | 39 wk | 3 | **Mortality**<br>=12: 1F (thrombocytopenia) 2F(declining condition)<br>=20: 1M, 1F (thrombocytopenia), 1M (proinflammatory effects, 2M (declining condition)<br><br>**Clinical observations**<br><br>**Haematology**<br>≥6: Sporadic cases of very low PLT, ↑IgG, ↑IgM,<br><br>**Serum chemistry**<br>≥6: ↑Cytokines/Chemokines MCP-1 and MIP-1β<br>=20: ↓Complement C3<br><br>**Gross Pathology**<br>≥6: ↑spleen weight, enlarged liver, enlarged kidney<br><br>**Histopathology**<br>≥3: basophilic granules kidney, lymph |

| | | | | |
|---|---|---|---|---|
| | | | | nodes and liver, mononuclear cell infiltrate in injection site kidney and liver, hypertrophy of Kupffer cells, sinusoidal histiocyte hypertrophy lymph nodes, haemorrhage and oedema at injection site<br>≥6: vacuolation proximal tubules<br><br>**Recovery**<br>≥12: mononuclear cell infiltrate in kidney, basophilic granules in liver and lymph nodes, Kupffer cell hypertrophy, lymph node sinusoidal histiocyte hypertrophy |
| 304801-AS02 (GLP) | Cynomolgus monkey: 5 or 7/s/g | 0-4-8-12-40 (loading IV, subsequent SC, SC only @40) | 13 wk | **Mortality**<br>=12: 1M<br><br>**Clinical observations**<br><br>**Haematology**<br>≥12: ↓Alb,<br>=40: ↑monocytes<br><br>**Serum chemistry**<br>≥8: Complement Bb,<br>=40:↑APPT, ↑MIP-1β, ↑MCP-1<br><br>**Histopathology**<br>≥4:mononuclear cell infiltrate in kidney and injection site, tubular vacuolation in kidney, basophilic granules in kidney, lymph nodes and liver, Kupffer cell hypertrophy<br>≥12: Mononuclear cell infiltrates in liver, sinusoidal histiocyte hypertrophy lymph nodes<br>=40:tubular regeneration, tubular casts<br><br>**Recovery**<br>≥12: basophilic granules in lymph nodes |

## *Genotoxicity*

An overview of the genotoxicity studies is presented in **Table 10**.

**Table 10.** Overview of genotoxicity studies with volanesorsen

| **Type of test / study ID / GLP** | **Test system** | **Concentrations / Concentration range / Metabolising system** | **Results Positive / negative / equivocal** |
|---|---|---|---|
| 304801-IS02/Gene mutations in bacteria/GLP | Salmonella strains | 1.5-5000µg/plate, - S9 | Negative |
| 304801-IS03/Gene mutations in mammalian cells/GLP | L5178Y/TK+/- | 100-500µg/ml +/- S9 | Negative |
| 304801-AS05/Chromosomal aberrations in vivo/GLP | Mouse, micronuclei in bone marrow | 1000-2000 mg/kg | Negative |

## *Carcinogenicity*

The carcinogenic potential of volanesorsen was assessed in 2-yr repeated dose studies in mice (Study No. 304801-AS15) and rats (Study No. 304801-AS16). An overview of these studies is presented in **Table 13** and the tumour findings from them in **Tables 11** and **12**.

**Table 11**. Overview of carcinogenicity studies performed with volanesorsen

| Study ID /GLP | Dose/Route | Exposure (AUC) | Species/No. of animals | Major findings |
|---|---|---|---|---|
| 304801-AS15/GLP | 0-6-25-40 and 25 murine ATO | | CD-1 mouse, 70/s/g | **Non-neoplastic findings**<br>≥6: ↓Ery, ↓HB, ↑Neut, ↑Lympho, ↑RDW %, ↑TNF-α, liver mass or nodules, enlarged pituitary, cardiomyocyte degeneration, atrial/ventricular dilatation, mononuclear cell infiltrate in heart, macrophages in heart and valves, liver alterations (cellular), extramedullary haemotopoiesis liver, mononuclear cell infiltration, liver necrosis single cell, liver and spleen basophilic granulation, injection site inflammation, mammary gland lobular hyperplasia, mammary gland mononuclear cell infiltration and macrophages<br><br>≥25: ↓BW, ↓TG, ↑gluc, |
| 304801-AS16 | 0-0.2-1-5mg/kg | | Rat Crl:CD(SD) | **Non-neoplastic findings**<br>NA |

**Table 12.** Tumour findings in mouse carcinogenicity study 304801-AS15

| Tumour findings | Gender | Control | Low dose | Mid dose | High dose | Mid dose (Mouse ASO) |
|---|---|---|---|---|---|---|
| Liver adenoma (benign) | Male | 9 | 15 | 30 | 13 | 17 |
| | Female | 3 | 3 | 5 | 6 | 4 |
| Hepatocellular tumors (carcinoma) | Male | 5 | 4 | 4 | 2 | 0 |
| | Female | 0 | 0 | 1 | 0 | 0 |
| Harderian gland adenoma (benign) | Male | 17 | 11 | 6 | 4 | 24 |
| | Female | 2 | 8 | 7 | 0 | 12 |
| Hemangiosarcoma | Male | 2 | 11 | 12 | 10 | 10 |
| | Female | 4 | 9 | 9 | 3 | 2 |
| Histiocytic sarcoma | Male | 2 | 7 | 5 | 1 | 5 |
| | Female | 0 | 8 | 6 | 3 | 8 |
| Bronchiolar adenoma | Male | 19 | 14 | 6 | 4 | 16 |
| | Female | 7 | 6 | 8 | 7 | 5 |
| Bronchiolar carcinoma | Male | 12 | 16 | 1 | 1 | 19 |
| | Female | 6 | 6 | 4 | 2 | 8 |
| Pituitary pars distalis adenoma (benign) | Male | 0 | 2 | 0 | 0 | 0 |
| | Female | 3 | 3 | 9 | 3 | 7 |
| Mammary gland adenocarcinoma | Female | 5 | 6 | 6 | 2 | 8 |
| Malignant lymphoma | Male | 9 | 5 | 3 | 6 | 5 |
| | Female | 18 | 17 | 9 | 3 | 25 |

**Table 13.** Tumour findings in rat carcinogenicity study 304801-AS16

| Tumour findings | Gender | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|---|
| Adrenal pheochromocytoma | Male | 10 | 9 | 18 | 3 |
| | Female | 5 | 4 | 3 | 1 |
| Lung histiocytoma (2nd) | Male | 0 | 0 | 0 | 4 |
| | Female | 0 | 0 | 0 | 0 |
| Skin fibrous histiocytoma, malignant | Male | 0 | 0 | 7 | 39 |
| | Female | 0 | 0 | 5 | 32 |
| Skin fibrosarcoma | Male | 1 | 0 | 1 | 5 |
| | Female | 0 | 1 | 1 | 1 |
| Thyroid C-cell adenoma benign | Male | 6 | 5 | 12 | 4 |
| | Female | 9 | 6 | 13 | 7 |
| Pituitary gland pars distalis benign adenoma | Male | | | | |
| | Female | 57 | 61 | 55 | 50 |

## *Reproduction Toxicity*

Reproductive and developmental toxicity studies were conducted in mice (Segment I/II/III), and rabbits (Segment II) (Study Nos. 304801-AS07, -AS08, -AS09 and -AS14). Dams were treated on alternate days during gestation to insure adequate exposure to the developing conceptus (Lebrec, Cowan et al. 2011, Cavagnaro, Berman et al. 2014). Thus, the total weekly dose in the mouse Seg I/II study (87.5 mg/kg/wk) and the rabbit Seg II (52 mg/kg/wk) were comparable to the high doses used in the general toxicology studies.

**Table 14.** Reproduction toxicity studies with volanesorsen

| Study type/ Study ID / GLP | Species; Number Female/ group | Route & dose* | Dosing period | Major findings | NOAEL (mg/kg/wk &AUC) |
|---|---|---|---|---|---|
| 304801-AS07<br>Fertility and embryo/ fetal development<br>GLP | Mouse<br>25/sex/dose<br>6/sex/dose for TK of liver | 304801:<br>0, 10.5, 35, 87.5 mg/kg/wk<br>SC<br>440670:<br>10.5 mg/kg/wk<br>SC | M: 4 wks prior – 6 wks<br>F: 2 wks prior – GD15 | Fertility:<br>**=87.5**: ↓ sperm count<br><br>F1:<br>**=87.5**: ↑ skeletal variations (14th rudimentary rib, 14th supernumary rib, split sternebra, asymmetric sternebra, wavy rib) | Fertility:<br>M: 35<br>F: 87.5<br><br>Development:<br>F0: 35 |
| 304801-AS08<br>Fertility and embryo/ fetal development<br>GLP | Mouse<br>10/sex/dose | 440670:<br>0, 35, 87.5 mg/kg/wk<br>SC | M: 4 wks prior – 6 wks<br>F: 2 wks prior – GD15 | Fertility:<br>**≥35**: ↑ abnormal sperm<br><br>F1:<br>**≥35**: ↑ skeletal variations (14th rudimentary rib, 14th supernumary rib, cervical rib) | Fertility:<br>M: 10.5<br>F: 87.5<br><br>Development:<br>F0: 10.5 |
| 304801-AS09P<br>Embryo-fœtal development<br>Non-GLP DRF | Rabbit<br>5F/dose | 304801:<br>0, 21, 52.5, 122.5 mg/kg/wk<br>SC | 3 weeks (no mating) | **≥52.5**: ↓ BW gain, ↑AST, ALT, discoloured kidneys<br>**=122.5**: ↑ kidney and spleen weight | F0: 21 |
| 304801-AS09<br>Embryo-fœtal development<br>GLP | Rabbit<br>22F/dose | 304801:<br>0, 10.5, 21, 52.5 mg/kg/wk<br>SC | GD6 - 29 | F1:<br>**=52.5**: ↓ BW gain GD 14-24, ↓ FC<br><br>F0:<br>**=52.5**: 4 abortions/early delivery, ↓ foetal BW | F0: 21<br>F1: 21 |
| 304801-AS17<br>Peri & postnatal Development | Mouse<br>46F control<br>30F/dose | 304801:<br>0, 10.5, 35, 87.5 | GD6 – LD20# | F0:<br>**≥10.5**: ↑ kidney weight<br>**≥35**: ↑ liver and spleen | F0: N.D.<br>F1: N.D.<br>F2: 87.5 |

| | | | |
|---|---|---|---|
| GLP | 26F 440670 | mg/kg/wk SC<br>440670:<br>87.5 mg/kg/wk SC | weight<br><br>F1:<br>**≥10.5**: delayed vaginal opening<br>**=87.5**: ↓ BW (304801 only)<br><br>F2:<br>No effects |

* Dosing every other day, expressed as dose/week

\# Weekly dosing during lactation

Exposure to the dams was confirmed by measuring maternal liver concentrations. The concentration of volanesorsen was also measured in placental tissue, but there was little drug measured (detected only at 1/10th the maternal liver concentration at the mid and high dose levels) and none was detected in foetal liver.

Maternal toxicity observed in mice consisted of decreases in mean PLT count, as well as, increases in spleen weight, circulating monocytes, and serum cytokines at the high dose only. Mice treated with the murine apoC-III inhibitor, mRNA levels reduced to 17 and 36% of control in male and female mice, respectively. This reduction in apoC-III RNA correlated with triglyceride levels that were 40% of control, an effect that was ascribed to the intended pharmacologic effect.

Maternal toxicity in rabbits was characterized by reductions in body weight and food consumption (Study No. 304801-AS09). Four dams treated with 52.5 mg/kg/wk aborted or delivered early, presumably attributable to the decreased food consumption. Decreases in fetal body weight observed at 52.5 mg/kg/wk were considered to be secondary to decreased maternal body weight and food consumption, rather than a direct effect of volanesorsen on foetal development.

An assessment of pre- and postnatal toxicity was performed using subcutaneous administration of volanesorsen or the murine-specific apoC-III inhibitor (ISIS 440670) on alternate days to pregnant female mice at doses up to 87.5 mg/kg/wk from Day 6 of gestation to Day 21 postpartum. There were increases in spleen, kidney, and liver weights of the treated dams. Volanesorsen was detected in expressed milk at a concentration of ≤0.6 µg/ml. There were also no adverse effects on the reproductive function of the F1 generation. There were no effects in mice treated with the murine-specific inhibitor.

## *Toxicokinetic data*

An overview of toxikokinetic data are summarised in **Table 15**.

**Table 15.** Overview of volanesorsen toxikokinetic data

| Species | Route | Dose (mg/kg) | Study Day | Steady State $AUC_{0-48h}$ (µg*h/mL) | Study Number |
|---|---|---|---|---|---|
| Mouse | SC | 3 | 42 | 4.81 | 304801-APK01 |
| | | 30 | 42 | 112 | |
| | | 100 | 42 | 473 | |
| | | 6 | 92 | 13.2 | 304801-AS15 |
| | | 25 | 92 | 102 | |
| | | 40 | 92 | 226 | |
| Rat | SC | 0.2 | 92 | 0.295 | 304801-AS16 |
| | | 1 | 92 | 3.39 | |
| | | 5 | 92 | 48.0 | |
| Monkey | SC | 4 | 91 | 92.0 ± 29.8 | 304801-AS02 |
| | | 8 | 91 | 208 ± 52.4 | |
| | | 12 | 91 | 403 ± 143 | |
| | | 40 | 91 | 1890 ± 447 | |
| | | 3 | 273 | 43.0 ± 17.8 | 304801-AS11 |
| | | 6 | 273 | 264 ± 160 | |
| | | 12 | 273 | 434 ± 218 | |
| | | 20 | 273 | 1096 ± 423 | |

Data for monkey studies are presented as mean ± standard deviation.
Plasma PK parameters for mice and rats were calculated based on pooled mean profiles obtained from sparse sampling.

## *Local Tolerance*

Formal studies evaluating local tolerance were not conducted however the injection sites were evaluated as part of the repeat-dose studies in mice, rats and monkeys which did not reveal any evidence of substantive irritation.

## *Other toxicity studies*

### *Studies on impurities*

The potential toxicity of volanesorsen and related impurities was evaluated in CD-1 mice after 13 weeks of SC treatment. Three (3) different formulations of impurity mixtures were evaluated (ISIS 304801 Impurity Mixture 1, 2, and 3).

There were no volanesorsen-related changes in mortality, clinical signs, ophthalmology andmacroscopic observation in this study. Decreases in body weight, body weight gain and/or food consumption were observed in females at receiving 10 or 30 mg/kg/wk Impurity Mixture groups compared to volanesorsen at the same dose. Accumulation of basophilic granules was observed in multiple tissues in both sexes and across treatment groups. These changes were considered test article-related, but not adverse, as they were attributable to a known class effects of antisense oligonucleotide administration. Increased mixed cell infiltration in the liver and extramedullary hemopoiesis in the spleen was observed in both sexes at all 10 and 30 mg/kg/wk groups. This change was considered test article-related but not adverse. Overall, there were no discernible histological differences among the test article treated groups with volanesorsen or associated Impurity Mixtures. There were no additional toxicities that could be attributed to any of the 3 impurity mixtures evaluated.

### *Other studies*

Because of the observed platelet effects in monkeys, the applicant evaluated platelet function and plasma concentrations of volanesorsen after a 13-week dosing period when administered SC to cynomolgus monkeys. There was no effect on PLT function as measured by aggregometry or the PFA-100 instrument following 5 or 10 mg/kg/wk for 13 weeks.

In addition, the applicant assessed *in vitro*, if volanesorsen directly activated platelets from normal human donors at clinically-relevant concentrations. Given the intended patient population (FCS) for volanesorsen, analysis was also conducted using purified chylomicrons and VLDL to examine their potential concentration-dependent effect on PLT activation. The positive control, ADP, produced the expected concentration dependent increase in PAC-1 and CD62P, demonstrating PLT activation by ADP. Clinically-relevant concentrations of volanesorsen used in this experiment ranged from 0.4 to 4 µM. The highest concentration is 2- to 4 times the plasma Cmax with a dose of 300 mg/wk volanesorsen administered to patients in clinical trials. There was no significant increase in either activation marker for volanesorsen. There was a concentration-dependent increase in PLT activation markers in PLTs exposed to chylomicrons, but not VLDL. A 2- to 10-fold increase in PLT activation was observed from samples from two donors.

## 2.3.5. Ecotoxicity / environmental risk assessment

**Table 16.** Summary of main study results

| **Substance (INN / Invented Name):** volanesorsen | | | |
|---|---|---|---|
| **CAS-number (if available): 915430-78-3 (free acid); 1573402-50-2 (sodium salt)** | | | |
| ***PBT screening*** | | Result | **Conclusion** |
| *Bioaccumulation potential*- log *K*ow | Based on representative phosphorothioate oligonucleotide | -1.65 in pH 5,<br>-1.89 in pH 7,<br>-1.74 in pH 9 | Potential PBT (N) |
| **PBT-statement :** | The compound is not considered as PBT nor vPvB | | |
| ***Phase I*** | | | |
| **Calculation** | **Value** | **Unit** | **Conclusion** |
| $PEC_{surface\ water}$, refined $F_{pen}$ | 0.0002145, 0.00001 | µg/L | > 0.01 threshold (N) |
| Other concerns (e.g. chemical class) | | | N |

## 2.3.6. Discussion on non-clinical aspects

Binding of ISIS 301804 to APOC-III mRNA has been shown in vitro, resulting in reduced mRNA and protein levels. The applicant mentions that ISIS 301804 is highly selective based on a bioinformatic analysis of the human transcripts

No targets with only 1 mismatch were identified, and there were only 3 targets with 2 mismatches. These were RAC1 (ras-related C3 botulinum toxin), RAC1P2 (ras-related C3 botulinum toxin substrate 1 pseudogene 2), and STIM2 (stromal interaction molecule 2). The half maximal inhibitory concentration (IC50) of ISIS 304801 against apoC-III mRNA was > 200 fold lower than the IC50 against the 3 expressed off-targets. At a concentration that produced near maximal reduction of apoC-III mRNA (20 µM), no significant change was seen in the expressed putative off-targets.

Due to difference in the potency/optimisation of volanesorsen towards the target apoC-III mRNA from human and monkey origin, the relationship between volanesorsen plasma and liver exposure and PD activity cannot directly be translated to man. Dose levels of volanesorsen < 10 mg/kg were not tested in the monkey to support the proposed clinical dose of 300 mg /week (and even lower dose) based on mg/kg extrapolation. However, by applying an inhibitory Emax model the Applicant showed that the dose level of 5 mg/kg is anticipated to result in comparable efficacy in the monkey (as assessed by liver Apo-CIII mRNA & plasma Apo-CIII Protein levels) as the dose of 10 mg/kg and therefore to support efficacy at the intended posology as well as at lower dose levels in view of the non-linear dose relationship. The PD study conducted in the monkey is supportive for a potential therapeutic benefit at 300 mg/kg as well as at a reduced dose level in case of thrombocytopenia.

The pharmacokinetics, metabolism and elimination were studied primarily in mice, rats, and monkeys. Monkey is considered the most relevant species to humans based on the general pharmacokinetic behavior of 2'-MOE ASOs (plasma AUC, Cmax, and clearance).

In general, the absorption of volanesorsen following single and multiple SC administration was fast in mice, rats and monkeys with a $T_{max}$ similar to humans. Thereafter, volanesorsen declined quickly in a multi-phasic manner indicating a fast distribution to the tissues, of which, depending on the species,

liver and/or kidney accumulated most. Following single or multiple SC dosing a greater than dose-proportional increase in exposure was found. In mouse and monkey, as in humans, there was no clear evidence of plasma AUC accumulation following repeated dosing of volanesorsen although plasma trough concentrations strongly increased (4-15-fold). In both liver and kidney, volanesorsen accumulation was less than the dose increase. Elimination of volanesorsen from tissue was slow and tissue half-life was found to be 25 to 32 days. This is in line with the plasma terminal elimination half-life, which was 20 to 36 days in monkeys. In humans a similar plasma terminal half-life (~32d) was found. Depending on the dose and time of treatment, the monkey liver and kidney tissue concentrations were 11- to 53-fold higher than the maximum plasma concentration ($C_{max}$). Volanesorsen plasma concentrations did not reveal a gender dependent PK and was highly protein bound (>97%). The subcutaneous bioavailability of volanesorsen in healthy humans and monkey was high.

Volanesorsen is not readily transported to the embryo or foetus and poorly secreted into milk. Excretion was very slow and the majority of radioactivity was excreted in urine with a minimal amount of radioactivity recovered in faeces and with 19% still in tissues and carcass after 56 days. Metabolites of volanesorsen were identified in plasma, liver, kidney, urine, and feces samples. The metabolite profile generally appeared similar across the investigated species (mouse, rat, monkey, and human). Volanesorsen is metabolized initially by endonuclease-mediated hydrolysis at various positions within the central gap of the parent compound, followed by subsequent exonuclease (3' and 5')-mediated hydrolysis of the deoxynucleoside-ends of the formed hemimers. The abundance of the metabolites of volanesorsen, including the 19-mer, is low in all the matrices investigated as compared to the parent molecule. The potential for off-target and/or PD effect is unlikely for shorter nucleotide metabolites due to lack of hybridization as well as for longer-nucleotide metabolites because of their low concentration.

Volanesorsen toxicity in animals is mainly characterized by intrinsic effects of antisense oligonucleotides. Adverse effects in the target organs of toxicity, skin, liver, kidney and mandibular and mesenteric lymph nodes are identified. Heart is also a target organ for toxicity in rodent and is considered treatment related. Complement activation is seen in monkeys, a known effect of antisense oligonucleotides and considered species specific.

Based on the data obtained from the *in vivo* primary PD studies conducted in different animal models of hypertriglyceridemia suggested that the extent of the decrease in circulating triglycerides induced by volanesorsen, is likely dependent on the level of plasma triglycerides at baseline.

In the rhesus monkey, the decrease in TG following volanesorsen administration was demonstrated in hypertriglyceridemic (HTG) animals exclusively. In those animals maintained under fructose diet the plasma TG levels (+/- 150-200 mg/dL) were increased at least 3-fold over baseline. Conversely, in the normo-triglyceridemic cynomolgus monkeys repeated sc administration of volanesorsen for 13 or 39 weeks did not induce a statistically significant change in plasma TG as compared to untreated animals (mean plasma TG ~ 40-50 mg/dL). However, the applicant's claim cannot be confirmed at this stage as whether any change in TG levels would have occurred in lean rhesus monkey following administration of volanesorsen has not been investigated.

Cardiac degeneration/inflammation observed in the mouse was considered as a pro-inflammatory class-effect of 2'-MOE ASOs in rodents that were considered to be species specific. This was supported by the different anatomy of the murine myocardium compared to monkey and the high background incidence of cardiomyopathy in mice. Importantly there were no cardiac toxicity findings in the monkey following repeated administration of volanesorsen. Moreover, the thorough QT study and the findings from the clinical studies do not indicate a possible cardiotoxic effect in humans.

A decrease in platelet count was noted in all tested animal species and included thrombocytopenia in individual monkeys. Low platelet counts in monkeys correlated with bruising and increased APPT and is considered a clinically relevant effect as thrombocytopenia was also observed in clinical trials. In a

subset of monkeys with PLT < 50,000 cells/µL, there was no apparent induction of PLT activation makers (CD62P). Furthermore, in vitro PLT activation experiments showed there was no direct activation of PLT as determined by CD62P and Pac-1 staining at concentrations 2 to 3-fold greater than the plasma Cmax value at 300 mg in humans (4 µM or 28 µg/ml). There was no toxicity to hematopoietic organs including bone marrow. Anti-volanesorsen antibodies were measured in the majority of animals but were not increased in animals with low platelets. Anti-platelet antibodies were not detected in 7 out of 8 affected monkeys. Nevertheless, the applicant considers that clearance of platelet is the most likely explanation for the observed thrombocytopenia. In addition, inflammatory markers (in particular IL-6 and neutrophil counts) appeared to be increased in several affected monkeys, suggesting that the inflammatory response, in part, may drive the observed changes.

Two-year carcinogenicity studies were conducted in mice and rats. Test article-related neoplastic findings were above the historical control ranges, and it is likely that the neoplasms are secondary to chronic inflammation. However, given the high mortality due to toxicity of volanesorsen, the value of these studies is limited. In the rat study, treatment related neoplastic lesions were primarily observed as dose dependent increases of masses in the region of the injection site, which corresponded with malignant fibrous histiocytoma. These were attributed to inflammatory reactions after repeated SC injection, but are not considered to be clinically relevant because malignant fibrous histiocytoma are known to occur spontaneously in rats, and rodents are particularly susceptible to the development of skin sarcoma after chronic irritation and inflammation.

Taking into account the lack of a genotoxic effect of volanesorsen and the likelihood that most tumours were secondary to chronic inflammation, volanesorsen has little carcinogenic risk for patients, despite the early termination of study groups in the mouse and rat study.

Pups from dams treated with the high dose volanesorsen but not the active ISIS 440670 had reduced body weight at birth, which continued to be lower than animals from the other dose groups throughout the study duration. The female pups from dams treated with both ASO's at all doses however, did have a delayed vaginal opening indicating delayed sexual maturation. The lower birthweight of the F1 generation in the 87.5mg/kg/week volanesorsen group was below historical control values. Decreased weight was attributed to maternal toxicity. Vaginal opening in treatment groups were within historical controls and were likely statistically significant because control group animals fell in the low end of the range. Preputial separation in the high dose group falls marginally over the historical control maximum value (29.5 days in the 87.5 mg/kg/week group vs 29.1 days. This did not result in adverse effects on fertility nor reproductive parameters. There were no effects in mice treated with the murine-specific inhibitor, indicating that reducing apoC-III had no untoward effects on foetal development or maturation

Volanesorsen was detected in expressed milk at a concentration of ≤0.6 µg/mL, these concentrations are very low (>800 fold lower than effective tissue concentrations in maternal liver). Due to the poor oral bioavailability of volanesorsen, it is considered unlikely that these low milk concentrations would result in systemic exposure from nursing.

The calculated PECsurface water for volanesorsen is below the action limit of 0.01 µg/L and therefore volanesorsen is not expected to pose a risk to the environment.

## 2.3.7. Conclusion on the non-clinical aspects

The pharmacologic, pharmacokinetic, and toxicological characteristics of volanesorsen alfa have been adequately characterised from a non-clinical perspective.

The main hazard identified in animals is a reduction in platelet count leading to thrombocytopenia, which has also been observed in humans in the clinical trials.

Non-clinical data did not reveal any special hazard for humans based on conventional studies of safety pharmacology, genotoxicity, carcinogenicity or toxicity to reproduction and development.

## *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 | study population | description | dose (mg) + route | # subjects |
|---|---|---|---|---|
| CS1 | healthy volunteers | Phase 1 single and multiple ascending dose ranging study | Single-dose cohorts (on D1): 50 mg, 100 mg, 200 mg or 400 mg SC Multiple-dose cohorts (SC on D1, 3, 5, 8, 15 and 22): 50 mg, 100 mg, 200 mg or 400 mg SC | Volanesorsen: 25 Placebo: 8 |
| CS13 | healthy volunteers | Phase 1 thorough QT/QTc study | 4-way crossover with 4 single doses of 300 mg SC, 300 mg 2-hour IV infusion, volanesorsen placebo, and 400 mg moxifloxacin PO | Total: 52 Volanesorsen: 49 |
| CS2 | patients with severe or uncontrolled hypertriglyceridemia | Phase 2 dose-ranging study | 100 mg, 200 mg, or 300 mg QW SC for 13 weeks | Volanesorsen: 64 Placebo: 24 |
| CS4 | patients with type 2 diabetes mellitus | Phase 2 study | 300 mg QW SC for 13 weeks with 3 doses administered on Week 1 | Volanesorsen: 10 Placebo: 5 |
| CS6 | patients with familial chylomicronemia syndrome | Phase 3 study | 300 mg QW SC for 52 weeks | Volanesorsen: 33 Placebo: 33 |
| CS16 | patients with hypertriglyceridemia | Phase 3 study | 300 mg QW SC for 26 weeks or 300 mg QW SC then 300 mg Q2W SC post Week 13 for 26 weeks | Volanesorsen: 75 Placebo: 38 |
| CS7 | patients with familial chylomicronemia syndrome | Phase 3 open label study | 300 mg QW SC for 52 weeks with an option to extend to additional 52 weeks | Volanesorsen: 67 as of 30 June 2018 |

## 2.4.2. Pharmacokinetics

A total of 7 clinical studies have been conducted with volanesorsen (50-400 mg) to identify the PK of volanesorsen conducted in healthy adults, patients with familial chylomicronemia syndrome, patients with hypertriglyceridemia and patients with diabetes mellitus. A population PK model of volanesorsen was developed using the pooled data from 6 of the 7 clinical studies. An exposure-response analysis was conducted to characterize the relationship of volanesorsen exposure with efficacy and safety. Furthermore, immunogenicity of volanesorsen was evaluated in studies CS2, CS4, CS6, CS7 and CS16.

### *Absorption*

The pharmacokinetics of volanesorsen after SC administration were investigated in the single and multiple dose ascending study CS1 and are summarised in **Table 17** (single dose) and **Table 18** (multiple dose).

**Table 17.** Summary of pharmacokinetic parameters of volanesorsen after a single SC dose in healthy subjects studies CS1 and CS19 (gMean (% CV) or median (range)

| dose (mg) | # of subjects | $AUC_{0-24h}$ (µg*h/mL) | $C_{max}$ (µg/mL) | $t_{max}$ (h) | $CL_{0-24h}/F$ (L/h) | $MRT_{0-24h}$ (h) |
|---|---|---|---|---|---|---|
| 50 | 3 | 9.01 (42) | 1.12 (49) | 2.0 (2.0-4.0) | 6.5 (54) | 6.4 (14) |
| 100 | 3 | 22.2 (24) | 2.19 (33) | 3.0 (3.0-4.0) | 4.7 (23) | 7.0 (16) |
| 200 | 3 | 34.6 (44) | 2.84 (45) | 4.0 (3.0-6.0) | 6.9 (56) | 8.1 (12) |
| 300 | 46 | 128 (29.1) | 11.8 (36.3) | 3.8 (1.6-10.1) | 2.6 (30.7) | 7.9 (9.1) |
| 400 | 3 | 143 (24) | 13.0 (18) | 3.0 (2.0-6.0) | 2.9 (27) | 7.6 (1.3) |

**Table 18.** Pharmacokinetic parameters of volanesorsen after multiple SC doses in healthy subjects dosed on Day 1, 3, 5, 8, 15 and 22 weekly, Study CS1 (gMean (%CV) and median (range)

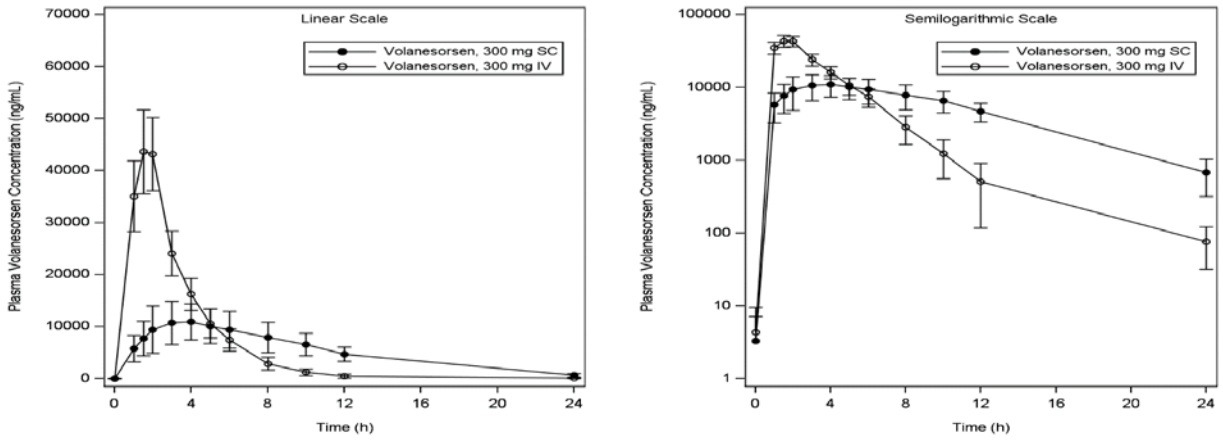
| dose (mg) | # of subjects | Day | $AUC_{0-24h}$ (µg*h/mL) | $C_{max}$ (µg/mL) | $C_{trough}$ (ng/mL) | $t_{max}$ (h) | $CL_{0-24h}/F$ (L/h) | $MRT_{0-24h}$ (h) |
|---|---|---|---|---|---|---|---|---|
| 50 | 3 | 1<br>22 | 4.11 (89)<br>8.30 (31) | 0.66 (56)<br>0.89 (27) | 3.45 (30) | 2.0 (1.5-2.0)<br>3.0 (3.0-8.0) | 19.2 (64)<br>6.5 (37) | 4.9 (24)<br>6.6 (8.9) |
| 100 | 3 | 1<br>22 | 20.9 (26)<br>24.5 (38) | 2.05 (48)<br>2.9 (49) | 19.3 (26) | 4.0 (3.0-6.0)<br>3.0 (3.0-4.0) | 5.0 (23)<br>4.5 (34) | 7.3 (13)<br>7.1 (12) |
| 200 | 3 | 1<br>22 | 49.1 (3.4)<br>50.6 (13) | 4.01 (20)<br>4.12 (17) | 79.2 (7.2) | 2.0 (1.5-3.0)<br>3.0 (3.0-4.0) | (4.1 (3.5)<br>4.0 (12) | 8.6 (13)<br>9.0 (6.9) |
| 400 | 4/3 | 1<br>22 | 128 (31)<br>121 (33) | 10.2 (30)<br>9.6 (37) | 98.2 (64) | 3.5 (1.5- | 3.4 (29) | 8.7 (12)<br>8.8 (16) |

| | | |
|---|---|---|
| | 4.0) 4.0 (3.0-6.0) | 3.6 (36) |

Following SC administrationin healthy subjects, volanesorsen was absorbed rapidly into the systemic circulation withmedian $t_{max}$ ranging from 2 to 4 hours. After reaching $C_{max}$, plasma volanesorsen concentrations declined in a multi-phasic fashion, with an initial, relatively rapid disposition phase that dominated the plasma clearance, followed by a slower elimination phase. The elimination half-life was 2 to 4 weeks with a half-life of ~2 weeks at the 200 and 400 mg dose.

The bioavailability of volanesorsen was investigated in study CS13 (**Figure 5**). The observed bioavailability after SC administration is approximately 80% and most likely higher because an AUC of 0 to 24 hours was used and volanesorsen has a half-life of >2 weeks.

**Figure 5.** Mean (± standard deviation) plasma concentration of volanesorsen versus time following single SC and IV 300 mg volanesorsen dose (Study CS13).




Population PK analysis identified SC injection site as a statistically significant covariate influencing the bioavailability with a 13% lower bioavailability in the arm compared to abdomen. In addition, SC injection in the thigh led to a 12% higher exposure compared to the abdomen. This does not lead to a clinically-relevant difference in exposure.

## *Distribution*

The volume of distribution of volanesorsen is ~251 L in healthy subjects and ~300 L in patients with familial chylomicronemia syndrome.

Volanesorsen has a high plasma protein binding as determined by an ultrafiltration method (study **304801-IS04**). At clinically relevant concentrations ($C_{max}$ = ~110 µg/ml and $C_{trough,ss}$ is 0.133 µg/ml), the plasma protein binding decreases from >99% to 98.0%, indicating that the free fraction increase from <1% to 2%.

The blood-to-plasma ratio of volanesorsen the blood-to-plasma ratio was ~0.56 over a dose range of 0.05 µg/ml to 5 µg/ml.

### *Elimination*

The plasma elimination half-life ranged from 11.7 days to 31.2 days with shorter half-lives observed for the 200 and 400 mg dose (study CS1).

Volanesorsen is metabolised via endonuclease hydrolysis at various positions within the deoxyphosphorothioate gap of volanesorsen followed by subsequent 3'- and 5'-exonuclease hydrolysis of the exposed deoxynucleoside ends of the formed metabolites. These metabolites are pharmacologically not active The chain-shortened oligonucleotide metabolites of volanesorsen are mainly excreted in urine, mainly as 7-mer to 5-mer metabolites.

No human mass balance study was performed due to the long elimination half-life (2 to 4 weeks) and the slow excretion of radioactivity in the mass balance studies in animals. For theses reasons absence of a mass balance study in humans is considered acceptable. Following IV administration of volanesorsen, urinary excretion of parent compound over a period of 0 to 24 hours is higher compared to CS administration (4.6% versus 0.4%) (study CS13).

Urinary excretion of parent compound within the first 24 hours after a single SC dose of volanesorsonaccounted for less than 1% of administered dose and was generally independent of dose level (studies CS1 and CS13). Urinary excretion as parent in the second 24-hour interval (24-48h) after a single SC dose was generally lower compared to the first 24 hours (study CS1). Volanesorsen is excreted slowly in urine, <1% up to 2.8% of the administered dose over 24 hours post multiple SC administrations was excreted as volanesorsen (study CS1). The urinary excretion of volanesorsen-equivalent total oligonucleotide over 24 hours post multiple SC administrations of 400 mg amounted to 16.2 ± 11.7% of the dose. Furthermore, urinary excretion of volanesorsen over a 24-hour interval on Day 22 following multiple SC administration was higher than that seen on Day 1, likely due to contributions from preceding doses and ranged from 0.5% to 2.5% of the last dose administered.

The renal clearance ($CL_R$) was 2 orders of magnitude lower than the apparent total body clearance (CL/F).

### *Dose proportionality and time dependencies*

Dose proportionality was assessed on the $C_{max}$ and AUC after single-dose and repeat-dose administration in healthy volunteers (study CS1). The $C_{max}$ increased approximately proportionally with dose at doses ranging from 50 to 400 mg (slope ranged from 1.08 to 1.28 using linear regression analyses of the log-transformed data) following both single and multiple doses. The AUC values increased slightly greater than proportionally over the same dose range (slope ranged from 1.26 to 1.61). However, the AUC increased nearly dose proportionally in the dose range of 100 to 400 mg on Day 22. Furthermore, the half-life of volanesorsen remained constant within the evaluated dose range and did not change with repeated dose administration.

Time dependencies were investigated in healthy volunteers (study CS1**, Table 19**) and patients (Study CS2, **Table 20**)

**Table 19.** PK of volanesorsen in healthy volunteers after multiple over single SC dose (study CS1)

| dose (mg) | $AUC_{0-24h}$ ratio | $C_{max}$ ratio | $C_{trough}$ ratio |
|---|---|---|---|
| 50 | 2.51 | 1.44 | ND |
| 100 | 1.14 | 1.41 | 8.8 |
| 200 | 1.02 | 1.03 | 14.3 |
| 400 | 0.95 | 0.92 | 7.9 |

**Table 20.** PK of volanesorsen in patients after multiple over single SC dose (study CS2).

| dose (mg) | $AUC_{0-24h}$ ratio | $C_{max}$ ratio | $C_{trough}$ ratio |
|---|---|---|---|
| 100 | 1.36 | 2.16 | 10.6 |
| 200 | 1.25 | 0.79 | 10.9 |
| 300 | 0.96 | 1.03 | 6.9 |

Steady state is reached after 13 to 19 weeks once weekly 300 mg volanesorsen SC which results in a $C_{trough,ss}$ of 127 ng/mL (range of 51.3-195 ng/mL), a $C_{max,ss}$ of 8.92 µg/mL (range of 4.9-15.0 µg/ml), and an $AUC_{0-\tau,ss}$ of 136 µg × h/ml (range of 72.3-236 µg × h/ml).

***Inter- and intrasubject variability***

The predicted inter-individual variability in immunogenicity-negative patients using the population PK model and was ~36% for the $C_{max,ss}$ and $AUC_{0-168,ss}$ and 58% for the $C_{trough,ss}$. The intra-individual variability in immunogenicity-negative patients as estimated by the residual variability from the population PK model was 27.9%. Intersubject variability was higher in in immunogenicity-positive patients.

## *Pharmacokinetics in the target population*

Pharmacokinetics of volanesorsen was investigated in patients with familial chylomicronemia syndrome (studies **CS2**, **CS6** and **CS7**), but also in patients with hypertriglyceridemia (studies **CS2** and **CS16**) and patients with type 2 diabetes mellitus (study **CS4**). The PK in patients with familial chylomicronemia syndrome was reasonably similar to that in healthy volunteers. Also patients with high triglyceride without diabetes and patients with high triglyceride and diabetes had a similar PK to that in healthy volunteers.

Plasma trough concentrations of volanesorsen monitored during the treatment and post-treatment follow-up period were similar between different patient groups (data not shown). The terminal elimination phase following the last SC dose of volanesorsen showed a half-life of ~2 to 5 weeks over the dose range studied. Steady-state was approached approximate at the end of the 13-week treatment period with $C_{max,ss}$ and $AUC_{0-24,ss}$ values of 7.1 µg/ml and 94 µg*h/ml, respectively (study CS2). $C_{trough,ss}$ values range are ~150 ng/ml after a dose of 300 mg volanesorsen once weekly. The $C_{trough,ss}$ for 300 mg biweekly is ~50% less than that for 300 mg once weekly dosing (study CS16 and PopPK modelling).

## *Special populations*

The effect of weight, gender, race, age and impaired renal function on the PK of volanesorsen was investigated using Population PK (report ISIS304801-PPK01). Furthermore, reduced dose frequency to 300 mg bi-weekly (300 mg every two weeks) for patients experiencing platelet decreases was modelled (report ISIS304801-PPK01). The analysis dataset consisted of 256 subjects (74 healthy volunteers, 41 familial chylomicronemia syndrome patients, 50 high triglyceridemia patients with diabetes, and 91 high triglyceridemia patients without diabetes) and a total of 2871 plasma concentrations. The model was a 2-compartment, linear model with first order absorption (for SC) and first order elimination from the central compartment. The Population PK model appears to be

sufficiently validated and suitable to predict the effect of age, gender, race, and disease on the PK of volanesorsen.

***Impaired renal function***

Renal function as represented by baseline eGFR, calculated by the MDRD equation, was tested as a covariate on clearance of volanesorsen in the population PK analysis. Among 256 subjects included in the population PK analysis, 137 subjects had normal renal function (eGFR ≥90 mL/min/1.73 $m^2$), while 112 subjects fell in the mild impairment group at Baseline (eGFR 60 to <90 mL/min/1.73 $m^2$), 7 subjects in the moderate impairment group (eGFR 30 to <60 mL/min/1.73 m2), and none in the severe impairment group.

Mild to moderate renal impairment had no statistically significant effect on the PK of volanesorsen. The effect of severe renal impairment on the PK of volanesorsen was not investigated, but is not expected to affect the PK of volanesorsen.

The limited number of patients with moderate or severe renal impairment is because they were excluded from the studies per the protocol exclusion criteria (creatinine clearance calculated by Cockcroft-Gault formula <50 or 60 ml/min).

***Impaired hepatic function***

No subjects with hepatic impairment were included in the PK studies of volanesorsen. However, no effect on the PK is expected from a decreased hepatic function, since volanesorsen is mainly metabolised by tissue nucleases.

***Gender***

Of the 256 subjects included in the population PK analysis, 166 were male and 90 were female. Sex has no statistically significant effect on the clearance of volanesorsen (clearance was 9.4% higher in males compared to females).

**Race**

Among the 256 subjects included in the population PK analysis, 202 patients were Caucasian, 37 were Black, 13 were Asian, and 4 were Other. Race was determined to have no statistically significant effect on the clearance of volanesorsen (clearance was 13% lower in African-American and 1.3% lower in Asians).

**Body weight**

The effect of weight on the PK of volanesorsen was investigated over a range of 37 to 140 kg. No clinically significant effect was observed on the PK of volanesorsen over this weight range; the Cmax and AUC were 16 %and 12% higher in the 37 to 76 kg body weight group and 10%to 8% lower in the 94 to 140 kg body weight group compared to the 76 to 94 kg body weight group, respectively.

***Age***

A total of 25 patients ≥65 years of age were treated with volanesorsen. No clinically significant effect on the pharmacokienics was observed for patients aged ≥65 years of age versus patients <65 years of age. Limited information on the pharmacokinetics is available for subjects >75 years (N=2), but the pharmacokinetics appears similar compared to subjects <65 years of age.

***Bi-weekly dosing regimen***

Reduced dose frequency to 300 mg biweekly (300 mg every two weeks) was modelled to help patients experiencing platelet decreases to retain these patients on volanesorsen. $C_{trough}$ after 1 year reduced from 127 ng/ml (51.3-295 ng/ml) when dosed once weekly to 58.0 n/ml (23.1-137 ng/ml) when dosed

biweekly. The simulated steady-state $C_{trough}$ after once weekly dosing was in good agreement with the observed Week 13 mean steady-state $C_{trough}$ of 119 ng/ml in FCS patients (study CS6).

***Immunogenicity***

Antibodies to volanesorsen were formed in 30% of the patients with familial chylomicronemia syndrome treated with volanesorsen (study **CS6**). In the other studies, the formation of anti-drug antibodies ranged from 16% to 40% (studies **CS2**, **CS4**, and **CS16**). The formation of the antibodies was characterised by a late onset (median onset was 6 months) and low antibody titres. Once formed the anti-drug antibodies were generally sustained. No consistent trend between duration of treatment, dose level and immunogenicity incidence was identified. The presence of anti-drug antibodies increased $C_{trough}$ levels of volanesorsen up to 19-fold (ranges from 2-fold to 19-fold).

### *Pharmacokinetic interaction studies*

Volanesorsen is not a substrate of CYP enzymes or drug transporters in *in vitro* studies.

Volanesorsen at a range of 0.1-100 µM was investigated as a CYP inhibitor (study 304801-IS07) or CYP inducer (study 304801-IS06). At these concentrations, it did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 nor did it cause induce CYP1A2, CYP2B6, or CYP3A4 at either the mRNA level or enzyme activity level.

Study 304801-IS05 investigated the potential of volanesorsen to act as an inhibitor of various drug transporters, and was found not to inhibit BCRP, P-glycoprotein, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 and BSEP at clinically relevant concentrations (1.6 µM).

The population PK analysis showed that co-administration with fibrates, other lipid modifying agents, HMG CoA reductase inhibitors, or platelet aggregation inhibitors (excluding heparin) did not result in a clinically-relevant alteration in the PK of volanesorsen.

## 2.4.3. Pharmacodynamics

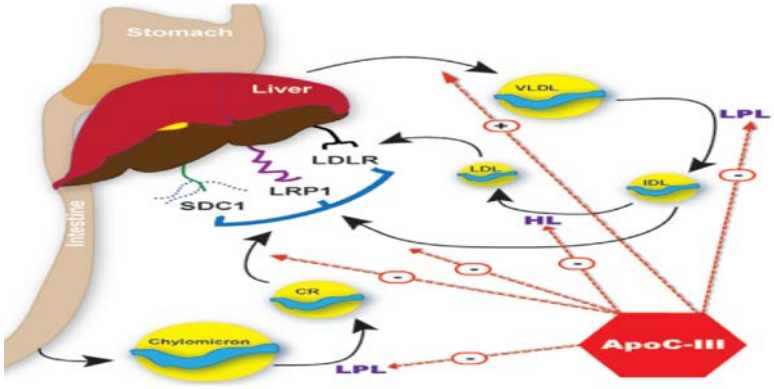
### *Mechanism of action*

Volanesorsen is a 2′-*O*-2-methoxyethyl (2′-MOE) antisense oligonucleotide (ASO) inhibitor of the molecular target apoC-III, a key regulator of TG metabolism.

Antisense technology interrupts the protein production process by degrading the target messenger ribonucleic acid (mRNA) and thus reducing the translation of the specific protein. Antisense inhibitors (ASOs) are designed to be sequence specific with a high binding affinity to their unique target mRNA. Volanesorsen is designed to bind to a specific segment within the 3′-untranslated region of the human apoC-III mRNA and promote a reduction in apoC-III protein.

Initially, apoC-III was believed to regulate plasma TG levels through inhibition of LPL activity. However, since volanesorsen therapy also reduced TG in subjects who genetically lack any functional LPL activity, ApoC-III also regulates TG levels through non-LPL mediated pathways, specifically, through inhibition of hepatic receptor-mediated clearance pathways mediated by the low-density lipoprotein cholesterol (LDL-C) and low density lipoprotein receptor-related protein 1 (LRP-1) receptors (**Figure 6**).

**Figure 6.** ApoC-III Regulation of Lipoprotein Metabolism




*Abbreviations: ApoC-III = apolipoprotein C-III; CR = chylomicron remnant; HL = hepatic lipase; IDL=intermediate-density lipoprotein; LCAT = lecithin cholesterol acyltransferase; LDL = low-density lipoprotein; LDLR = LDL receptor; LPL = lipoprotein lipase; LRP1 = low density lipoprotein receptor-related protein 1; SDC1 = syndecan 1; VLDL = very low-density lipoprotein.*

FCS is primarily a consequence of impaired triglyceride-rich lipoprotein (TRL) clearance, manifested by the severe elevation in plasma TG, chylomicrons, and/or very low-density lipoproteins (VLDL). Levels of the apoC-III protein, which reside on the surface of apoB containing lipoproteins and high density lipoproteins, are elevated in patients with FCS and the majority of apoC-III is associated with TG-rich lipoproteins (TLRs) in these patients. Volanesorsen therapy causes suppression of the ApoC-III protein, which leads to reductions in plasma TG in FCS patients.

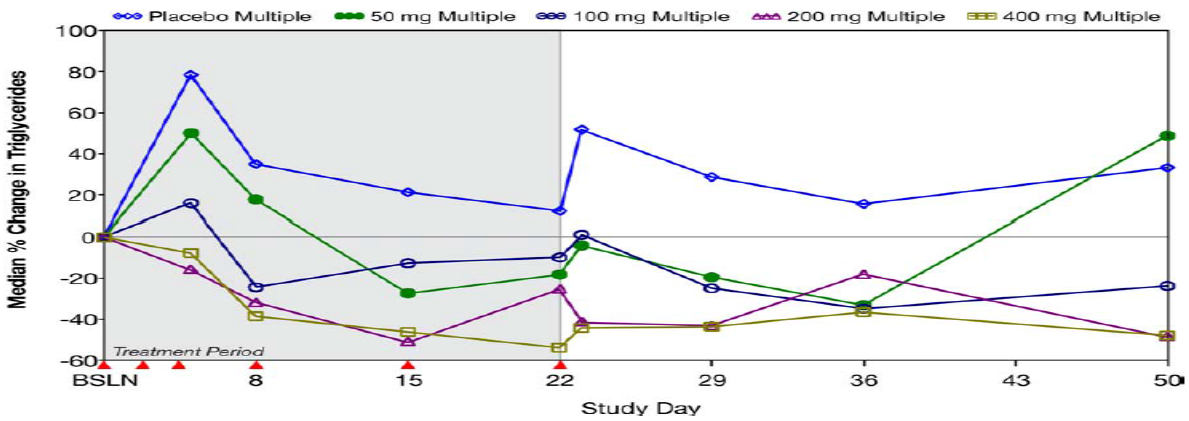
## *Primary and Secondary pharmacology*

*Healthy subject pharmacodynamics in a single- and multiple-ascending dose study*

The proof-of concept first in human study (study CS1) was conducted in 33 healthy subjects.

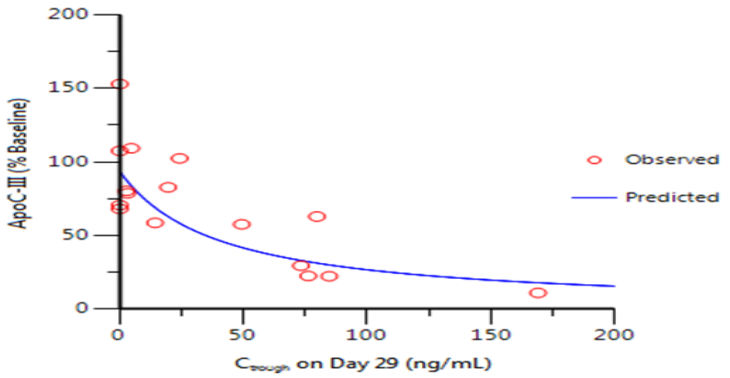
Volanesorsen administered by SC injection on alternate days during the first week (Days 1, 3 and 5) and then once-weekly for the next 3 weeks (Days 8, 15 and 22), resulted in dose-dependent substantial reductions in ApoC-III of 20%, 17%, 71%, and 78% on Day 29, 1 week after the last dose, at doses of 50, 100, 200, and 400 mg, respectively , which was associated with dose-dependent reductions in TG of 20%, 25%, 43%, and 44%, respectively (**Figure 7**).

**Figure 7.** Dose-dependent reductions in triglycerides (study CS1)



An apparent exposure-response relationship with respect the plasma trough concentration (expected to be in equilibrium with target tissue exposure) and apoC-III level was described with an inhibitory effect $E_{max}$ model (**Figure 8**). The model-estimated plasma trough concentration that produced 50% of maximum drug-induced inhibitory effect ($IC_{50}$) for apoC-III was 39.9 ng/mL.

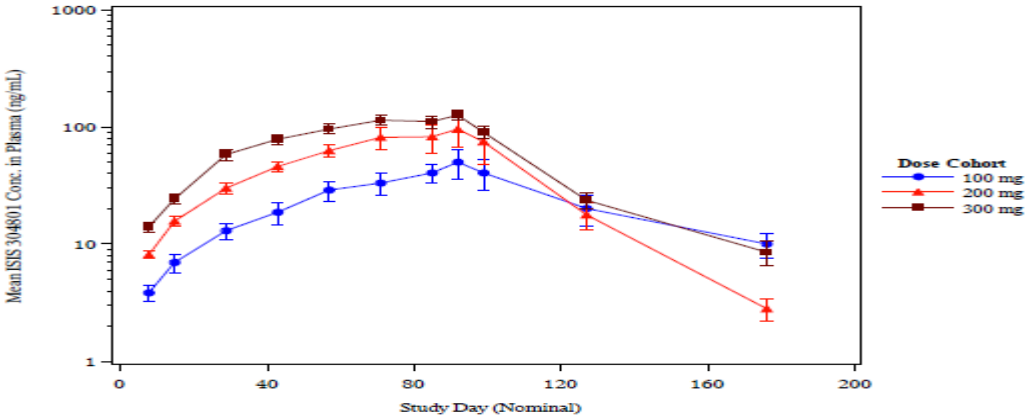
**Figure 8.** Relationship between Volanesorsen Plasma Trough Concentrations and ApoC-III on Day 29 in Multiple-Dose Cohorts in Study CS1




*Exposure-response relationship in phase 2*

In the phase 2 study CS2, a randomized, double-blind placebo-controlled dose response study, the PK/PD relationship of 100, 200, and 300 mg volanesorsen administered weekly has clearly been demonstrated. Plasma trough concentrations were dose-dependent and increased gradually with time, achieving steady state at 13 weeks (**Figure 9**).

**Figure 9.** Mean (± Standard Error) Plasma Trough and Post-Treatment Concentrations of Volanesorsen in Patients with Hypertriglyceridemia following Once-Weekly Subcutaneous Administration for 13 Weeks in Study CS2




At the end of 13 weeks treatment, plasma concentrations of volanesorsen decreased slowly with a half-life of ~2 to 5 weeks, which was independent of the dose. Volanesorsen monotherapy was associated with dose dependent reductions in apoC-III of -40%, -64%, and -80% (**Figure 10**) and TG reductions of -31%, -58%, and -71% **Figure 11**) at doses of 100, 200, and 300 mg, respectively. The plasma trough concentrations at steady-state, a surrogate of target tissue exposure, were positively correlated with these dose-dependent reductions in ApoC-III and TG (**Figure 12**). Highest levels of ApoC-III and TG reduction were achieved with the 300 mg volanesorsen dose.

The exposure-response relationship was further evaluated using an inhibitory effect $E_{max}$ model. The model-estimated plasma $IC_{50}$ were 36.3 and 44.5 ng/ml for the ApoC-III and TG respectively.

**Figure 10.** Dose-Dependent Prolonged Reduction in Fasting ApoC-III in Patients with Hypertriglyceridemia after Volanesorsen Monotherapy

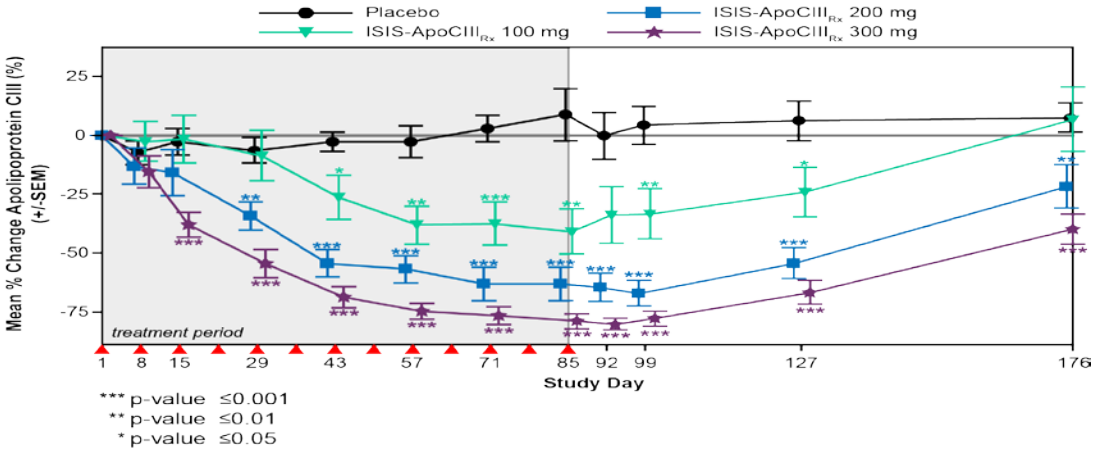



**Figure 11.** Dose-Dependent Prolonged Reduction in Triglycerides in Patients with Hypertriglyceridemia after Volanesorsen Monotherapy

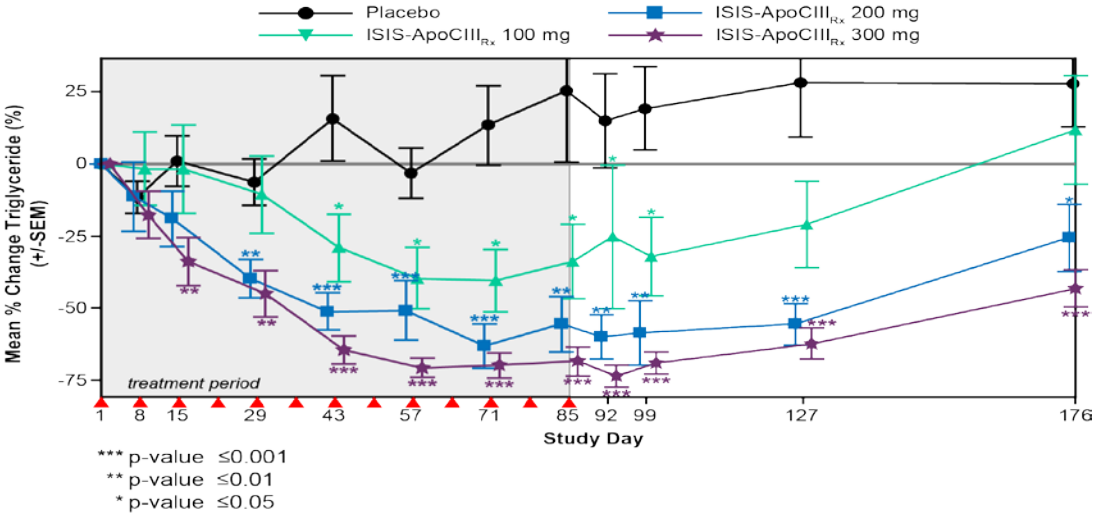
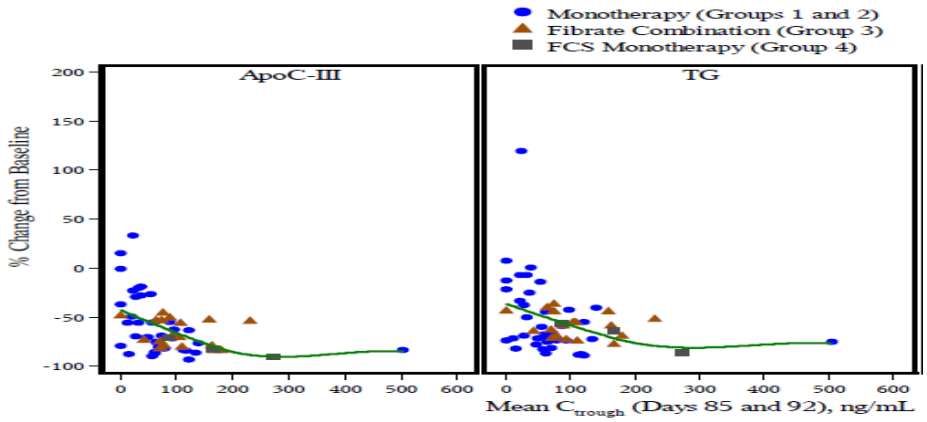


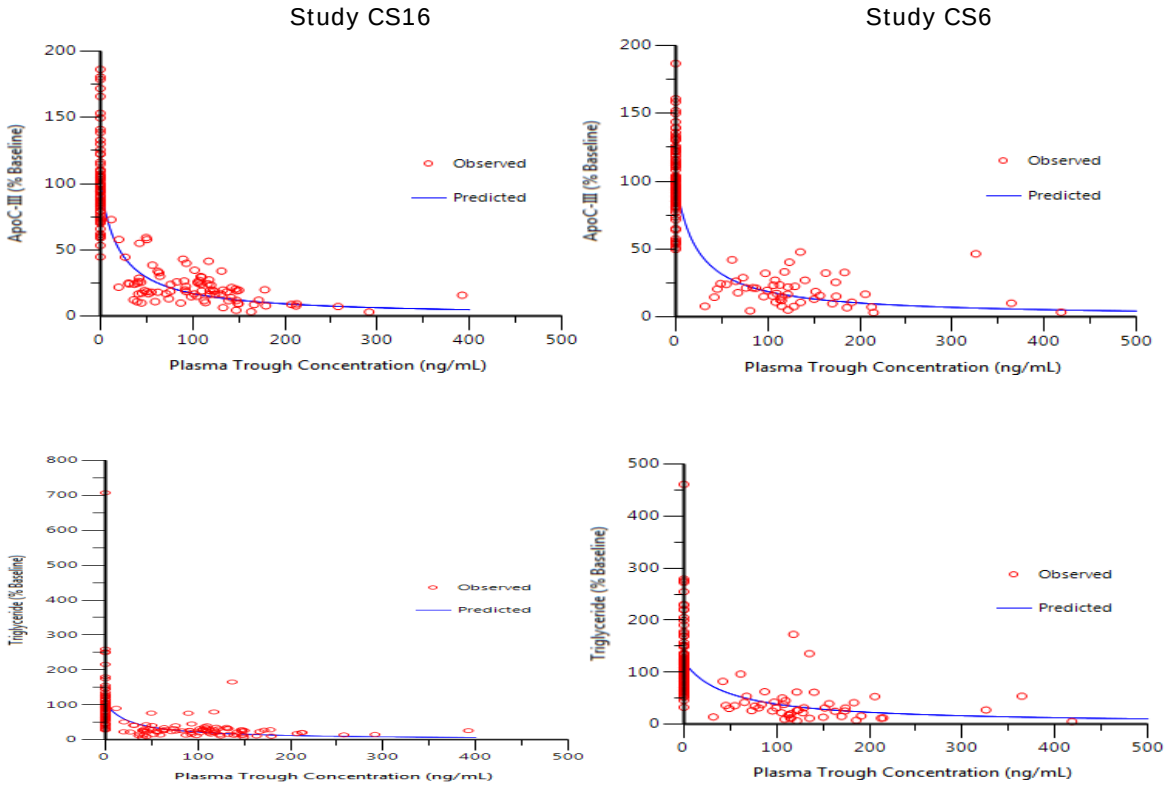

**Figure 12.** Correlation of Volanesorsen Plasma Trough Concentration at Steady-State with Percent Change from Baseline in ApoC-III and TG in Study CS2



*Exposure-response relationship in phase 3*

Similar exposure-response relationships as compared with the phase study CS2 were observed in the the two phase 3 studies, *i.e* study CS6 conducted in patients with FCS and in study CS16 conducted in patients with hypertriglyceridemia. For each of studies CS6 and CS16, time-matched $C_{trough}$ and apoC-III and TG data at Months 3, 6, and 12 in immunogenicity-negative patients were pooled and fit to an inhibitory effect $E_{max}$ model assuming a complete inhibition of apoC-III and TG at infinite $C_{trough}$ as was done for Phase 1 and 2 studies. The model-estimated plasma $IC_{50}$ values were 21.0 ng/mL (study CS16) and 23.0 ng/mL (study CS6) for apoC-III and 26.6 ng/mL (study CS16) and 47.3 ng/mL (study CS6) for TG (**Figure 13**).

**Figure 13.** Inhibitory Effect Emax Model of Percent of Baseline ApoC-III (Top) and TG (Bottom) as a Function of Observed Plasma Trough Concentrations of Volanesorsen in Study CS16 and Study CS6




*PK/PD modelling*

The exposure-TG response relationship was further characterized using population PK model-predicted $C_{trough}$ values and observed TG responses in individual FCS patients in study CS6. The results of the % change in TG vs. $C_{trough}$ analysis at Month 3 show that all four $C_{trough}$ quartiles of volanesorsen achieve a mean reduction of 65-75% from Baseline, indicating a robust TG response across the range of exposure resulting from the 300 mg weekly dose.

*Immunogenicity*

In the phase 3 study CS6, of the 33 volanesorsen-treated patients, 10 (30%) patients tested positive for ADA during the study (vs 3% in placebo). ADA-positive patients in the volanesorsen group had similar TG reduction (-75.6% at Month 3, -69.1% at Month 6 and -39.2% at Month 12) as those who remained ADA-negative (-70.2% at Month 3, -58.6% at Month 6 and -50.3% at Month 12).

In the phase 3 study CS16, of the 75 volanesorsen-treated patients, 12 (16%) patients tested positive for ADA during the study (vs 5 in placebo). Similarly, ADA-positive patients in the volanesorsen group

had similar TG reduction (-73.0% at Month 3, -57.7% at Month 6) as those who remained ADA-negative (-72.7% at Month 3, -68.6% at Month 6).

The formation of the antibodies was characterised by a late onset (median onset was 6 months) and low antibody titres. Once formed the anti-drug antibodies were generally sustained. No consistent trend between duration of treatment, dose level and immunogenicity incidence was identified. The presence of anti-drug antibodies increased $C_{trough}$ levels of volanesorsen up to 19-fold (ranges from 2-fold to 19-fold), but did not affect the terminal elimination half-life (studies CS6 and CS16).

## 2.4.4. Discussion on clinical pharmacology

***Pharmacokinetics***

Following subcutaneous injection, peak plasma concentrations of volanesorsen are typically reached in 2 to 4 hours. The absolute bioavailability of volanesorsen following a single subcutaneous administration is approximately 80% (most likely higher because an AUC of 0 to 24 hours was used and volanesorsen has a half-life of >2 weeks).

Following a dose of 300 mg once weekly in patients with FCS, the estimated geometric mean (coefficient of variation % of geometric mean) steady-state $C_{max}$ is 8.92 µg/ml (35%), $AUC_{0-168h}$ is 136 µg*h/ml (38%), and $C_{trough}$ is 127 ng/ml (58%) in patients who remain negative for anti-drug antibody. An alternative dosing regimen of 300 mg volanesorsen every two weeks results in a $C_{trough,ss}$ of approximately 58.0 ng/ml with $C_{max}$ and AUC similar compared to the once weekly dosing regimen.

Single- and multiple-dose pharmacokinetics of volanesorsen in healthy volunteers and patients with hypertriglyceridemia have shown that the $C_{max}$ of volanesorsen is dose-proportional over a dose range of 100 to 400 mg and the AUC is slightly more than dose-proportional over the same dose range. Steady-state was reached approximately 3 months after starting volanesorsen. Accumulation in $C_{trough}$ was observed (7- to 14-fold) and little or no increase in $C_{max}$ or AUC was observed following weekly SC administration over a dose of 200 to 400 mg. Volanesorsen was rapidly and widely distributed to tissues following subcutaneous or intravenous administration. The estimated steady-state volume of distribution ($V_{ss}$) in patients with FCS is 330 L. Volanesorsen is highly bound to human plasma proteins (>98%) and the binding is concentration independent.

Elimination involves both metabolism in tissues and excretion in urine. Urinary recovery of the parent drug was limited in humans with < 3% of administered subcutaneous dose recovered within 24 hours post dose. The parent drug and 5- to 7-mer chain-shortened metabolites accounted for approximately 26% and 55% of oligonucleotides recovered in urine, respectively. Following subcutaneous administration, terminal elimination half-life is approximately 2 to 5 weeks.

*In vitro* studies show that volanesorsen is not a substrate or inhibitor of P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporting polypeptides (OATP1B1, OATP1B3), bile salt export pump (BSEP), organic cation transporters (OCT1, OCT2), or organic anion transporters (OAT1, OAT3).

Volanesorsen is not a substrate for CYP metabolism, and is metabolised in tissues by endonucleases to form shorter oligonucleotides that are then substrates for additional metabolism by exonucleases. Unchanged volanesorsen is the predominant circulating component.

*In vitro* studies indicate that volanesorsen is not an inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 or inducer of CYP1A2, CYP2B6, or CYP3A4.

Taking into account the *in vitro* study results, the potential for drug-drug interactions with volanesorsen was considered low and therefore the lack of formal drug-drug interaction studies was acceptable.

Special Populations

*Renal impairment*

A population pharmacokinetic analysis suggests that mild and moderate renal impairment has no clinically relevant effect on the systemic exposure of volanesorsen. The effect of severe renal impairment on the pharmacokinetics of volanesorsen was not investigated, but is not expected to affect the PK of volanesorsen. *Hepatic impairment*

The pharmacokinetics of volanesorsen in patients with hepatic impairment is unknown. However, no effect on the PK is expected from a decreased hepatic function, since volanesorsen is mainly metabolised by tissue nucleases.

*Age, sex, weight, and race*

Based on the population pharmacokinetic analysis, age, body weight, sex, or race has no clinically relevant effect on volanesorsen exposure. There are limited data available in subjects >75 years of age.

***Pharmacodynamics***

Volanesorsen is an antisense oligonucleotide designed to inhibit the formation of apoC-III, a protein that is recognised to regulate both triglyceride metabolism and hepatic clearance of chylomicrons and other triglyceride-rich lipoproteins. The selective binding of volanesorsen to the apoC-III messenger ribonucleic acid (mRNA) within the 3′ untranslated region at base position 489-508 causes the degradation of the mRNA. This binding prevents translation of the protein apoC-III, thus removing an inhibitor of triglyceride clearance and enabling metabolism through an LPL-independent pathway.

The proof of concept study for volanesorsen showed that the dose response for decrease in ApoC-III over time correlated well with the dose responses for a decrease in TG. Furthermore, these reductions gradually returned to baseline after the last dose, consistent with the long terminal elimination half-life of volanesorsen (~2-5 weeks).

Furthermore, results from the PK/PD model support the clinical use of the proposed 300 mg volanesorsen once weekly dosing regimen. Modelling and simulation results are supportive of starting with a lower dose with option to increase the dose particularly for patients of >70kg who are underexposed to the drug and are at lower risk of experiencing thrombocytopenia based on modelling and simulation results.

Relatively high incidences in anti-drug antibodies (ADA) were observed in the phase 3 studies. The presence of anti-drug antibodies increased $C_{trough}$ levels of volanesorsen up to 19-fold (ranges from 2-fold to 19-fold), however, the presence of antibodiesdid not seem to affect the efficacy of volanesorsen since approximately similar reductions in TG in ADA-positive and ADA-negative were found.

The formation of binding antibodies to volanesorsen appeared to increase total $C_{trough}$ by 2- to 19-fold.

## 2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology of volanesorsen is considered to have been adequately characterised from the submitted data and no additional measures are considered necessary.

## *2.5. Clinical efficacy*

### 2.5.1. Dose response study

Study CS2 was a phase 2, randomized, double-blind, placebo-controlled, dose response study of volanesorsen administered SC weekly for 13 weeks in subjects with severe or uncontrolled hypertriglyceridemia.

Study CS2 consisted of 4 patient groups in three treatment arms:

- In the monotherapy arm (n=57), groups 1 and 2 received volanesorsen as monotherapy and were randomized to 3 dose cohorts (100, 200, or 300 mg) or placebo with group 2 consisting of a PK group.
- In the fibrate combination arm (group 3; n= 28) patients on a stable dose of fibrate were randomized to 2 dose cohorts (200 or 300 mg) or placebo
- In the FCS arm (group 4), all three FCS patients received 300 mg volanesorsen open-label

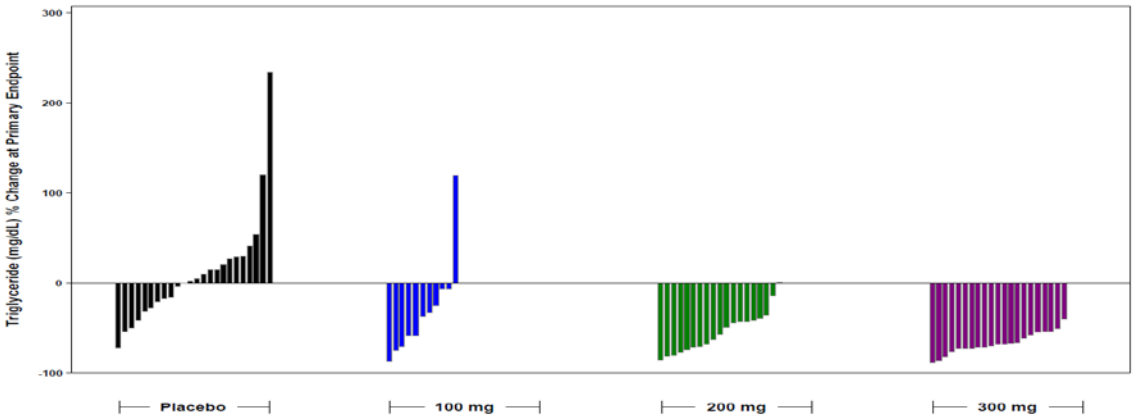
The primary objective was to evaluate the dose/response pharmacodynamic effects versus placebo on mean percent change from baseline in fasting total apoC-III levels with the primary analysis taking place after all patients in group 1 and 2 completed the Day 92 visit (week 13). Secondary objectives were comprised of PD effect on fasting ApoC-III associated with a variety of lipid parameters, PD effects over time, safety, tolerability and PK evaluation.

Pharmacodynamic results from this study are summarised in **Table 21** and individual patient responses in **Figure 14**.

**Table 21.** Pharmacodynamic effects in ApoC-III and TG by patient group and study treatment in Study CS2, Per-Protocol Set

| | **Groups 1 and 2 (Monotherapy)** | | | | **Group 3 (Fibrate Combination)** | | | **Group 4 (FCS)** |
|---|---|---|---|---|---|---|---|---|
| | | Volanesorsen | | | Fibrate + | Fibrate + Volanesorsen | | Volanesorsen |
| | Placebo (n=16) | 100 mg (n=11) | 200 mg (n=13) | 300 mg (n=11) | Placebo (n=8) | 200 mg (n=8) | 300 mg (n=10) | 300 mg (n=3) |
| **ApoC-III** | | | | | | | | |
| Baseline mean (mg/dL) | 22.16 | 22.39 | 23.09 | 22.64 | 19.04 | 15.49 | 18.26 | 24.6 |
| Mean primary endpoint (mg/dL) | 21.86 | 12.03 | 7.62 | 4.39 | 17.74 | 6.09 | 5.11 | 4.11 |
| Mean percent change (SD) from baseline at primary endpoint | 4.2 (41.7) | -40.0 (32.0) P=0.008 | -63.8 (22.3) P<0.001 | -79.6 (9.3) P<0.001 | -2.2 (25.2) | -60.2 (12.5) P<0.001 | -70.9 (13.0) P<0.001 | -81.3 (9.8) |
| **Triglycerides** | | | | | | | | |
| Baseline mean (mg/dL) | 523 | 591 | 642 | 559 | 457 | 282 | 394 | 1844 |
| Mean primary endpoint (mg/dL) | 547 | 312 | 235 | 139 | 372 | 141 | 134 | 546 |
| Mean percent change from baseline at primary endpoint | 20.1 (71.9) | -31.3 (56.8) P=0.015 | -57.7 (28.3) P=0.001 | -70.9 (14.1) P<0.001 | -7.7 (33.8) | -51.0 (13.5) P=0.008 | -63.9 (8.9) P=0.002 | -68.8 (15.6) |

**Figure 14.** Waterfall Plot of Individual Patient TG Response to Volanesorsen Treatment by Dose in Study CS2




The reductions observed in the different groups were sustained for 2 to 6 weeks after the last dose, consistent with the drug's long terminal elimination half-life. Exploratory PK/PD analysis showed good correlation between the plasma volanesorsen trough concentrations at steady-state and serum apoC-III protein and TG levels in all patient groups studied.

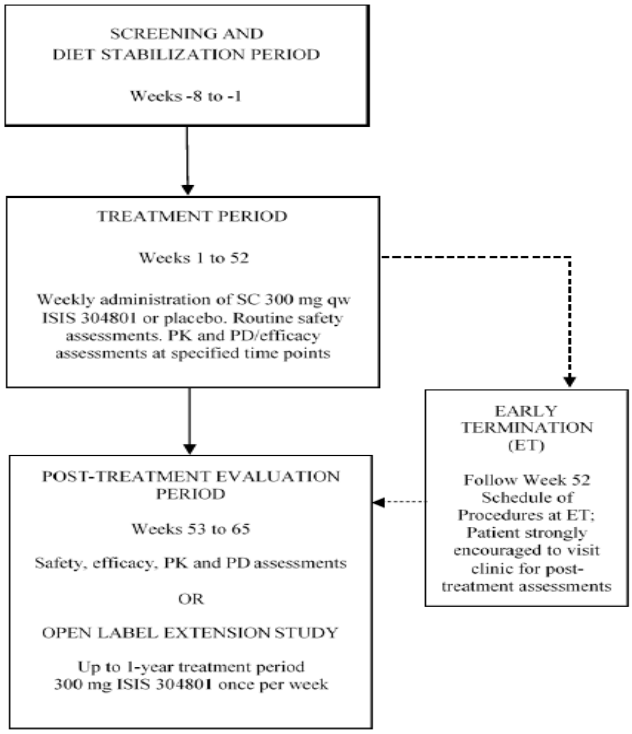
## 2.5.2. Main study

**Study CS6**: A randomized, double-blind, placebo-controlled, phase 3 study of ISIS 304801 administered subcutaneously to patients with familial chylomicronemia syndrome (FCS)

### *Methods*

**Figure 15** provides a schematic of the overall study design.

**Figure 15.** CS6 study design and treatment schema




*Note: Volanesorsen is referred to as ISIS 304801 in this figure*
*Note: Open-label extension study (ISIS 304801-CS7) study was extended. Following the Week 52 visit,*

*patients will have the option of participating in an expanded access program or continuing dosing for up to an additional 52 weeks until an expanded access program is approved and available in their country. Patients not participating in an expanded access program will enter a 13-week post-treatment evaluation period.*
*Abbreviations: PD = pharmacodynamic; PK = pharmacokinetic; qw = once weekly*

## *Study Participants*

Inclusion criteria (selection):

- Age≥ 18 years at the time of informed consent
- History of chylomicronemia as evidenced by documentation of lactescent serum (a creamy top layer after ultracentrifugation of a fasting blood sample) or documentation of fasting TG measurement≥ 880 mg/dL (10 mmol/L)
- A diagnosis of FCS (Type 1 hyperlipoproteinemia) by documentation of at least one of the following:

  a) Confirmed homozygote, compound heterozygote, or double heterozygote for known loss-of-function mutations in Type 1-causing genes (such as LPL, APOC2, GPIHBP1, or LMF1)

  b) Post heparin plasma LPL activity of≤20% of normal
- Fasting TG ≥ 750 mg/dL (8.4 mmol/L) at Screening.
- History of pancreatitis (defined as a documented diagnosis of acute pancreatitis or hospitalization for severe abdominal pain consistent with acute pancreatitis and for which no alternate diagnosis was made). Patients without a documented history of pancreatitis were also eligible but their enrollment was capped at 28% (i.e., ≤ 20 of the 70 planned patients).
- Willing to follow a diet comprising ≤ 20g fat per day during the study.

Exclusion criteria (selection):

- Diabetes mellitus with any of the following:

  a) Newly diagnosed within 12 weeks of Screening

  b) Glycated hemoglobin (HbA1c) ≥ 9.0% at Screening

  c) Recent change in anti-diabetic pharmacotherapy (change in dosage or addition of new medication within 12 weeks of Screening [with the exception of ± 10 units of insulin])

  d) Anticipated need to change dose or type of medication during the treatment period of the study (with the exception of ± 10 units of insulin)

e) Current use of glucagon-like peptide 1 (GLP-1) agonists

- Severe hypertriglyceridemia other than due to FCS
- Acute pancreatitis within 4 weeks prior to Screening
- History within 6 months of Screening of acute or unstable cardiac ischemia (myocardial infarction, acute coronary syndrome, new onset angina, stroke, transient ischemic attack or unstable congestive cardiac failure requiring a change in medication) or major surgery within 3 months of Screening
- LDL-C> 130 mg/dL at Screening
- Uncontrolled hypertension (blood pressure> 160/100 mmHg)

- History of bleeding diathesis or coagulopathy or clinically-significant abnormality in coagulation parameters at Screening
- History of heart failure with New York Heart Association score greater than Class II
- Hepatic laboratory values at screening: Total bilirubin > upper limit of normal (ULN); Alanine aminotransferase (ALT) > 2.0 × ULN; Aspartate aminotransferase (AST) > 2.0 × ULN
- Renal laboratory values at screening: persistently positive (2 out of 3 consecutive tests ≥ 1+) for protein on urine dipstick; Persistently positive (2 out of 3 consecutive tests ≥ 1+) for protein on urine dipstick; estimated creatinine clearance < 50 ml/min.
- Use of any of the following:

  a) Statins, omega-3 fatty acids (prescription or over-the-counter [OTC]), or fibrates unless on a stable dose for at least 3 months prior to Screening and dose and regimen expected to remain constant during the treatment period. Patients taking OTC omega-3 fatty acids should have made every effort to remain on the same brand throughout the study.

  b) Nicotinic acid or derivatives of nicotinic acid within 4 weeks prior to Screening

  c) Systemic corticosteroids or anabolic steroids within 6 weeks prior to Screening unless approved by the Sponsor's Medical Monitor

  d) Atypical antipsychotic medications (e.g., olanzapine and clozapine) unless on a stable dose for at least 3 months prior to Screening and dose and regimen expected to remain stable throughout the study

  e) Antihypertensive medication unless on a stable dose for at least 4 weeks prior to Screening and dose and regimen expected to remain constant during the treatment period

  f) Glybera gene therapy within 2 years prior to Screening

  g) Oral anticoagulants (e.g., warfarin, dabigatran, rivaroxaban, and apixaban), unless on a stable dose for at least 4 weeks prior to Screening and regular clinical monitoring was performed

  h) Tamoxifen, estrogens or progestins, unless on a stable dose for at least 4 months prior to Screening and dose and regimen expected to remain constant during the treatment period

  i) Plasma apheresis within 4 weeks prior to Screening or planned during the study

  j) Prior exposure to volanesorsen

  k) Any other medication unless stable at least 4 weeks prior to Screening (Occasional or intermittent use of OTC medications was allowed at Investigator's discretion)

### *Treatments*

According to the randomized assignment, each individual patient was to be administered 300 mg volanesorsen or placebo SC as a single 1.5 ml injection, once weekly for Weeks 1 to 52.

Self-administration was allowed after appropriate training of patient and/or caregiver.

Treatment with Study Drug (volanesorsen or placebo) was to be paused or permanently discontinued if any of the pre-defined stopping criteria were met:

- ALT or AST > 8×ULN, which was confirmed
- ALT or AST > 5×ULN, which was confirmed and persisted for ≥ 2 weeks
- ALT or AST > 3×ULN (or the greater of 2× baseline value or 3×ULN if the baseline value was > ULN), which was confirmed and total bilirubin > 2×ULN or international normalized ratio (INR) > 1.5
- ALT or AST > 3×ULN (or the greater of 2× baseline value or 3×ULN if the baseline value was > ULN), which was confirmed, and the new appearance (i.e., onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia (> ULN) felt by the Investigator to be potentially related to hepatic inflammation
- Serum creatinine increase that fulfilled all of the following criteria: ≥ 0.3 mg/dL (26.5 μmol/L) and ≥ 40% above baseline creatinine values and > ULN
- Proteinuria, dipstick 2 + (confirmed by dipstick retest and then further confirmed by a quantitative total urine protein measurement of > 1.0 g/24-hour)
- Estimated creatinine clearance calculated according to the formula of Cockcroft and Gault ≤ 40 ml/min that was confirmed by a 24-hour urine collection
- The possible dosing re-initiation or follow-up schedule for any events meeting either of these criteria were determined by the Investigator in consultation with the Sponsor Medical Monitor or designee.

Actions to be taken in the event of a low platelet count are summarised in **Table 22**.

**Table 22.** Actions in Patients with Low Platelet Count in Study CS6

| Platelet Count on | Drug Dose | Monitoring |
|---|---|---|
| Normal range, > 140,000/mm³ | No action | Monitor every 2 weeks |
| 100,000-140,000/mm³ | No action | Closer observation<br>Monitor every 1-week until stable[a] |
| 75,000-100,000/mm³ | Permanently Reduce dose frequency to 300 mg every 2 weeks or reduce dose to 150 mg weekly | Closer observation<br>Monitor every 1-week |
| 50,000-75,000/mm³ | If occurs while on dose of 300 mg every 2 weeks or 150 mg every week then permanently discontinue Study Drug, otherwise dose pause.<br>When platelet count returns to > 100,000/mm³ restart dosing at dose frequency of 300 mg every 2 weeks or 150 mg weekly **only if approved by Sponsor Medical Monitor** | Closer observation<br>Monitor every 2-3 days until 2 successive values show improvement<br>Consider discontinuation of antiplatelet agents/NSAIDS/anticoagulant medication |
| 25,000-50,000/mm³ | If occurs while on dose of 300 mg every 2 weeks or 150 mg every week then permanently discontinue Study Drug, otherwise dose pause. When platelet count returns to > 100,000/mm³ restart dosing at dose frequency of 300 mg every 2 weeks or 150 mg weekly **only if approved by Sponsor Medical Monitor** | Closer observation<br>Monitor daily until 2 successive values show improvement then monitor every 2-3 days until platelet count stable<br>Discontinue antiplatelet agents/NSAIDS/anticoagulant medication while platelet count < 50,000/mm³ if possible |

| Platelet Count on | Drug Dose | Monitoring |
|---|---|---|
| < 25,000/mm³ | Permanently discontinue Study Drug | Closer observation:<br>Monitor daily until 2 successive values show improvement then monitor every 2-3 days until platelet count stable.<br>Steroids recommended [b]<br>Consider need for hospitalization and referral to hematologist<br>Discontinue antiplatelet agents/NSAIDS/anticoagulant medication while platelet count < 50,000/mm³ if possible |

*Note: This includes the clarification updates provided to the sites in a Safety Update letter on 28 June 2016 after the*
*CS6 protocol amendment was submitted*
*Abbreviations: NSAIDS = nonsteroidal antiinflammatory drugs*
*a At least 3 consecutive values measured weekly that are stable as determined by the Sponsor medical monitor and > 100,000/mm3*
*b Recovery in platelet count may be accelerated by administration of high dose steroids. Treatment guidelines for immune thrombocytopenia (Provan et al. 2010) recommend Dexamethasone 40 mg daily for 4 days every 2-4 weeks for 1-4 cycles; Prednis(ol)one 0.5-2 mg/kg/d for 2-4 weeks then taper; or Methylprednisolone 30 mg/kg/day for 7 days (note: may require continuation with oral steroids after methyl prednisolone)*

## *Objectives*

The primary objective of the study was to evaluate the efficacy of volanesorsen (300 mg once weekly) as compared to placebo on the percent change in fasting TG from Baseline to Month 3 in patients with FCS.

The secondary objectives of this study were to evaluate the efficacy of volanesorsen as compared to placebo on the following:

• Postprandial TG change from Baseline

• Absolute change from Baseline in fasting TG

• Proportion of patients who achieve fasting TG < 750 mg/dL

· Proportion of patients who achieve ≥ 40% fasting TG reduction from Baseline

• Patient reported abdominal pain

  o Frequency

  o Severity

• Composite of episodes of acute pancreatitis and patient-reported abdominal pain

• Change from Baseline in hepatosplenomegaly as assessed by MRI

## *Outcomes / endpoints*

The primary endpoint was the percentage change in fasting TG from Baseline to the primary analysis time point at the end of Month 3, where the value was defined as the average of Week 12 (Day 78) and Week 13 (Day 85) fasting assessments.

Secondary Endpoints

The secondary endpoints were rank prioritized and tested according to position in the hierarchy using a sequential closed testing procedure:

1. Treatment response rate, where a patient with fasting plasma TG < 750 mg/dL at the primary analysis time point (3 months) was defined as a responder. Only the subset of patients with baseline fasting plasma TG ≥ 750 mg/dL was included in this population

2. Percent change from baseline to the 6-month time point in fasting TG

3. Percent change from baseline to the 12-month time point in fasting TG

4. Average of maximum intensity of patient reported abdominal pain during the treatment period

5. Postprandial TG area under the curve (AUC)(0-9h) change from Baseline to on-treatment measures (between Week 13 and Week 19)

6. Treatment response rate, where a patient who achieves fasting TG ≥ 40% reduction from Baseline at the primary analysis time point is defined as a responder

7. Absolute change from Baseline to the primary analysis time point in fasting TG

8. Frequency of composite of episodes of acute pancreatitis and patient reported abdominal pain during the treatment period

9. Change from Baseline in hepatic volume as assessed by MRI at Week 52

Exploratory endpoints included:

- Percent change from baseline in fasting apolipoprotein B-48 (apoB-48) and chylomicron TG as measured in the primary analysis time point
- Percent change from baseline in postprandial apoB-48 and chylomicron TG
- Percent change from baseline in fasting apoC-III as measured in the primary analysis time point
- Percent change from baseline in other fasting lipid measurements, including non-HDL-C, apoB, HDL-C, apoA-1, VLDL-C and LDL-C

## *Sample size*

Based upon prior clinical trial experience with volanesorsen, it is estimated that the standard deviation of the percent change in total TG is approximately 46%. With 22 volanesorsen 300 mg once-weekly patients and 22 placebo patients there would be approximately 80% power to detect a 50% difference in TG levels between treatment groups at an alpha level of 0.01, assuming 60% reduction in the volanesorsen treatment patients and 10% reduction in the placebo patients. Approximately 70 patients will be randomized and dosed in this study.

## *Randomisation*

Using an Interactive Voice/Web-Response System (IXRS), eligible, consenting patients were randomized 1:1 to receive volanesorsen or placebo and stratified for:

• Prior history of pancreatitis vs. no history of pancreatitis

• Receiving concurrent fibrate and/or prescription omega-3 fatty acid vs. not receiving these drugs

History of pancreatitis was defined as a documented diagnosis of acute pancreatitis or hospitalization for severe abdominal pain consistent with acute pancreatitis and for which no alternate diagnosis was made.

A permuted block schedule was used.

### *Blinding (masking)*

This was a double-blind study. Placebo and active drug infusions were visually and physiological indistinguishable.

### *Statistical methods*

The primary efficacy analysis was the comparison of the percent change from baseline to the primary analysis time point in fasting TG between ISIS 304801 300 mg once weekly group and placebo group in the Full Analysis Set. The data are analyzed using an ANCOVA model with the two randomization stratification factors (presence or absence of pancreatitis; presence or absence of concurrent omega-3 fatty acids and/or fibrates and T2DM status and log-transformed baseline TG as covariates.

A responder analysis was performed as the key sensitivity analysis. The responder analysis was the comparison of proportion of patients that achieved ≥ 40% reduction in triglyceride from baseline to the primary analysis time point between the 2 groups. The data are analysed using a logistic regression model with the two randomization stratification factors and log-transformed baseline triglyceride as covariates. In order to examine whether the response is consistent over a range of response thresholds, responder analysis with a series of response thresholds (50%, 60%, 70% reduction) is also conducted.

The primary analysis time point is at the end of Month 3 where the value is defined as the average of Week 12 (Day 78) and Week 13 (Day 85) fasting assessments. The Month 6 analysis time point is at the end of Month 6 where the values defined as the average of Week 25 (Day 169) and Week 26 (Day 176) fasting assessments. The Month 12 analysis time point is at the end of Month 12 where the values defined as the average of Week 50 (Day 344) / Week 51 (Day 351) and Week 52 (Day 358) fasting assessments.

The baseline for fasting lipid measurements was defined as the average of Day 1 pre-dose assessment and the last measurement prior to Day 1. The baseline for other measurements is defined as the last non-missing assessment prior to the first dose of Study Drug.

The primary efficacy endpoint was analysed using Wilcoxon rank-sum test for sensitivity analysis, and the treatment effect will be estimated using Hodges-Lehmann estimator. The primary efficacy endpoint was also analysed using the nonparametric Wei-Johnson method for sensitivity analysis.

Patients with a missing primary endpoint have TG value multiply imputed using an imputation model that contains the following variables: baseline TG, TG values at post-baseline visits and the 2 stratification factors, and the multiple imputation will be stratified by treatment (Schafer 1997; Schafer 1999). Any patient who discontinued early from the Treatment Period was strongly encouraged to attend applicable landmark visits including Week 4, Week 8, Weeks 12 and 13 during which TG were collected and used in the primary analysis. The multiple imputations method was not implemented in the primary analysis since there was no missing TG value at the primary endpoint. Missing data were imputed according to protocol for the Months 6 and 12 analyses.

## *Results*

## Participant flow

Patient disposition is summarized in **Table 23.**

**Table 23.** Patient disposition in Study CS6

| Disposition | Placebo n (%) | Volanesorsen 300 mg n (%) | All Patients n (%) |
|---|---|---|---|
| Patients Screened | | | 130 |
| Screen Failures | | | 63 |
| Patients Randomized | 34 | 33 | 67 |
| Patients Dosed | 33 (97.1) | 33 (100.0) | 66 (98.5) |
| Patients Who Completed the Study Treatment | 32 (94.1) | 19 (57.6)[a] | 51 (76.1) |
| Patients Who Terminated from the Study Treatment | 2 (5.9) | 14 (42.4) | 16 (23.9) |
| Before Week 13 | 1 (2.9) | 2 (6.1) | 3 (4.5) |
| After/On Week 13 and Prior to Week 26 | 1 (2.9) | 7 (21.2) | 8 (11.9) |
| After Week 26 | 0 (0.0) | 5 (15.2) | 5 (7.5) |
| Main Reason for Termination | | | |
| Investigator judgment | 0 (0.0) | 1 (3.0) | 1 (1.5) |
| Voluntary withdrawal | 1 (2.9) | 4 (12.1) | 5 (7.5) |
| Adverse event or SAE | 0 (0.0) | 9 (27.3) | 9 (13.4) |
| Other | 1 (2.9) | 0 (0.0) | 1 (1.5) |
| Patients Who Entered the Post-Treatment Follow-up | 29 (85.3) | 29 (87.9) | 58 (86.6) |
| Completed the Post-Treatment Follow-up | 4 (11.8) | 10 (30.3) | 14 (20.9) |
| Terminated from the Post-Treatment Follow-up | 16 (47.1) | 14 (42.4) | 30 (44.8) |
| Main Reason for Termination | | | |
| Voluntary withdrawal | 0 (0.0) | 6 (18.2) | 6 (9.0) |
| Other | 1 (2.9) | 2 (6.1) | 3 (4.5) |
| Patients Who Enrolled Over to the Open-Label Extension study by the 06 Jan 2017 cutoff date | 19 (55.9) | 10 (30.3) | 29 (43.2) |

## Recruitment

Study Start: 19 December 2016 (First patient screened)

Study Finish: 28 March 2017 (Last patient, last visit)

## Conduct of the study

The original protocol, dated 06 June 2014, was amended 8 times. The most important one was Protocol Amendment 6 which was released on 06 June further to reports of two SAEs of Grade 4 platelet count. An enhanced platelet monitoring plan (**Table 24)** was implemented following an Urgent Safety Measures letter dated 27 May 2016.

## Baseline data

**Table 24.** Summary of Patient Demographics in Study CS6 – Full Analysis Set (N = 66)

| DEMOGRAPHIC AND DISEASE CHARACTERISTIC: | PLACEBO (N = 33) | VOLANESORSEN (N = 33) | ALL PATIENTS (N = 66) |
|---|---|---|---|
| **Age at Informed Consent (year)** | | | |
| Mean (SD) | 46 (14) | 47 (13) | 46 (13) |
| **Age Group (years), n (%)** | | | |
| Age <65 years old | 31 (93.9) | 30 (90.9) | 61 (92.4) |
| Age ≥65 years old | 2 (6.1) | 3 (9.1) | 5 (7.6) |
| **Age at FCS Diagnosis (years)** | | | |
| n | 28 | 18 | 46 |
| Mean (SD) | 26 (15 ) | 28 (22) | 27 (18 ) |
| **Sex n (%)** | | | |
| Female | 19 (57.6) | 17 (51.5) | 36 (54.5) |
| Male | 14 (42.4) | 16 (48.5) | 30 (45.5) |
| **Ethnicity - n (%)** | | | |
| Hispanic or Latino | 7 (21.2) | 7 (21.2) | 14 (21.2) |
| Not Hispanic or Latino | 26 (78.8) | 26 (78.8) | 52 (78.8) |
| **Race - n (%)** | | | |
| White | 29 (87.9) | 24 (72.7) | 53 (80.3) |
| Asian | 4 (12.1) | 7 (21.2) | 11 (16.7) |
| Other Race | 0 | 2 (6.1) | 2 (3.0) |
| **Geographic Location - n (%)** | | | |
| Europe | 18 (54.5) | 18 (54.5) | 36 (54.5) |
| North America | 14 (42.4) | 11 (33.3) | 25 (37.9) |
| Other | 1 (3.0) | 4 (12.1) | 5 (7.6) |
| **Body Weight (kg)** | | | |
| Mean (SD) | 67.42 (20.16) | 72.91 (21.46) | 70.16 (20.85) |
| **BMI (kg/m²)** | | | |
| Mean (SD) | 24.1 (4.7) | 25.9 (6.5) | 25.0 (5.7) |
| **Fasting Triglycerides (mg/dL)** | | | |
| Mean (SD) | 2152 (1153) | 2267 (1259) | 2209 (1199) |
| **Documented Diagnosis of Acute Pancreatitis - n (%)** | | | |
| Yes | 26 (78.8) | 24 (72.7) | 50 (75.8) |
| No | 7 (21.2) | 9 (27.3) | 16 (24.2) |
| If No, Severe Abdominal Pain? | | | |
| Yes | 0 (0.0) | 2 (22.2) | 2 (12.5) |
| No | 7 (100.0) | 7 (77.8) | 14 (87.5) |
| **Lipemia Retinalis - n (%)** | | | |
| Yes | 9 (27.3) | 5 (15.2) | 14 (21.2) |
| No | 24 (72.7) | 28 (84.8) | 52 (78.8) |
| **Eruptive Xanthomas Prior to Screening - n (%)** | | | |
| Yes | 9 (27.3) | 6 (18.2) | 15 (22.7) |
| No | 24 (72.7) | 27 (81.8) | 51 (77.3) |
| **History of Type II Diabetes - n (%)** | | | |
| Yes | 4 (12.1) | 6 (18.2) | 10 (15.2) |
| No | 29 (87.9) | 27 (81.8) | 56 (84.8) |
| **Glybera Treatment History - n (%)** | | | |
| Yes | 5 (15.2) | 2 (6.1) | 7 (10.6) |
| No | 8 (84.8) | 31 (93.9) | 59 (89.4) |
| If Yes, how many years has it been? | | | |
| n | 5 | 2 | 7 |
| Mean (SD) | 8 (1) | 8 (1) | 8 (1) |
| **Confirmation for Type 1 Phenotype Based on Genetic Sequencing Results - n (%)** | | | |
| n | 25 (75.8) | 25 (75.8) | 50 (75.8) |
| LPL | 24 (96.0) | 17 (68.0) | 41 (82.0) |
| APOA5 | 1 (4.0) | 1 (4.0) | 2 (4.0) |
| GPIHBP1 | 2 (8.0) | 5 (20.0) | 7 (14.0) |
| LMF1 | 0 (0.0) | 2 (8.0) | 2 (4.0) |
| APOC2 | 0 (0.0) | 1 (4.0) | 1 (2.0) |

## Numbers analysed

All 66 patients took at least 1 dose of study drug and had a baseline TG assessment and therefore were included in the safety set and the Full Analysis Set (FAS).

The Per Protocol Set, which excluded patients with protocol deviations that would be expected to affect efficacy and PD assessments, was comprised of 31 (93%) patients in the volanesorsen group and 31 (91%) patients in the placebo group. Four patients, in whom a pathogenic mutation in a type-1 gene was not identified, entered the study based on low LPL activity. Subsequently, the laboratory data for two of these patients could not be verified and although these were not considered protocol deviations (i.e. they were not due to patient or investigator action or error), the data from these 2 patients were excluded from the PPS analysis. The other two patients were excluded from the PPS due to ineligible LPL activity results because a pre-heparin sample was used for LPL activity analysis. These were considered major protocol violations.

The PK population used for the assessment of plasma volanesorsen concentration comprised all 33 (100%) patients who were randomized to receive volanesorsen.

## Outcomes and estimation

Treatment with volanesorsen 300 mg weekly demonstrated a statistically significant reduction in triglyceride levels in patients with FCS at Month 3 (primary endpoint) (**Table 25**). Results for change in triglyceride levels are also presented for months 6 and 12.

**Table 25,** Percent Change from Baseline to Month 3, 6 and 12 in Fasting Triglycerides (mg/dL) in Study CS6: - Full Analysis Set

| TG Analysis Endpoint | Statistic | Placebo (N = 33) | Volanesorsen 300 mg (N = 33) |
|---|---|---|---|
| | N | 33 | 33 |
| Baseline (mg/dL)[a] | Mean (SD) | 2152 (1153) | 2267 (1259) |
| **Month 3 Primary Endpoint** | | | |
| Month 3 (mg/dL)[b] | Mean (SD) | 2367 (1315) | 590 (497) |
| % Change from Baseline[c] | LS Mean (95% CI) | 17.6 (-4.0, 39.2) | -76.5 (-97.4, -55.5) |
| % Change from baseline vs placebo (95%CI) | | **-94.1** (-121.7, -66.6) p<0.0001 | |
| **Month 6 Endpoint** | | | |
| Month 6 (mg/dL)[b] | Mean (SD) | 2423 (1007) | 815 (600) |
| % Change from Baseline[c] | LS Mean (95% CI) | 25.3 (4.1, 46.5) | -52.5 (-82.0, -22.9) |
| % Change from baseline vs placebo (95%CI) | | **-77.8** (-106.4, -49.1) p<0.0001 | |
| **Month 12 Endpoint** | | | |
| Month 12 (mg/dL)[b] | Mean (SD) | 2307 (1290) | 1178 (948) |
| % Change from Baseline[c] | LS Mean (95% CI) | 8.9 (-19.7, 37.5) | -40.2 (-86.1, 5.7) |
| % Change from baseline vs placebo (95%CI) | | **-49.1** (-94.7, -3.5) p=0.0347 | |

*Abbreviations: CI=confidence interval; LS = least squares; SD=standard deviation;*

[a] *The baseline for fasting triglycerides was defined as the average of Day 1 pre-dose assessment and the last measurement prior to Day 1 pre-dose assessment. If one of the two measurements was missing, then the other measurement was assigned as the baseline value.*

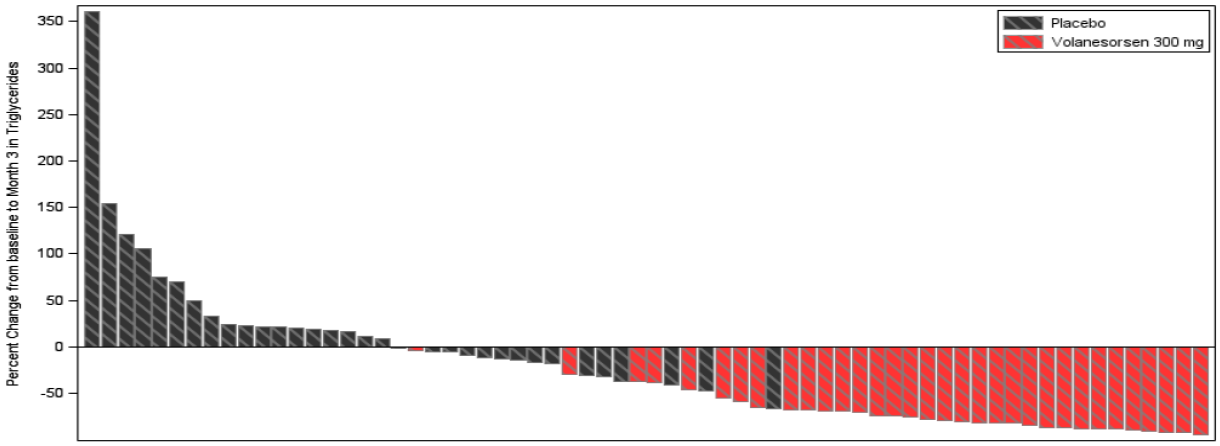
[b] *The Month 3 endpoint was defined as the average of Week 12 (Day 78) and Week 13 (Day 85) fasting assessments. The Month 6 and Month 12 endpoint were defined as the average of Week 25 (Day 169) and*

*Week 26 (Day 176) and Week 50 (Day 344/Week51 (Day 351) and Week 52 (Day 358) fasting assessment, respectively. If one visit was missing, then the other visit was used as the endpoint.*

[c] *The LS means, confidence intervals, and p-values were from an ANCOVA model with percent change from Baseline as the dependent variable, presence of pancreatitis and presence of concurrent omega-3 fatty acids and/or fibrates, and treatment group as factors, and log-transformed baseline as a covariate based on observed data*

Individual patient responses are presented in **Figure 16**.

**Figure 16.** Waterfall Plot of Percent Change in Fasting Triglycerides from baseline to Month 3 - Full Analysis Set




*Secondary endpoints*

Treatment response analysis was performed (**Table 26**) with, a responder defined as a patient with fasting plasma TG < 750 mg/dL at the primary analysis time point (3 months) .

The threshold of TG < 750 mg/dL was chosen as at this point chylomicron accumulation becomes significant and is widely considered to present a threshold above the risk of pancreatitis is increased.

**Table 26.** Treatment Response Rate Analysis: With Fasting Triglycerides Threshold in Study CS6- Full Analysis Set

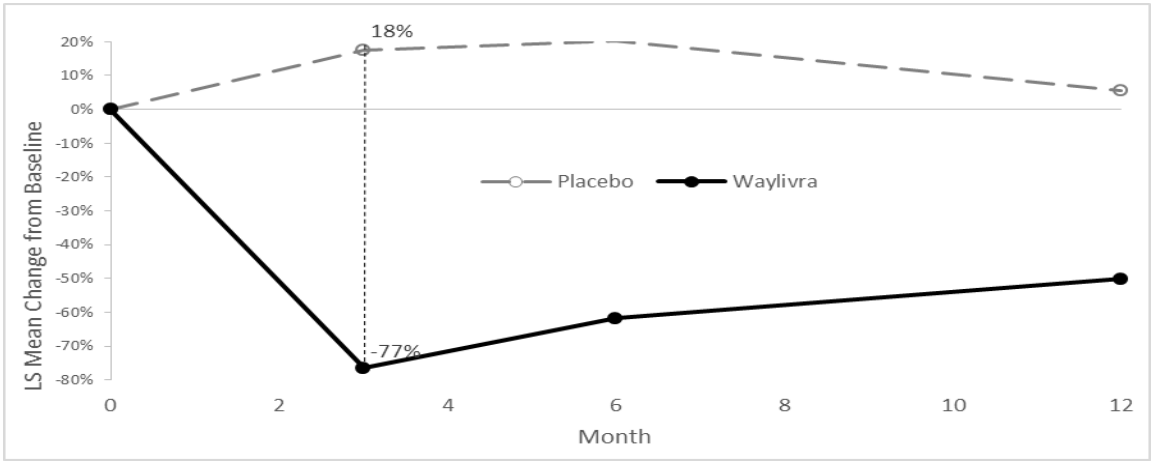
| | **Placebo (N=33)** | **Volanesorsen 300 mg (N=33)** |
|---|---|---|
| **Responder Category / Statistic** | | |
| **Month 3 Endpoint:** | | |
| Patients with baseline TG ≥750 mg/dL | 31 | 30 |
| Endpoint TG <750 mg/dL | 3 (9.7%) | 23 (76.7%) |
| Endpoint TG <500 mg/dL | 0 (0.0%) | 15 (50.0%) |
| Endpoint TG <880 mg/dL | 3 (9.7%) | 24 (80.0%) |
| Endpoint TG <1000 mg/dL | 4 (12.9%) | 24 (80.0%) |
| **Month 6 Endpoint:** | | |
| Patients with baseline TG ≥750 mg/dL | 31 | 30 |
| Endpoint TG <750 mg/dL | 0 (0.0%) | 14 (46.7%) |
| Endpoint TG <500 mg/dL | 0 (0.0%) | 9 (30.0%) |
| Endpoint TG <880 mg/dL | 1 (3.2%) | 14 (46.7%) |
| Endpoint TG <1000 mg/dL | 1 (3.2%) | 17 (56.7%) |
| **Month 12 Endpoint:** | | |
| Patients with baseline TG ≥750 mg/dL | 31 | 30 |
| Endpoint TG <750 mg/dL | 2 (6.5%) | 11 (36.7%) |
| Endpoint TG <500 mg/dL | 0 (0.0%) | 8 (26.7%) |
| Endpoint TG <880 mg/dL | 3 (9.7%) | 11 (36.7%) |
| Endpoint TG <1000 mg/dL | 4 (12.9%) | 12 (40.0%) |

| | Placebo (N=33) | Volanesorsen 300 mg (N=33) |
|---|---|---|
| **Key Exploratory treatment response efficacy endpoints** | | |
| **Month 3 Endpoint Analysis:** | | |
| Responder: ≥40% Reduction | 3 (9.1%) | 29 (87.9%) |
| Responder: ≥50% Reduction: | 1 (3.0%) | 28 (84.8%) |
| Responder: ≥60% Reduction: | 1 (3.0%) | 26 (78.8%) |
| Responder: ≥70% Reduction: | 0 (0.0%) | 21 (63.6%) |
| **Month 6 Endpoint Analysis:** | | |
| Responder: ≥40% Reduction: | 1 (3.0%) | 24 (72.7%) |
| Responder: ≥50% Reduction: | 0 (0.0%) | 20 (60.6%) |
| Responder: ≥60% Reduction: | 0 (0.0%) | 20 (60.6%) |
| Responder: ≥70% Reduction: | 0 (0.0%) | 15 (45.5%) |
| **Month 12 Endpoint Analysis:** | | |
| Responder: ≥40% Reduction: | 3 (9.1%) | 21 (63.6%) |
| Responder: ≥50% Reduction: | 1 (3.0%) | 18 (54.5%) |
| Responder: ≥60% Reduction: | 0 (0.0%) | 14 (42.4%) |
| Responder: ≥70% Reduction: | 0 (0.0%) | 8 (24.2%) |

*Percent change in TG at Month 6 and 12 and over time*

A graphic display of % change in fasting TG at each time point is provided in **Figure 17**.

**Figure 17.** Mean (±SEM) of Percent Change in Fasting Triglycerides (mg/dL) Over Time in Study CS6-Full Analysis Set




*Difference= LS Mean of [Waylivra % Change – Placebo % Change] (ANACOVA model)*
*p-value from Shapiro-Wilk normality test*
*p-value < 0.0001 at Month 3 (primary efficacy endpoint), 0.0002 at Month 6 and p-value =0.0034 at Month 12*

*Effect on patient-reported abdominal pain*

During treatment, 15 (46%) patients in the volanesorsen group and 14 (42%) patients in the placebo group reported abdominal pain (**Table 27**).

**Table 27.** Summary of Patient Reported Abdominal Pain During Treatment Period with Next Observation Carried Forward Imputation in Study CS6 – Full Analysis Set (N=66)

| Treatment Period Category | Placebo (N = 33) | Volanesorsen 300 mg (N = 33) |
|---|---|---|
| Patients with Any Reported Abdominal Pain Event(s) During On-Treatment Period - n (%) | 14 (42.4) | 15 (45.5) |
| Average of Maximum Intensity of Reported Abdominal Pain During On-Treatment Period, n | 33 | 33 |
| Mean (SD, SEM) | 0.36 (0.79, 0.14) | 0.38 (0.83, 0.14) |
| Median (P25, P75) | 0.00 (0.00, 0.42) | 0.00 (0.00, 0.18) |
| Minimum, Maximum | 0.00, 3.27 | 0.00, 3.12 |
| Mean 95% CI | 0.08, 0.64 | 0.09, 0.68 |
| p-value[a] | 0.8959 | |
| Worst Maximum Intensity of Reported Abdominal Pain During On-Treatment Period, n | 33 | 33 |
| Mean (SD, SEM) | 2.70 (3.50, 0.61) | 2.33 (3.04, 0.53) |
| Median (P25, P75) | 0.00 (0.00, 6.00) | 0.00 (0.00, 5.00) |
| Minimum, Maximum | 0.00, 10.00 | 0.00, 9.00 |
| Mean 95% CI | 1.45, 3.94 | 1.26, 3.41 |
| Worst Maximum Intensity of Weekly Reported Abdominal Pain During On-Treatment Period – n (%) | | |
| 0 | 19 (57.6) | 18 (54.5) |
| 1-3 (mild) | 1 (3.0) | 4 (12.1) |
| 4-6 (moderate) | 5 (15.2) | 6 (18.2) |
| 7-10 (severe) | 8 (24.2) | 5 (15.2) |

*Effect on frequency of composite episodes of acute pancreatitis and patient reported abdominal pain during the on-treatment period*

Twelve (36%) patients in the volanesorsen group and 13 (39%) patients in the placebo group had adjudicated acute pancreatitis and/or moderate/severe abdominal pain on treatment. Review of the per-patient per year incidence of episodes of acute pancreatitis and/or reported moderate/severe abdominal pain did not reveal any statistically significant differences between the volanesorsen (2.73) and placebo groups (2.04) (p = 0.6131).

*Effect on acute pancreatitis events (exploratory endpoints)*

Prior to study treatment, 13 (39%) patients in the volanesorsen group experienced 30 adjudicated pancreatitis events and 10 (30%) patients in the placebo group experienced 23 such events. On treatment, 1 (3%) volanesorsen-treated patient suffered 1 event of pancreatitis and 3 (9%) placebo-treated patients suffered 4 events of pancreatitis (p=0.6132). Review of the per patient per year incidence of pancreatitis events did not show statistically significant differences between the volanesorsen and placebo groups, but the study was not powered for this analysis.

An analysis of patients with a history of recurrent pancreatitis events ≥ 2 events in the 5 years prior to Study Day 1) was performed to focus on higher risk patients who might have a detectable difference in event rate. The results of this analysis showed a significant reduction in pancreatitis attacks in volanesorsen-treated patients compared to placebo treated patients (p=0.0242). In the volanesorsen

group, of the 7 patients who had 24 adjudicated pancreatitis attacks in the prior 5 years, none of the patients experienced a pancreatitis attack during the 52 week treatment period. In the placebo group, of the 4 patients who had 17 adjudicated pancreatitis attacks in the prior 5 years, 3 patients experienced 4 pancreatitis attacks during the 52-week treatment period.

*Other Lipid parameters (exploratory endpoints)*

Results in the mean changes in other fasting lipid parameters at Month 3 are summarised in **Table 28**.

**Table 28.** Summary of Fasting Lipid Parameters (mg/dL) in Study CS6 - Full Analysis Set (N=66)

| | Placebo (N = 33) | | | | Volanesorsen 300 mg (N = 33) | | | |
|---|---|---|---|---|---|---|---|---|
| Time Point | n | Observed Value Mean (SD, SEM) | Change from Baseline Mean (SD, SEM) | % change from Baseline Mean (SD, SEM) | n | Observed Value Mean (SD, SEM) | Change from Baseline Mean (SD, SEM) | % change from Baseline Mean (SD, SEM) |
| **Fasting Chylomicron-TG (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 1785 (1149, 200) | NA | NA | 33 | 1913 (1216, 212) | NA | NA |
| Month 3 Endpoint[a] | 33 | 1991 (1279, 223) | 206 (1189, 207) | 37.7 (112.4, 19.6) | 33 | 436 (480, 83) | -1477 (1141, 199) | -76.6* (22.1, 3.8) |
| **ApoB-48 (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 9.25 (5.96, 1.04) | NA | NA | 33 | 11.18 (7.14, 1.24) | NA | NA |
| Month 3 Endpoint[a] | 33 | 9.92 (6.90, 1.20) | 0.67 (3.95, 0.69) | 16.4 (60.1, 10.5) | 33 | 2.59 (2.38, 0.41) | -8.59 (6.44, 1.12) | -75.3* (22.3, 3.9) |
| **Fasting ApoC-III (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 28.94 (13.08, 2.28) | NA | NA | 33 | 31.42 (15.29, 2.66) | NA | NA |
| Month 3 Endpoint[a] | 33 | 30.70 (16.11, 2.80) | 1.76 (9.87, 1.72) | 6.3 (28.0, 4.9) | 33 | 4.58 (2.79, 0.49) | -26.84 (14.87, 2.59 | -83.8* (9.5, 1.7) |
| **Fasting HDL-C (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 17 (4, 1) | NA | NA | 33 | 17 (4, 1) | NA | NA |
| Month 3 Endpoint[a] | 33 | 17 (5, 1) | 1 (3, 1) | 4.9 (22.0, 3.8) | 33 | 25 (11, 2) | 8 (8, 1) | 44.8* (41.8, 7.3) |
| **Fasting LDL-C (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 28 (13, 2) | NA | NA | 33 | 28 (19, 3) | NA | NA |
| Month 3 Endpoint[a] | 33 | 29 (18, 3) | 1 (14, 2) | 7.4 (42.7, 7.4) | 33 | 61 (39, 7) | 33 (30, 5) | 139.4* (124.8, 21.7) |
| **Fasting ApoB (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 69.38 (19.78, 3.44) | NA | NA | 33 | 64.69 (19.45, 3.39) | NA | NA |
| Month 3 Endpoint[a] | 33 | 70.41 (22.74, 3.96) | 1.03 (12.09, 2.10) | 2.2 (18.4, 3.2) | 33 | 75.85 (27.13, 4.72) | 11.16 (22.03, 3.84) | 19.8 (34.8, 6.1) |
| **Fasting VLDL-C (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 41 (29, 5) | NA | NA | 33 | 40 (34, 6) | NA | NA |

| Time Point | Placebo (N = 33) | | | | Volanesorsen 300 mg (N = 33) | | | |
|---|---|---|---|---|---|---|---|---|
| | n | Observed Value Mean (SD, SEM) | Change from Baseline Mean (SD, SEM) | % change from Baseline Mean (SD, SEM) | n | Observed Value Mean (SD, SEM) | Change from Baseline Mean (SD, SEM) | % change from Baseline Mean (SD, SEM) |
| Month 3 Endpoint[a] | 33 | 42 (36, 6) | 1 (17, 3) | 9.0 (79.6, 13.9) | 23 | 13 (10, 2) | -27 (27, 5) | -65.0* (25.4, 5.2) |
| **Fasting non-HDL-C (mg/dL)** | | | | | | | | |
| Baseline[a] | 33 | 267 (125, 22) | NA | NA | 33 | 276 (135, 23) | NA | NA |
| Month 3 Endpoint[a] | 33 | 287 (134, 23) | 20 (113, 20) | 14.1 (48.7, 8.5) | 33 | 131 (51, 9) | -144 (127, 22) | -44.6* (28.6, 5.0) |

*Effect on acute pancreatitis events (exploratory endpoints)*

Prior to study treatment, 13 (39%) patients in the volanesorsen group experienced 30 adjudicated pancreatitis events and 10 (30%) patients in the placebo group experienced 23 such events. On treatment, 1 (3%) volanesorsen-treated patient suffered 1 event of pancreatitis and 3 (9%) placebo-treated patients suffered 4 events of pancreatitis (p=0.6132). Review of the per patient per year incidence of pancreatitis events did not show statistically significant differences between the volanesorsen and placebo groups,

An analysis of patients with a history of recurrent pancreatitis events (≥ 2 events in the 5 years prior to Study Day 1) was performed to focus on higher risk patients who might have a detectable difference in event rate. The results of this analysis showed a significant reduction in pancreatitis attacks in volanesorsen-treated patients compared to placebo treated patients (p=0.0242). In the volanesorsen group, of the 7 patients who had 24 adjudicated pancreatitis attacks in the prior 5 years, none of the patients experienced a pancreatitis attack during the 52 week treatment period. In the placebo group, of the 4 patients who had 17 adjudicated pancreatitis attacks in the prior 5 years, 3 patients experienced 4 pancreatitis attacks during the 52-week treatment period.

## Ancillary analyses

Not applicable.

## Summary of main study

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

**Table 29.** Summary of Efficacy for trial CS6

| **Title:** A randomized, double-blind, placebo-controlled, phase 3 study of ISIS 304801 administered subcutaneously to patients with familial chylomicronemia syndrome (FCS) | |
|---|---|
| Study identifier | ISIS 304801-CS6 (Study CS6) |
| Design | Randomized, multicenter, double-blind, placebo-controlled study |

| | | | |
|---|---|---|---|
| | Duration of main phase:<br>Duration of Run-in phase:<br>Duration of Extension phase: | | 52 weeks<br>Up to 8 weeks, including a diet stabilization period of at least 6 weeks<br>52 weeks |
| Hypothesis | Superiority | | |
| Treatments groups | volanesorsen | | 300mg volanesorsen SC as single 1.5 ml injection once weekly (dosing could be adjusted to every 2 weeks or dose pause based on platelet decreases) for 52 weeks (n= 33 FCS patients) |
| | placebo | | Placebo SC as a single 1.5 ml injection once weekly (dosing could be adjusted to every 2 weeks or dose pause based on platelet decreases) for 52 weeks (n= 34 FCS patients) |
| Endpoints and definitions | Primary endpoint | % change from baseline in TG at Month 3 | percentage change from baseline in TG at the end of Month 3, defined as the average of Week 12 (Day 78) and Week 13 (Day 85) fasting assessments |
| | Secondary endpoint | % change in TG at Month 6 and 12 | Percent change from baseline in TG at Month 6 and 12 month |
| | Secondary endpoint | Treatment response rate at Month 3, 6 and 12 | The percentage of patients with fasting plasma TG < 750 mg/dL at the primary analysis time point Month 3 and at Month 6 and 12 |
| | Secondary endpoint | ≥40% TG Reduction at Month 3 | The percentage of patients achieving target reductions of fasting plasma TG ≥ 40% |
| | Secondary endpoint | Abdominal pain | Episodes and severity of abdominal pain during the on-treatment period |
| | Secondary endpoint | Acute pancreatitis | The number of patients with acute pancreatitis attacks during the on-treatment period |
| | Secondary endpoint | Abdominal pain and acute pancreatitis | Composite episodes of acute pancreatitis and patient reported moderate/severe abdominal pain during the on-treatment period |
| | Exploratory endpoint | Acute pancreatitis in patients with prior treatment events | The number of acute pancreatitis attacks on study treatment in patients with a history of recurrent pancreatitis events (≥ 2 events in the 5 years prior to Study Day 1) |
| Database cut-off date | 18 January 2017 | | |
| **Results and Analysis** | | | |
| **Analysis description** | **Primary Analysis** | | |

| | | | |
|---|---|---|---|
| Analysis population and time point description | Full analysis set (representing modified intention-to-treat<br><br>Month 3, defined as the average of Week 12 (Day 78) and Week 13 (Day 85) | | |
| Descriptive statistics and estimate variability | Treatment group | Volanesorsen | Placebo |
| | Number of subject | 33 | 33 |
| | % change from baseline in TG at Month 3 | -76.5 (-97.4, -55.5) | 17.6 (-4.0, 39.2) |
| Effect estimate per comparison | Primary endpoint | Comparison groups | Volanesorsen vs Placebo |
| | | % change from baseline vs placebo | -94.1 |
| | | 95% CI | (-121.7, -66.6) |
| | | P-value | p<0.0001 |
| **Analysis description** | **Secondary analysis** | | |
| Analysis population | Full analysis set (representing modified intention-to-treat | | |
| Descriptive statistics and estimate variability | Treatment group | **Volanesorsen** | **Placebo** |
| | Number of subject | 33 | 33 |
| | % change from baseline in TG at Month 6 | -52.5 (-82.0, -22.9) | 25.3 (4.1, 46.5) |
| | | % change from baseline vs placebo | -77.8 |
| | | 95% CI | (-106.4, -49.1) |
| | | p-value | P<0.0001 |
| | % change from baseline in TG vs placebo at Month 12 | -40.2 (-86.1, 5.7) | 8.9 (-19.7, 37.5) |
| | | % change from baseline vs placebo | -49.1 |
| | | 95% CI | (-94.7, -3.5) |
| | | p-value | P=0.0347 |
| | Treatment response rate TG < 750 mg/dL at Month 3 | 76.7% | 9.7% |
| | | Odds Ratio (95%CI) | 186.16 (12.86, N/A) |
| | | p-value | P=0.0001 |
| | Treatment response rate TG < 750 mg/dL at Month 6 | 46.7% | 0% |
| | | Odds Ratio (95%CI) | 59.36 (3.67, 959.47) |
| | | p-value | P=0.0040 |
| | Treatment response rate TG < 750 mg/dL at Month 12 | 36.7% | 6.5% |
| | | Odds Ratio (95% CI) | 44.55 (3.28, 604.46) |
| | | p-value | P=0.0043 |
| | Treatment response rate ≥40% TG Reduction at Month 3 | 87.9% | 9.1% |
| | | p-value | P<0.0001 |
| | Events of abdominal pain | 15 patients (46%) | 14 patients (42%) |
| | | P value | P=0.8959 |

| | | | |
|---|---|---|---|
| | Average of maximum intensity of reported abdominal pain | 0.38 | 0.36 |
| | | P value | P=0.8959 |
| | Events of acute pancreatitis | 1 patient (3%) experienced 1 event | 3 patients (9%) experienced 4 events |
| | | p-value | P=0.6132 |
| | Composite episodes of abdominal pain and acute pancreatitis | 12 patients (36%) | 13 patients (39%) |
| | | p-value | 0.6131 |
| **Analysis description** | Exploratory analysis | | |
| | Acute pancreatitis in patients with prior treatment events | 0 | 3 patients experienced 17 adjudicated event |
| | | p-value | 0.0242 |

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

Not applicable.

## Clinical studies in special populations

Clinical studies included 4 patients with FCS aged 65 or over treated with volanesorsen in randomised control studies (phase II study CS2, 1 patient; CS6 3 patients), and 6 patients aged 65 and over in the open-label extension study (CS7). No overall differences in safety or effectiveness were observed between these patients and younger patients, however data are limited in this subpopulation.

### *Supportive studies*

**Study CS16**, was a randomized, double-blind, placebo-controlled Phase 3 Study of volanesorsen administered subcutaneously to patients with hypertriglyceridemia. Patients were included in the study if they had fasting TG≥500 mg/dL (≥5.7 mmol/L) at Screening. The study design was similar to that of CS6, but the treatment period was limited to 26 instead of 52 weeks. Actions to be taken in patients with low platelet counts were the same as in study CS6. In study CS16, even though the target population was patients with severe hypertriglyceridemia, based on Amendment 2, 7 patients with documented FCS were also enrolled in study CS16.

A total of 114 patients were enrolled for a 26-week treatment period with the dose frequency reduced to 300 mg every 2 weeks after 13 weeks of treatment (exemptions were given to patients who completed $\geqslant$ 5 months of treatment as of 27 May 2016) per a protocol amendment.

A total of 39 patients were switched to every 2 weeks treatment due to Protocol Amendment 4 (14 volanesorsen treated and 5 placebo patients at Week 13 and 12 volanesorsen-treated and 8 placebo patients at various timepoints after Week 13 up to and including Week 21). Three additional patients dose adjusted during the study (2 for AEs and 1 for platelet count). An additional 8 patients were assigned to the biweekly dose group because they missed 4 or more doses in the last 3 months of the treatment period, including those who discontinued early and did not meet the minimum dose requirement for the weekly group. Baseline charactersitcs of the patients included in this study are summarsied in **Table 30**.

**Table 30.** Summary of Patient Demographics in Study CS16 – Full Analysis Set (N = 113)

| Demographic and Disease Characteristic | Placebo (N = 38) | Volanesorsen | | | All Patients (N=113) |
|---|---|---|---|---|---|
| | | 300 mg weekly cohort (N=25) | 300 mg every 2 weeks post W13 cohort (N=50) | Volanesorsen Total (N=75) | |
| **Age at Informed Consent (year)** | | | | | |
| Mean (SD) | 53 (10) | 50 (9) | 51 (11) | 50 (10) | 51 (10) |
| **Age Group (years), n (%)** | | | | | |
| Age <65 years old | 34 (89.5) | 23 (92.0) | 44 (88.0) | 67 (89.3) | 101 (89.4) |
| Age ≥65 years old | 4 ( 10.5) | 2 ( 8.0) | 6 ( 12.0) | 8 ( 10.7) | 12 ( 10.6) |
| **Sex n (%)** | | | | | |
| Female | 8 ( 21.1) | 5 ( 20.0) | 14 ( 28.0) | 19 ( 25.3) | 27 ( 23.9) |
| Male | 30 ( 78.9) | 20 ( 80.0) | 36 ( 72.0) | 56 ( 74.7) | 86 ( 76.1) |
| **Ethnicity - n (%)** | | | | | |
| Hispanic or Latino | 1 ( 2.6) | 1 ( 4.0) | 0 | 1 ( 1.3) | 2 ( 1.8) |
| Not Hispanic or Latino | 37 ( 97.4) | 24 ( 96.0) | 50 (100.0) | 74 ( 98.7) | 111 ( 98.2) |
| **Race - n (%)** | | | | | |
| White | 33 ( 86.8) | 25 (100.0) | 47 ( 94.0) | 72 ( 96.0) | 105 ( 92.9) |
| Asian | 3 ( 7.9) | 0 | 1 ( 2.0) | 1 ( 1.3) | 4 ( 3.5) |
| American Indian or Alaska Native | 0 | 0 | 1 (2.0) | 1 (1.3) | 1 (0.9) |
| Other Race | 1 ( 2.6) | 0 | 1 ( 2.0) | 1 ( 1.3) | 2 ( 1.8) |
| **Body Weight (kg)** | | | | | |
| Mean (SD) | 89.67 (18.57) | 94.81 (18.41) | 97.04 (22.01) | 96.30 (20.78) | 94.07 (20.23) |
| **BMI (kg/m²)** | | | | | |
| Mean (SD) | 30.3 (4.4) | 30.7 (3.8) | 32.1 (6.4) | 31.6 (5.7) | 31.2 (5.3) |
| **Fasting Triglycerides (mg/dL)** | | | | | |
| Mean (SD) | 1414 (1253) | 1046 (560) | 1251 (838) | 1183 (759) | 1261 (955) |
| **Type II Diabetes Mellitus** | | | | | |
| n (%) | 12 (31.6) | 9 (36.0) | 19 (38.0) | 28 (37.3) | 40 (35.4) |

One of the randomized patients was discontinued prior to treatment for failure to meet the eligibility criteria and thus is only included in the volanesorsen 'total' and 'all patients' group. Overall, 29 (25%) of the 113 patients were reported to have prematurely discontinued from study treatment; no FCS patients discontinued from treatment prematurely. Across treatment groups, 11% of placebo patients and 33% of patients treated with volanesorsen discontinued treatment prematurely. The most common reason for discontinuation of treatment was AE or SAE (20% in the volanesorsen group (n=15) and 8% in the placebo group (n=3). The majority (33%) of the AEs leading to discontinuation were adverse events at the injection site. There were no deaths reported during the study. Of the 113 treated patients, 74.6% patients completed the study drug treatment (67.1% and 89.5% of the volanesorsen and placebo group, respectively). Ninety-eight percent of patients entered the post-

treatment follow-up period at the end of study. Sixteen (14%) of the patients on post-treatment follow-up were terminated early with the most common reason for early termination being voluntary withdrawal (8%). Other reasons for early termination of the post-treatment follow-up were AE (2%), enrollment in the open label extension (1%), and other (4%).

Treatment with volanesorsen 300 mg weekly demonstrated a statistically significant reduction in TG levels in both patients with severe hypertriglyceridemia (SHTG) and in patients with FCS at Month 3. Weekly treatment with volanesorsen decreased TG levels with -72.1% (absolute TG reduction of 889 mg/dL) compared to a -2.9% change in the placebo group; this 69% difference between groups was statistically significant ($p < 0.0001$). In the FCS patients, volanesorsen 300 mg weekly resulted in a mean absolute reduction of 1511 mg/dL, which correlates to a reduction of -73% from baseline after 3 Months of treatment, compared with a mean increase of 70% in placebo-treated FCS patients (N=2).

At the end of the 26-week treatment period, 35% of the patients receiving volanesorsen had the dose reduced to 300 mg every 2 weeks. The dose reduced patients had a 62% reduction in TGs and the weekly patients had a 78% reduction from baseline, both statistically significant ($p<0.0001$).

The primary objective of the open-label extension study **CS7** was to further evaluate the safety and efficacy of dosing and extended dosing with volanesorsen in patients with FCS. Three patient groups were enrolled:

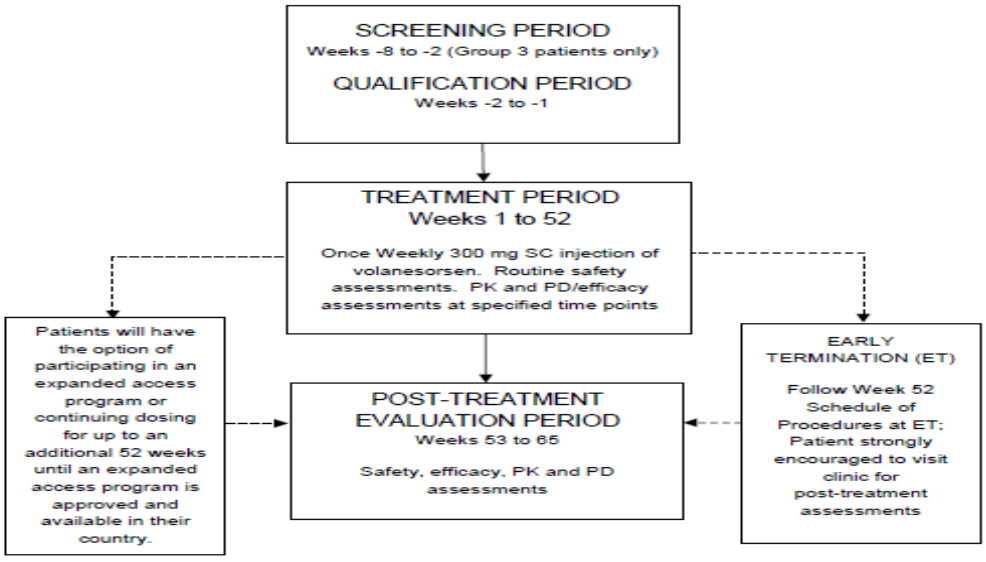
Group 1: FCS patients rolling over from study CS6 (index study)

Group 2: FCS patients rolling over from study CS16 (index study)

Group 3: FCS patients who did not participate in either the CS6 or CS16 index studies.

For patients in Group 3, inclusion criteria were similar to those of study CS6. The study design is presented in **Figure 18.**

**Figure 18.** Study design and treatment schema of study CS7




In study CS7, all patients were to have the dose frequency reduced to 300 mg every 2 weeks after 13 weeks of treatment in advance (exceptions were given to patients who completed ≥ 5 months of dosing as of 27 May 2016) per Amendment 4 of the protocol dated 06 June 2016. The primary efficacy endpoints of study CS7 are summarized below:

- Percent change and absolute change from Baseline in fasting total ApoC-III, TG and other lipid measurements including total cholesterol, non-HDL-C, apoB, HDL-C, apoA-1 (apoA-1), VLDL-C, and LDL-C, at month 3, 6 and 12.
- Frequency and severity of patient reported abdominal pain during the treatment period,
- Change from Baseline in Quality of Life (QOL) questionnaires (EQ-5D, SF-36),
- Independently adjudicated acute pancreatitis event rate,
- Frequency of other symptoms: eruptive xanthoma, lipemia retinalis.

Patient disposition in this study are summarised in **Table 31**.

**Table 31**. Study CS7 Patient Disposition and Status as of the June 20, 2018 Cut-off Date

| | Treatment Naïve Group | CS6-Volanesorsen | CS16-Volanesorsen | Overall |
|---|---|---|---|---|
| Patients Enrolled | 51* | 14 | 3 | 68* |
| Patients Treated | 50 (98.0%) | 14 (100%) | 3 (100%) | 67 (98.5%) |
| Patients Who Terminated from the Study Treatment | 12** (23.5%) | 5 (35.7%) | 0 | 17** (25.0%) |
| Main Reason for Termination | | | | |
| Investigator judgement | 0 | 0 | 0 | 0 |
| Voluntary withdrawal | 5 (9.8%) | 1 (7.1%) | 0 | 6 (8.8%) |
| Adverse event | 6 (11.8%) | 4 (28.6%) | 0 | 10 (14.7%) |
| Other | 0 | 0 | 0 | 0 |

* One (Patient 2181-7057) of 51 enrolled patients in the treatment-naive group did not have records of dose administration as of 31 Dec 2017 data cut-off, thus number of treated patients was reduced by 1 compared to number of enrolled patients.
**One patient terminated study treatment to enter the early access program.

The results on TG lowering effect are presented in **Table 32**.

**Table 32.** Summary of Fasting Triglycerides (Mean (SD, SEM), mg/dL) Over Time in Study CS7

| Time Point | Treatment-naïve Group (Open-label Study Baseline[a], N=50) | | | CS6-volanesorsen (Index Study Baseline[a], N=14) | | | CS16-volanesorsen (Index Study Baseline[a], N=3) | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | Observed Value | % change from Baseline in CS7 | n | Observed Value | % change from Baseline in CS6 | n | Observed Value | % change from Baseline in CS16 |
| Baseline[a] | 50 | 2317 (1193, 169) | - | 14 | 2641 (1228, 328) | - | 3 | 2288 (1524, 880) | - |
| Month 3 | 42 | 792 (589, 91) | -60.4 (36.4, 5.6) | 14 | 1266 (812, 217) | -49.2 (34.8, 9.3) | 3 | 855 (651, 376) | -64.9 (9.1, 5.3) |
| Month 6 | 41 | 979 (683, 107) | -46.6 (34.0, 5.3) | 13 | 1248 (927, 257) | -54.8 (23.8, 6.6) | 2 | 1558 (193, 137) | -39.1 (26.2, 18.5) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month 12 | 18 | 1287 (904, 213) | -34.9 (40.8, 9.6) | 10 | 1411 (1143, 361) | -47.2 (37.1, 11.7) | 0 | NC | NC |
| Month 15 | 7 | 1071 (560, 211) | -30.1 (45.9, 17.4) | 3 | 1751 (1850, 1068) | -61.4 (29.5, 17.0) | 0 | NC | NC |
| Month 18 | 3 | 884 (826, 477) | -31.3 (8.4, 4.9) | 1 | 239 | -82.0 | 0 | NC | NC |

[a] Baseline values for treatment-naïve group were taken from the open-label study CS7 and baseline for CS6-volanesorsen and CS16-volanesorsen groups were taken from the respective index study.
NC = not calculated

## 2.5.3. Discussion on clinical efficacy

### *Design and conduct of clinical studies*

Study CS2, was a randomized, double-blind, placebo-controlled, 13-week dose response study conducted in patients with severe hypertriglyceridemia, was initiated based on the results of the proof-of-concept first-in-human study CS1. Study CS2 consisted of 4 cohorts in three treatment arms (monotherapy, concomitant fibrate therapy and patient with FCS) in which several doses of volanesorsen have been investigated; In the monotherapy arm (n=57), volanesorsen was investigated at doses of 100, 200 and 300 mg once weekly, in the fibrate arm (n=28) at doses of 200 and 300 mg once weekly and in the FCS arm (n=3) at the dose of 300 mg once weekly. The overall design and duration of study was considered acceptable for a dose finding study.

The applicant has conducted several studies in different types of patients, i.e. patients with FCS and patients with (severe) hypertriglyceridemia. The primary efficacy data obtained in the proposed target population are derived from the pivotal phase 3 study CS6, a placebo-controlled, 52-week study in patients with FCS. Additional data come from the phase 3 study (CS16) in (severe) hypertriglyceridemia, and an ongoing open label extension study of 52 weeks (CS7) in which FCS patients could be included from study CS6 and CS16 or treatment naïve FCS patients.

General inclusion/exclusion criteria of study CS6 and CS7 seem appropriate for the claimed indication of FCS.

The double-blind treatment period of 52 weeks in the pivotal Study CS6 in FCS patients is provides the pivotal data to evaluate efficacy, although the ongoing open-label extension Study CS7 with a treatment duration of at least 52 weeks may provide more insight on the long term efficacy of volanesorsen. The treatment duration of 26 weeks in Study CS16 is sufficient to provide supportive efficacy data in in patients with hypertriglyceridemia, although the treatment period may be considered minimal considering that steady state has only been reached after 13 weeks of therapy. A follow-up period of 13 weeks off-treatment of Study CS6 can be considered appropriate for information on the maintenance of the effect and the safety of volanesorsen.

Initially, volanesorsen was to be administered once weekly for each individual patient in the phase 3 studies. Due to two serious adverse events of thrombocytopenia observed in Study CS6, a dosing algorithm based on platelet counts has been implemented in the protocols of all phase 3 studies. According to the dosing algorithm, dose adjustments to 300 mg every 2 weeks or dose interruptions can be implemented for patients experiencing platelet decrease in order to reduce the risk of progression to more severe thrombocytopenia. In Study CS6, all dose adjustments, if necessary,

occurred after month 3, whereas in Study CS16, all patients were to have the dose frequency reduced to 300 mg every 2 weeks after 13 weeks of treatment in advance (exceptions were given to patients who completed ≥ 5 months of dosing as of 27 May 2016).

The primary objective of the phase 3 studies was to establish the effect of volanesorsen on reduction of TG levels. The primary endpoint was therefore chosen as the percentage change in fasting TG from baseline to the primary analysis time point at the end of month 3, where the value was defined as the average of Week 12 (Day 78) and Week 13 (Day 85) fasting assessments. This is considered appropriate to establish the initial TG lowering effect and can be considered sufficiently long given that steady state of $C_{trough}$ of volanesorsen is reached at that time period and maximum reductions in ApoC-III and TG may be expected at this time point. However, for maintenance of effect change in TG (full analysis set) at 6 and 12 months are also considered important, while long-term maintenance of effect should be further established based on the open label long term CS7 study.

The reduction of TG provides biomarker results on the effects of volanesorsen while the clinical implications of these effects were assessed in secondary endpoints of intensity and frequency of abdominal pain, and especially episodes of acute pancreatitis, although it is expected that events will be few with the number of patients included. Other supportive analyses include effects on other parameters of the lipid profile, among some others both including laboratory measurements as other associated clinical aspects and could provide further inside on the treatment effect.

The sample size calculation in the pivotal study was considered appropriate. Sample size was determined ensuring sufficient power for the primary endpoint and based upon prior clinical trial experience. The stratified randomisation was also considered appropriate as the different stratification factors may affect the primary and secondary endpoints. Appropriate secure blinding principles of measurements and evaluation were applied of assessment of laboratory values by an independent laboratory and an external independent monitoring committee to review the safety data to avoid a possible increased risk.

The applicant had sought scientific advice from the CHMP on the clinical development for this product. The advice pertained to the appropriate patient population to be included in the pivotal study, and the selection of endpoints which could support the claimed indication.

The applicant followed the CHMP advice with respect to the proposed baseline TG threshold for inclusion in the pivotal study and the primary endpoint % change from baseline in fasting TG at 13 weeks. Furthermore, recommendations with respect to an open-label extension of up to 52 weeks, stratification by the presence of background therapy and standardised meals were also followed for this application.

## *Efficacy data and additional analyses*

Phase 2 dose finding study

In the monotherapy arm, volanesorsen therapy showed a dose-dependent ApoC-III reduction from baseline of 40%, 64%, and 80% at doses of 100, 200, and 300 mg once weekly, respectively, which was associated with dose-dependent TG reduction of 31%, 58%, and 71%, respectively. The fibrate therapy arm (-60% and -71% for ApoC- III and -51% and -64% for TG at 200 and 300 mg, respectively) and the FCS arm (-81.3% and -68.8% for ApoC-III and TG at 300 mg respectively) showed similar levels of reduction in ApoC-III and TG as the monotherapy arm. As the highest levels of ApoC-III and TG reduction were achieved with 300 mg once weekly and the 300 mg dose administered once weekly for 13 weeks was well tolerated, volanesorsen 300 mg weekly dose was selected for the phase 3 clinical program.

Phase 3 evaluation

Overall, the baseline data in Study CS6 are representative of a patient population with FCS with very high levels of TG of 2209 mg/ml and 75.8% of the patients with history of acute pancreatitis. Furthermore, a total of 50 (76%) patients had confirmed mutations in type 1 causing genes of which the majority reported mutations in the LPL gene (82%) and 36 out of 66 (55%) patients tested had post heparin plasma LPL activity of ≤ 20% of normal.

Patients in study CS16 represent a different population with an approximately 40% lower level of TG (1261 mg/dL versus 2209 mg/dL), and a higher BMI (30 vs 25). In this study CS16 only 7 patients with FCS were enrolled.

In the pivotal study CS6 in FCS patients, volanesorsen demonstrated a substantial reduction of -77% (2267 mg/dL to 590 mg/dL) in TG compared to an increase of 18% for placebo, resulting in a 94% difference at month 3 ($p<0.0001$). All patients responded to volanesorsen treatment with the exception of one patient, which was considered a non-responder. Also, a much higher percentage of patients in the volanesorsen group achieved target TG levels of < 750 mg/dL compared with the placebo group (77% compared with 10%, respectively). The target level of TG < 750 mg/dL was chosen as at this level chylomicron accumulation becomes significant and it is thought that above this threshold acute pancreatitis risk is increased. Likewise, for patients with hypertriglyceridemia in study CS16 volanesorsen resulted in a substantial TG reduction of 72% from baseline (TG of 1183 mg/dL to 294 mg/dL) compared with 3 % in placebo at Month 3 with a difference of 69% ($p<0.0001$). Moreover, the TG reduction from baseline to month 3 for 5 FCS patients (72%) in study CS16 was comparable to the TG reduction found in study CS6.

Treatment with volanesorsen also resulted in favourable outcomes in other lipid parameters including chylomicron-TG, ApoC-III, ApoB-48, VLDL-C and non-HDL-C along with increases in HDL-C, however an increase in LDL-C was observed. It is agreed with the Applicant that the increased LDL-C, which remained below the ULN, was accompanied by a decrease in non-HDL-C, suggesting an overall anti-atherogenic effect. The changes in lipid parameters observed in patients with hypertriglyceridemia in Study CS16 were comparable with those in the FCS population in Study CS6.

Despite these positive findings, treatment with volanesorsen was associated with substantial number of discontinuations in study CS6 (42%, 14/33 volanesorsen vs 5.9%, 2/33 placebo)), and this was mainly due to adverse events (AE) (9 patients, including 5 patients with decreased platelet count). Moreover, eleven patients (10 volanesorsen and 1 in placebo) discontinued voluntary, which could likely be associated with the burden of the need for intense platelet monitoring. Further, tolerability issues were apparent due to the need of frequent dose adjustment to every 2 weeks (10 (30%) volanesorsen vs 0 placebo), or dose interruptions (11 (33%) volanesorsen group versus 6 (18%) placebo), after a dose algorithm and frequent platelet monitoring protocol was implemented during the study. From the 19 volanesorsen-treated FCS patients (58%) who completed the study, more than half of the patients (n=13) had their dose adjusted, whereas only 6 patients completed the study without dose adjustments.

In study CS16, the most common reason for discontinuation of treatment was AE (20% in the volanesorsen group (n=15) and 8% in the placebo group (n=3), of which the majority were injection site reaction (33%)). Also in this study, 13 patients (12%; 9 volanesorsen and 4 in placebo) discontinued on their own volition and could be due to the burden of need for intense monitoring.

Within the open-label study CS7, the dose algorithm and platelet monitoring was applied from the beginning. However, data until June 2018 showed a discontinuation rate due to AEs of 27% (18 patients, including 9 patients which discontinued due to thrombocytopenia). Discontinuations due to

other reasons remain unknown and could further inflate the overall discontinuation rate. Overall, the high percentage of discontinuations from study treatment may limit the possibility for long term treatment for most of the patients.

The diminished TG-lowering effect after 3 months (-77%) to -53% and -40% from baseline, respectively, at month 6 and 12 appears to be associated with these dose adjustments and dose interruptions needed due to the occurrence of platelet count decreases. For instance, for the very few patients who completed the study without dose adjustments (n=6) TG reduction versus baseline at month 6 and 12 were higher with -80% and -76% compared to patients who completed the study with dose adjustments (n=13) (-52% and -54% at Month 6 and 12). As a result, less patients compared with the Month 3 time point maintained the target level of < 750 mg/dL after treatment with volanesorsen at Month 6 and Month 12 (77%, 47% and 37% in the volanesorsen group and 10%, 0% and 6.5% in the placebo group at Month 3, 6, and 12, respectively).

Comparably, in study CS16, patients with post 13 weeks obliged dose reduction (n=50) to every 2 weeks showed less pronounced TG-lowering effects (-62%) compared to patients on 300mg once weekly during the study (n=25; -78%). Of the 19 patients that completed one year on volanesorsen in study CS6, 14 have continued therapy in the open-label phase (3 on weekly 300 mg dose). The very limited open-label data demonstrate a maintenance of effect with percent change in fasting TG from baseline to 18 months (n=9) and 24 moths (n=3) of -53.1% and -60.8% (as per date cut off 31 Aug 2017).

To improve tolerability and reduce treatment discontinuation an initial weekly dose with subsequent biweekly dosing after 3 months and possible up-titration after 6 months when further lowering of TG is needed is being proposed. This is further discussed in the clinical safety section of this report.

The assessment of the clinical impact of the TG lowering effect of volanesorsen is challenging, mainly due to the limited clinical data available (acute pancreatitis) and/or a lack of clear effects for other clinical related outcomes including abdominal pain. For the secondary endpoint of acute pancreatitis, a trend toward less frequent occurrence of these events was shown, with 1 event in 1 patient in the volanesorsen group compared with 4 events in 3 patients in the placebo group (p=0.6132). A similar positive trend could also be observed in study CS16 where 3 placebo patients had 5 events of acute pancreatitis during 26 weeks of treatment compared to none in the volanesorsen group (p=0.036). Furthermore, an exploratory analysis in study CS6 of patients with prior frequent acute pancreatitis events (≥ 2 events in the 5 years prior to treatment) showed for the 7 patients who had 24 adjudicated pancreatitis attacks in the prior 5 years, no events during the 52 week treatment period for volanesorsen while for the 4 placebo patients who had 17 adjudicated pancreatitis attacks in the prior 5 years, 3 patients experienced 4 pancreatitis attacks during the 52-week treatment period.

For abdominal pain, in study CS6 volanesorsen treatment did not affect the secondary endpoint of the overall number of patients who reported abdominal pain (15 patients (46%) volanesorsen and 14 patients (42%) placebo) and the overall mean maximum abdominal pain intensity score (0.38 vs 0.36). Also, for the combined adjudicated acute pancreatitis and/or moderate/severe abdominal pain on treatment no clear difference was shown (12 (36%) patients in the volanesorsen group and 13 (39%) patients in the placebo group). However, fewer patients in the volanesorsen group (5 [15%]) reported severe abdominal pain events in comparison with the placebo group (8 [24%]) while on treatment, but not for moderate abdominal pain (6 [18%] vs 5 [15%]). In exploratory analyses, for those with abdominal pain at baseline (7 and 10 for volanesorsen and placebo) a trend towards a reduction in frequency of abdominal pain events (3 vs 11 events per patient per year) after 7-12 months, and a reduction in episodes of moderate to severe abdominal pain (2 vs 5 events per patient per year in month 4-12; 2 vs 7 in month 7-12) and worst abdominal pain intensity (3.1 vs 5.4 month 4-12; 2.4 vs 5.4 month 7-12) was observed.

However, as commented by the Expert group the reported effect of volanesorsen TG levels is of clinical relevance and in clinical practice should result in a reduction of the incidence of pancreatitis. It is likely that longer follow up and exposure in a larger number of patients would be needed to further be able to assess the clinical impact of maintained TG reductions on acute pancreatitis events.

The CHMP considering the totality of the data and the comments raised by the Expert Group concluded that it would be appropriate to restrict the population that could be treated with volanesorsen to patients to patients with genetically confirmed FCS and at high risk for pancreatitis, in whom response to diet and triglyceride lowering therapy has been inadequate. Furthermore, secondary causes of hypertriglyceridemia (e.g. uncontrolled diabetes, hypothyroidism) should be excluded or appropriately addressed prior to initiation of treatment.

The CHMP noted that this restricted indication better reflected the studied population in the clinical trials for which stringent criteria had been applied to define FCS. Furthermore, and in view of the high discontinuation rates which to a large extent were due to the reduced platelet counts (See also Clinical Safety section) it was considered necessary to restrict the indication as a last line treatment option and only for patients most likely to benefit the most from treatment.

To address the concerns of the CHMP, the applicant submitted a revised SmPC during the assessment procedure, restricting the therapeutic indication of the medicinal product.

## *Additional expert consultation*

An Ad-Hoc Expert Advisory Group was asked to provide their view on the following issues:

**Question 1**

**What is the view of the AHEG on the clinical relevance of the reported efficacy of volanesorsen on the level of serum triglycerides in patients with FCS, also taking into account the treatment discontinuations over time.**

The expert group considered that the reported effect of volanesorsen on serum triglyceride levels is of clinical relevance, even though this did not result in a statistical significant reduction in the incidence of pancreatitis. The group noted that the reported incidence of pancreatitis was considerably lower than what would have been expected in patients with FCS. The group observed that a number of patients included in the pivotal trial had previously been treated with Glybera and considered this as a potential explanation for this observation due to residual effect on pancreatitis prevention carried over from the previous treatment.

The group noted that the applicant's proposal for the dosing regimen was lower than the one used in the pivotal clinical trial. Therefore, the triglyceride-lowering efficacy with the proposed dose is likely to be less than the one observed in Study CS06 and additional data would be required to confirm its efficacy (and the assumed reduction of the risk of thrombocytopenia).

**Question 2**

**What is the view of the AHEG on the reported occurrence of thrombocytopenia taking into consideration the potentially safety risk (e.g. severe bleeding events).**

The group agreed that volanesorsen treatment is associated with a clear risk of thrombocytopenia. Of particular concern to the group was the fact that these events appear to be unpredictable in terms of patient susceptibility and time to onset and that in some cases low platelet count persisted more than what is typically observed for drug induced thrombocytopenia. The group was also concerned that the mechanism of action for the thrombocytopenia remains largely unknown even though it was acknowledged that reduced platelet counts has been observed with other anti-sense oligonucleotides.

Even though no major bleedings have been observed in the clinical studies performed to date, the group considered that the observed events of thrombocytopenia in the studies in a real-life setting would be associated with an increased risk of bleeding. At this stage, with the data currently available, it is not possible to estimate the magnitude of this risk.

**Question 3**

**Does the AHEG consider the current proposed measures of a 2 weeks platelet monitoring schedule, with optional 'at home' blood sampling, and additional patient and care-giver education sufficient, feasible and manageable to mitigate the consequences of thrombocytopenia for an intended life-long treatment?**

The group considered that the proposals of the applicant for platelet monitoring are overall adequate to minimise this risk. Even though the onset of thrombocytopenia is not possible to predict, the initial two-weekly interval for monitoring was considered appropriate by the group. The drops in platelet counts observed in the trials were generally gradual. Any trend for decrease in platelets in patients therefore should be able to be identified with the proposed monitoring before reaching levels that could lead to serious bleeding events.

The experts agreed with the feasibility of the proposals and supported a home blood sampling scheme provided by the applicant.

However, the group noted that a significant number of patients in the pivotal trial were receiving anti-platelet drugs. As such use can also be expected in real-life due to the increased cardiovascular risk in these patients appropriate risk minimisation measures for patients with concomitant use of anti-platelet should be implemented.

**Question 4**

**The AHEG is asked whether a subgroup of patients with FCS, in the situation of high unmet medical need, could likely be identified in whom a high expected benefit could be anticipated despite the safety profile and the high degree of discontinuations as currently observed.**

The group advised that the most appropriate target population for volanesorsen would be patients with a definite diagnosis of FCS, as described for instance in a recent paper by Stroes et al (Diagnostic algorithm for familial chylomicronemia syndrome, Atherosclerosis Suppl., 23 (2017), pp. 1–7).

In addition the experts considered that treatment should be reserved only for patients who are not able to control their levels of triglycerides by other means (i.e. diet or pharmacological interventions). The group also emphasised that treatment should only be initiated once secondary causes of hypertriglyceridemia have been excluded.

## 2.5.4. Conclusions on the clinical efficacy

Volanesorsen demonstrated a substantial effect on TG during the first 3 months of therapy, which is slightly diminished during the rest of the treatment period up to 12 months. This could be due to the need for dose reductions and dose interruptions. Nevertheless, the effect could be maintained during this extended treatment period for patients able to remain on therapy. Further information on the long-term efficacy of volanesorsen will be collected through the planned Registry.

A treatment effect on clinical endpoints was hampered due to the limited data available. A trend towards a clinical effect on pancreatitis could be observed, while any effect on abdominal pain was not

observed. However, it should be expected that the significant reduction in TG observed in the clinical trials would translate to a reduction in the events of pancreatitis in clinical practice in the long term.

Therefore, it was concluded that volanesorsen is an effective treatment option for patients with FCS.

## *2.6. Clinical safety*

### *Patient exposure*

The safety data submitted in support of the use of volanesorsen is combined from a number of different sources as outlined below:

- the primary safety data for patients in the proposed indication are derived from Study CS6;
- study CS16 provides additional randomized, controlled data in a related patient population, patients with HTG;
- safety data from the Phase 3 studies: Study CS6, and Study CS16 provides additional randomized, controlled data in a related patient population (including some patients with HTG;
- safety data from patients with FCS from the pivotal and support studies (CS7 and CS16). This pooled analysis consisted of 86 patients and is the most representative analysis set for the overall safety profile of volanesorsen in the intended target population;

The above datasets have different data lock-points as they include different studies.

The overall exposure to volanesorsen in controlled to studies is provided in **Table 33**.

**Table 33**. Exposure to volanesorsen in controlled studies, data cut-off 31 Dec 2017

| | **CS6** | | **CS16** | | **Pooled**[a] | |
|---|---|---|---|---|---|---|
| **Exposure Parameter** | **Placebo (N = 33)** | **Volanesorsen (N = 33)** | **Placebo (N = 38)** | **Volanesorsen (N = 75)** | **Placebo (N = 71)** | **Volanesorsen (N = 108)** |
| Treatment Duration (Weeks)[b] | | | | | | |
| N | 33 | 33 | 38 | 75 | 71 | 108 |
| Mean (SD, SE) | 50.24 (4.98, 0.87) | 38.20 (16.09, 2.80) | 22.82 (6.58, 1.07) | 19.40 (8.94, 1.03) | 35.57 (14.97, 1.78) | 25.15 (14.43, 1.39) |
| Median (P25, P75) | 51.14 (50.71, 51.43) | 49.43 (22.14, 51.00) | 25.14 (24.14, 25.29) | 24.14 (14.14, 25.14) | 25.43 (25.14, 51.14) | 24.64 (18.36, 25.57) |
| Min, Max | 23.29, 54.14 | 8.14, 53.14 | 0.14, 29.14 | 0.14, 27.57 | 0.14, 54.14 | 0.14, 53.14 |
| Mean 95% CI | 48.48, 52.01 | 32.50, 43.91 | 20.66, 24.98 | 17.35, 21.46 | 32.02, 39.11 | 22.39, 27.90 |
| 0 - 13 Weeks | 0 | 3 (9.1) | 4 (10.5) | 17 (22.7) | 4 (6.4) | 20 (17.3) |
| > 13 - 26 Weeks | 1 (3.0) | 6 (18.2) | 32 (84.2) | 56 (74.7) | 33 (52.1) | 62 (52.3) |
| > 26 Weeks | 32 (97.0) | 24 (72.7) | 2 (5.3) | 2 (2.7) | 34 (41.5) | 26 (30.4) |
| Number of Administration of Study Drug | | | | | | |
| N | 33 | 33 | 38 | 75 | 71 | 108 |
| Mean (SD, SE) | 49 (6, 1) | 35 (15, 3) | 21 (7, 1) | 18 (8, 1) | 34 (15, 2) | 23 (13, 1) |
| Median (P25, P75) | 51 (49, 52) | 41 (20, 48) | 24 (19, 26) | 19 (13, 26) | 26 (23, 51) | 21 (15, 26) |
| Min, Max | 24, 53 | 5, 53 | 1, 30 | 1, 26 | 1, 53 | 1, 53 |
| Mean 95% CI | 47, 51 | 29, 40 | 19, 24 | 16, 19 | 31, 38 | 20, 25 |
| Number of Patients with Dose Reduction, n % | 0 | 10 (30.3) | 13 (34.2) | 29 (38.7) | 13 (20.7) | 39 (35.4) |
| Number of Patients with Dose Pause, n % | 6 (18.2) | 11 (33.3) | 3 (7.9) | 10 (13.3) | 9 (12.0) | 21 (21.2) |

**Adverse events**

In controlled studies, 985 TEAEs were reported in 32 (97%) patients in the volanesorsen group and 227 TEAEs were reported in 31 (94%) patients in the placebo group at the time of the data cut-off. The most common AEs for patients receiving volanesorsen were events related to local tolerability and decreases in platelet counts. The majority of AEs (734 out of 985 events; 75%) were mild in severity.

Local cutaneous reactions at the injection site (LCRIS; defined as those AEs at the injection site presenting as either pain, tenderness, erythema, pruritus, or swelling occurring on the day of injection and persisting for at least 2 days), occurred following 12% of injections in volanesorsen-treated patients; most LCRIS were mild and none were severe, and most resolved. Twenty (61%) volanesorsen-treated patients experienced at least 1 LCRIS during the study; no placebo-treated patients experienced these types of events.

Twelve events of platelet count decreased were reported in 10 (30%) patients in the volanesorsen group and 5 thrombocytopenia events were reported in 4 (12%) patients in the volanesorsen group, compared with 1 event of platelet count decreased in 1 (3%) patient in the placebo group; these AEs were generally mild in severity.

Bleeding events were reported in 16 (49%) patients in the volanesorsen group compared with 4 (12%) patients in the placebo group. Nearly half of these events were local to the injection site, which may be a result of the known transient effect of ASOs on the tenase complex. Additional bleeding events occurring in more than 5% of patients were epistaxis in 5 (15%) patients, petechiae in 4 (12%) patients, and vaginal haemorrhage in 2 (6%) patients. There were no major or severe bleeding events. There was no apparent relationship between bleeding events and platelet levels or the use of concomitant antiplatelet or anticoagulant medications. Local injection reactions, platelet count decreased/thrombocytopenia, and bleeding events are further discussed in the section other significant events

Other most commonly reported AEs included abdominal pain (27% volanesorsen vs 21% placebo), headache (21% vs 15%), fatigue (21% vs 9%), erythema (18% vs 9%), nausea (18% vs 6%), nasopharyngitis (15% vs 21%), asthenia (15% vs 9%), vomiting (15% vs 9%), diarrhea (15% vs 6%), myalgia (15% vs 3%), and epistaxis (15% vs 0%) in volanesorsen vs placebo.

A summary of the most commonly reported adverse events in the placebo-controlled phase 3 group is summarised in **Table 34.**

**Table 34.** On-Treatment Adverse Events Experienced by > 10% of Pooled Volanesorsen Patients (Excluding AEs at the Injection Site) – Placebo Controlled Phase 3 Group

| | CS6 | | CS16 | | Pooled[b] | |
|---|---|---|---|---|---|---|
| Preferred Term[a] | Placebo (N = 33) n (%) | Volanesorsen (N = 33) n (%) | Placebo (N = 38) n (%) | Volanesorsen (N = 75) n (%) | Placebo (N = 71) n (%) | Volanesorsen (N = 108) n (%) |
| Nasopharyngitis | 7 (21.2) | 5 (15.2) | 5 (13.2) | 12 (16.0) | 12 (16.3) | 17 (15.7) |
| Fatigue | 3 (9.1) | 7 (21.2) | 4 (10.5) | 9 (12.0) | 7 (10.0) | 16 (15.6) |
| Abdominal pain | 7 (21.2) | 7 (21.2) | 0 | 8 (10.7) | 7 (8.4) | 15 (14.8) |
| Diarrhoea | 2 (6.1) | 5 (15.2) | 4 (10.5) | 10 (13.3) | 6 (8.8) | 15 (14.1) |
| Thrombocytopenia | 0 | 4 (12.1) | 2 (5.3) | 10 (13.3) | 2 (3.2) | 14 (12.9) |
| Platelet count decreased | 1 (3.0) | 10 (30.3) | 2 (5.3) | 3 (4.0) | 3 (4.4) | 13 (14.4) |
| Headache | 5 (15.2) | 7 (21.2) | 4 (10.5) | 6 (8.0) | 9 (12.4) | 13 (13.2) |
| Nausea | 2 (6.1) | 6 (18.2) | 1 (2.6) | 7 (9.3) | 3 (4.0) | 13 (12.8) |

*Abbreviations: AE = adverse event*
*Note: An On-treatment AE was defined as any AE that occurred from the first dose of the study drug through 28 days post the last dose of study drug.*
[a] *Patients reporting more than 1 instance of a preferred term were counted only once for the total incidence and for each treatment. Preferred terms are sorted in descending frequency by the pooled volanesorsen column.*
[b] *Pooled proportion was adjusted by study using the CMH approach.*

Adverse drug reactions (ADRs), or AEs related or possibly related to drug exposure, in patients with FCS were determined by evaluating AEs that occurred with a ≥ 1% overall incidence rate and that 1) occurred with a ≥ 1% difference between the volanesorsen-treated group and the placebo group, and/or 2) had an apparent dose response trend. Medical judgment was further applied, taking into consideration the extent to which the AE was consistent with the pharmacology of the drug and the consistency of the pattern of symptoms across studies. Adverse drug reactions were identified that occurred in patients who were treated in the completed double-blind, placebo-controlled study CS6. Additionally, any noteworthy AEs not meeting the ≥ 1% difference between the volanesorsen-treated group vs the placebo group, or those occurring at an incidence rate of < 1% during the double-blind, placebo-controlled phase were also evaluated.

Related on-treatment AEs (excluding AEs at the injection site) occurring in > 5% of patients in the pooled volanesorsen group are presented in **Table 35.**

**Table 35.** On-Treatment Adverse Events Potentially Related to Study Drug Experienced by > 5% of Pooled Volanesorsen Patients (Excluding AEs at the Injection Site )– Placebo Controlled Phase 3 Group (N = 179)

| | CS6 | | CS16 | | Pooled[b] | |
|---|---|---|---|---|---|---|
| **Preferred Term[a]** | **Placebo (N = 33) n (%)** | **Volanesorsen (N = 33) n (%)** | **Placebo (N = 38) n (%)** | **Volanesorsen (N = 75) n (%)** | **Placebo (N = 71) n (%)** | **Volanesorsen (N = 108) n (%)** |
| Thrombocytopenia | 0 | 4 (12.1) | 1 (2.6) | 10 (13.3) | 1 (1.6) | 14 (12.9) |
| Platelet count decreased | 1 (3.0) | 10 (30.3) | 2 (5.3) | 3 (4.0) | 3 (4.4) | 13 (14.4) |
| Fatigue | 1 (3.0) | 4 (12.1) | 1 (2.6) | 5 (6.7) | 2 (2.8) | 9 (8.8) |
| Myalgia | 1 (3.0) | 5 (15.2) | 0 | 4 (5.3) | 1 (1.2) | 9 (9.2) |
| Asthenia | 1 (3.0) | 4 (12.1) | 1 (2.6) | 4 (5.3) | 2 (2.8) | 8 (8.0) |
| Headache | 1 (3.0) | 4 (12.1) | 2 (5.3) | 4 (5.3) | 3 (4.4) | 8 (8.0) |
| Low density lipoprotein increased | 0 | 0 | 0 | 7 (9.3) | 0 | 7 (5.6) |
| Nausea | 1 (3.0) | 3 (9.1) | 0 | 3 (4.0) | 1 (1.2) | 6 (6.0) |
| Pruritus | 1 (3.0) | 3 (9.1) | 0 | 3 (4.0) | 1 (1.2) | 6 (6.0) |

*Abbreviations: AE = adverse event*
*Note: An On-treatment AE was defined as any AE that occurred from the first dose of the study drug through 28 days post the last dose of study drug.*
[a] *Patients reporting more than 1 instance of a preferred term were counted only once for the total incidence and for each treatment. Preferred terms are sorted in descending frequency by the pooled volanesorsen column.*
[b] *Pooled proportion was adjusted by study using the CMH approach and the adjustments are based on actual number of patients in the treatment period.*

In the ongoing open label extension study CS7 (as per cut-off date June 2018; n=67), the most common AEs for patients receiving volanesorsen were local injection site reaction (21.7%-44.2%), pyrexia (15%), fatigue (12%), and asthenia and chills (10% each), nasopharyngitis (34%), influenza (15%), and urinary tract infection (12%), abdominal pain (25%), nausea (24%), diarrhea (13%), vomiting (10%), cough and arthralgia (18% each); headache (16%); pain in extremity (13%); and, dizziness, myalgia, and oropharyngeal pain (10% each). Most commonly treatment related AEs (excluding local injection site reactions) were platelet count decreased (18%), arthralgia and chills

(10%), thrombocytopenia (9%), headache (9%), nausea (8%), pyrexia (8%), abdominal pain (6%), asthenia (6%), fatigue (6%), and myalgia (6%).

The frequency of adverse reactions reported in FCS patients, by System Organ Class are summarised in **Table 36**.

**Table 36.** Summary of adverse reactions in clinical studies in patients with FCS (N=86), data cut-off June 2018

| **System Organ Class** | |
|---|---|
| Blood and lymphatic system disorders | Thrombocytopenia (10, 12%)<br>Leukopenia (2, 2%)<br>Eosinophilia (1, 1%)<br>Immune thrombocytopenic purpura (1, 1%)<br>Spontaneous haematoma (1, 1%) |
| Immune system disorders | Immunisation reaction (3, 3%)<br>Hypersensitivity (1, 1%)<br>Serum sickness-like reaction (1, 1%) |
| Metabolism and nutrition disorders | Diabetes mellitus (1, 1%) |
| Psychiatric disorders | Insomnia (1, 1%) |
| Nervous system disorders | Headache (8, 9%)<br>Hypoaesthesia (1, 1%)<br>Presyncope (1, 1%)<br>Retinal migraine (1, 1%)<br>Syncope (2, 2%)<br>Dizziness (1, 1%)<br>Tremor (1, 1%) |
| Eye disorders | Conjunctival haemorrhage (1, 1%)<br>Vision blurred (1, 1%) |
| Vascular disorders | Haematoma (3, 3%)<br>Hypertension (1, 1%)<br>Haemorrhage (1, 1%)<br>Hot flush (1, 1%) |
| Respiratory, thoracic and mediastinal disorders | Epistaxis (3, 3%)<br>Cough (1, 1%)<br>Dyspnoea (2, 2%)<br>Nasal congestion (1, 1%)<br>Pharyngeal oedema (1, 1%)<br>Wheezing (1, 1%) |
| Gastrointestinal disorders | Nausea (8, 9%)<br>Diarrhoea (4, 5%) |

| | |
|---|---|
| | Dry mouth (1,1 %)<br>Gingival bleeding (1, 1%)<br>Mouth haemorrhage (1, 1%)<br>Parotid gland enlargement (1, 1%)<br>Vomiting (4, 5%)<br>Abdominal pain (4, 5%)<br>Abdominal distension (1, 1%)<br>Dyspepsia (1, 1%)<br>Gingival swelling (1, 1%) |
| Skin and subcutaneous tissue disorders | Erythema (4, 5%)<br>Pruritus (4,5 %)<br>Urticaria (3, 3%)<br>Hyperhidrosis (2, 2%)<br>Rash (3, 3%)<br>Petechiae (1, 1%)<br>Ecchymosis (1, 1%)<br>Night sweats (1, 1%)<br>Papule (1, 1%)<br>Skin hypertrophy (1, 1%)<br>Swelling face (1, 1%) |
| Musculoskeletal and connective tissue disorders | Myalgia (8,9%)<br>Arthralgia (6, 7%)<br>Pain in extremity (5, 6%)<br>Arthritis (2, 2%)<br>Back pain (2, 2%)<br>Musculoskeletal pain (2, 2%)<br>Neck pain (2, 2%)<br>Muscle spasms (1, 1%)<br>Joint stiffness (1, 1%)<br>Myositis (1, 1%)<br>Pain in jaw (1, 1%)<br>Polymyalgia rheumatica (1, 1%) |
| Renal and urinary disorders | Haematuria (1, 1%)<br>Proteinuria (1, 1%) |

| | |
|---|---|
| General disorders and administration site conditions | Injection site erythema (67, 78%)<br>Injection site pain (38, 44%)<br>Injection site pallor (37, 43%)<br>Injection site swelling (25, 29%)<br>Injection site pruritus (22, 26%)<br>Injection site discolouration (19, 22%)<br>Injection site induration (17, 20%)<br>Injection site bruising (10, 12%)<br>Injection site oedema (10, 12%)<br>Asthenia (8, 9%)<br>Fatigue (8, 9%)<br>Injection site haematoma (7, 8%)<br>Injection site reaction (6, 7%)<br>Injection site urticaria (5, 6%)<br>Injection site warmth (5, 6%)<br>Chills (5, 6%)<br>Pyrexia (4, 5%)<br>Injection site dryness (4, 5%)<br>Injection site haemorrhage (4, 5%)<br>Injection site hypoaesthesia (4, 5%)<br>Injection site vesicles (3, 3%)<br>Malaise (2, 2%)<br>Feeling hot (2, 2%)<br>Influenza-like illness (2, 2%)<br>Injection site discomfort (2, 2%)<br>Injection site inflammation (2, 2%)<br>Injection site mass (2, 2%)<br>Pain (2, 2%)<br>Injection site paraesthesia (1, 1%)<br>Injection site scab (1, 1%)<br>Injection site papule (1, 1%)<br>Oedema (1, 1%)<br>Non-cardiac chest pain (1, 1%)<br>Vessel puncture site haemorrhage (1, 1%) |

| | |
|---|---|
| Investigations | Platelet count decreased (34, 40%)<br>Blood creatinine increased (1, 1%)<br>Blood urea increased (1, 1%)<br>Creatinine renal clearance decreased (1, 1%)<br>Transaminases increased (1, 1%)<br>White blood cell count decreased (1, 1%)<br>Haemoglobin decreased (1, 1%)<br>Hepatic enzyme increased (1, 1%)<br>International normalised ratio increased (1, 1%) |
| Injury, poisoning and procedural complications | Contusion (3, 3%) |

## *Serious adverse event / deaths / other significant events*

In study CS6 and CS16, serious AEs were reported on treatment in 13 (13%) patients in the volanesorsen group and 8 (11%) patients in the placebo group. Three SAEs in 3 (3%) patients in the volanesorsen group were considered potentially related to study drug, and none in the placebo group. The potentially related SAEs included the 2 reports of thrombocytopenia (CS6) and a report of serum sickness (CS16). In study CS6, the 2 (6%) grade 4 thrombocytopenia (< 25,000/$mm^3$) led to treatment discontinuation, and subsequently, a more intensive platelet monitoring plan was implemented.

In study CS6, other SAEs in the volanesorsen group were abdominal pain in 1 (3%) patient (adjudicated as pancreatitis), cyst in 1 (3%) patient, cholangitis and drug-induced liver injury (following diclofenac) in 1 (3%) patient, ankle fracture in 1 (3%) patient, and dehydration in 1 (3%) patient; except for severe dehydration, these events were moderate in severity and none were considered related to study treatment. The ankle fracture and dehydration both resulted in interruption of study treatment. No action was taken with study treatment for the other SAEs and all were reported as resolved.

In Study CS16, 4 on-treatment potential MACE cases were adjudicated (none in study CS6). One patient of these 4 was found to be not applicable (event determined to be non-cardiac chest pain); 2 were found to be elective re-vascularization, and thus non-coronary events, and 1 MACE was adjudicated as stroke. All were unrelated to study drug and occurred in patients treated with volanesorsen with established cardiovascular disease or symptoms of cardiovascular disease.

In the ongoing study CS7, thirteen (19%) patients (10 treatment naïve, 2 volanesorsen from CS6, 1 from CS16) reported a serious adverse event (SAE). Also, 4 cases of pancreatitis were observed and 1 case of proteinuria. The cases of juvenile idiopathic arthritis, platelet count decreased, 1 event of thrombocytopenia, and proteinuria were considered related to study drug.

### *Deaths*

No deaths were reported during the clinical development program.

***Other significant events***

***Platelet counts***

In study CS6, mean decreases in platelets were observed over time for patients in the volanesorsen group as compared to those in the placebo group with lowest level of 134,000/$mm^3$ (Week 25) and 207,000 $mm^3$ (Week 48), respectively; and mean change from baseline of -48,000/$mm^3$ and 25,000/$mm^3$, respectively. Similar findings could be observed for study CS16.

In study CS6, 75% for volanesorsen vs 24% for placebo had a decrease below normal level of platelet count (< 140,000/$mm^3$, **Table 37**).

**Table 37.** Summary of Confirmed Nadir Post-Baseline Platelet Counts in Study CS6 – All Patients (N = 66)

| | **Placebo (N = 33) n (%)** | **Volanesorsen (N = 33) n (%)** | **All Patients (N = 66) n (%)** |
|---|---|---|---|
| Patients with baseline platelet counts[a] | 33 | 32[c] | 65 |
| Number (%) patients with confirmed nadir platelet count < 140,000/$mm^3$ | 8 (24.2) | 24 (75.0) | 32 (49.2) |
| Number (%) patients with confirmed nadir platelet count < 100,000/$mm^3$ | 0 (0.0) | 15 (46.9) | 15 (23.1) |
| Confirmed Nadir Platelet Count Post-Baseline[b] | | | |
| 100,000/$mm^3$ to < 140,000/$mm^3$ | 8 (24.2) | 9 (28.1) | 17 (26.2) |
| 75,000 to < 100,000/$mm^3$ | 0 (0.0) | 6 (18.8) | 6 (9.2) |
| 50,000 to < 75,000/$mm^3$ | 0 (0.0) | 6 (18.8) | 6 (9.2) |
| 25,000 to < 50,000/$mm^3$ | 0 (0.0) | 1 (3.1) | 1 (1.5) |
| 0 to < 25,000/$mm^3$ | 0 (0.0) | 2 (6.3) | 2 (3.1) |

[a] *The baseline of platelets was defined as the average of all pre-dose values.*

[b] *A confirmed value was based on a consecutive lab value within 7 days. If that value was in the same or worse category the initial value was confirmed. If the consecutive value was in a better category than the initial value was confirmed using the consecutive value category. If there was no retest within 7 days, the initial value was presumed confirmed.*

[c] *Baseline platelet count could not be obtained in 1 patient because of hemolysis.*

In study CS6, platelet count decreased AEs was 10 (30%) vs 1 (3%), and thrombocytopenia was 4 (12%) vs none, respectively, and were generally mild in severity and managed with dose pauses and adjustments. In study CS16, this was 3 (4%) vs 2 (5%) and 10 (13%) vs 2 (5%). As no overlap exists between the two definitions, this results in study CS6 24/33 volanesorsen-treated patients (75%) had confirmed post-baseline platelet count below the LLN (140,000/$mm^3$), and TEAE of 'thrombocytopenia/ platelet count decreased' was reported in 14 patients (42.4%). In study CS16 results were 28/75 volanesorsen-treated patients (37%) had a confirmed post-baseline platelet count below the LLN (140,000/$mm^3$), and TEAE of 'thrombocytopenia/ platelet count decreased' was reported in 13 patients (17%).

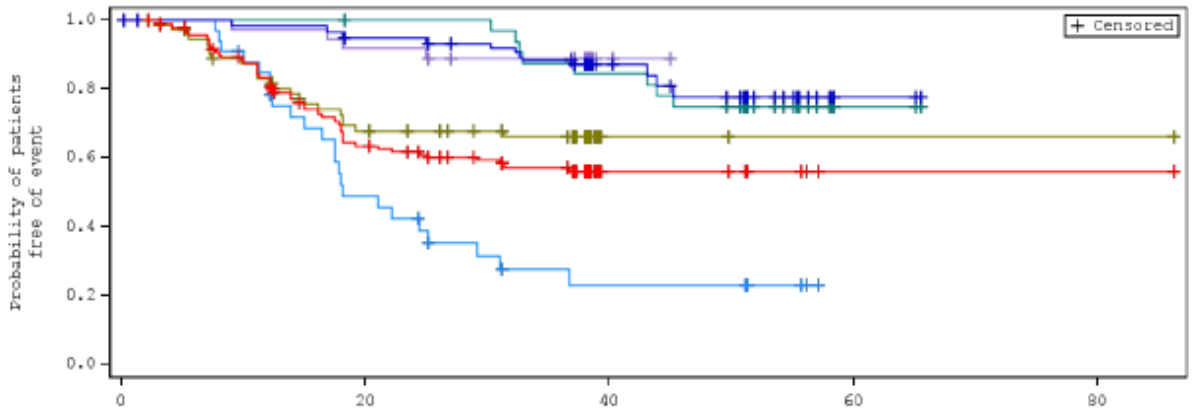
In study CS16, the proportion of patients according to categories of platelet count reductions were not different between patients with 300 mg weekly dosing and 300 mg biweekly dosing (**Table 38**). Mean platelet count decrease was -52 (x10^9/L) at week 13, -65 at week 26 for 300 mg weekly (n=25) from a 238 (SD 69) baseline level and -72 and -67 for a selection of patients from the 300 mg biweekly group (n=15 of 27 treated during end of study) from a 247 (SD 83) baseline level.

**Table 38.** Summary of Confirmed Nadir Post-Baseline Platelet Counts in Study CS16 – All Patients (N = 113)

| | Placebo (N=38) n (%) | Volanesorsen | | | All Patients (N=113) n (%) |
|---|---|---|---|---|---|
| | | 300 mg Weekly (N=25) n (%) | 300 mg Biweekly post Wk 13 (N=50) n (%) | Total (N=75) n (%) | |
| Number of patients with baseline[a] platelet counts ≥140,000/mm3[a] | 38 | 25 | 50 | 75 | 113 |
| Number of patients with any 2 occurrences of platelet count < 140,000/mm$^3$ | 4 (10.53) | 6 (24.00) | 15 (30.00) | 21 (28.00) | 25 (22.12) |
| Number of patients with any single occurrence of platelet count < 100,000/mm$^3$ | 1 (2.63) | 3 (12.00) | 6 (12.00) | 9 (12.00) | 10 (8.85) |
| Number of patients with any 2 occurrences of platelet count <140,000/mm$^3$ or with any single occurrence of platelet count < 100,000/mm$^3$ | 4 (10.53) | 7 (28.00) | 16 (32.00) | 23 (30.67) | 27 (23.89) |
| Confirmed Nadir Platelet Count Post-Baseline[b] | | | | | |
| 100,000 to < 140,000/mm$^3$ | 5 (13.16) | 8 (32.00) | 13 (26.00) | 21 (28.00) | 26 (23.01) |
| 75,000 to < 100,000/mm$^3$ | 1 (2.63) | 3 (12.00) | 3 (6.00) | 6 (8.00) | 7 (6.19) |
| 50,000 to < 75,000/mm$^3$ | 0 | 0 | 2 (4.00) | 2 (2.67) | 2 (1.77) |
| 25,000 to < 50,000/mm$^3$ | 0 | 0 | 1 (2.00) | 1 (1.33) | 1 (0.88) |
| 0 to < 25,000/mm$^3$ | 0 | 0 | 0 | 0 | 0 |

Platelet count reductions were observed immediately after initiation but mostly between 3 to 6 months in the phase 3 studies (**Figure 20**). This decline reached a plateau where the group mean platelet count was approximately 30% lower than the baseline value. Three of the 5 patients who had platelet reductions to < 50,000/mm$^3$ experienced the events within 13 weeks, the other 2 after 26 weeks, and were not associated with major or severe bleeding, while 1 had a non-major bleeding of moderate and mild injection site haemorrhages. Two patients with platelet counts < 25,000/mm$^3$ had epistaxis non-major bleed, being mild and not requiring specific treatment or intervention. Platelet counts returned to normal in all patients following dose pause or discontinuation of study drug and/or administration of corticosteroids. One of the patients with platelet count < 25,000/mm$^3$ was also treated with intravenous immunoglobulin. Four more cases were identified during the open-label CS7 study (and thus after dose algorithm implementation) that led to treatment discontinuation (see further below). Overall, platelet count decreased events were reported in 12 (18%) patients and thrombocytopenia was reported in 6 (9%) patients in the ongoing -study CS7.

**Figure 19.** Kaplan-Meier Analysis of Time to First Event of Any 2 Occurrences of Platelet Count < 140,000/mm3 or Any Single Occurrence of Platelet Count <100,000/mm3 during the On-study Period - Safety Set Placebo Controlled Phase 3 Group

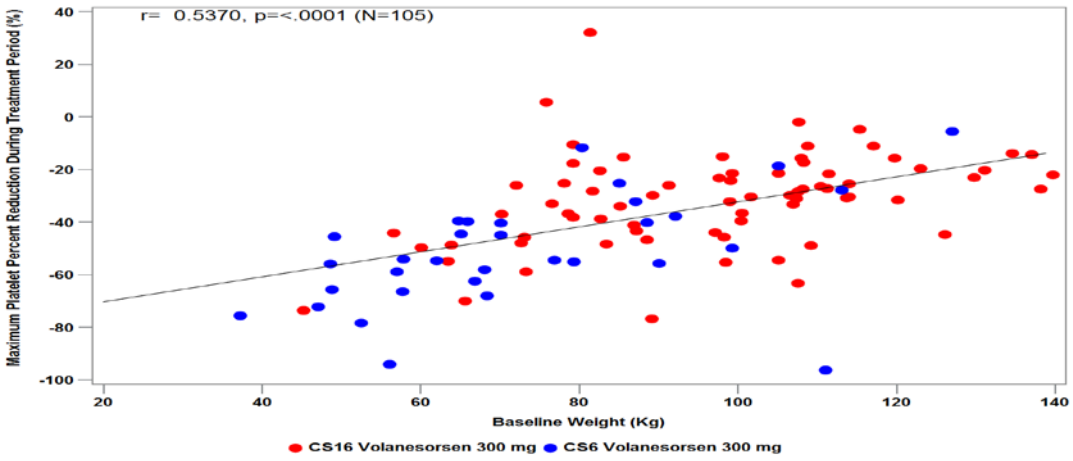



| | Subjects | Event | Censored | Median survival | 95% CI |
|---|---|---|---|---|---|
| Placebo CS6 | 33 | 8 | 25 | NR | NR, NR |
| Volanesorsen CS6 | 33 | 23 | 10 | 18.14 | 15.00, 29.14 |
| Placebo CS16 | 38 | 4 | 34 | NR | NR, NR |
| Volanesorsen CS16 | 75 | 23 | 52 | NR | NR, NR |
| Placebo Pooled | 71 | 12 | 59 | NR | NR, NR |
| Volanesorsen Pooled | 108 | 46 | 62 | NR | 25.14, NR |

In the open-label study CS7 (as of December 2017), median time to first dose reduction was 92 days and median time to first dose pause was 180 days. Median number of dose pauses per patients were 2 with a median time of 16 days per patient during a median follow up of 247 days in the treatment-naïve group and 736 days in the CS6-volanesorsen group.

A statistical association between platelet count percent decrease and body weight was observed (p < 0.0001) (**Figure 21**), suggesting that patients on the lower weight spectrum may have an increased risk of platelet reductions. However, in CS7, only a weak correlation with body weight was observed (data not shown).

**Figure 20.** Correlation Between Maximum Percent Reduction in Platelets During the On Treatment Period Versus Baseline Weight (Studies CS6 and CS16, N = 105)




A population PK analysis showed that all 4 body size metrics (body weight, lean body mass, body mass index, and body surface area) were statistically significant but moderate effect on the clearance of

volanesorsen, (data not shown). In addition, patients with a body weight of less than 70 kg reach a lower nadir platelet value than those patients with bodyweights over 70 kg (**Table 39**).

**Table 39.** Dose, Platelet Reduction, and TG Reduction as a Function of Body Weight in Study CS6 Completers

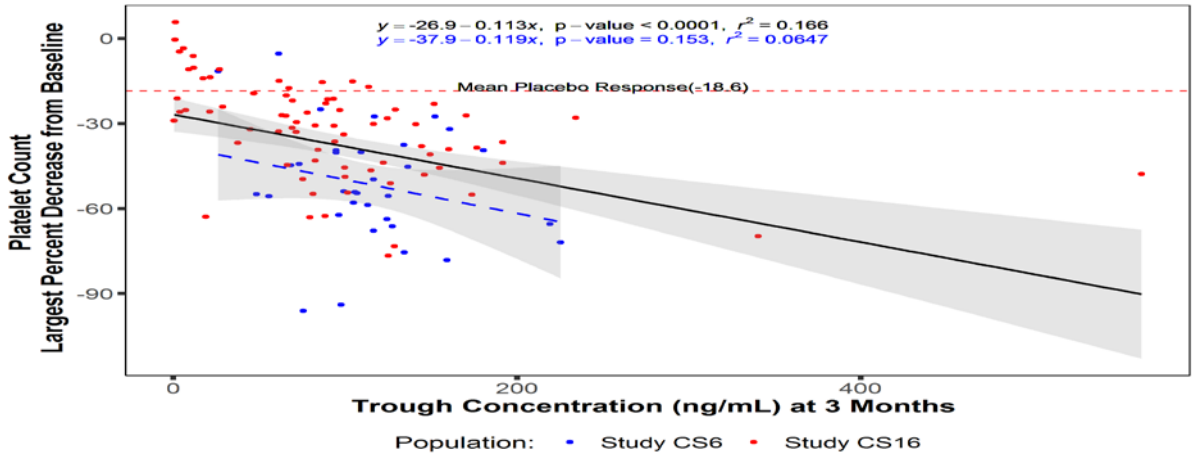
| **Dose, Platelet Reduction, and TG Reduction as a Function of Body Weight in Study CS6 Completers** | **Body Weight < 70 kg (N=11)** | **Body Weight ≥ 70 kg (N=8)** |
|---|---|---|
| Average of average weekly dose in the last 6 months (mg) | 218.2 | 282.7 |
| Average platelet count at baseline ($10^9$/L) | 209 | 217 |
| Average nadir platelet count ($10^9$/L) | 76 | 126 |
| Average nadir % change in platelet count from baseline (%) | -62.3 | -43.3 |
| Average TG at baseline (mg/dL) | 2286 | 2666[a] |
| Average TG at Month 12 (mg/dL) | 935 | 873[a] |
| Average TG % change from baseline at Month 12 (%) | -60.3 | -72.1[a] |

*Abbreviations: TG = triglyceride*

[a] *Excluded patient 1690-1165 (70 kg body weight) whose Baseline, Month 3, Month 6, and Month 12 TG levels were 1367, 1320, 2362, and 1856 mg/dL corresponding to % TG change from baseline at Month 3, Month 6, and Month 12 of -3%, +73%, +36%, respectively, despite continued dose administration.*

A regression analysis showed a (modest) relationship between Ctrough, 3 months (**Figure 22**) and $AUC_{0\text{-}3\ months}$ (data not shown) and largest decrease in platelet count.

**Figure 21.** Largest Percent Decrease in Platelet Count Vs. Steady-state Exposure - Pooled Studies CS6 and CS16



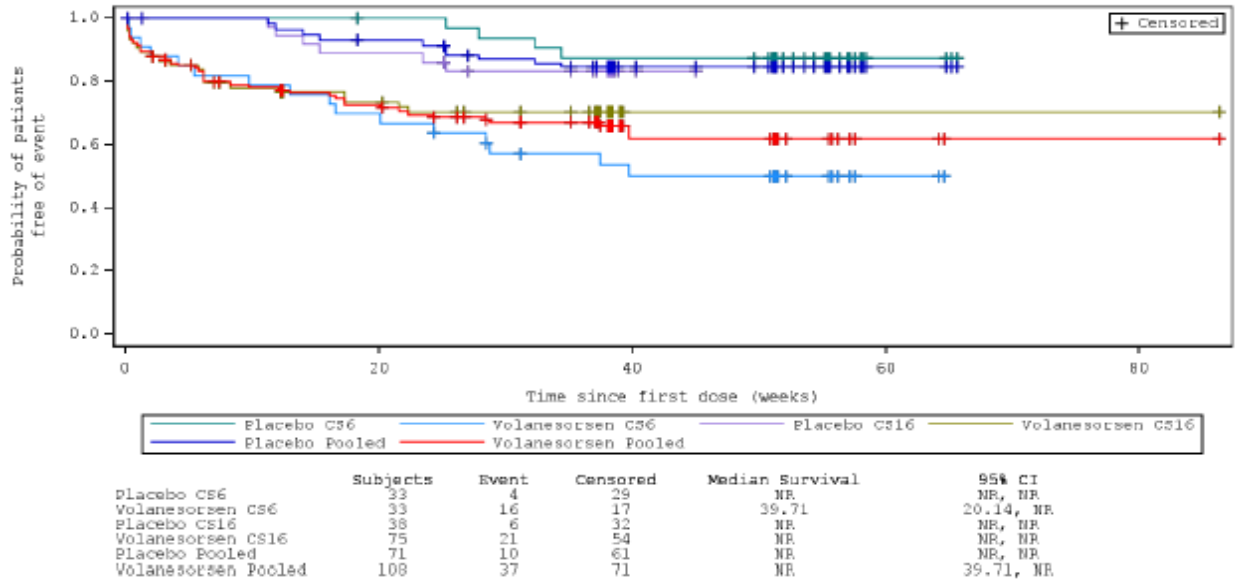
***Bleeding events***

In Study CS6, 20 patients experienced 50 bleeding events (16 patients had 45 events in the volanesorsen group and 4 patients had 5 events in the placebo group); 20 (40%) of the 50 bleeding events were reported at the injection site; there appears to be no correlation between platelet counts and bleeding events; and no increased risk of bleeding events with concurrent antiplatelet or anticoagulation medications.

The most commonly reported bleeding events in the volanesorsen group (excluding the injection site events and laboratory abnormalities) were epistaxis in 5 (15%) patients, petechiae (4 (12%)) and vaginal hemorrhage 2 (6%), versus none in placebo. All bleeding events were mild; none were reported as SAEs, none were major bleedings, and none led to treatment discontinuation.

Most patients experienced bleeding events within 13 weeks of therapy (44 (30%) ≤ 13weeks, 16 (12.5%) > 13-26 weeks, and 17 (3.4%) > 26 weeks for volanesorsen; 8 (8.3%), 5 (5.6%), 4 (4.9%) for placebo) in the combined phase 2 and 3 studies. This is also displayed graphically for the combined phase 3 studies (**Figure 23).**

**Figure 22.** Kaplan-Meier Analysis of Time to Onset of On-study Bleeding Adverse Events - Safety Set Placebo Controlled Phase 3 Group



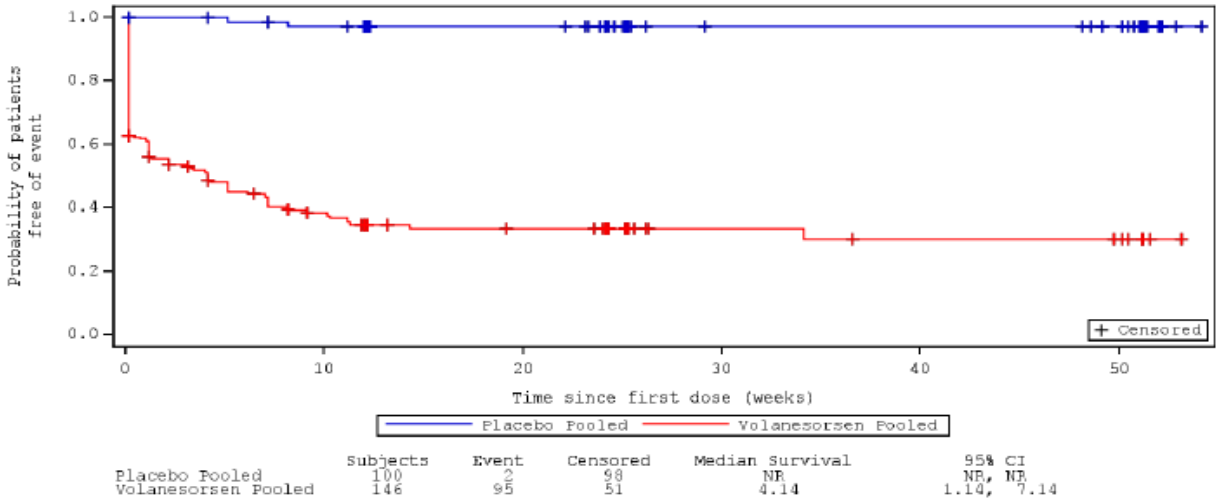

| | Subjects | Event | Censored | Median Survival | 95% CI |
|---|---|---|---|---|---|
| Placebo CS6 | 33 | 4 | 29 | NR | NR, NR |
| Volanesorsen CS6 | 33 | 16 | 17 | 39.71 | 20.14, NR |
| Placebo CS16 | 38 | 6 | 32 | NR | NR, NR |
| Volanesorsen CS16 | 75 | 21 | 54 | NR | NR, NR |
| Placebo Pooled | 71 | 10 | 61 | NR | NR, NR |
| Volanesorsen Pooled | 108 | 37 | 71 | NR | 39.71, NR |

***Injection site reactions***

In Study CS6, 20 (61%) volanesorsen-treated patients vs none in placebo experienced at least 1 injection site reaction, (lasting > 2 days). The most commonly reported AEs at the injection site in the volanesorsen group were injection site erythema (17 patients, 52%), injection site pain (8 patients, 24%), and injection site pruritus (5 patients, 15%), and injection site swelling (3 patients, 9.1%).

In study CS6 and CS16, a total of 68 (63%) of 108 patients (663 total events) for volanesorsen and 2 (3%) of 71 patients (3 total events) for placebo had an injection site reaction. Median time to onset for injection site reactions in the phase 3 studies for volanesorsen group was 4.3 weeks, indicating that when these events first occur, it is early on in therapy (**Figure 24),** and incidence appeared to decrease over time.

**Figure 23.** Kaplan-Meier Analysis of Time to Onset of On-Study Local Cutaneous Reactions at Injection Site - Safety Set Placebo Controlled Phase 2 and 3 Group




**Immunological events**

Relatively high incidences in anti-drug antibodies (ADA) were observed (10 patients (30%) in volanesorsen vs 1 patient (3%) in placebo in study CS6 and 12 patients (16%) in volanesorsen vs 2 patients (5%) in placebo in study CS16. The median time of onset in these ADA-positive patients was Study Day 179.5 and 165 for study CS6 and CS16, respectively. The presence of ADA was not associated with a different safety outcome, since patients with ADA had similar patient characteristics and AE frequencies as patients without ADA.

In study CS6, the demographics of patients who tested positive for ADA were similar to those who were negative. Six of the 10 (60%) ADA-positive patients in the volanesorsen group discontinued treatment as compared to 8 of 23 (35%) ADA-negative patients which discontinued treatment. However, 4 ADA-positive patients in the volanesorsen group discontinued treatment prior to seroconversion and 2 patients after seroconversion, suggesting that the presence of ADA is unlikely to have affected patient disposition.

The most commonly reported AEs for volanesorsen in both antibody-positive and antibody-negative patients were events related to local tolerability. Nine of 10 (90%) ADA-positive patients and 20 of 23 (87%) IM-negative patients reported these events. There was no notable difference in the incidence of LCRIS between ADA-positive (7 of 10 patients, 70%) and ADA-negative patients (13 of 23 patients, 57%). Overall, ADA development did not appear to be associated with a higher occurrence of AEs. There were no notable differences in the levels of platelet count (IM-positive: 38%; 3 of 8), IM negative (78%; 18 of 23) for confirmed platelet counts < 140,000 /mm$^3$), ALT, AST, creatinine clearance, hsCRP, complement C5a, and complement Bb between ADA-positive patients and antibody-negative patients. There was no increased incidence of platelet count decrease, ALT elevations above 3×ULN, and ALP elevations above 2×ULN) in the antibody-positive patients.

***Flu-like reactions***

In the phase 3 studies (CS6 and CS16), 4 (4%) of 108 patients in the volanesorsen group experienced a total of 6 flu-like reactions (FLRs). These included 3 patients who experienced an AE of influenza-like

illness (2 in CS6 and 1 in CS16) and 1 patient who experienced pyrexia/feeling hot with arthralgia and myalgia (non-FCS patient in Study CS16). These FLRs represent isolated events not representative of a systemic reaction associated with volanesorsen administration.

### *Laboratory findings*

A summary of laboratory abnormalities (other than decreased platelets) I n study CS6 and CS16, are presented below.

- Creatinine: Three patients had any shifts from normal to high; 2 patients received volanesorsen (1 each in Study CS6 and CS16) and experienced a shift from normal (< 1.5 µmol/L) to 2.1 2.5 µmol/L while on treatment and the third received volanesorsen in Study CS16 and had a shift from normal to 1.5 1.7 µmol/L for creatinine.
- BUN: shifts in BUN while on treatment were experienced by both patients groups. A total of 11 of 108 patients in the volansorsen group and 7 of 71 patients in the placebo group had shifts from ≤ 26 mg/dL to ≥ 27 mg/dL for BUN.
- ALT: In the volanesorsen group, 9 of 108 patients had on-treatment shifts in ALT from <2.5 × ULN to >2.5 × ULN; 1 each of these patients had shifts to > 5× ULN and >10 × ULN. Both of the values > 5× ULN were confirmed within 7 days. No volanesorsen-treated patient experienced elevations in bilirubin to > 2 × ULN. One patient in the placebo group had a shift from normal (< 1.1 × ULN) to the 5.1 10 × ULN category for ALT, which was confirmed.
- AST: In the volanesorsen group, 4 of 108 patients had on-treatment shifts in AST from <2.5 × ULN to >2.5 × ULN; 1 each of these patients had shifts to > 5× ULN and >10 × ULN. All shifts > 2.5 × ULN were confirmed within 7 days for the volanesorsen group. One patient in the placebo group had a shift from normal (< 1.1 × ULN) to the 5.1 10 × ULN category for AST and another had a shift to the 2.6 5.0 × ULN category; the shift >5 × ULN was not confirmed.

No events related to increased ALT, AST, ALP, or bilirubin increases led to discontinuation of treatment.

The patient-based incidence rate of on-study events of ALT > 3 × ULN was 8.9 (95% CI: 3.49, 22.84) per 100 patient-years in the pooled volanesorsen group and 2.2 (95% CI: 0.31, 15.56) per 100 patient-years in the pooled placebo group.

Volanesorsen treatment did not affect haemoglobin or leukocytes.

### *Safety in special populations*

The number of AEs experienced by younger patients (< 65 years) was higher than older patients (3249 vs 202 AEs, respectively), but the proportion of patients experiencing AEs was similar between volanesorsen and placebo (98% vs 90% for < 65 years and 100% vs 100% for ≥ 65 years, respectively).

When including patients from the Phase 2 studies, it appears as though a higher proportion of older patients experienced bleeding events (55% of volanesorsen patients) compared to younger patients (39% of volanesorsen patients). All platelet reductions to < 50,000/$mm^3$ were experienced by patients < 65 years old.

### *Safety related to drug-drug interactions and other interactions*

In the Phase 3 studies CS6 and CS16, 44 of 108 volanesorsen-treated patients were receiving antiplatelet drugs and the incidence of bleeding events does not seem to be higher in these patients.

### *Discontinuation due to adverse events*

***Discontinuations due to injection site reactions***

Placebo-Controlled Phase 3 Group

Permanent discontinuations of study drug due to AEs at the injection site were reported in 10 patients in the volanesorsen group (1 in CS6, 9 in CS16), but not in the placebo group; in most cases multiple specific AEs at the injection site contributed to discontinuation in each patient. Specific reasons for discontinuation due to AEs at the injection site in the pooled volanesorsen group included injection site erythema (7 patients, 6%), injection site pain (7 patients, 6%), injection site swelling (3 patients, 2%), injection site discoloration (2 patients, 2%), injection site edema (2 patients, 2%), and injection site discomfort, injection site pruritus, injection site rash, and injection site warmth in 1 patient each (< 1%).

***Discontinuations due to decrease in platelet counts***

Across Studies CS6, CS16, and CS2, 5 patients had platelet reductions to < 50,000/$mm^3$, 2 of which were declines to < 25,000/$mm^3$, 3 of which led to permanent discontinuation of study treatment (**Table 40).** In Study CS6, 2 of these events were considered serious (declines to < 25,000/mm3) and resulted in treatment discontinuation. In addition to these SAEs, 1 non-serious AE of thrombocytopenia and 2 non-serious AEs of decreased platelet count led to permanent discontinuation from study treatment; these patients are described briefly below.

**Table 40.** Summary of Patients with Platelet Decreases to < 50,000/mm3 (Studies CS2, CS6, and CS16)

| Study | Preferred Term | Platelet Nadir n/ $mm^3$ | Time to Onset | Duration | Associated Bleeding Events | Treatment discontinuation /interruption/ resumption |
|---|---|---|---|---|---|---|
| **Platelet Decreases to < 50,000/$mm^3$** | | | | | | |
| CS6 | Platelet count decreased | 40,000 | 85 days | 61 days | None | 92 days interrupted<br>155 days resumed |
| CS16 | Thrombocytopenia | 41,000 | 51 days | 14 days | Injection site hemorrhage | 51 days interrupted<br>72 days resumed<br>99 days discontinued (after platelet 44,000/$mm^3$) |
| CS6 | Thrombocytopenia | 8,000 | 257 days | 36 days | Epistaxis and petechiae | Days 256 discontinued* |
| CS6 | Thrombocytopenia ; platelet count decreased | 15,000 | 134 days | 32 days | Epistaxis, spontaneous hematoma, conjunctival hemorrhage | Days 127 discontinued |
| CS2 | NA | 49,000 | 92 days | Unknown | None | Received all doses in the dosing period |
| **Milder platelet reductions leading to study discontinuation** | | | | | | |
| CS6 | | 56,000 | 176 days | 141 days | Epistaxis, mouth hemorrhage, gingival bleeding | 176 days interrupted<br>317 days resumed<br>344 days discontinued (event of platelet count decreased) |
| CS6 | Platelet count decreased was reported as a moderate AE | 75,000 | 127 days | 56 days (in between levels increasing) | None | 134 days interrupted<br>141 days resumed<br>183 days interrupted<br>190 days discontinued (platelet count decreased) |
| CS6 | Thrombocytopenia | 62,000 | 142 days | 28 days | | 106 days discontinued (platelet count decreased) |

*[*]hospitalized and began treatment with methylprednisolone and immunoglobulin human normal infusion*
*** treatment with prednisolone.*

In the ongoing open label extension study CS7 (as per cut-off date June 2018), 4 cases of severe thrombocytopenia (**Table 41**) were reported and led to treatment discontinuation. These cases included:

- One case occurred around Day 85 of volanesorsen treatment in the open-label study CS7 and while the patient was on vacation during the December 2016 / January 2017 holidays and missed her regular weekly platelet count blood draws.
- An AE of platelet count < 50,000/mm$^3$ (nadir of 37,000/mm$^3$) was reported in a treatment-naïve patient after approximately 80 days of volanesorsen treatment in the open-label extension study CS7 while the patient was being treated according to the treatment algorithm and monitoring schedule.
- Another patient, coming from the placebo CS6, reported a platelet nadir of 28,000/mm$^3$ on study Day 159.

**Table 41.** Patients with Platelet Count Reductions < 50,000/mm3 and Recovery (CS2, CS6, CS16, CS7)

| Study | Nadir Platelet count (/mm$^3$) | Initial monitoring frequency per protocol | Dose regimen at onset | Time to decline from > 100,000/mm$^3$ to Nadir (days) | Time to Recover from Nadir to > 50,000/mm$^3$ (days) | Time to Recover from Nadir to > 140,000/mm$^3$ |
|---|---|---|---|---|---|---|
| CS2 | 49,000 | Q6W | Weekly | 102 | 11 | NA* |
| CS16 | 41,000 | Q6W | Weekly | 28 | 7 | 14 |
| CS6 | 40,000 | Q6W | Weekly | 57 | 21 | 40 |
| CS6 | 8,000 | Q6W | Weekly | 48 | 11 | 45 |
| CS6 | 15,000 | Q6W | Weekly | 55 | 9 | 27 |
| CS7 | 28,000 | Q2W | Q2W | 19 | 3 | 4 |
| CS7 | 15,000 | Q2W | Weekly | 22 | 6 | 11 |
| CS7 | 37,000 | Weekly | Q2W | 22 | 5 | 12 |
| CS7 | 17,000 | Weekly | Q2W | 15 | 6 | 15 |

All these severe cases of thrombocytopenia used corticosteroid treatment to recover. Eighteen (27%) patients in study CS7 discontinued study treatment because of AEs. Other AEs leading to discontinuation of volanesorsen treatment in study CS7 were (reported in one patient for each) moderate vasculitis, moderate sensory loss, and mild injection site rash, severe chills, severe neck pain, and severe pain in jaw, severe serum sickness-like reaction, severe juvenile idiopathic arthritis, severe vomiting, severe headache, moderate abdominal pain, mild contusion, moderate diarrhea, mild nausea, and mild blurred vision, moderate myalgia and moderate chills, mild fatigue and mild myalgia, severe proteinuria and moderate anxiety.

### *Study CS13 – Thorough QT Study*

Study CS13 was a randomized, placebo-controlled, 4-period crossover tQTc study in healthy volunteers to determine if volanesorsen administered as a single therapeutic dose (300 mg SC) and a single

supra-therapeutic dose (300 mg IV) delayed cardiac repolarization as determined by the measurement of QT/QTc interval when compared with moxifloxacin as positive control. A total of 52 healthy male and female subjects were enrolled in this 4-way crossover study.

Treatment with volanesorsen did not prolong QTc to a clinically significant degree nor did it affect other ECG parameters including heart rate, PR, or QRS intervals. The largest mean effect on ΔΔQTcF at any post-dose time-point was 3.0 msec (90% CI: 0.8 to 5.2) after SC dosing and 1.8 msec after IV dosing.

The QT effect of 400 mg moxifloxacin confirmed the study's assay sensitivity with mean ΔΔQTcF at the predefined time-points (2, 3, and 4 hours) of 9.0 msec, 11.0 msec, and 11.6 msec, respectively, with all lower bounds of the 90% CI above 5 msec.

### *Post marketing experience*

Not applicable.

## 2.6.1. Discussion on clinical safety

For the target population, a limited number of 86 FCS patients have been treated with volanesorsen. These include patients from the pivotal CS6 study and the ongoing open label single arm extension study CS7: with 30 placebo treated patients, 14 patients previously treated with volanesorsen from study CS6, 3 previously treated in study CS16, and 20 complete naïve patients included. Placebo controlled data for the FCS patients is limited to 267 days of treatment with volanesorsen and this is shorter than the 352 days for placebo. Additional placebo controlled data come from the CS16 study, which included a very limited number of FCS patients (5 FCS on volanesorsen and 2 FCS on placebo), though 75 vs 38 patients with HTG (high triglycerides). The overall safety database is therefore relatively limited but acceptable in the context of a rare and orphan disease like FCS.

The most commonly reported AEs were injection site reactions. The most commonly observed in the phase 3 studies were injection site erythema (79% vs 4%), injection site pain (49% vs 7%), injection site swelling (37% vs 4%), injection site pruritus (31% vs 0%), and injections site discoloration (27% vs 0%) for volanesorsen vs placebo. And these events occurred mostly at initiation of therapy (≤13 weeks 63%, 13-26 weeks 17%, and > 26 weeks 8% in the overall placebo controlled pool) and were mild or moderate in severity. None of the injection site AEs were severe, but not all of these events resolved (5 patients CS6 and 8 patients in CS16). To minimise such reactions, it is important to rotate sites for injection. Sites for injection include the abdomen, upper thigh region, or outer area of the upper arm. If injected in the upper arm, the injection should be administered by another person. Injection should be avoided at the waistline and other sites where pressure or rubbing may occur from clothing. This medicinal product should not be injected into tattoos, moles, birthmarks, bruises, rashes, or areas where the skin is tender, red, hard, bruised, damaged, burned, or inflamed.

In the placebo-controlled phase 2 and 3 studies, eighteen (13%) patients in the volanesorsen group experienced 24 serious adverse events (SAEs) and 9 (9%) patients in the placebo group experienced 16 SAEs. However, the most reported and only considered as related to treatment SAEs was thrombocytopenia (Grade 4 thrombocytopenia in 2 patients (6%) in study CS6 and 4 in the ongoing open label study CS7).

Four MACE events found in study CS16 were not considered related to study drug. Any QT prolonging or other arrhythmic potential of volanesorsen was considered unlikely based on the absence of any pro-arrhythmic effect in *in vitro*, preclinical studies, the Thorough QT study and phase 3 ECG data.

Overall, higher frequencies of AEs were observed for volanesorsen than for placebo with local tolerability (82% vs 14% in FCS patients and 96% vs 32% in non-FCS for volanesorsen vs placebo) and decreases in platelet count (75% vs 24% in FCS patients for volanesorsen vs placebo (platelet count < 140,000/$mm^3$)) being the most noticeable adverse effects, although most were mild or moderate in severity. Bleeding events were also commonly reported (36% vs 14% of the patients with approximately 3 events vs 1 event per patient in the phase 3 placebo controlled studies). Approximately half of them occurred at the injection site. Other most reported AEs (excluding AEs at injection site) were fatigue (16% volanesorsen vs 10% placebo), abdominal pain (15% vs 8%), diarrhoea (14% vs 9%), and nausea (13% vs 4%).

Due to observed severe platelet count reductions in 2 patients in study CS6 protocol amendments were performed for a strict dose algorithm and platelet count monitoring and for study CS16 the protocol was amended at week 13 such that patients had to be treated with biweekly 300 mg volanesorsen instead of weekly 300 mg volanesorsen. For volanesorsen, reduced levels of platelet counts were seen early after start of treatment but were most notable during month 3-6. In the pivotal study (CS6) a larger number of patients had reduced platelet counts for volanesorsen compared to placebo (75% vs 24% < 140,000/$mm^3$;28% vs 24% 100,000/$mm^3$-140,000/$mm^3$; 19% vs 0% 75,000/$mm^3$-100,000/$mm^3$; 19% vs 0% 50,000/$mm^3$-75,000/$mm^3$and 3 vs 0 patients < 50,000/$mm^3$). Also, thrombocytopenia (13% vs 2%) and platelet count decreased (14% vs 4%) were reported as common treatment related adverse events during the placebo controlled phase 3 studies. Large proportions of patients had their dose reduced to biweekly dosing (n=10; 30% in study CS6 (vs 0 placebo), n=29; 38% in study CS16; n=6; 21% in study CS7). For study CS6, dose reductions all occurred between week 26-46. A substantial proportion of patients had their dose interrupted (n=11; 33% in study CS6, n=10; 13.3% in study CS16) primarily due to AEs or lab values and this was substantially more than in the placebo arm (n=6; 18% in study CS6, n=3; 8% in study CS16). For treatment naïve patients entering the open-label study CS7 the data presented suggest similar results.

Despite the new dose algorithm and monitoring interventions rare occurrences of sudden severe drops in platelet counts were still observed. Two serious events of thrombocytopenia occurred before algorithm implementation and 4 more cases were identified during the open-label CS7 study (after dose algorithm implementation) all leading to treatment discontinuation. It is reassuring however, that for the thrombocytopenia cases in CS7, platelet levels recovered to > 50000/m3 within 3 to 6 days, although corticosteroid therapy was needed in all severe cases of thrombocytopenia.

Currently there are no predictive factors of such events. The applicant provided some data to demonstrate that for many patients the decrease in platelet count was dose-dependent. Data on steady state exposure in relation to platelet count categories of maximum reduction in platelet count, and baseline weight versus maximum reduction in platelet count show some moderate correlations, and data according to body weight <70 kg show a larger reduction in platelet count with the lower body weight category.

None of the severe reductions in platelet counts were associated with major or severe bleedings, although non-major bleedings (hemorrhage and epistaxis in 1 patient each in study CS6) were observed.

Bleeding events were observed more for volanesorsen than placebo with 36% vs 14% of the patients with approximately 3 events vs 1 event per patient in the phase 3 placebo controlled studies. Most events were reported at initiation of therapy (≤ 13 weeks 29.9% patients; 13-16 weeks 12.5%; > 26 weeks 13.4% in the placebo controlled studies). This may indicate that bleeding events are unlikely to be directly associated to the observed reduction in platelet counts as this primarily appeared to occur later during the study. Moreover, approximately 40% of the bleedings were local at the injection site (in study CS6). This is explained by the applicant as due to a transient disruption of the contact

pathway, while antisense oligonucleotides are known to affect the tenase complex, although the last effect is more considered a systemic effect. Local bleeding events mostly observed were injection site bruising (15% vs 1.6%), injection site haemorrhage (7% vs 0%), and injection site hematoma (4% vs 1.6%). Any major bleedings were not observed, while non-major bleedings of 8 (24%) vs 4 (12%) patients vs placebo in study CS6 were observed. No major bleedings have occurred in the study CS7 to date.

Volanesorsen patients were more likely to discontinue treatment than patients treated with placebo throughout the clinical program (25 (17%) volanesorsen vs 2 (2%) placebo due to AE in phase 2/3 studies). Discontinuations due to AEs were frequently observed with the treatment of volanesorsen (27% (n=9) in study CS6, 19% (n=14) in study CS16) vs placebo (0% in study CS6, 2 (5%) in study CS16) and mostly related to injection site reaction or platelet count reduction/thrombocytopenia. Similar discontinuation rates due to AEs were observed in study CS7.

Apart from discontinuation due to AEs, voluntary discontinuations were also observed. Discontinuations for reduced platelet count or thrombocytopenia was found in 15% (n=5) in study CS6 and 1.3% (n=1) in study CS16, and discontinuation associated with injection site reaction were 3% (n=1) in study CS6 and 12% (n=9) in study CS16 with volanesorsen treatment vs none in placebo. Several of the patients who discontinued due to low platelet levels were rechallenged with study treatment before they permanently discontinued study treatment. Most study discontinuations occurred early during study, within the first 13 weeks, in particular for injection site reactions, and thus selection of those patients who can tolerate treatment is apparent early on treatment. Discontinuations due to low platelet levels, especially the previously mentioned severe cases, appear unpredictable.

It is clear from the available data that the reduction of platelet levels in association with volanesorsen is of particular concern and was also acknowledged by the Ad-Hoc Expert Advisory Group. In order to minimise this risk the applicant modified the initially proposed dosing frequency which should be reduced after 3 months of treatment from once weekly to once every 2 weeks. This proposal is line with the amended protocol of study CS16.

Moreover a number of monitoring and treatment recommendations have been introduced to minimise the potential adverse events of low platelet count. Monitoring of platelets should be done every 2 weeks for patients with normal counts, weekly for counts between 75-139 $x10^9$/L, every 2-3 days for counts between 50-74 $x10^9$/L and daily for lower values. For patients with platelet counts between 50-99$x10^9$/L, treatment should be paused for≥4 weeks and resumed after platelet levels reach ≥ 100 $x10^9$/L. If platelet counts are less than 50 $10^9$/L treatment should be discontinued and use of glucocorticoids is recommended. Before initiation of treatment, platelet count should be measured. If the platelet count is below 140 $x10^9$/L another measurement should be taken approximately a week later to reassess. If platelet counts remain below 140 x $10^9$/L upon a second measurement, treatment should not be initiated. Finally volanesorsen is contraindicated in patients with chronic or unexplained thrombocytopenia.

The constant monitoring and subsequent dose alteration or stopping raises question regarding patient compliance to treatment even though it is acknowledged that FCS patients are probably highly motivated due to the impact of the disease in their quality of life. In the open-label study, for 2 patients (out of 50 treatment naïve patients) the burden of frequent monitoring could be related to patient withdrawal, and for 2 patients to frequent monitoring and study burden in general (as of December 2017). Moreover, even though the strictest algorithm was implemented, still 9 patients discontinued due to platelet count decreases in OLE study CS7, with 4 patients with severe thrombocytopenia (as of June 2018).

The CHMP considered that even though the risk of thrombocytopenia appears to be relatively well characterised, current data cannot be considered comprehensive. The impact of the proposed dosing

algorithm in reducing this risk and adherence to the platelet monitoring / dose adjustment algorithm remain unknown. Further data will therefore be required and the applicant will conduct a post-authorisation safety study, using a registry to monitor the incidence of thrombocytopenia and potential bleeding once the product has been authorised.

In addition to these aspects, the CHMP also considered that a long term follow-up of patients within the registry will be needed even if the specific uncertainties underlying the need for the PASS mentioned above are addressed. It is expected therefore, that following the study completion the follow-up of the patients will continue through the registry.

In addition, educational materials will be available for potential prescribers, patients and carers and will outline the importance of platelet monitoring and dose frequency adjustment on signs and symptoms of bleeding, and on the need to seek immediate treatment.

Volanesorsen is an ASO (antisense oligonucleotide) which have been associated with class concerns such as injection site reactions, thrombocytopenia, liver toxicity, renal toxicity, and immunological effects (flu like symptoms, antidrug antibodies).

Flu-like symptoms were adverse events of special interest as these have been observed with previous antisense oligonucleotides. These events were limited with 4 (4%) of 108 patients in the volanesorsen group experiencing a total of 6 FLRs over the course of study treatment. These included 3 patients who experienced an AE of influenza-like illness (2 in CS6 and 1 in CS16) and 1 patient who experienced pyrexia/feeling hot with arthralgia and myalgia (non-FCS patient in Study CS16).

In terms of laboratory abnormalities an increase in creatinine was observed in 3 patients (2 on study treatment). Further, a case of severe proteinuria leading to hospitalisation and discontinuation of study drug was reported in study CS7. This strengthens the potential accumulation of volanesorsen in the kidney based also on non-clinical PK data and kidney findings in the toxicology studies. Therefore, the renal safety profile should be monitored during treatment and has been included in the RMP as an important potential risk. Monitoring for evidence of nephrotoxicity by routine urine dipstick is recommended on a quarterly basis. In the case of a positive assessment, a broader assessment of renal function, including serum creatinine and a 24-hour collection to quantify the proteinuria and assess creatinine clearance, should be performed. Treatment should be discontinued if: proteinuria of ≥ 500 mg/24 hour is recorded, or an increase in serum creatinine ≥ 0.3 mg/dL (26.5 µmol/L) that is >ULN is recorded, or creatinine clearance estimated by the CKD-EPI equation is≤30 mL/min/1.73m2. Treatment should also be discontinued for any clinical symptoms or signs of renal impairment pending the previous confirmatory assessment.

Some cases of increase in ALT or AST were observed in volanesorsen users (15 patients >2.5 × ULN). Taking into account its mechanism of action which may lead to accumulation of triglycerides in liver cells, the theoretical risk of accumulation of volanesorsen in the liver based on non-clinical PK, and the fact that hepatotoxicity has appeared to be an important issue for other ASOs, hepatotoxicity is included in the RMP as an important potential risk. Monitoring for hepatotoxicity through serum liver enzymes and bilirubin should be assessed on a quarterly basis. Treatment should be discontinued if there is a single increase in ALT or AST > 8 x ULN, or an increase > 5 x ULN, which persists for ≥ 2 weeks, or lesser increases in ALT or AST that are associated with total bilirubin > 2 x ULN or INR > 1.5. Treatment should also be discontinued for any clinical symptoms or signs of hepatic impairment or hepatitis.

Although patients treated with volanesorsen were frequently tested positive for antidrug-antibodies (ADA) (30% in study CS6 (vs 3% placebo) and 16% in study CS16 (vs 5% placebo)), this did not appear to be associated with any particular safety concern. However, taking into account, the limited long-term data and the known potential serious risks such antibodies are associated with,

immunogenicity and hypersensitivity have been included in the RMP as important potential risks and will be prospectively assessed in the post-marketing setting.

The post-authorisation safety study designed to characterise the risk of thrombocytopenia will also investigate the severity of immunogenicity/immunological events, hepatotoxicity, renal toxicity and severe injection site reactions.

There is limited experience in patients > 65 years of age, which does not allow for any robust conclusion on the safety aspects of volanesorsen in these older patients. For the data observed, comparable number of events could be observed in comparison to the younger age group, except that bleeding events were higher in the older age group (55% vs 39%).

### *Additional expert consultations*

See discussion on clinical efficacy.

### *Additional efficacy data needed in the context of a conditional MA*

Taking into account the totality of the available data, the CHMP was of the view that comprehensive data on the product are not available, due to the small size of the clinical trials and the uncertainties around the proposed dosing algorithm and whether it can effectively minimise the risk of thrombocytopenia in FCS patients.

The CHMP was therefore of the view that a conditional marketing authorisation should be granted subject to a specific obligation to conduct a study in order to evaluate the impact and adherence in clinical practice of the treatment algorithm on the risk of thrombocytopenia.

### *Conclusions on the clinical safety*

Despite the limited size of the safety database, due to the rarity of FCS, the overall safety profile of volanesorsen has been adequately characterised. The main safety concern identified is that of thrombocytopenia for which a number of additional risk minimisation measures will be implemented and monitored through a post-authorisation safety study based on a registry. The other potential risks are considered manageable with warnings in the product information.

The CHMP considers the following measure necessary to address the missing safety data in the context of a conditional MA:

- A Post-Authorisation Safety Study based on a Registry in order to evaluate the safety of Waylivra on thrombocytopenia and bleeding (including incidence rate, severity and outcomes) in FCS patients according to the dose recommendation and dose algorithm and investigate adherence with platelet monitoring and dose adjustment requirements.

  The applicant will also ensure the long term follow-up of patients in the Registry

## *2.7. Risk Management Plan*

### *Safety Concerns*

| **Summary of Safety Concerns** | |
|---|---|
| Important identified risks | Thrombocytopenia<br>Injection site reactions |

| | |
|---|---|
| Important potential risks | Immunogenicity<br>Hepatotoxicity<br>Nephrotoxicity |
| Missing information | Use in pregnancy and lactation<br>Use in patients with hepatic impairment<br>Use in patients with severe renal impairment<br>Long-term safety<br>Use in elderly |

## *Pharmacovigilance Plan*

| Study / Status | Summary of Objectives | Safety Concerns Addressed | Milestones | Due dates |
|---|---|---|---|---|
| **Category 2** - Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances authorisation | | | | |
| WAYLIVRA™ Product Registry / Planned | To evaluate the safety of Waylivra™ on thrombocytopenia and bleeding in FCS patients according to the dose recommendation and dose algorithm in the Summary of Product Characteristics | Thrombocytopenia | Protocol Submission to PRAC | Within 1 month of EC Decision |
| | To determine real-world incidence rates and severity of immunogenicity/immunological events, hepatotoxicity, renal toxicity, and severe injection site reactions | Immunogenicity, hepatotoxicity, nephrotoxicity, injection site reactions | | |
| | To describe the real-world adherence with platelet monitoring and dose adjustment requirements per the WAYLIVRA SmPC | Thrombocytopenia | Interim Reports | Annual Updates |
| | To determine real-world incidence rates and severity of thrombocytopenia, and associ-ated bleeding events, overall and by event grading (including moderately severe thrombocytopenia defined as platelet count 50 to 74 x $10^9$/L) | Thrombocytopenia | | |
| | To describe the safety profile of WAYLIVRA in patients with renal or hepatic impairment | Use in patients with hepatic impairment; Use in patients with severe renal impairment | Study Report | Q3 2026 |
| | To describe the safety profile of WAYLIVRA in elderly patients (ages ≥65 years) who receive WAYLIVRA, | Use in elderly<br>Long-term safety | | |
| | To describe the long-term safety profile of WAYLIVRA | Use in pregnancy | | |
| | To describe outcomes for WAYLIVRA-exposed pregnancies; specifically, gestational outcomes (e.g., live birth, spontaneous abortion, etc.) and major congenital malformations observed at pregnancy conclusion. | | | |

### *Risk Minimisation Measures*

| Safety Concern | Risk Minimisation Measures |
|---|---|
| **Important identified risks** | |
| Thrombocytopenia | **Routine Risk Minimisation Measures**<br>• **SmPC**<br>This risk minimisation measure is addressed in Sections 4.2, 4.3, 4.4, and 4.8. Prescription only medicine.<br>• **PIL**<br>The patient information leaflet contains information on the risk of thrombocytopenia, importance of adherence with platelet monitoring requirements and how to recognise bleeding events requiring medical attention.<br>**Additional Risk Minimisation Measures**<br>• **Education**<br>Education to healthcare providers, patients and carers on the SmPC and Patient Information Leaflet (PIL) thrombocytopenia safety messages, and recommended risk minimisation actions, will be undertaken by the company. The importance of platelet monitoring, dose adjustments and early recognition by patients/carers of signs and symptoms of bleeding, and need to seek immediate treatment will be emphasised. The educational materials will also contain information regarding participation in the WAYLIVRA™ Product Registry. The product will be shipped along with the Prescriber Kit containing educational materials for the treating physician, dispensing pharmacist and the patient/carer, and with each product shipment. |
| Injection site reactions | **SmPC**<br>Information on injection site reactions is provided in Section 4.2 and 4.8<br>**PL**<br>Section 4 |

| Safety Concern | Risk Minimisation Measures |
|---|---|
| **Important potential risks** | |
| Immunogenicity | **SmPC**<br>Information provided in Section 4.4 on risk of immunogenicity.<br>**PL**<br>Sections 2 and 4 |
| Hepatotoxicity | **SmPC**<br>Information provided in Section 4.4 on risk of hepatotoxicity.<br>**PL**<br>Sections 2 and 4 |

| Safety Concern | Risk Minimisation Measures |
|---|---|
| Nephrotoxicity | **SmPC**<br>Information provided in Section 4.4 on risk of nephrotoxicity.<br>**PL**<br>Sections 2 and 4 |

| Safety Concern | Risk Minimisation Measures |
|---|---|
| **Missing information** | |
| Use in pregnancy and lactation | **SmPC**<br>Use in pregnancy and lactation is discussed in Section 4.6.<br>Prescription only medicine.<br>**PL**<br>Section 2 |
| Use in patients with hepatic impairment | **SmPC**<br>Use in patients with hepatic impairment is discussed in Sections 4.2 and 5.2.<br>Information provided in Section 4.4 on risk of hepatotoxicity.<br>Prescription only medicine. |
| Use in patients with severe renal impairment | **SmPC**<br>Use in patients with severe renal impairment is discussed in Section 4.2 and 5.2.<br>Information provided in Section 4.4 on risk of nephrotoxicity.<br>Prescription only medicine. |
| Long-term safety | **None** |
| Use in elderly | **SmPC**<br>Use in elderly patients is discussed in Sections 4.2, 5.1, and 5.2.<br>Prescription only medicine. |

### *Conclusion*

The CHMP and PRAC considered that the risk management plan version 1.8 is acceptable.

## *2.8. Pharmacovigilance*

### *Pharmacovigilance system*

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

### *Periodic Safety Update Reports submission requirements*

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

## *2.9. New Active Substance*

The applicant compared the structure of volanesorsen 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 volanesorsen 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, WAYLIVRA (volanesorsen) 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;
- It is approved under a conditional marketing authorisation [REG Art 14(7)]

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.

#### *Third party intervention during the evaluation of Waylivra*

LPLD Alliance, a registered charity working in support of people affected by Familial Chylomicronaemia Syndrome (FCS) sent two letters to the CHMP describing the impact of the disease in the quality of affected patients and the impact that Waylivra has had on patients that participated in the trials.

The CHMP took note of the letters submitted by LPLD Alliance.

In order to obtain a real-life perspective in relation to the use of volanesorsen, two of the trustees of the charity participated in the Ad-Hoc Expert Advisory for Waylivra.

# 3. Benefit-Risk Balance

## *3.1. Therapeutic Context*

### 3.1.1. Disease or condition

Volanesorsen is proposed to be indicated for the treatment of familial chylomicronemia syndrome (FCS), a genetic based orphan disease characterised by extremely high serum triglycerides (TG) (> 880 mg/dL, 10 mmol/L). These are carried primarily in chylomicrons (dietary lipids). FCS affects an estimated 3000-5000 patients globally (1-2 in a million). The most serious effects are severe abdominal pain and acute pancreatitis, which can occasionally lead to death. Other effects are cognitive impairment (''brain fog'') and muscle and joint pain. Approximately 65-80% of patients with FCS will experience at least one episode of acute pancreatitis, with the majority experiencing recurrent episodes. Aim of therapy is to reduce triglyceride (TG) levels preferably to such levels (< 750 mg/dL) that occasional cases of acute pancreatitis (and abdominal pain) can be reduced or avoided.

### 3.1.2. Available therapies and unmet medical need

Patients with FCS are on strict dietary fat restriction. Traditional lipid-lowering medications used to treat hypertriglyceridemia (HTG), such as fibrates, statins and fish oils, niacin (not registered anymore in EU) and off-label lomitapide are only minimally effective in patients with FCS. Gene therapy (Glybera (INN:alipogene tiparvovec)) was approved for FCS patients but restricted to those with LPL deficiency, who suffer from severe or multiple pancreatitis attacks and detectable levels of LPL protein with a genetic confirmed testing. However, this product has been withdrawn from use in the European Union since October 2017 following the marketing authorisation holder's decision not to apply for a renewal.

### 3.1.3. Main clinical studies

Volanesorsen has been investigated in a randomized, double-blind, placebo-controlled, 52-week phase 3 study in 66 patients with FCS (Study CS6). Patients were selected based on genetic testing, TG levels (mean of 2209 (SD 1199) mg/ml at baseline) and FCS disease complications. 75.8% of patients having a history of acute pancreatitis, 76% with confirmed mutations in type 1 LPL gene mutations and 55% patients tested post heparin plasma LPL activity of ≤ 20% of normal. Also, genetic abnormal phenotypes of other genes related to LPL function (APOC2, LMF1, APOA5, and GPIHBP1) were identified.

Volanesorsen 300 mg was administered once a week, with the opportunity to reduce dose to biweekly for patients experiencing platelet decreases. A protocol amendment was introduced during the trial, after two serious cases of thrombocytopenia occurred. A dosing algorithm based on thrombocyte levels was introduced that described dose adjustments up to a dose interruption for a maximum of 52 weeks. The primary endpoint was TG reduction after 3 months of treatment. Important secondary endpoints included maintenance of effect on TG levels until 12 months and the occurrence of acute pancreatitis and abdominal pain, amongst some others. All dose adjustments occurred after 3 Months of treatment.

Volanesorsen was further investigated in a supportive randomized, double-blind, placebo-controlled, 26-week phase 3 study in 113 patients with high TG levels (1261 mg/dL; 7 were FCS,). Volanesorsen 300 mg was administered once a week for a maximum of 26 weeks, with all patients reduced to biweekly dosing after 13 weeks of treatment after a dose algorithm that was also introduced during this study. The primary endpoint was TG reduction after 3 months of treatment.

An open-label single arm study including FCS patients from both studies above is ongoing (Study CS7).

## 3.2. Favourable effects

A triglyceride lowering effect was demonstrated in the pivotal study during the first 3 months. In the pivotal study CS6 in FCS patients (n=66), volanesorsen demonstrated a substantial 77% reduction in TG levels (2267 mg/dL to 590 mg/dL) compared to an increase of 18% with placebo, resulting in a 94% difference at month 3 (p<0.0001). In the volanesorsen group 77% of patients achieved target TG levels of < 750 mg/dL versus only 10% in the placebo group.

A trend of reduced events of acute pancreatitis in patients treated with volanesorsen was observed (1 event compared to 4 in placebo treated patients.

Treatment with volanesorsen also resulted in favourable outcomes in other lipid parameters including chylomicron-TG, ApoC-III, ApoB-48, VLDL-C and non-HDL-C and HDL-C.

TG levels in patients in the supportive study CS16 were reduced by 72% from baseline (TG of 1183 mg/dL to 294 mg/dL) in the volanesorsen group compared to 3% in the placebo group after 3 months (difference of 69% (p<0.0001)).

The limited open-label data demonstrate a maintenance of effect with percent change in fasting TG from baseline to 18 months total (n=9) and 24 months (n=3) of -53.1% and -60.8%.

## 3.3. Uncertainties and limitations about favourable effects

In the pivotal study CS6, the TG lowering effect decreased over time, partially at least due to the introduction of dose interruptions and dose adjustments. The TG-lowering effect was diminished from -77% to -53% (6 months) and -40% (12 months) from baseline. The percentage of patients reaching target levels (< 750 mg/dL) was also reduced at these time points (77%, 47% and 37% in the volanesorsen group vs 10%, 0% and 6.5% in the placebo group). Smaller TG reductions were observed in patients who had their dose adjusted (n=13; -52% and -57% after 6 and 12 months) versus those without dose adjustments (n=6; - 80% and -76% after 6 and 12 months) with fewer patients achieving TG values of < 750 mg/dL after 12 months of treatment, 46.2% vs 60% on 300 mg weekly dose.

The results on clinically relevant endpoints of acute pancreatitis favoured volanesorsen but very few cases were observed. This was further supported by exploratory data in patients who had prior frequent acute pancreatitis events (pre-treatment 7 patients, 24 events with 0 on-treatment in volanesorsen; pre-treatment 4 patients, 17 events with 3 patients, 4 events on-treatment in placebo; p=0.0242). The incidence of pancreatitis in volanesorsen-treated patients in the combined Study CS6 and Study CS7 (including exposure from Study CS6) was 0.03 events per patient-year, compared to 0.13 events per patient-year in the placebo group of Study CS6.

However, no impact on other clinical (secondary) endpoints (abdominal pain, quality of life) was observed in study CS6. Abdominal pain was observed in 15 patients (46%) treated with volanesorsen and 14 patients (42%) with placebo; and with a similar overall mean maximum abdominal pain intensity score (0.38 vs 0.36). Also, for the combined endpoint of adjudicated acute pancreatitis and/or moderate/severe abdominal pain on treatment no clear difference was shown (12 (36%) patients in the volanesorsen group and 13 (39%) patients in the placebo group). However, fewer patients in the volanesorsen group (5 [15%]) reported severe abdominal pain events in comparison with the placebo group (8 [24%]), but not for moderate abdominal pain (6 [18%] vs 5 [15%]).

The long-term efficacy potential of volanesorsen for every FCS patient is uncertain. A large proportion of patients discontinued treatment in study CS6 primarily due to AEs, but several patients also discontinued on their own volition during treatment and post-treatment follow-up. This could be

associated with the burden of the need for frequent platelet monitoring. Moreover, only 42% of patients treated with volanesorsen in study CS6 were enrolled in the open label extension study CS7 and in which discontinuation rates remained high.

A limited number of FCS patients ≥ 65 years of age was included (5 patients (7.6%)). These limited data show a similar efficacy in patients ≥ 65 years of age compared with patients ≤ 65 years of age.

Similar to the pivotal study in the supportive study CS16, a lower efficacy was shown for patients with post 13 weeks dose adjustment (n=50; TG -62%) to every 2 weeks compared to the TG reduction for patients on 300 mg once weekly (n=25; TG -78%). Further, exploratory data in study CS16 on pancreatitis events also showed a similar trend as in the pivotal study with none occurring in volanesorsen treated patients vs 5 events in 3 patients in placebo; p=0.036.

## *3.4. Unfavourable effects*

In the pivotal study CS6, a high frequency of injection site reactions (lasting > 2 days) was observed for volanesorsen (61%) vs none in placebo, with none being severe. The injection site reactions occurred mostly in the first 13 weeks of therapy.

Volanesorsen treatment was frequently associated with a decrease in platelet counts (68% vs 15% in FCS patients for volanesorsen vs placebo (any 2 occurrences of platelet count < 140,000/$mm^3$)). Although most thrombocytopenias were mild or moderate in severity they frequently led to discontinuation of study treatment (15% (n=5) in study CS6 - 9% (n=6) in study CS7). Thrombocytopenia occurred already after start of treatment but more often later between day 99 and 344. Moreover, dose reductions to every 2 weeks (10 (30%) volanesorsen in study CS6) and dose interruptions (11 (33%) volanesorsen group versus 6 (18%) placebo) were frequently needed (a dose algorithm and monitoring rules were introduced during the study at 13 weeks). Only 6 (out of 33) volanesorsen treated patients in study CS6 completed the study without dose adjustments. Severe drops in platelet counts are observed (2 patients before dose algorithm implementation, 4 during the open-label study CS7 after implementation), all leading to treatment discontinuation.

Bleeding events were also commonly reported (49% vs 12% of the patients with approximately 3 events vs 1 event per patient), most occurred after < 13 weeks of treatment, but none were severe. Approximately half of them occurred at the injection site. A very limited number of flu-like symptoms occurred (observed with previous antisense oligonucleotides) with 2 patients in study CS6 with influenza-like illness. Other frequently reported AEs (excluding AEs at injection site) were headache (21% volanesorsen vs 15% placebo), nasopharyngitis (15% vs 21%), fatigue (21% volanesorsen vs 9% placebo), erythema (18% vs 9%), and nausea (18% vs 6%), amongst others.

In the supportive study CS16 similar safety issues were observed as in the pivotal study CS6. In study CS16, also a higher frequency of injection site reactions was observed for volanesorsen vs placebo (96% vs 32%), which led to a high frequency of discontinuation of study treatment (9 patients (12%)) than in study CS6. Further, 21 patients (28%) in the volanesorsen group vs 4 patients (11%) in the placebo group experienced platelet count decreases (any 2 occurrences of platelet count < 140,000/mm3), which led to discontinuation of study treatment of one patient (1,3%). Likewise to study CS6, a higher frequency in bleeding events was observed for volanesorsen compared with placebo (28% vs 16%).

Compared to study CS6, as of December 2017, in the ongoing open-label study CS7, a lower number of discontinuations of 27% in treatment naïve patients (n=50), and 35% in previous volanesorsen treated patients was observed (n=14). For the treatment naïve patients a lower number of voluntary withdrawals were observed 10% (n=5), and 12% (n=6) discontinued due to AEs, 3 due to severe thrombocytopenia and 3 due to other AEs. However, as of June 2018, a higher discontinuation rate of 18 patients (27%) who discontinued (25% potentially related to study drug) due to an adverse event

was observed, while overall discontinuation rates remain unknown and likely further increase the overall discontinuation rate.

## 3.5. Uncertainties and limitations about unfavourable effects

The mild likely dose dependent (and possible weight dependent) thrombocytopenia appears reasonably well managed with dose reductions, dose interruptions and platelet monitoring. Those patients completing the one-year period (of whom 13 completed the study period on a every 2-week dosing schedule) platelet levels could be stabilised/maintained reasonably within the 100-140.000/$mm^3$ range with some occasionally dropping to the 75000/$mm^3$ level or slightly below. However, the alternative implemented dosing algorithm was not able to prevent some sudden severe drops in platelet counts (4 during the open-label study CS7 after implementation of the new dosing algorithm). None of the severe reductions in platelet counts were associated with major or severe bleeding, although non-major bleedings (single events of hemorrhage and epistaxis) were observed. It is not clear whether reductions in platelet counts in real life could result in bleeding events.

The complicated dosing algorithm and cumbersome platelet monitoring could potentially lead to compliance issues, despite the motivation of FCS patients due to the severe complications of this disease. Adherence to clinical recommendations and characterisation of incidence and clinical consequences of thrombocytopenia will be monitored in a post-authorisation safety study.

There is some discrepancy between the number of discontinuations due to decreased platelet counts or injection site reactions between studies CS6 and CS16. In the pivotal study CS6 most discontinuations were due to AE of platelet count decreased (15% (n=5) in study CS6, 1.3% (n=1) in study CS16) while in study CS16 patients discontinued treatment primarily due to injection site reactions (3% (n=1) in study CS6 and 12% (n=9) in study CS16). Patients in study CS6 are likely to be more symptomatic and consequently have a greater motivation to continue the study, which may explain this difference.

Few cases of increase in creatinine were observed (3 patients (2 on study treatment) in study CS6 and CS16). Some patients in study CS6 and CS16 with increases in ALT or AST levels were also observed. Liver toxicity has been observed previously for oligonucleotides. Patients who tested positive for antidrug-antibodies (ADA) were frequent (30% in study CS6 (vs 3% placebo and 16% in study CS16 (vs 5% placebo)). The clinical data did not show any differences in safety profile or TG level reductions. Immunogenicity will be monitored as part of the imposed PASS.

Volanesorsen, like other ASOs, accumulates in renal tissue and renal inflammatory effects have previously been associated with oligonucleotides. Liver toxicity has also been observed previously for oligonucleotides. Given the known class effects of ASOs and the limited databases available, any potential risk of hepatotoxicity and renal toxicity cannot be excluded. Frequent monitoring is proposed in the SmPC and these risks will be prospectively assessed this in the post-marketing setting.

## 3.6. Effects Table

**Table 42.** Effects Table for Waylivra in the treatment of Familial chylomicronemia syndrome(data cut-off: June 2018)

| Effect | Short description | Unit | Volanesorsen | Control | Uncertainties / Strength of evidence | References |
|---|---|---|---|---|---|---|
| **Favourable Effects** | | | | | | |
| Reduction in TG levels | % change from baseline at Month 3 | % (95%CI) | -76.5 (-97.4,-55.5) | 17.6 (-4.0, 39.2) | Statistical significant difference : 94.1 (-121.7, -66.6), p< .0001<br><br>Diminished effect at 6 (-52.5% vs 25.3%) and 12 | CSR CS6 |

| Effect | Short description | Unit | Volanesorsen | Control | Uncertainties / Strength of evidence | References |
|---|---|---|---|---|---|---|
| | | | | | months (-40% vs 8.9%)<br><br>Responders defined as TG <750 mg/dL at Month 3 also favours volanesorsen (77% vs 10%, P=0.0001) | |
| Pancreatitis | On-treatment acute pancreatitis | n | 1 | 4 (in 3 patients) | Limited event numbers, P=0.6132<br>No difference in patients with moderate/severe abdominal pain or acute pancreatitis (36 (n=12) vs 39% (n=13)) | |
| **Unfavourable Effects** | | | | | | |
| Platelet counts | Any 2 occurrences of platelet counts <140,000mm$^3$) | % | 68 | 15 | Clinically relevant difference; 3 severe cases in study CS6 (2 before dose algorithm change), 4 cases in open-label CS7 study while following dose algorithm. | CSR CS6 |
| | leading to discontinuation | (%, (n/N) | 15 (5/33) | 0 | | |
| Bleeding events | | n (%) | 16 (48.5) | 4 (12.1) | 40% of the bleeding events were reported at the injection site; no major or severe bleeding events were reported. | |

Abbreviations: TG: triglycerides, CI: Confidence interval,

## *3.7. Benefit-risk assessment and discussion*

### 3.7.1. Importance of favourable and unfavourable effects

Treatment with volanesorsen (Waylivra) is targeted at patients with familial chylomicronemia syndrome (FCS). These patients have extremely high levels of TG (and chylomicrons) which are associated with several clinical symptoms including abdominal pain and acute pancreatitis. It is believed that TG levels below a threshold of approximately 10 mmol/L ((880 mg/dL); ESC guideline) will reduce these events of acute pancreatitis.

Volanesorsen treatment demonstrated a clear significant reduction on TG levels even below the threshold levels for risk of acute pancreatitis. Current studies (52 weeks for the pivotal study in FCS patients and 26 weeks for the high TG level patients) were most likely too small and too short to draw a firm conclusion on clinically relevant endpoints such as reduction in pancreatitis. Evaluation of the long term treatment effect is further compounded by the large number of patients who discontinued treatment as a result of tolerability issues. However, some trend towards an effect could be shown on the very limited number observed for acute pancreatitis. The Ad-Hoc Expert Advisory Group also considered that the pharmacodynamic effect of volanesorsen on serum triglyceride levels is of clinical relevance, despite the limited available data on pancreatitis.

Treatment with volanesorsen is clearly associated with reduced platelet counts which after the occurrence of 2 serious cases of thrombocytopenia in the clinical trials, led to a strict dose and monitoring algorithm in both phase 3 studies. Following implementation of this dose algorithm after 3

months, many dose reductions and dose interruptions were needed, which diminished the anticipated efficacy, but a substantial TG lowering effect was still maintained. Although the dose algorithm and intense platelet monitoring appears to keep the platelet levels reasonably within a slightly lower than normal range, several cases of unpredictable severe thrombocytopenia in some of the patients could not be prevented. Also, in terms of treatment management the need for frequent platelet monitoring and subsequent dose amendments and dose pauses is complex and can be a large burden to patients and related health care systems. Several patients appear to have discontinued Volanesorsen because of this, despite the lack of an alternative to control their disease.

Additionally, injection site reactions occurred frequently and also resulted in discontinuations, although these occurred early after start of treatment and thus already could select out patients intolerable to antisense oligonucleotide treatment of volanesorsen. Bleeding events were also observed, but appear mainly associated to the injection site reaction while currently not being observed in the severe thrombocytopenia cases. Effects on kidney and liver are known for ASOs and its mechanism of action may lead to accumulation in the kidney and liver.

Current data are limited to one year but further information on the impact of the proposed dosing algorithm to minimise the risk of thrombocytopenia, and the other risks associated with volanesorsen treatment will be evaluated through a PASS based on a registry. Additional long term safety data will continue to be provided as part of the long term follow-up of patients though the registry.

### 3.7.2. Balance of benefits and risks

Volanesorsen can reduce TG levels to a considerable extent. Even though not demonstrated, it is expected that this will translate into fewer pancreatitis and abdominal pain events. Furthermore, there is a rare but serious risk for severe thrombocytopenia with the potential of severe bleeding events. A complex mitigation strategy of intense platelet monitoring and dose algorithm including dose adjustments and dose pauses is needed to keep the platelet levels within an acceptable range and to be able to timely identify serious thrombocytopenia events. Patients at risk of serious thrombocytopenia cannot be identified a-priori.

Taken together, the CHMP considered that the indication of volanesorsen should be restricted to those patients with genetically confirmed FCS and at high risk of pancreatitis in whom response to diet and triglyceride lowering therapy has been inadequate.

### 3.7.3. Additional considerations on the benefit-risk balance

#### *Conditional marketing authorisation*

As comprehensive data on the new dosing algorithm and its effect on the risk of thrombocytopenia and the efficacy of the product in clinical practice are not available, a conditional marketing authorisation was proposed by the CHMP during the assessment, after having consulted the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment of a seriously debilitating nature of the disease and is designated as an orphan medicinal product.

Furthermore, the CHMP considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data. It is currently not known

whether the proposed dosing recommendations and platelet monitoring will be adhered to in clinical practice and whether this will be sufficient to effectively manage the risk of thrombocytopenia. A synopsis for a study detailing how to address these questions including timelines for the completion of this study has been submitted. The CHMP has reviewed this proposal and considers that the design of this study is feasible and will likely provide adequate information to address its objectives.

- Unmet medical needs will be addressed, as there are currently no satisfactory treatments in this indication.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. The demonstrated substantial reduction in levels of triglyceride, which is expected to lead in a reduction on the incidence of potentially life-threatening pancreatitis, and the lack of any other approved therapeutic option in this condition outweighs the risk inherent in the fact that additional data are still required.

### *3.8. Conclusions*

The overall B/R of WAYLIVRA is positive.

Divergent position is appended to this report.

## 4. Recommendations

### *Similarity with authorised orphan medicinal products*

The CHMP did not conclude on the similarity of Waylivra to Glybera, as the Marketing Authorisation of Glybera expired during the evaluation procedure of Waylivra.

### *Outcome*

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

adjunct to diet in adult patients with genetically confirmed familial chylomicronemia syndrome (FCS) and at high risk for pancreatitis, in whom response to diet and triglyceride lowering therapy has been inadequate.

The CHMP therefore recommends the granting of the conditional 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 launch of Waylivra in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The objective of the programme is to provide information on the risks of thrombocytopenia and bleeding; advise on platelet monitoring and provide details about the dose frequency adjustment algorithm.

The MAH shall ensure that in each Member State where Waylivra is marketed, all healthcare professionals, patients and carers who are expected to prescribe, dispense and use Waylivra have access to/are provided with the following educational package:

- Physician educational material

- Patient information pack

- **The physician educational material** should contain:
  - The Summary of Product Characteristics
  - Guide for healthcare professionals

- **The Guide for healthcare professionals** shall contain the following key elements:
  - Relevant information on thrombocytopenia and severe bleeding
  - Details of the population at higher risk for thrombocytopenia and bleeding (e.g. those with weight less than 70 kg), and patients for which Waylivra is contraindicated (i.e. patients with chronic or unexplained thrombocytopenia)
  - Platelet monitoring recommendations including dosage adjustment recommendations, both before and during treatment.
  - That patients should be made aware of the possibility of thrombocytopenia and to seek medical attention immediately in case of signs of bleeding. Patients must be reminded to read the patient leaflet and the patient/carer guide.
  - Information about the FCS disease registry and the PASS study and the importance of contributing to those studies.

- The **patient information pack** should contain:
  - Patient information leaflet
  - A patient/carer guide

- **The Patient/carer guide** shall contain the following key messages:
  - Relevant information on thrombocytopenia and severe bleeding
  - Importance of monitoring platelet levels
  - Possible need for dose adjustments or treatment pauses based on platelet test results
  - Need to be aware of and alert to the signs of thrombocytopenia and the importance of seeking immediate assistance from a health professional
  - Information about the FCS disease registry and the PASS study and encouragement to participate in those studies.
  - Reporting of any adverse drug reaction to a health professional

## *Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation*

This being a conditional marketing authorisation and pursuant to Article 14a of Regulation (EC) No 726/2004, the applicant shall complete, within the stated timeframe, the following measures:

| Description | Due date |
|---|---|
| **Non-interventional PASS**: the applicant should conduct and submit the results of a study based on a Registry in order to evaluate the safety of Waylivra on thrombocytopenia and bleeding (including incidence rate, severity and outcomes) in FCS patients according to the dose recommendation and dose algorithm and investigate adherence with platelet monitoring and dose adjustment requirements.<br><br>The applicant will ensure the long term follow-up of patients in the Registry. | Q3 2026 |

### *Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States*

Not applicable.

These conditions fully reflect the advice received from the PRAC.

### *New Active Substance Status*

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

## Appendix

1. Divergent positions to the majority recommendation

## APPENDIX

## DIVERGENT POSITION DATED 28 February 2019

DIVERGENT POSITION DATED 28 February 2019

WAYLIVRA EMEA/H/C/004538/0000

The undersigned member(s) of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Waylivra in the following indication:

*Waylivra is indicated as an adjunct to diet in adult patients with genetically confirmed familial chylomicronemia syndrome (FCS) and at high risk for pancreatitis, in whom response to diet and triglyceride lowering therapy has been inadequate.*

The reasons for divergent opinion are the following:

Whilst a large effect of Waylivra on triglycerides (TG) levels in patients with familial chylomicronemia syndrome (FCS) was observed at month 3, a benefit in clinical outcomes of interest like abdominal pain, pancreatitis and quality of life, has not been shown in the clinical trials. The effect on TG levels decreased over time likely due to the dose adjustment for safety concerns.

Few FCS patients were exposed to Waylivra but a number of adverse events were reported, like local tolerability events (including skin discoloration), reduction in platelet count, bleeding events, abdominal pain, diarrhoea and nausea. The sudden and unexpected severe drops in platelet count observed in some patients after the implementation of the dose adjustment in the clinical study are particularly worrying and suggest that the proposed dose adjustment and the intensive monitoring cannot avoid the platelet fall and its potential clinical consequences. This together with the uncertainties related to the long-term administration of Waylivra are of relevance considering the lifelong nature of this treatment.

Taking all these aspects into account, the benefit risk balance of Waylivra is considered negative. There are doubts that the proposed PASS can solve the uncertainties previously mentioned.

Concepcion Prieto Yerro