13 November 2025
EMA/CHMP/303940/2025
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Dawnzera

International non-proprietary name: Donidalorsen

Procedure No. EMEA/H/C/006554/0000

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List of abbreviations

ACE	angiotensin-converting enzyme
ADA	Anti-drug antibodies
AECT	Angioedema Control Test
AE-QoL	Angioedema Quality of Life
ASO	Antisense oligonucleotide
BMI	Body mass index
C1	Complement component C1
C1-INH	C1-inhibitor
CSR	Clinical study report
DSC	Differential Scanning Calorimetry
ER	Emergency room
FDA	Food and Drug Administration
HAE	Hereditary angioedema
HAE-1	Hereditary angioedema type I
HAE-2	Hereditary angioedema type II
HAE-QoL	Hereditary Angioedema Quality of Life
HDPE	High Density Polyethylene
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPC	In-process control
ISP	Injection Site Pain
ISIS 721744	Donidalorsen; antisense inhibitor of prekallikrein
ITT	Intent-to-treat
MID	Minimal important difference
Min	Minimum
MMRM	Mixed effects model with repeated measures
mRNA	Messenger RNA
MS	Mass Spectrometry
nC1-INH-HAE	Hereditary angioedema with normal C1-inhibitor (previously known as type III)

NMR	Nuclear Magnetic Resonance
OLE	Open-label extension
P25	25th percentile
P75	75th percentile
PAA	Plasma proenzyme activation
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
Ph. Eur.	European Pharmacopoeia
PKa	Plasma kallikrein
PK	Pharmacokinetic(s)
PKK	Prekallikrein
PLG	Plasminogen
PP	Polypropylene
PRO	Patient-reported outcome
QoL	Quality of life
SAP	Statistical Analysis Plan
SCE	Summary of Clinical Efficacy
SD	Standard deviation
SEM	Standard error of the mean
siRNA	Small interfering ribonucleic acid
SmPC	Summary of Product Characteristics
SPS	Solid Phase Synthesis
TSQM-II	Treatment Satisfaction Questionnaire for Medication, version II
USP	United States Pharmacopoeia
UV	Ultraviolet
WPAI+CIQ:SHP	Work Productivity and Impairment questionnaire plus Classroom Impairment Questions: Specific Health Problem

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Otsuka Pharmaceutical Netherlands B.V. submitted on 20 November 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Dawnzera, 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 21 March 2024.

Dawnzera, was designated as an orphan medicinal product EU/3/24/2898 on 19 February 2024 in the following condition: treatment of hereditary angioedema.

The applicant applied for the following indication *'Dawnzera is indicated for routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older'*.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

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

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

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0246/2024 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0246/2024 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

Following the CHMP positive opinion on this marketing authorisation and at the time of the review of the orphan designation by the Committee for Orphan Medicinal Products (COMP), this product was removed from the Union Register of designated orphan medicinal products on 24 November 2025. More information on the COMP's review can be found in the orphan withdrawal assessment report published under the 'Assessment history' tab on the Agency's website:

https://www.ema.europa.eu/en/medicines/human/EPAR/Dawnzera.

1.4.1. Similarity

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

medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. New active substance status

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

1.6. Protocol assistance

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

Date	**Reference**	**SAWP co-ordinators**
22 July 2021	EMA/SA/0000061997	Brigitte Schwarzer-Daum and Clemens Mittmann

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

• Adequacy of the nonclinical package to support Phase 3 study initiation and MAA, including a waiver to perform an embryo-foetal development toxicity study in rabbit and a 2-year rat carcinogenicity study

• Acceptability of the clinical pharmacology program to support initiation of the Phase 3 study and MAA, including a waiver for a human mass balance study

• Clinical package supporting an MAA for prevention of recurrent HAE in adults and children from 12 to less than 18 years of age, including:

- Design elements of the global Phase 3 pivotal study ISIS 721744-CS5, including target population and underlying enrolment criteria, dosage and dose frequency, choice of HAE attack rate over 25 weeks versus placebo as primary endpoints, secondary endpoints

- Proposed statistical analysis for primary and secondary endpoints

- Acceptability of a single pivotal study and proposed size of the safety database

1.7. Steps taken for the assessment of the product

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

Rapporteur: Finbarr Leacy Co-Rapporteur: Paolo Gasparini

The application was received by the EMA on	20 November 2024
The procedure started on	27 December 2024
The CHMP Rapporteur's first Assessment Report was circulated to all	17 March 2025

CHMP and PRAC members on	
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	31 March 2025
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	31 March 2025
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 April 2025
The applicant submitted the responses to the CHMP consolidated List of Questions on	16 July 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	25 August 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	4 September 2025
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	18 September 2025
The applicant submitted the responses to the CHMP List of Outstanding Issues on	13 October 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	29 October 2025
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 Dawnzera on	13 November 2025
The CHMP adopted a report on similarity of Dawnzera with Takhzyro and Ekterly on (see Appendix on similarity)	13 November 2025
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	13 November 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Hereditary angioedema (HAE) is a rare and potentially life-threatening autosomal dominant inherited disease of vascular permeability. Patients with HAE present with unpredictable, recurrent, painful, and often debilitating swelling episodes referred to as attacks, which affect subcutaneous tissue (face, upper or lower extremities, genitals), abdominal organs (stomach, intestines, bladder), and the upper airway (larynx, tongue) (Petersen et al. 2024; Wilkerson and Moellman 2023; Bernstein 2018). Symptoms of this lifelong disease usually appear within the first 2 decades of life, most often by puberty, and may increase in severity after puberty (Bernstein 2018).

2.1.2. Epidemiology

This rare disease has an estimated prevalence of approximately 1 in 50,000 individuals worldwide (Sinnathamby et al. 2023) with no known differences in prevalence among ethnic groups (Neako et al. 2001).

2.1.3. Aetiology and pathogenesis

For most patients, HAE is caused by mutations in the **complement 1 (C1) inhibitor (C1-INH) gene** (SERPING1). C1-INH, a member of the serpin family of serine protease inhibitors, is a primary regulator of multiple proteases including prekallikrein (PKK) and coagulation Factor XIIa, as well as several complement proteases (Zuraw 2008). C1-INH is responsible, in part, for the control of the kallikrein-kinin pathway, also known as the contact system. The biological role of the contact system is to initiate and participate in the pathophysiological responses to injury, mainly in the processes of coagulation and inflammation. Upon activation of the contact system, blood coagulation may be triggered and proinflammatory products such as bradykinin are generated (Wu 2015). Bradykinin is a powerful vasodilator nanopeptide, which increases vascular permeability by binding to its cognate receptor (the bradykinin B2 receptor) on vascular endothelial cells. Loss of C1-INH function results in unregulated production of Factor XIIa, leading to uncontrolled activation of plasma PKK into kallikrein. This enzyme can then cleave high-molecular weight kininogen, releasing uncontrolled bradykinin, which allows fluid leakage into the extracellular space and results in angioedema (Wedner 2020, Kaplan and Joseph 2010).

2.1.4. Clinical presentation, diagnosis and stage/prognosis

The 2 main types of HAE include type I (international nomenclature: HAE-C1INH-Type1) (hereafter referred to as HAE-1) and type II (international nomenclature: HAE-C1INH-Type2) (hereafter referred to as HAE-2) in approximately 85% and 15% of cases, respectively (Sinnathamby et al. 2023). **Hereditary angioedema-1 (HAE-1)** is caused by C1-INH mutations that occur throughout the SERPING1 gene and result in truncated or misfolded proteins that are not efficiently secreted, with decreases in both antigenic and functional levels of C1-INH.

Hereditary angioedema-2 (HAE-2) is caused by C1-INH mutations that usually involve Exon 8 at or near the active site, resulting in a mutant protein that is secreted, but dysfunctional; thus, antigenic C1-INH levels are normal but functional C1-INH levels are low (Zuraw 2008).

In rare cases, patients have a **third type of HAE**, nC1-INH-HAE (international nomenclature: HAE-nC1INH) (formerly referred to as HAE type III), is a rare genetic disease with similar phenotype to HAE-C1-INH but different genetic background, in which antigenic and functional C1-INH levels are normal (Sinnathamby et al. 2023). This form is characterized by specific genetic mutations in the FXII, the plasminogen (PLG), the angiopoietin-1, the kininogen 1, myoferlin, and heparan sulfate-glucosamine 3-O-sulfotrasnferase 6 gene, or due to an unknown cause and genetic background (Maurer et al. 2022). There is clinical evidence that bradykinin may play a role in nC1-INH-HAE, especially in HAE-FXII and HAE-PLG (Bork et al 2017; Bork et al 2020), as mutations are linked to the kallikrein-kinin systems (Bork et al. 2020). Limited data are available regarding the prevalence of nC1-INH-HAE. Certain types of nC1- INH-HAE (HAE-FXII, -PLG, -ANGPT1, and KNG1) have so far mainly been reported in European patients including countries like Germany, France, Spain and Italy (Bork et al. 2020). A survey estimated the prevalence of HAE-FXII in Germany at 1 in 400,000 (Bork et. al. 2015).

HAE is a heterogeneous disease with a fluctuating and unpredictable course (Bygum et al. 2017). **For patients with HAE, severity of symptoms and incidence of swelling attacks are variable and unpredictable.** Minor trauma and stress are frequent precipitants of swelling episodes, although many attacks occur without an apparent trigger. A classic HAE attack worsens slowly but relentlessly over the first 24 hours, then gradually subsides over the subsequent 48 to 72 hours. The arms, legs, hands, feet, and abdomen are the most common sites of swelling. Attacks may start in one location and then spread to another before resolving (Nzeako et al. 2001). Attacks affecting the abdomen or oropharynx can be associated with significant morbidity and mortality. All patients with HAE are at risk for life-threatening episodes of laryngeal angioedema, which may cause fatal asphyxiation (Henao et al. 2016). Over 50% of patients with HAE have had at least 1 episode of laryngealangioedema during their lifetime (Bork et al. 2006). Together with the unpredictable nature and severity of the disease, including hospitalisation and death, HAE symptoms result in substantial physical, emotional, and economic burden on patients and their families (Lumry et al. 2010).

Diagnosing HAE can be challenging given the large heterogeneity of this patient population, the lack of knowledge by physicians given the low prevalence of the disease, sometimes nonspecific symptoms, and the fact that specific genetic mutations account only partially for the occurrence of HAE (Lara Marquez et al. 2018; Henao et al. 2016).

In addition to presentation of disease symptoms, tools for diagnosis of HAE-1 or HAE-2 include measurements of C1-INH function, and of C4 and C1 levels in serum/plasma (Maurer et al. 2022). The combined use of all 3 tests, if available, allows for a higher accuracy in diagnosing HAE. **A combination of low C4 and low C1 INH function has been shown to be 98% specific for C1-INH deficiency** in the tested population (Gompels et al. 2002).

2.1.5. Management

Both the US HAE Association Medical Advisory Board (Busse et al. 2021), and the World Allergy Organization (WAO), in collaboration with the European Academy of Allergy and Clinical Immunology (EAACI; Maurer et al. 2022), recommend a 3-method approach for the management of HAE, including on-demand treatment for acute swelling attacks, short-term or situational prophylactic treatment with the intent of minimizing the risk

of an attack during exposure situations (i.e., surgical trauma, dental surgery, etc), in addition to long-term prophylactic treatment with the goal of reducing the number of HAE attacks, and, in turn, increasing the quality of life (QoL) for the patient. The indication to initiate long-term prophylaxis in adolescents follows the same guidelines as in adults (Maurer et al. 2022), to achieve complete control of the disease, and to "normalize patients' lives" (Maurer et al. 2022).

Long-term prophylaxis treatment is essential to achieving this goal of reducing HAE attacks with limited burden of treatment (Betschel et al. 2023). Current long-term prophylactic regimens for HAE include plasma-derived C1-INH concentrate (administered either intravenously or subcutaneously), monoclonal antibodies directed against plasma kallikrein (pKa), a small molecule inhibitor of pKa, and attenuated androgens. These options reduce the number and severity of attacks due to HAE, although breakthrough attacks still occur.

2.2. About the product

Donidalorsen is a 2'-O-methoxyethyl–modified **antisense oligonucleotide (ASO) conjugated** to a triantennary N-acetylgalactosamine (GalNAc3) moiety that causes ribonuclease H1 (RNase H1) mediated **degradation of prekallikrein (PKK) mRNA** through selective binding to PKK mRNA, which results in reduced production of PKK protein. PKK is a pro-enzyme for plasma kallikrein, which results in the release of bradykinin, a potent vasodilator causing swelling and pain in HAE. In patients with HAE, C1 inhibitor (C1-INH) deficiency or dysfunction leads to excessive plasma kallikrein activity, bradykinin generation, and angioedema attacks. Donidalorsen lowers PKK concentration, preventing excessive bradykinin production in patients with HAE.

The proposed indication: "for routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older."

The proposed posology:

The recommended starting dose in adult and adolescent patients aged 12 years and older patients is 80 mg donidalorsen by subcutaneous injection. Doses should be administered once monthly.

A dosing interval of 80 mg once every 2 months may be considered if the patient is well controlled (e.g., attack free) for at least 3 months while receiving Dawnzera.

2.3. Type of application and aspects on development

The applicant obtained CHMP scientific advice (EMA/SA/0000061997) in 2021.

In this SA in regard to the clinical development the following aspects were discussed:

Study design:

- The use of placebo as a comparator in the study investigating the prophylactic effect of donidalorsen on recurrent attacks of HAE was considered acceptable considering that acute medications to treat attacks were allowed as concomitant treatment.
- The treatment period of 25 weeks was considered acceptable for the evaluation of the frequency of attacks however an open-label extension study after the controlled treatment period was also recommended

Target population:

- In general, the proposed inclusion criteria were endorsed including the requirement for a minimum of 2 investigator-confirmed HAE attacks during the 8 weeks screening period. Nevertheless, it was highlighted that as chronic prophylaxis medications except for a stable dose of androgens were disallowed, this may result in recruiting a less severe population of patients.

Dose:

- Although the justification for the proposed dose (i.e. 80 mg every 4 weeks) was considered reasonable, further considerations were required for the frequency of dosing and why 80 mg and not 60mg dose was selected for the phase 3 study even though a similar efficacy on PKK was observed in the study performed in healthy subjects.
- The data to justify dosing in the paediatric population were considered as limited.

Primary endpoint:

- The selection of the time-normalized HAE attack rate compared to placebo was considered as clinically meaningful and accepted. It was noted that the sample size was based on a HAE attack rate of approximately 2 attacks per month in the placebo however this could be relatively high, given the selection criteria of participants.

Secondary endpoints:

- The proposed secondary endpoints were endorsed.

Statistical analysis:

- The treatment policy estimand being the primary estimand of interest for the primary endpoint was endorsed. It was highlighted to the applicant that the treatment policy estimand using an appropriate imputation method (e.g. imputation using the placebo recurrent event rate) for unobserved recurrent events after premature treatment discontinuation is preferred to be the primary estimand of interest. Sensitivity analyses and supplementary analyses based on other estimands (e.g. the hypothetical estimand censoring patients at time of premature treatment discontinuation) can also be provided to support results of the primary estimand.
- It was highlighted that as the study is intended to be the only pivotal study to support MAA, the requirements of the CPMP/EWP/2330/99 guideline apply.

Number of patients to be recruited:

- A small number of patients planned to be recruited was noted and it was suggested to increase the number of patients included in the pivotal trial and assess the possibility to generate more data on long term efficacy and safety.

Although, minimum numbers, as outlined in ICH E1 (overall 300 – 600 patients. 100 patients at least one year) are not expected in a rare disease like HAE1 and 2, larger numbers than those proposed were considered to be feasible.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as solution for injection in pre-filled pen containing 80 mg of donidalorsen. The product contains the sodium salt.

Other ingredients are: sodium dihydrogen phosphate (E 339), disodium hydrogen phosphate (E 339), sodium chloride, water for injections, hydrochloric acid (E 507), and sodium hydroxide (E 524).

The product is available in a single-use Type I glass syringe with a stainless steel staked needle, rigid needle shield, and siliconised chlorobutyl elastomer plunger stopper. The filled primary container and a pen are assembled to a pre-filled pen (autoinjector) as described in section 6.5 of the SmPC.

2.4.2. Active Substance

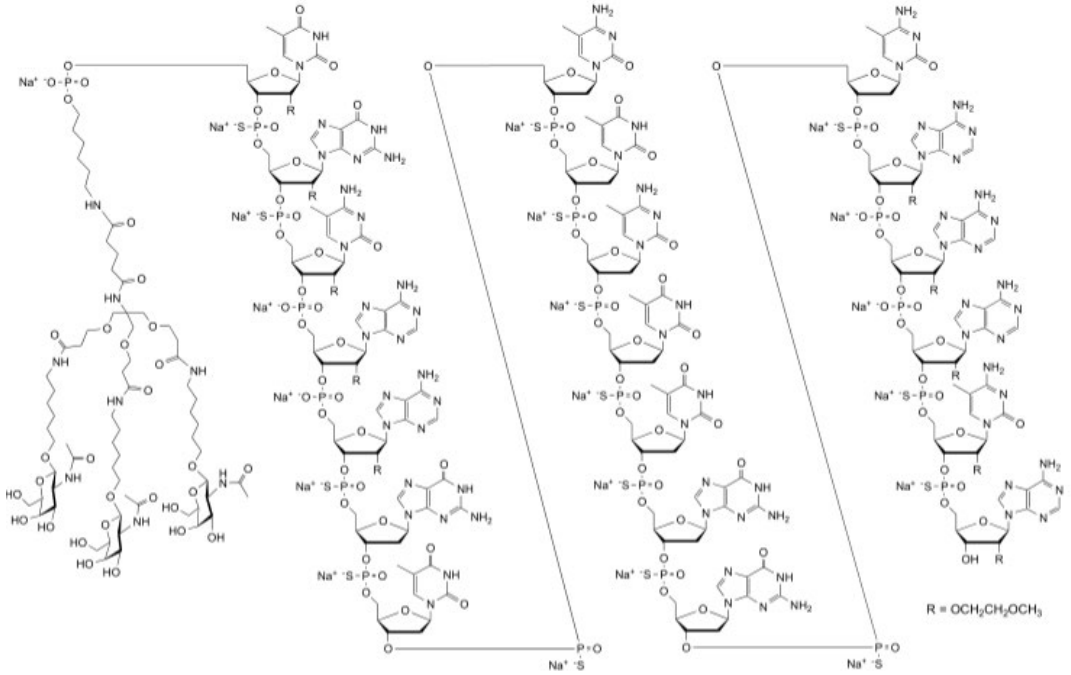
2.4.2.1. General information

Donidalorsen sodium is a synthetic antisense oligonucleotide conjugated to a triantennary N-acetylgalactosamin (GalNAc) ligand via an aminohexyl linker.

The donidalorsen sequence can be written in shorthand as follows: *5′-THA-AH$_O$^{Me}U$_S$G$_S$^{Me}C$_O$A$_O$A$_S$G$_S$T$_S$^{Me}C$_S$T$_S$^{Me}C$_S$T$_S$T$_S$G$_S$G$_S$^{Me}C$_S$A$_O$A$_O$A$_S$^{Me}C$_S$A-3′.* The underlined residues are the 2′-MOE nucleosides. The locations of phosphorothioate and phosphate diester linkages are designated by S and O, respectively. AH designates the position of the aminohexyl linker; THA is 5-[(tris{3-[6-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)hexylamino]-3-oxopropoxymethyl})methyl]amino-5-oxopentanoyl. 2-*O*-(2-methoxyethyl)methyluridine. The nucleobases are U (Uracil), G (Guanine), C (Cytosine), A (Adenine) and T (Thymine).

The molecular formula of donidalorsen sodium is $C_{296}H_{415}N_{83}O_{151}P_{20}S_{15}Na_{20}$. It has a molecular weight of 9112.27 D and the following structure:

Figure 1. Active substance structure



The chemical structure of donidalorsen sodium was elucidated by a combination of methods.^{1}H and ^{13}C are used to determine the structure with supportive COSY/HMBC/HSQC 2D NMR and ^{31}P NMR confirms the ratio of phosphorothioate diester internucleotide linkages, alkyl phosphate diester linkage and internucleotide phosphate diester linkages are as expected.

High resolution mass spectrometry confirms the monoisotopic mass of donidalorsen sodium to within 2 ppm. The nucleotide sequence is confirmed by both failure sequence in which the crude MMT-on aminohexyl intermediate material obtained from solid phase synthesis is analyses for failure (capped) sequences and MS/MS for which interpretation of the fragmentation data with theoretical and observed masses are presented. This confirms the primary nucleotide sequence including modifications. Elemental analysis is consistent with the proposed composition of donidalorsen sodium and confirms the amount of counterion present.

In terms of physiochemical characterisation of donidalorsen, UV spectrometry is used to determine the ultraviolet absorption spectrum, DVS to characterise its hygroscopicity and DCS and TGA to demonstrate it is an amorphous solid. The solubility of donidalorsen is also characterised.

The primary structure of donidalorsen sodium has been adequately characterised and the sequence of nucleotides confirmed. The secondary structure of donidalorsen sodium has been characterised through calculations based on Watson-Crick base pairing rules. However, due to its short length, donidalorsen sodium exists in a random coil and does not adopt a preferred, stable secondary structure. This conclusion is supported by the results of CD analysis. The specificity of the conjugation to the aminohexyl linker and not the amine groups of the nucleic acids has been confirmed through experimentation.

The active substance is a white to yellow amorphous solid. It is freely soluble in water and aqueous sodium acetate buffer (pH 3), slightly soluble in methanol, and insoluble in acetone, ethanol, acetonitrile, isopropyl alcohol, and chloroform.

Donidalorsen sodium is hygroscopic and gains and loses moisture as a function of relative humidity.

The absolute configuration of each 2-deoxy-D-ribose unit is (1*R*, 3*S*, 4*R*). The absolute configuration of each 2-*O*-(2-methoxyethyl)-D-ribose unit is (1*R*, 2*R*, 3*R*, 4*R*). The absolute configuration of each galactosamine unit is (1*R*, 2*R*, 3*R*, 4*R*, 5*R*). The absolute configuration at the phosphorus atom of each phosphorothioate diester is undefined; hence, donidalorsen sodium is a mixture of 2^{15} diastereoisomers.

The stereochemistry of the nucleotide 2'-MOE 2'-deoxy residues of donidalorsen sodium is determined by the phoshoramidite starting materials. Potential anomerisation propensity is concluded not a risk. Likewise, the stereochemistry of the GalNAc conjugate is determined by the THA8 starting material. Using an alternative technique that involved sampling the growing nucleotide chain after each coupling step of the synthesis and IP-HPLC analysis, the ratios of R/S isomers at each internucleotide linkage were determined and an estimation of the diastereomeric distribution was calculated. Considering the complexity of the stereochemistry of the active substance this is generally considered acceptable. The experiment was performed twice and the data concluded that the diastereomeric distribution was reproducible across the two runs. Based on the low abundance of any individual diastereomer no adverse impact on the biological/pharmacological activity is expected by minor variations in the stereochemical distribution.

Polymorphism has not been observed for donidalorsen sodium.

2.4.2.2. Manufacture, characterisation and process controls

The active substance is manufactured by one manufacturing site. A valid QP declaration for the site is provided.

Donidalorsen sodium is synthesised chemically by solid phase synthesis (SPS). The manufacturing process consists of eight distinct unit operations; (1) SPS followed by cleavage and deprotection, (2) reversed phase (RP) chromatography, (3) detritylation, (4) conjugation, (5) strong anion exchange (SAX) chromatography, (6) ultrafiltration and diafiltration (UF/DF), (7) thin film evaporation (TFE) and (8) freeze-drying. A typical batch size is defined.

The SPS step is comprised of 4 sequential reactions; deprotection, coupling, oxidation/thiolation and capping that are repeated for 21 cycles to add each nucleotide (as phosphoramidite starting materials (SM)) in succession as per the intended sequence. Subsequent process steps downstream from the SPS include purification, conjugation to GalNAc moiety, concentration and drying steps.

Adequate in-process controls are applied during the synthesis. Relevant process parameters (critical process parameters (CPPs) and process parameters (PPs)) are provided. For the CPPs both set points and PARs are provided and appropriately justified.

The steps that impact the critical quality attributes CQAs of the active substance were identified as critical steps. The CQAs are: purity, impurities and sodium counterion. As water content is not a CQA of the active substance, the lyophilisation step is not considered critical.

The specifications and control methods for three intermediate products, starting materials and reagents have been presented. Omission of specifications for the other intermediates of the process has been justified on the basis that the CQAs they impact are all controlled in either the subsequent stage intermediate or the final active substance.

The starting materials (SM) defined for the process are the nucleoside phosphoramidites, MMT-aminohexyl phosphoramidite (linker) and THA8 (triantennary N-acetylgalactosamin (GalNAc) ligand). The names and

addresses of the SM suppliers are provided. Multiple suppliers are registered for each starting material and all synthetic routes are included in the dossier. Justifications for each of the SM is provided in line with ICH Q11. Structures of each of the SM are presented which identify the parts of the molecules incorporated into the final active substance structure. The absolute configuration of each of the nucleoside phosphoramidites is also provided.

The synthesis of THA8 GalNAc SM is described from a number of chemical transformations back and all reagents/catalysts and solvents used are identified.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised. Impurities fall under two broad categories a) product-related impurities and non-oligonucleotide impurities. Product related impurities are further categorised into three main groups; 1) those originating from the starting materials; 2) product related impurities formed during the manufacturing process; and 3) oligonucleotide degradation products.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. In general, the method of synthesis has not changed significantly throughout the manufacturing history and donidalorsen sodium has been manufactured by the same sequence of steps for all batches presented and used in clinical development. For each step in the process, the changes that were applied though the development are described. Changes introduced have been presented in sufficient detail and have been justified.

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

The active substance packaging complies with Commission Regulation (EU) 10/2011, as amended and relevant Ph. Eur. chapters.

2.4.2.3. Specification

The active substance specification includes tests for: appearance (visual inspection), identification by Mass (IP-HPLC-UV-MS), identification by sequence confirmation (duplex melting temperature (Tm)), identity and quantity of counterion (ICP-OES), assay (IP-HPLC-UV-MS), purity (IP-HPLC-UV-MS), specified oligonucleotide impurities (IP-HPLC-UV-MS), unspecified oligonucleotide Impurities (IP-HPLC-UV-MS), total oligonucleotide degradation products (IP-HPLC-UV-MS), total oligonucleotide impurities (IP-HPLC-UV-MS), residual solvents (acetonitrile): GC, bacterial endotoxins (USP<85>, Ph. Eur. 2.6.14, JP 4.01), microbial examination of nonsterile products TAMC, TYMC (USP<61>, Ph. Eur. 2.6.12, JP 4.05I), and water content (Karl-Fisher).

The active substance specifications are based on the active substance critical quality attributes (CQA). The CQA identified are: appearance, identity, assay, purity, oligonucleotide impurities – non degradation products, oligonucleotide impurities –degradation products, residual solvents, elemental impurities, other process-related impurities, bacterial endotoxins, and total aerobic microbial count; total yeast and mould.

The active substance specification was developed according to the principles delineated in International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines Q6A Specifications, and Q3A, Q3B, Q3C, and Q3D Impurities and is also in line with the recommendations of

the EMA Draft Guideline on the Development and Manufacture of Oligonucleotides. Prior knowledge and experience manufacturing and analysing antisense oligonucleotides was also relied on and detailed in the dossier.

The specification comprises tests designed to assess the critical quality attributes (CQA) of donidalorsen sodium.

Assay is determined by IP-HPLC-UV using a weight-based assay against a standard of known purity and concentration. It is expressed in terms of the anhydrous substance and is corrected for impurities that co-elute with the main donidalorsen sodium peak. The limit for assay is based on the limit for purity and analytical measurement variability. This approach is considered acceptable and the assay limits are justified.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set. The same IP-HPLC-UV-MS method conditions are used to determine identification (by mass), assay, purity and impurities. Assay is determined by IP-HPLC-UV using a weight-based assay against a standard of known purity and concentration. It is expressed in terms of the anhydrous substance and is corrected for impurities that co-elute with the main donidalorsen sodium peak.

The levels of each impurity/group of impurities quantified in nine batches of donidalorsen sodium are presented along with the qualification level and corresponding toxicology study number as appropriate. Impurities that are not qualified due to low levels present are controlled at 1.5% and unidentified impurities are controlled at 1.0% which is considered acceptable. The impurities are controlled in the drug substance as per the IP-HPC-UV-MS method with impurities that separate chromatographically from the main peak of donidalorsen, detected and quantified using UV analysis and those that co-elute by MS. For each impurity / impurity group discussed representative chromatograms from the corresponding UV and/or MS analysis are provided.

The IP-HPC-UV-MS method used to control impurities in donidalorsen sodium cannot identify/control the product related impurities 3'-5' and 2'-3' isomer impurities originating from the phosphoramidite starting materials. This approach has been justified based on the control limits applied in the corresponding starting materials and a theoretical calculation concluding a worst-case amount in the final active substance. Toxicological studies on a batch of donidalorsen prepared from SMs with higher levels of impurities concluded no significant differences.

Many of the product-related impurities are controlled in groups as per the donidalorsen sodium specification (e.g. Full-length $(P{=}O)_1$, Total (n-1), Total (n+1) etc.) and based on the impurity characterisation data, this approach is justified with UV/MS spectra incapable of distinguishing between many impurities of a given group. This approach is further justified and found acceptable.

Justification for omission if tests for elemental impurities is based on a risk assessment performed in line with ICH Q3D and batch data. Omission of controls for process impurities is based on purge calculations and batch data and biological assay is not required.

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 testing has been presented.

Batch analysis data on commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

2.4.2.4. Stability

Stability data from three commercial scale batches of active substance are stored in the intended commercial package for up to 24 months under long term conditions (-20 ± 5 °C) and 6 months under accelerated conditions (5 ± 3 °C). They were also stored and for up to 3 months under stress conditions (30 ± 2 °C/70 ± 5% RH). The differences in the manufacturing process used compared to the proposed commercial process/manufacturer are considered relatively minor and the batches of the active substance from two manufacturers are considered comparable. Additional stability data from the proposed commercial manufacturer are also available on three PPQ stability batches stored at -20 °C for 18 months and at 5 °C for six months. In addition to registration and PPQ stability data, 60 months and 6 months of supporting stability data gathered at -20 °C and 5 °C, respectively, are available. Further supportive stability data has been provided.

The following parameters were tested: appearance, assay, purity, impurities, water content, BET and microbiological purity. The parameters tested are the same as for release. The analytical methods used were the same as for release and were stability indicating.

All tested parameters were within the specifications.

Photostability testing following the ICH guideline Q1B was performed on one batch. Results indicate some degradation of donidalorsen sodium after exposure predominantly as an increase in full-length (P=O)1 and corresponding loss in purity. However, results remain within specification limits and special precautions to protect donidalorsen sodium from the effects of light during manufacturing, handling, and finished product formulation activities are not required.

Results on stress conditions on temperature, light, acidic, basis and oxidative conditions were also provided. For each of the conditions, the main impurities are identified, and degradation pathways are proposed. Mass balance results indicate that the IP-HPLC-UV-MS method used to determine purity and impurity levels is appropriately stability indicating. Significant degradation was observed for all the conditions studied. on

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 30 months when stored at -20 ± 5 °C in the proposed container.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Donidalorsen solution for injection (Dawnzera) is a sterile, single-dose, ready-to use parenteral solution of donidalorsen intended for subcutaneous (SC) administration. The formulation contains 100 mg/mL donidalorsen free acid (105 mg/mL donidalorsen administration) in a sodium phosphate buffer, at a target of pH 7.4. Sodium chloride is added to achieve an isotonic solution and osmolality of approximately 290 mOsm/kg. The solution appears clear and colourless to yellow. The finished product is packaged with 0.8 mL deliverable volume in a 1 mL long (1 mL L) staked needle glass prefilled syringe (PFS) closed with a siliconized butyl rubber plunger stopper. The PFS is assembled into a pre-filled pen (autoinjector) as the final finished product. The pre-filled pen delivers a single dose of 80 mg donidalorsen.

The finished product and description are appropriately described including the use of nitrogen (controlled as per USP quality standards) as a processing aid. There are no overages in the finished product manufacture.

The pharmaceutical development of the product is based on a quality target product profile (QTPP) in accordance with ICHQ8(R2), and its elements are defined in the dossier.

The applicant applied prior knowledge of the process and platform data regarding the nature of the process. A description of product specific and platform studies has been included for each step of the process. The use of platform studies is acceptable for oligonucleotide finished products and the applicant has provided a brief discussion on the platform studies in relation to the manufacture of the finished product but also in other sections of the dossier where it is used as a justification not limited to but including formulation of the finished product, oligonucleotide structure, selection of dosage form, criteria for inclusion and exclusion of an oligonucleotide finished product, clear identification e.g. information on sequences of all included oligonucleotides.

Donidalorsen sodium is a synthetic oligonucleotide with a molecular weight of 9112.27 Da (8672.66 Da for the free acid). Due to its polyanionic nature, the drug substance is freely water soluble at physiological pH, making it simple to formulate in an aqueous solution. The active substance is an amorphous solid; therefore, there is no concern regarding differential dissolution rates of polymorphs.

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 excipients consist of water for injection (WFI), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, and sodium hydroxide or hydrochloric acid, as appropriate. The finished product formulation is isotonic, and stability studies demonstrate adequate buffering capacity to maintain the pH within the specification limit.

The formulation was kept essentially consistent through the stages of the finished product development. The clinical presentation for Phase 1 through Phase 3 clinical studies was donidalorsen 100 mg/mL solution for injection in a sodium phosphate buffer at neutral pH with sodium chloride added as a tonicity modifier for a physiologically acceptable osmolality. The bulk finished product solution was sterile filtered and aseptically filled into vials and administered subcutaneously with a disposable sterile syringe.

The commercial formulation has been developed in a pre-filled pen format to deliver 80 mg donidalorsen, administered by injecting 0.8 mL of 100 mg/mL donidalorsen solution for injection.

The 100 mg/mL vial formulation was slightly adapted for the PFS formulation with a fill volume of 0.8 mL for the 80 mg dose. Release and stability results demonstrate the clinical and commercial formulations are comparable in regard to physicochemical stability.

The commercial formulation in the pre-filled pen configuration was assessed in a clinical study to demonstrate bioequivalence to the corresponding clinical vial dose. The pre-filled pen configuration is also being used in a Phase 3 open-label extension study.

The finished product manufacturing process consists of dissolving the active substance in a phosphate buffered saline vehicle to yield the bulk finished product solution, which undergoes bioburden reduction filtration (BRF), sterile filtration, and aseptic filling to produce the sterile bulk PFS. The finished product formulation and manufacturing process used throughout the course of clinical trials were largely unchanged. A neutral pH sodium phosphate buffered saline vehicle was used for all finished product formulations. Minor differences between the PFS and vial finished product manufacturing processes were appropriately justified.

A justification for the use of sterile filtration and the feasibility of terminal sterilisation has been provided. The choice of sterile filtration with aseptic filling has therefore been appropriately justified as per EMA/CHMP/QWP/850374/2015.

The primary packaging is a single-use Type I glass syringe with a stainless steel staked needle, rigid needle shield, and siliconised chlorobutyl elastomer plunger stopper. The material complies with Ph.Eur. and EC requirements. Interactions of the finished product formulation with potential syringe incompatibilities (e.g., tungsten, silicone oil, ethylene oxide residuals) have not been observed, as demonstrated by the stability data. The choice of the container closure system has been validated and is adequate for the intended use of the product. The syringe is assembled into a pre-filled pen and a valid notified body opinion has been provided. The applicant has also included relevant details on the pre-filled pen in the dossier. The syringe is compatible with the pre-filled pens. The components of the primary packaging are supplied as sterile ready-to-use. The staked needle syringes are sterilised via ethylene oxide in accordance with ISO 11135 to a sterility assurance level of 10^{-6} and the plunger stoppers are sterilised by gamma or steam sterilisation in accordance with ISO 11137 and ISO 17665 respectively. Information in line with "Guideline on quality documentation for medicinal products when used with a medical device (EMA/CHMP/QWP/BWP/259165/2019) has been provided and is acceptable.

Studies to confirm the suitability along with the safety of the container have been described including glide force and container closure integrity. The studies show that the container closure is suitable and the critical quality attributes of the product are not affected on stability while safety has also been appropriately demonstrated. Reference to photostability studies has been made and there has been no significant change in the quality attributes of the drug product when stored under normal conditions and exposed to light. Biocompatibility data has been presented in section 3.2.R and this data has been referenced in section P.7 of the dossier.

Extractables and leachables have been tested and test results have been provided for each relevant component. The results show that all detected extractables are as expected for the materials used in the primary container components. A glass delamination study was undertaken and no sign of delamination was observed.

The applicant has provided a sufficient discussion which indicates that the finished product is appropriately sterilised and the sterility of the finished product is maintained during assembly into the pre-filled pen as well as during storage.

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured and primary packaged by one manufacturing site. The finished product manufacturing, pre-filled pen assembly and finished product testing sites are appropriately authorised and hold valid GMP certificates.

The manufacturing process consists of eight main steps:

1. Compounding of the vehicle
2. Bioburden reduction filtration of the vehicle
3. Compounding of the bulk finished product concentrate
4. Compounding of the bulk finished product solution
5. Bioburden reduction filtration of the bulk finished product solution
6. Double inline (redundant) sterile filtration and aseptic filling of the bulk finished product solution into the syringe

7. 100% visual inspection of the prefilled syringe (PFS) batch

8. Assembling of the PFS into the autoinjector.

The process is considered to be a non-standard manufacturing process.

Relevant CPP's and IPCs have been defined and are acceptable. The applicant has also provided the critical hold-times for the manufacture of the finished product.

PPQ/process validation batches were manufactured, two at the lower end of the proposed batch size range and one at the upper end of the proposed batch size range. The process has been suitably validated and all relevant data has been provided in the dossier. Autoinjector assembly process validation data for multiple sites has been provided and found acceptable. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

Hold time challenge data and microbiological testing to support the hold times have been provided in this section and where data is provided the hold times are suitably justified.

Appropriate validation of the PVDF 0.22 µm filters used in the manufacturing process has been undertaken with relevant data provided including data on bacterial viability and recovery, filter leachables and filter compatibility.

Results of the media fill runs have also been provided and based on the data provided for the aseptic filling process is appropriately validated for a maximum filling time for routine production of 48 hours.

The post-filtration bioburden reduction filtration limits of ≤ 100 CFU/10 mL and sterile filtration limits of ≤10 CFU/100 mL for TAMC and TYMC are acceptable.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (Ph. Eur. 2.9.20, JP 6.06), degree of coloration (USP <631>, Ph. Eur. 2.2.2, JP 2.65), clarity and degree of opalescence (Ph. Eur. 2.2.1), identification sequence confirmation by melting temperature (duplex melting temperature (Tm)), identification by mass (IP-HPLC-UV-MS), assay (IP-HPLC-UV-MS), purity (IP-HPLC-UV-MS), specified oligonucleotide degradation products (IP-HPLC-UV-MS), unspecified oligonucleotide degradation products (IP-HPLC-UV-MS), any single unspecified degradation product (IP-HPLC-UV-MS), total oligonucleotide degradation products (IP-HPLC-UV-MS), pH (USP <791>, Ph. Eur. 2.2.3, JP 2.54), osmolality (vapor pressure USP <785>), particles /container of size ⩾10 µm (USP <788>, Ph. Eur. 2.9.19, JP 6.07 JP 2.54), particles /container of size ⩾25 µm (USP <788>, Ph. Eur. 2.9.19, JP 6.07), bacterial endotoxins (USP <85>, Ph. Eur. 2.6.14, JP 4.01), sterility (USP <71>, Ph. Eur. 2.6.1, JP 4.06), container closure integrity (dye ingress, USP <1207>), uniformity of dosage units (USP <905>, Ph. Eur. 2.9.40, JP 6.02), activation force (mechanical force and time analysis, ISO 11608-5), injection time (mechanical force and time analysis, ISO 11608-5), delivered volume (ISO 11608-1, USP <697>).

The finished product is released on the market based on the release specifications, through traditional final product release testing.

The specification parameters proposed and the relevant limits for each parameter described are acceptable and have been suitably justified per relevant compendial chapters, ICH guidelines and batch data.

The applicant has outlined the observed degradation impurities in the finished product each of which are appropriately controlled in the active substance specification both at release and on stability. A number of potential degradation impurities from thermal and photolytic stress are also described and appropriately discussed.

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

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary.

Omission of testing for deamination has been accepted based on stability data provided as deamination will not occur when the product is stored under recommended conditions.

The omission of control of primary function autoinjector tests for cap removal force, needle length and needle cover lockout displacement have also been sufficiently justified based on the data provided.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay testing has been presented.

Batch analysis results are provided for commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification. Additional supportive data on batches used in clinical and supporting stability studies were also provided.

2.4.3.4. Stability of the product

Stability data on three commercial scale batches of finished product stored for up to 24 months under long term conditions (2 - 8 ºC) and for up to six months under accelerated conditions (25 ± 2°C/60 ± 5% RH) according to the ICH guidelines were provided. The batches of finished product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing. Supporting stability data was also provided.

Samples were tested for assay, purity and impurities, sterility, endotoxins, container closure integrity, particulate matter, appearance, degree of colouration, clarity/opalescence and pH along with autoinjector tests activation force, injection time and delivered volume. The analytical methods and acceptance criteria for the parameters controlled in both the release and shelf-life specification are the same. The analytical procedures used are stability indicating.

All data complies with the specifications and no significant changes are observed for any parameters over the time-period for which the data has been provided. A shelf-life of 36 months is proposed based on the data currently provided this shelf-life is acceptable.

Results from particulate matter testing performed during finished product stability studies support the conclusion that a propensity to agglomerate is not a risk for donidalorsen sodium.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Results are all within specification and levels of degradation in both the pre-filled pen and samples in carton are comparable. The data provided indicates the product is stable in the pre-filled pen when exposed to light and does not point to any light sensitivity. However, the product includes a storage condition that it should be protected from light, this is stated by the applicant to be included as a precautionary measure.

Forced degradation studies have also been provided conducted by exposing samples to 6 million lux hours of cool white-fluorescent lamp light and1000-watt hours/m2 of ultraviolet lamp light and the data provided shows that the drug product solution did show some susceptibility to photodegradation during forced degradation studies however the storage condition is accepted. Forced thermal degradation results are also included which show degradation under conditions of 80 °C for 4 weeks. The product is stored at 2 - 8 °C.

The stability data support the proposed allowance for the end-user to store the finished product for up to 6 weeks at a temperature less than 30°C. To aid compliance with this, a section is included on the outer packaging where the disposal date, calculated from the moment the product is stored outside the refrigerator, can be inserted.

Based on available stability data, the proposed shelf-life of 36 months when stored in a refrigerator (2 - 8 °C) as stated in the SmPC (section 6.3) is acceptable.

2.4.3.5. Adventitious agents

A material of animal origin is used in the manufacture of one of the active substance starting materials. It is concluded that the material acceptable for use because it complies with the requirements in EMA/410/01 Rev. 3 as a negligible risk for transmitting animal spongiform encephalopathy agents per the controls at place through the qualified vendor.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Donidalorsen sodium is a synthetic antisense oligonucleotide conjugated to a triantennary N-acetylgalactosamin (GalNAc) ligand via an aminohexyl linker. The oligonucleotide part contains 20 nucleotides with 2'-O-(2-methoxyethyl) sugar modifications on the first and last five nucleotides.

Dawnzera is a sterile, single-dose, ready-to use parenteral solution of donidalorsen intended for subcutaneous (SC) administration. The formulation contains 100 mg/mL donidalorsen free acid (105 mg/mL donidalorsen administration) in a sodium phosphate buffer, at a target of pH 7.4. Sodium chloride is added to achieve an isotonic solution. The solution appears clear and colourless to yellow. The finished product is packaged with 0.8 mL deliverable volume in a 1 mL long (1 mL L) staked needle glass prefilled syringe (PFS) closed with a siliconized butyl rubber plunger stopper. The PFS is assembled into a pre-filled pen constituting the finished product. The autoinjector delivers a single dose of 80 mg donidalorsen.

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.4.6. Recommendations for future quality development

None.

2.5. Non-clinical aspects

2.5.1. Introduction

Donidalorsen is a 2'-MOE ASO with a mixed backbone comprised of PS and PO internucleotide linkages conjugated to a $GalNAc_3$ moiety that causes RNase H1-mediated degradation of PKK mRNA, resulting in reduced production of PKK protein. Therefore, the formation of bradykinin and the downstream clinical manifestations of vascular permeability are reduced.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

In vitro and *in vivo* studies were conducted to evaluate the primary pharmacodynamics of donidalorsen.

In vitro: The efficacy and specificity of donidalorsen have been characterised in human cells *in vitro* using its unconjugated predecessor ASO, ISIS 546254, which has the same nucleobase sequence as donidalorsen, but a uniform PS backbone. ISIS 546254 is perfectly matched to only PKK mRNA in the human transcriptome and binds to a site free of meaningful sequence variation. *In silico* analysis was used to identify potential off-target effects. Transcriptome analysis revealed 7 genes with a mismatch of 2 bases. Of these 7 genes, only 4 were expressed in the tissue of preferential GalNAc3-conjugated ASO accumulation. While ISIS 546254 produced potent dose-dependent reductions in PKK mRNA in Hep3B cells (IC_{50}: 0.35 μM), the 4 off-target transcripts were either not significantly reduced by ISIS 546254 or were reduced but had greater than 20-fold higher IC_{50} values (9.1 and 8.8 μM for IFIH1 and PRKN, respectively) compared to PKK mRNA. A study in primary human hepatocytes compared the effects of donidalorsen and ISIS 546254 on PKK mRNA. Donidalorsen was demonstrated to be 46-fold more potent at inhibiting PKK mRNA than its unconjugated full PS backbone predecessor ASO ISIS 546254 (IC_{50} 8.73 μM vs 0.19 μM). These data provide a proof-of-concept for donidalorsen demonstrating it is effective at reducing PKK mRNA in hepatocellular cell lines and primary hepatocytes and it is selective for its target.

In vivo: Mice and cynomolgus monkeys were used as nonclinical species to examine the pharmacological effect of donidalorsen. Donidalorsen is not pharmacologically active in the mouse due to its lack of complementarity to the mouse PKK gene transcript. Therefore, a mouse-specific surrogate ASO, ISIS 482584, was used in five *in vivo* studies to elucidate the role of PKK in vascular permeability. These experiments demonstrate that ISIS 482584 treatment for 3-weeks results in a dose-dependent decrease of

PKK mRNA in the liver and protein in the plasma. These reductions in PKK expression resulted in decreases in basal plasma kallikrein levels, basal HMWK cleavage and basal vascular permeability with significance reached at 20 mg/kg/wk. These results confirm the role of PKK in the kinin-kallikrein system and the important function of PKK in the regulation of vascular permeability.

Treatment with captopril and C1-INH ASO enhanced vascular permeability in mice but did not increase PKK mRNA or protein expression. ISIS 482584 dose-dependently reduced PKK mRNA in captopril-treated mice, with significance reached at 10 mg/kg/wk, however this was only translated into a functional effect i.e. decrease vascular permeability, at ≥ 80 mg/kg/wk. Inhibition of PKK mRNA resulted in reduced plasma PKK protein and kallikrein levels and HMWK cleavage. Similar effects were evident in the C1-INH ASO-treated mice where ISIS 482584 (80 mg/kg/wk) reversed the increase in vascular permeability and reduced PKK mRNA and protein, kallikrein levels and HMWK cleavage. A non-targeting ASO was used as a control in C1-INH ASO model but did not have an effect. No non-targeting ASO control was used in the other studies, which used PBS as control.

On-target pharmacology of donidalorsen was evaluated in transgenic mice expressing human KLKB1/PKK gene and cynomolgus monkey. Single administration of donidalorsen to mice (2 males and 2 females) resulted in a robust dose- and time-dependent reduction in human PKK plasma protein that approached 91% at the highest dose of 10 mg/kg on day 14 after treatment. PKK levels were not fully restored at day 56 (20% below baseline). The Applicant did not evaluate vascular permeability or functional effects in this study. Pharmacology of donidalorsen was also evaluated in NHP as part of the 39-week repeat-dose toxicity study. The sequence targeted by donidalorsen contains one mismatch to the binding site in cynomolgus monkey PKK RNA. This study demonstrated that donidalorsen caused dose-dependent inhibition of hepatic PKK mRNA expression and reduction in plasma PKK protein activity up to 62% and 80%, respectively at day 93 for 10 mg/kg/wk group. It is noted that hepatic PKK was lower at day 93 versus day 275 for comparable doses (58% vs 35% and 60% vs 52% for 6 and 12 mg/kg/wk, respectively), which could indicate a tolerance to the pharmacological effect. However, a time-dependent reduction was evident in PKK protein activity for comparable doses. By day 185 reductions were at the nadir and remained low during treatment, recovering upon treatment cessation. Analysis of the recovery samples 26 weeks after the end of treatment for animals treated for 13- and 39-weeks with donidalorsen at 30 and 12 mg/kg/wk, respectively showed higher hepatic PKK mRNA expression (115% and 160%, respectively) and corresponding increase in PKK Plasma protein levels (196 % and 103%, respectively) than the pre-study baseline.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were provided by the Applicant. ASOs are predicted to be highly specific to their target sequences and secondary off-target actions are expected to be minimal.

2.5.2.3. Safety pharmacology programme

An *in vivo* safety pharmacology study conducted in the monkey indicated that donidalorsen had no adverse effects on cardiovascular, respiratory or neurobehavioural parameters at a dose of up to 30 mg/kg. Donidalorsen (300 µM) failed to block the hERG in HEK293 cells that express hERG. Both studies were GLP compliant. There were also no donidalorsen-related effects on renal function in monkeys in the repeat-dose toxicity study.

2.5.2.4. Pharmacodynamic drug interactions

Since the mechanism of action of donidalorsen is to inhibit translation of the PKK gene by specifically binding to its mRNA and promoting its degradation, the sequence-driven specificity of this binding greatly reduces the likelihood of pharmacological action on other gene products. No pharmacodynamic studies have been performed to evaluate possible interactions of donidalorsen with other drugs that may be co-administered.

2.5.3. Pharmacokinetics

2.5.3.1. Bioanalytical methods

Plasma PK of donidalorsen was evaluated in mice and monkeys using a validated hybridization ECL method, which did not discriminate between full-length GalNAc conjugated, partially conjugated and non-conjugated compound. Tissue kinetics of the unconjugated donidalorsen was evaluated in mice and monkeys using a validated UHPLC-MS/MS method. Select plasma, tissues, urine, and/or faeces across species (including mouse, rat, monkey, human) were further analysed to assess donidalorsen metabolism including each full-length ASO species (fully, partially conjugated or unconjugated), oligonucleotide metabolites associated with nuclease-mediated metabolism of donidalorsen (chain-shortened ASOs which may be fully, partially conjugated, or unconjugated), as well as the THA-linker-related metabolism. Bioanalytical methods for this analysis included a non-validated UHPLC-MS/MS, HPLC-UV-MS, or HPLC-HRMS methods. The radioactivity in samples from [^{3}H]donidalorsen-treated rats in the ADME study was measured by liquid scintillation counting (LSC) and whole-body distribution of radioactivity was quantified by QWBA. Unconjugated donidalorsen was measured using a non-validated hybridization ELISA method. Moreover, the formation of anti-donidalorsen antibodies was evaluated by a validated ELISA.

2.5.3.2. Absorption

Absorption of donidalorsen was evaluated in a 6-week repeat dose study in mice, a single dose ADME study with [^{3}H]donidalorsen in rats and a 9-month chronic monkey toxicology study. The PK profiles of donidalorsen were comparable across mouse, rats and monkeys. Donidalorsen appears to be well-absorbed following SC injection as shown by a t_{max} of 0.5, 1 and 1-4 in mice, rats and monkeys, respectively. Peak (C_{max}) and total ($AUC_{0\text{-}48h}$) plasma exposure were dose-dependent in mice and monkeys. Dose-proportional increase in C_{max} were evident in mice but greater than dose-proportional increase in C_{max} in monkeys and in $AUC_{0\text{-}48h}$ in mice and monkeys was observed over the dose range. After peak concentration, donidalorsen in plasma declined in a multiphasic manner with a rapid distribution phase which dominated the clearance. This was attributed predominantly to extensive and rapid distribution into tissues ($MRT_{0\text{-}48h}$: range of 3 to 10 h in monkeys) and was followed by a slower elimination phase ($t_{½}$: 2 to 5 weeks in monkeys). Plasma exposure was generally similar after single and repeated doses in mice and monkeys, suggesting similar kinetics with little to no accummulation in plasma. Incidence of ADAs was evaluated in the chronic monkey study. There were no notable differences in mean plasma exposure or tissue exposure between IM-positive and IM-negative animals. Presence of ADAs did not have a notable impact on liver mRNA or PKK activity in monkeys. Monkey plasma PK is generally predictive of human plasma PK on a mg/kg dose basis for ASOs within this chemical class with similar t_{max} and $t_{1/2}$ in humans as monkeys. Plasma PK was only evaluated in male mice and rats, however no notable differences between male and female monkeys were observed.

2.5.3.3. Distribution

Extensive distribution of donidalorsen to tissue was observed in mice, rats and monkeys, resulting in high concentrations in liver, the target organ for pharmacology, and kidney. In mouse and monkey, concentrations in liver and kidney were dose-dependent throughout the administered dose range after SC administration. The extent of tissue exposure increase with dose was greater in the kidney compared to the liver in both species at higher doses (≥ 12 mg/kg in monkeys, ≥ 10 mg/kg in mice), consistent with the potential saturation of ASGPR-mediated uptake in hepatocytes at these dose levels. Near steady state exposure was achieved following the loading dose regimen within 2 weeks of treatment, while steady-state exposure was achieved by day 91. At the end of the 13- and 26-week recovery period in mice and monkeys, respectively, tissue concentrations of unconjugated donidalorsen were near limit of quantification in mice and only 1% ASO remained in monkeys, suggesting nearly complete clearance. No notable gender differences in tissue distribution were observed in mice and monkeys.

Following a single SC administration of [^{3}H]donidalorsen to rats, LSC analysis demonstrated that the highest concentrations of radioactivity were observed in the liver and kidney, at their t_{max} observed at 24- and 168-h post dose in the liver and kidney, respectively. Exposures in liver were >1000-fold above the mean circulating concentration in plasma at 24 h post-dose. Relative distribution to all other tissues was much lower (< 10-fold) compared to liver and kidney, with very little radioactivity evident in brain or spinal cord. The % of dose in the liver declined at a more rapid rate than observed in the kidney, suggesting more extensive metabolism in the rat liver. Tissue concentrations of radioactivity obtained by QWBA were consistent with the LSC results with maximum levels detected in the liver and kidney. No quantifiable radioactivity was associated with the brain or spinal cord.

In vitro binding of donidalorsen to plasma proteins and distribution to blood cells was evaluated. Donidalorsen is highly bound (≥ 96%) to plasma protein in human, monkey and mouse, limiting their urinary excretion. Plasma protein is length-dependent and shorter metabolites are commonly less plasma protein bound. Blood cell partitioning demonstrated that donidalorsen distributes mostly in the plasma compartment in human, monkey and rat.

Tissue distribution of donidalorsen in reproductive animals was evaluated in combined FEED/EFD study and PPND study in mice. These studies demonstrated dose-dependent exposure of donidalorsen in liver and kidneys in parental males and females. Concentrations were BLQ in foetal tissue, and very low in placenta (0.1 and 0.7% of the maternal liver exposure at 4 and 10 mg/kg, respectively), confirming that donidalorsen is not readily transported across the placenta. The concentration of donidalorsen in breast milk from lactating parental females was below the LLOQ in 5 mg/kg group and was < 3000-fold lower than the observed kidney or liver concentrations in the 10 and 20 mg/kg group.

2.5.3.4. Metabolism

The metabolism of donidalorsen was evaluated across species by determination of oligonucleotide-related and GalNAc-THA-linker-related metabolites. The oligonucleotide-related metabolism was generally similar across the species evaluated (mouse, rat, monkey and human), and consistent with nuclease-mediated metabolism in tissues and elimination in urine. In plasma, the most abundant oligonucleotide species detected was intact donidalorsen at the 2 h time point in rat, monkey and human plasma. The unconjugated form was the most abundant full-length ASO species trough samples in monkeys (10 mg/kg Q1W for 13 weeks), whereas human trough samples had no detectable levels of oligonucleotides (80 mg Q4W for 12 weeks), due to the less

frequent dosing. Low levels of chain-shortened metabolites of donidalorsen were present in tissue, but not in plasma, indicating rapid excretion of the shortmer metabolites.

When administered at higher toxicologically relevant doses (10 to 30 mg/kg), conjugated donidalorsen is the major component in mice and monkey urine samples, followed by unconjugated. However, only the unconjugated form was detected in human urine samples. The difference in metabolites between human and mouse/monkey is thought to be due to the higher doses in animals resulting in spill-over to kidneys following uptake saturation. Chain-shortened metabolites were identified in urine across species.

The linker-related metabolites are consistent across species. M8, M12 and M5 were the most abundant linker metabolites in human and monkey plasma. The metabolite concentrations are substantially lower than the parent drug concentrations (< 2%). These concentrations reduced over time and were minimal in pre-dose samples, suggesting that linker-related metabolites were minimally released to circulation and rapidly excreted in urine and faeces. Similar to plasma, the most abundant linker metabolites were M8, M12 and M5 in urine samples from human, monkey and mouse. The majority of linker-related metabolites were eliminated in monkey faeces with M8, M9, M11 and M5 being the most abundant. In the rat ADME study, M8 was the only linker metabolite measured in liver and kidney which showed highest levels 2 h post dose followed by a decrease of 40% in kidney and levels below the LLOQ in liver at 24 h.

2.5.3.5. Excretion

Excretion of donidalorsen was examined in rats following single SC administration of [^{3}H]donidalorsen. Excretion was a slow and gradual process with a recovery of 82.6% of radioactivity by day 56; the largest proportion recovered in faeces (57.5%). No studies were conducted with the ^{3}H label in the THA-linker moiety of donidalorsen to evaluate its excretion. However, references are made to 3 GalNAc-conjugated ASOs which use the same THA linker structure. These studies show that THA linker was extensively metabolized by oxidation, with approximately 10 or 25% of administered radio-labelled dose being recovered in urine and 85 or 71% in faeces in 24 h achieving near complete recovery. The same metabolites for donidalorsen were detected in urine samples from mouse, monkey and human studies demonstrating similarities in THA metabolism across species.

2.5.3.6. Pharmacokinetic drug interactions

Donidalorsen has a very low potential for involvement in CYP450-, transporter-, or plasma protein binding-mediated drug-drug interactions, and additional dedicated drug interaction studies were deemed not necessary. The drug interaction potential of the linker-related metabolite M8 was also evaluated as this is generally the metabolite with the highest relative amounts among the 11 linker-related metabolites. M8 has low potential for CYP450-mediated DDI. It is not a substrate for any transporter-mediated DDI but was shown to be an inhibitor of MATE1 with an IC_{50} of 62.7 µM. This is 60-fold lower than the expected liver and kidney concentrations of M8 following clinical dose, therefore the risk of transporter inhibition of MATE1 by M8 is not clinically relevant.

2.5.4. Toxicology

The nonclinical toxicology program for donidalorsen included studies in mice and monkeys with the appropriate design following the ICH M3(R2) requirements. All pivotal toxicology studies were fully compliant

with Good Laboratory Practice (GLP) regulations. The subcutaneous (SC) route of administration was used, as this is the intended therapeutic route in humans.

Studies conducted for the evaluation of donidalorsen includes repeat dose studies in CD-1 mice and cynomolgus monkeys of up to 26- and 39-week duration, respectively. Plasma and/or tissue pharmacokinetics were assessed as part of the toxicity evaluation. The genetic toxicity of donidalorsen has been investigated in a battery of in vitro and in vivo assays, and its tumorigenic potential has been evaluated in a 27-week carcinogenicity study in Tg.rasH2 mice, and a 2-yr carcinogenicity study in Sprague Dawley (SD) rats, which is ongoing. Reproductive and developmental toxicity studies were conducted in one species only, namely CD-1 mice, and including a combined fertility and embryofoetal development study and a pre- and post-natal development study. Local tolerance was evaluated in repeat dose toxicity studies. An impurity qualification study was conducted for mixed donidalorsen impurities.

2.5.4.1. Single dose toxicity

No single dose toxicity studies were conducted for donidalorsen in line with ICH M3 (R2). However, large doses (up to 2000 mg/kg) of donidalorsen have been characterized in the mouse micronucleus study. Furthermore, a single-dose safety pharmacology study in monkeys identified no untoward effects with single doses of up to 30 mg/kg. There were also no notable clinical effects seen following the first dose of 30 mg/kg in the repeat-dose monkey study.

2.5.4.2. Repeat dose toxicity

Mice and monkeys were selected as the main nonclinical safety assessment species based on their pharmacologic and pharmacokinetic characteristics. General toxicology studies for donidalorsen consisted of 13- and 26-week repeat-dose studies in mice followed by 13-week recovery period and 39-week repeat dose study in monkeys (with a 13-week interim necropsy) followed by 26-week recovery period for each treatment interval (13 and 39 weeks). Dose administration was by SC injection at doses up to 10 mg/kg/wk or 12 mg/kg/q2w in the 13- and 26-week mouse studies, respectively, or up to 30 and 12 mg/kg/wk for 13- and 39-week durations, respectively, in the monkey study. ISIS 722059 (mouse-active surrogate) at 4 mg/kg/q2w was included in the 26-week mouse study. Plasma and/or tissue pharmacokinetics were assessed as part of the toxicity evaluation in all repeat-dose studies.

In mice, donidalorsen was clinically well tolerated with no signs of overt toxicity including survival, clinical observations, body weight, food consumption, ophthalmologic endpoints, or cytokine/chemokines (26-week study). The test article-related findings were mostly similar between the 13- and 26-week studies and mostly related to ASO accumulation in tissues and hepatocyte turnover. The primary target organs for donidalorsen were the liver and kidney, with microscopic findings also present in skin/subcutis, injection sites, inguinal lymph nodes, and/or spleen (26-week study only). Liver findings included hepatocellular hypertrophy and cytoplasmic alteration, individual hepatocellular necrosis, increased hepatocellular mitotic figures, and/or vacuolated/granular macrophages (consistent with oligonucleotide uptake). The minimal to moderate hepatocellular hypertrophy, which associated with increased mean liver weights in animals administered donidalorsen, and with mild increased liver enzymes (AST, ALT, or ALP). Kidney findings consisted of minimal accumulation of basophilic granules in the proximal tubular epithelium. There were no accompanying degenerative alterations or necrosis associated with granule accumulation, and there were no changes in kidney function (e.g., serum creatinine and blood urea nitrogen). Pro-inflammatory effects included dose-dependent increases in vacuolated/granular macrophage accumulation at injection sites and increased

incidence and/or severity of extramedullary haematopoiesis in the spleen and lymphocytes in the inguinal lymph node. ISIS 722059 (at 4mg/kg/q2w) demonstrated statistically significant inhibition in hepatic *PKK* mRNA expression relative to controls at the end of the dosing phase (77% reduction from control) and was not associated with any toxicity findings.

Complete or partial reversibility was demonstrated for most effects in these studies and those that did persist were attributed to ASO uptake and accumulation in tissues and known proinflammatory effects in rodents. The NOAEL was set at the highest dose level tested, 10 mg/kg/week or 12 mg/kg/q2w for 13- and 26-week studies, respectively. Using monthly cumulative exposure based on AUC (derived from a 6-week PK study in mice), the NOAEL at 12 mg/kg/q2w (24 mg/kg/month) provides an approximate ≥ 4.8- or ≥ 9.6-fold margin over to the exposure at the monthly (every-4-week) or every-8-week clinical dose of 80 mg donidalorsen, respectively.

In monkeys, donidalorsen was clinically well tolerated in monkeys up to the highest doses tested (12 and 30 mg/kg/week), with no test article-related effects noted on survival, body weight, food consumption/inappetence, ophthalmoscopic or electrocardiographic examinations, urinalysis/urine chemistry, cytokines/chemokines, platelet aggregation or activation, complement C3, IgM, IgG, or macroscopic observations.

Coagulation changes consisted of minimal, transient prolongations in activated partial thromboplastin time (aPTT) at 30 mg/kg/week at the 4-hour post-dose collection on Days 1 and 91, which were partially to fully resolved at subsequent collection intervals. A potential test article-related minimal increase in mean complement Bb levels was observed at 4 hours post-dose on Day 1 in animals at 30 mg/kg/week. Slightly elevated mean Bb levels were also seen in other groups but comparable to control animals. The transient nature of the effect suggests it may be related to C_{max} and is not likely to be clinically relevant (observed at plasma C_{max} levels that were is 137-217-fold higher than clinical dose). A potential test article-related minimal increase in mean complement Bb levels was observed at 4 hours post-dose on Day 1 in animals at 30 mg/kg/week. This was also observed in other groups but at similar levels to control animals. Marginal Bb increases noted after repeated dosing but no changes in C3 were noted.

Donidalorsen-related clinical chemistry effects were limited to mild to moderate increases in C-reactive protein (CRP) in females at ≥ 12 mg/kg/week and a few individual males at 6 and 12 mg/kg/week. These changes were indicative of a minor, acute inflammatory stimulus, as related to the severity of injection site inflammation/infiltration, and/or perivascular and vascular inflammation. Liver and kidney weight changes were noted which were partially or completely reversible. Microscopic findings associated with oligonucleotide uptake included accumulation of basophilic granules in macrophages, minimal to moderate accumulation of basophilic granules within kidney tubular epithelial cells, hepatocyte hypertrophy, and accumulation of basophilic granules in hepatocytes and/or Kupffer cells. After 26 weeks recovery, basophilic granules were still observed in hepatocytes and lymph nodes but with reduced severity and/or incidence in animals at 12 and 30 mg/kg/wk.

Proinflammatory effects were mostly limited to the injection sites consisting of fibrosis and/or mononuclear infiltrates. pronounced inflammatory response and perivascular/vascular inflammation was noted in selected animals at ≥ 6 mg/kg/wk but showed no dose-dependency in incidence or severity. After an additional 26 weeks of dosing, there were also observations of mixed cell inflammation, haemorrhage, myofiber degeneration/ regeneration, and necrosis. This was largely reversible in the recovery period. Test article-related perivascular/vascular inflammation occurred in one or more tissues of one animal each at 6, 12, and 30 mg/kg/week at the interim necropsy (Day 93).

In the keyhole limpet hemocyanin (KLH) antigenic challenge, donidalorsen was associated with decreases in mean anti-KLH IgM at 6 and 12 mg/kg/week on Day 247 (~55% reduction from control) and at 6 mg/kg/week on Day 254 (37.7% reduction to control), which were driven primarily by very high values in a few control females. However, the anti-KLH IgM values in donidalorsen-treated group were comparable to control on Days 261, 268, and 275. A decrease in anti-KLH IgG was observed at 2, 6, and 12 mg/kg/week on Days 254, 261, 268, and 275, compared to controls (ranged from 24.6% to 73% reduction from control), which was driven by female cohort.

The most noteworthy finding in the monkey study was a sporadic incidence of severe platelet (PLT) reduction to < 25 × 10^3/µL, which occurred in one female monkey in the 12 mg/kg/week dose group on Day 65 and one male monkey in the 30 mg/kg/week dose group on Day 80. The PLT changes demonstrated reversibility in both animals upon steroid treatment or dose suspension. Doses (≥ 12 mg/kg/week or ≥ 48 mg/kg/month) associated with severe PLT reduction in monkeys were > 50-fold above the exposure level in patients receiving 80 mg monthly (every-4-week) or every-8-week donidalorsen based on cumulative AUC exposure in monkeys.

Donidalorsen produced dose-dependent decreases in hepatic PKK mRNA as well as dose- and time-dependent reductions in cynomolgus monkey plasma PKK activity. There were no toxicities associated with pharmacologic reduction of PKK (up to ~68 and 87% reduction in PKK mRNA and protein, respectively), and the hepatic mRNA and protein activity levels were restored to or above the control range after the recovery period. Anti-drug antibodies (ADAs) ranged from 12.5- 62.5% and did not impact the PK, PD or safety profile of donidalorsen. The NOAEL for donidalorsen was determined to be 6 mg/kg/week in monkeys, based on PLT reductions at ≥ 12 mg/kg/week, providing approximately 29- or 58-fold cumulative margin of safety over the monthly (every-4-week) or every-8-week dose at 80 mg donidalorsen.

2.5.4.3. Genotoxicity

Donidalorsen was evaluated for genotoxicity potential both *in vitro* and *in vivo*. Gene mutational activity was assessed *in vitro* by the Ames bacterial reverse mutation test, genotoxic potential in mammalian cells was investigated in the chromosomal aberration assay in Chinese Hamster Lung (CHL) cells and clastogenicity was investigated in the *in vivo* mouse bone marrow micronucleus assay. In the Ames assay and chromosomal aberration assay, no evidence of genotoxicity was observed at concentrations up to 5000 µg/plate and 500 µg/plate, respectively, with or without S9 mix. In the *in vivo* mouse bone marrow micronucleus assay, two SC administrations of donidalorsen at doses up to 2000 mg/kg did not induce an increase in micronucleated polychromatic erythrocytes (MNPCE). Under the conditions of these assays described, donidalorsen does not have genotoxic potential.

2.5.4.4. Carcinogenicity

The carcinogenicity assessment for donidalorsen includes a completed 27-week study in Tg.rasH2 mice and a 2-year study in SD rats that is ongoing with the final report due in Q4 2025. In the Tg.rasH2 study doses up to 20 mg/kg/q2w in males and 60 mg/kg/q2w in females were selected based on ALT changes and liver pathology identified in the dose-range finding study. ISIS 722059 was also included at 10 mg/kg/q2w to determine if the desired pharmacodynamics had any influence on carcinogenicity. There were no definitive donidalorsen- or ISIS 722059- related unscheduled deaths/euthanasia. There were no increased incidences of neoplasia in female mice and no increase in malignant tumours in male mice. Donidalorsen-related hepatocellular adenomas were present in two out of 25 male mice (8%, statistically significant to control) at

20 mg/kg/q2w, one out of 25 male mice (4%) at 5 mg/kg/q2w donidalorsen and 1/25 male mice (4%) at 10 mg/kg/q2w ISIS 722059. Donidalorsen-related basophilic foci of cellular alteration were present in the liver of males at ≥ 10 mg/kg/q2w. No plasma exposure data was available for this study, target tissue concentrations were included to demonstrate exposure.

In-life data from the 104-week carcinogenicity study in rats at doses of 1.5 and 5 mg/kg/q2w has been provided. No clear or definitive effects attributable to donidalorsen are noted in clinical findings and body weight changes up to Week 66 treatment. In addition, the incidence of mortality is generally similar between donidalorsen-treated and control animals.

2.5.4.5. Reproductive and developmental toxicity

The reproductive and developmental toxicity assessment for donidalorsen consisted of a combined fertility and developmental toxicity study in mice (combined FEED and EFD) and a pre- and postnatal development (PPND) study in mice, including a mouse-active surrogate group to evaluate any effects related to PKK inhibition. The total weekly doses in the mouse FEED/EFD study (1, 4 and 10 mg/kg/wk) were comparable to the 13-week repeat-dose toxicity study in mice while higher total weekly doses (5, 10 and 20 mg/kg/wk) were used in the PPND study due to the differential ALT responses in male and female mice.

No EFD study has been conducted in rabbits with donidalorsen based on Scientific Advice received from EMA. However, fertility and embryo-foetal development (FEED, EFD and PPND) studies in mice or rabbits were previously completed for the unconjugated donidalorsen, ISIS 546254, which has the same sequence and general chemistry (2′ -MOE-modified) as donidalorsen, but with a different backbone chemistry (full PS for ISIS 546254 vs. a mixed PS and phosphate diester [PO] backbone for donidalorsen). The combined fertility and development toxicity study in mice and EFD study in rabbits with ISIS 546254 were submitted in support of this application. ISIS 546254 did not produce any changes in combined FEED/EFD study in mice at doses up to 84 mg/kg/wk (Study No. 546254-AS04) or effects on EFD in rabbits at doses up to 75 mg/kg/wk.

No clear adverse effects on fertility or foetal development were observed in mice given up to the top dose of 10 mg/kg/wk donidalorsen. However, a number of observations were noted in this assessment. An increase in percent abnormal sperm was noted and considered non-adverse by the Applicant. A review of findings in the testes (degeneration of seminiferous tubules) and an apparent increase in incidence of skeletal malformations in the mouse surrogate treatment group were noted. No plasma exposure data for donidalorsen or the mouse surrogate were provided, exposure was described at the level of unconjugated donidalorsen in target tissues. Dose-dependent increase in exposure was confirmed in parental liver over the dose range of 1 to 10 mg/kg/wk. There was minimal exposure in the placenta (0.1 and 0.7% of the maternal liver exposure at 4 and 10 mg/kg/wk) and the donidalorsen concentrations in the foetal liver were BLQ. The NOAEL for fertility or foetal development in mice was determined to be the top dose at 10 mg/kg/wk. ISIS 722059 reduced PKK mRNA levels in liver up to 80% compared to control, and there were no developmental toxicity findings associated with the pharmacologic inhibition of hepatic PKK mRNA expression.

In the PPND study with donidalorsen, non-adverse maternal effects included minimal to mild increases in AST and ALT at doses ≥ 10 mg/kg/wk; minimal to mild increases in relative liver (≥ 5 mg/kg/wk), kidney (≥ 10 mg/kg/wk) and spleen weights (20 mg/kg/wk). There were no measurable effects on fertility and reproductive function in F0 females and behavioural, fertility, and reproductive function in the F1 offspring. The concentrations of donidalorsen in maternal mouse liver and kidney increased in a dose-dependent manner. The concentrations of donidalorsen in breast milk from lactating mice increased in a dose-dependent

manner at doses ≥ 10 mg/kg/wk and, but these concentrations of donidalorsen in breast milk were > 3000-fold lower than the observed tissue concentrations.

2.5.4.6. Toxicokinetic data

Exposure to donidalorsen was predominantly represented in the form of unconjugated donidalorsen tissue concentrations at target organs. In monkeys, the toxicokinetics and tissue distribution of donidalorsen indicate a rapid absorption phase followed by a prolonged elimination phase, with tissue exposure reflecting plasma concentrations. The liver and kidney were the primary sites of distribution, with a dose-dependent accumulation pattern. The recovery phase demonstrated significant clearance of the drug from plasma and tissues, consistent with the observed elimination half-life. The more-than-proportional increase in exposure and the distribution of the compound in kidney and liver indicate a non-linearity in the pharmacokinetics, which becomes more evident at higher doses, such as 12 and 30 mg/kg/week. This was attributed to the saturation of the asialoglycoprotein receptor-mediated uptake of the GalNAc-conjugated ASO at higher doses and is not considered to be clinically relevant given the absence of significant toxicities in the monkey.

Margins of exposure were derived based on the exposure achieved in the pivotal monkey and a single subcutaneous mouse study. The margin to the clinical dose from the NOAEL in the 39-week NHP study is ~29-fold when adjusted for monthly exposure and monthly dosing. In terms of general toxicity, the margin to the anticipated clinical exposure is sufficient in line with relevant guidance including ICH M3 (R2) and ICH S6 (R1).

2.5.4.7. Local Tolerance

Local tolerance was evaluated as part of repeat dose toxicity studies in mice and monkeys. There was no evidence of substantive irritation aside from expected infiltrates of granular macrophages and other inflammatory cells.

2.5.4.8. Other toxicity studies

Juvenile toxicity

A juvenile toxicity study was included in this submission to support the expansion of indications at a later stage. Mice were treated for 13 weeks beginning PND 21. Donidalorsen and ISIS 722059 were not associated with any treatment-related effects on survival, clinical observations, body weights and body weight gain, food consumption, sexual maturation, behavioural evaluations (motor activity, Morris water maze, acoustic startle), haematology and clinical chemistry parameters, femur length measurements or bone densitometry parameters using Peripheral Quantitative Computed Tomography (pQCT), organ weights, or macroscopic findings in males and females at any dose level evaluated. Both compounds demonstrated similar findings (basophilic granules in kidney, vacuolated macrophages at injection sites) and target organs (liver, kidney) in other mice studies. Kidney concentrations were greater than dose-proportionally higher than the liver. ISIS 722059 (at 4 mg/kg/q2w) produced 70.6% and 53.4% reductions in hepatic Klkb1/Pkk mRNA expression in male and female juvenile mice, respectively. The NOAEL was determined to be 12 mg/kg/q2w.

Impurities

An impurity qualification study was conducted for donidalorsen-associated impurities in CD-1 mice after 13 weeks administration. Microscopic and macroscopic findings were similar to that observed with donidalorsen

and donidalorsen-associated test article mixtures (TAMs - TAM #1 (MOE impurities) or TAM #2 (Dithioate impurity)). Clinical chemistry changes were mirrored in all test-article groups and changes were considered non-adverse and of similar character and severity across dose levels of donidalorsen, TAM #1 and TAM #2. Thus, the levels of these impurities are considered qualified at the levels present in the batches used in this toxicology study.

2.5.5. Ecotoxicity/environmental risk assessment

No ERA studies have been submitted for donidalorsen on the basis that the major components of donidalorsen are natural i.e. nucleotides and sugar and undergo rapid metabolism in the body. While donidalorsen is not in itself naturally occurring, and the GalNAc conjugate is synthetically derived, it can be considered a natural substance considering its composition as outlined. Thus, the absence of ERA studies for donidalorsen is acceptable.

2.5.6. Discussion on non-clinical aspects

The nonclinical data presented by the Applicant in support of the evaluation of donidalorsen is generally well-rounded, addressing the most salient aspects required for the development of an ASO.

Pharmacology

Over 1300 ASOs targeting various sites within human PKK mRNA were screened for their ability to inhibit PKK mRNA *in vitro.* ISIS 546254 was identified as one of the most potent and efficacious. Alignment data shows that the donidalorsen binding site on PKK mRNA is not fully conserved among species *In silico* analysis identified potential off-target transcripts in the human genome that may be affected by donidalorsen. The effects of ISIS 546254, not donidalorsen, on the expression of the potential off-target genes (ACACA, CTAGE5, IFIH1 and PARK2) were examined in cell-based assays in Hep3B cells. The four off-target transcripts were either not significantly reduced by ISIS 546254 or were reduced but had greater than 20-fold higher IC_{50} values compared to PKK mRNA. The relevance of examining effects on off-target transcripts in cell lines following transfection with ISIS 546254 and not in primary hepatocytes after donidalorsen treatment was questioned. While donidalorsen and ISIS 546254 differ in backbone (PS vs PS-PO) and GalNAc conjugation (with vs without), they share an identical nucleobase sequence. It is the partial sequence complementarity to unintended transcripts that is the primary mechanism underlying off-target RNA downregulation by ASOs. Accordingly, the set of partially complementary off-target RNAs predicted for both molecules is the same, and their off-target profiles are expected to be equivalent. Therefore, the IC_{50} differences between on- and off-target activity for donidalorsen in hepatocytes are presumed to be the same as for ISIS 546254 in Hep3B cells.

The Applicant was asked to discuss whether a 20-fold margin was sufficient to avoid potential off-target effects on these genes. At concentrations of ISIS 546254 yielding 80% to 90% knockdown of plasma kallikrein (KLKB1) mRNA, no statistically significant reductions in IFIH1 or PRKN mRNA were observed indicating that no meaningful modulation of IFIH1 or PRKN mRNA is anticipated in the liver or kidney at clinically relevant exposures. This is supported by the lack of relevant adverse effects in the repeat dose toxicity studies in monkeys at exposures 137-217-fold higher than expected clinical exposures at the planned therapeutic dose.

Mice and cynomolgus monkeys were considered suitable species for the pharmacology assessment because PKK is expressed in both species. A murine-specific ASO surrogate (ISIS 482584) was used in mice. It is

noted that the Applicant used ISIS 482584 (full PS non-conjugated) instead of ISIS 722059 (PS/PO conjugated) as the mouse-active ASO for these studies, however as both ASOs were shown to potently reduce PKK mRNA, albeit at different concentrations (482584 20 mg/kg: 70% reduction; 722059 4 mg/kg: 77% reduction), this is acceptable. ISIS 482584 was demonstrated to reduce hepatic PKK mRNA expression, plasma PKK protein levels and vascular permeability in normal mice and in two *in vivo* models of vascular permeability (captopril- and C1-INH ASO-treated mice). These experiments provide proof-of-concept that ASO-mediated inhibition of PKK leads to a reduction in vascular permeability. In all experiments, there were a modest decrease in body weight gain in treatment groups compared to the vehicle-treated group. Reductions in PKK expression of 50% or greater had a significant effect on basal vascular permeability, this reduction was achieved following a dose of at least 20 mg/kg/wk for 3 weeks. However, in captopril-induced model of vascular permeability, a reduction of 80% in PKK mRNA is required to observe a significant reduction in vascular permeability compared to control mice.

The on-target pharmacology of donidalorsen was evaluated in transgenic mice expressing the human KLKB1/PKK gene and in cynomolgus monkeys as part of the repeat dose toxicity study. Donidalorsen was demonstrated to result in a potent dose-dependent decrease in plasma PKK protein levels in mice and hepatic PKK mRNA and plasma PKK protein activity in monkeys.

After 26 weeks of treatment in monkeys, PKK activity levels were at their lowest and remained at these levels until cessation of treatment. Levels were fully restored after 26 weeks of recovery. Higher PKK mRNA and protein levels were evident in monkeys following the 26-week recovery period vs animals at pre-study baseline, suggesting a rebound effect. However, analysis in the recovery animals was limited by low n numbers and high data variability. In the recovery arms, PKK mRNA levels in the liver and PKK activity in plasma were not statistically different from control or baseline values and remained within the range of variability observed in vehicle-treated animals indicating no rebound effect. In response to an OC, the Applicant also clarifies that the PKK mRNA and PKK protein endpoints were normalised differently and therefore not directly comparable.

An *in vivo* safety pharmacology study conducted in the monkey indicated that donidalorsen had no adverse effects on cardiovascular, respiratory or neurobehavioural parameters. Furthermore, donidalorsen (300 µM) failed to block the hERG in HEK293 cells that express hERG.

Pharmacokinetics

The PK of donidalorsen was demonstrated to be generally consistent across species. Following SC administration, donidalorsen was rapidly absorbed into the systemic circulation. After C_{max}, donidalorsen concentrations in plasma declined in a multiphasic manner characterised by rapid distribution phase with a followed by a slower elimination phase with a terminal half-life of 2 to 5 weeks in monkeys. This rapid clearance phase is attributed to the extensive distribution of donidalorsen to tissues resulting in highest concentrations of donidalorsen in liver and kidney.

Concentrations in the liver and kidney were dose-dependent over the dose range in mice and monkeys. The extent of tissue exposure increase with dose was greater in the kidney compared to the liver in both species, suggesting saturation of tissue uptake at higher dose levels. Lower mean kidney and liver concentrations were observed in mouse compared to monkey at comparable dose levels, in line with other 2'-MOE ASOs (Geary et al. 2003; Yu et al. 2007). Exposures in the liver were >1000-fold above the mean circulating concentration in plasma at 24 h post-dose. Relative distribution to all other tissues was much lower, with maximum exposure less than 10-fold compared to liver and kidney. Very little radioactivity was associated

with the brain and spinal cord. Comparable tissue concentrations were evident at days 93 and 275 in monkeys, confirming achievement of steady-state tissue exposure by 3 months.

Plasma PK was unaltered following repeated dosing for up to 39 weeks in monkeys suggesting little or no accumulation in plasma Trough plasma concentrations were shown to be in equilibrium with tissues, and the estimated plasma half-life of 2-5 weeks was generally consistent with the estimated tissue half-life of 2-4 weeks. This was also evident in the plasma.

Tissue distribution of donidalorsen in reproductive animals showed very low concentrations of donidalorsen in the placenta while no measurable concentrations were detected in the foetus indicating donidalorsen is not readily transported to the foetus. Section 4.6 of the SmPC cautions the use of donidalorsen during pregnancy. This is acceptable. Donidalorsen could be detected in breast milk of lactating dams at concentrations > 3000-fold lower than the observed kidney or liver concentrations. Systemic exposure of donidalorsen from nursing dams is considered unlikely due to the lack of oral absorption of donidalorsen. However, section 4.6 of the SmPC states a decision must be made whether to discontinue breast-feeding or to discontinue/abstain from Dawnzera therapy taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman. This wording is acceptable. Donidalorsen showed extensive plasma protein binding of ≥ 96% across species including human, despite this donidalorsen did not displace other highly bound drugs. Donidalorsen preferentially partitioned into plasma. The metabolism of donidalorsen is similar across different species. The oligonucleotide portion of the molecule is metabolized by endo- and exo-nucleases to chain-shortened oligonucleotide molecules while the THA linker and GalNAc portion of molecule is hydrolyzed to sequentially remove the sugar moieties and the linker from the oligonucleotide portion. Donidalorsen as an ASO is not a substrate for cytochromes and is unlikely to be impacted by other drugs that are CYP inhibitors/inducers. DDI studies demonstrated that donidalorsen did not induce or interfere with CYP450 enzymes and did not serve as a substrate or inhibitor of human drug transporters. Donidalorsen lacks significant potential for DDI, which has been adequately addressed in SmPC section 4.5

Toxicology

Repeat dose toxicity

Studies conducted for the evaluation of donidalorsen include repeat dose studies in CD-1 mice and cynomolgus monkeys of up to 26- and 39-week duration, respectively. The studies involved treatment of donidalorsen at doses up to 10 mg/kg/wk or 12 mg/kg/q2w in the 13- and 26-week mouse studies, respectively. ISIS 722059 was also administered at 4 mg/kg/q2w in the 26 weeks study. Target organs for effects in mice were predominantly the liver and kidney. Microscopic findings including in the liver in the 13-week study included hepatocellular hypertrophy and cytoplasmic and vacuolated/granular macrophages (indicative of ASO uptake), concomitant increases in AST and ALT and liver weight. Similar observations were noted in the 26-week study, in addition to liver inflammation in females at all dose levels albeit within a broad historical control range (8-18% vs up to 47%). Liver effects were partially or completely reversed in the recovery period; liver enzyme changes have been identified clinically and are considered an on-target effect that may be more pronounced in this species. There were also reports of hepatocellular adenomas in select male animals with no dose relationship, likely due to an increased sensitivity of mice to the proinflammatory effects of ASO uptake and not due to a direct carcinogenic effect of donidalorsen. There is sufficient literature evidence to support the increased susceptibility of mice to the development of hepatocellular neoplasms and thus this finding is not considered clinically relevant. Minimal accumulation of basophilic granules was noted in the kidney in the 13-week study without any change in kidney function, these effects were absent in the 26-week study. ISIS 722059 induced 77% reduction in *PKK* mRNA in the

absence of any toxicity findings. The NOAEL in both studies was the top dose, 10 mg/kg/wk and 12 mg/kg/q2w in the 13- and 26-week mouse studies, respectively.

The liver and kidney were also target organs for donidalorsen in monkeys. Hepatocyte hypertrophy, accumulation of basophilic granules in hepatocytes and Kupffer cells were noted. Liver weight increases were also observed in males at the top dose and females at the mid dose. Some of these changes (basophilic granules, increased liver weight) persisted in recovery phase but were reduced and there were no corresponding changes in liver enzymes thus liver function was not impacted. Similar to mice, basophilic granules were noted in tubular epithelial cells however this was absent at the end of the recovery phase and no changes in kidney function were noted, similar to mouse.

Effects on the complement system were investigated in monkeys (not investigated in mice as rodents are not sensitive to complement activation by ASOs) to establish the exposure-response relationship of activation of the alternative complement pathway by donidalorsen. Complement Bb, a marker of pathway activation, was slightly elevated in mid- and high dose groups but there was no change in intact complement factor C3 levels, thus changes in complement activation were not considered clinically significant. Perivascular/vascular inflammation was noted in one monkey in each dose group however the incidence and severity suggest this is a background finding as complement responses are believed to be heightened in monkeys in response to ASOs. This conclusion is supported.

Thrombocytopenia (as defined by platelet reduction < 25 × 10^3/μL) was the most significant finding observed in monkeys, occurring in 2 animals, 1 female at 12 mg/kg/wk and 1 male at 30 mg/kg/wk. Both cases were resolved by steroid treatment or dosing holidays and were not observed in any other animals during the additional 26 weeks study (at max dose level of 12 mg/kg/wk). Platelet reductions have been observed in monkeys following treatment with other 2'MOE ASOs and it has been suggested that monkeys are more susceptible to these effects and thus the clinical relevance is questionable. The Applicant has highlighted the absence of any evidence of bone marrow toxicity or thrombosis in this study and postulate that innate immune cell activation to PS-modified ASOs may be involved in this effect, stimulating increased clearance of platelets and not due to direct effects of the oligonucleotide on platelets. It is also suggested that it is a Cmax-related effect and closely linked to dose and dose frequency. It must also be acknowledged that the complex disease background in HAE patients may contribute to the potential for platelet activation. The Applicant's position that this is strictly an ASO class effect is not based on a balanced weight of evidence, as no studies investigating the effect of donidalorsen on platelet numbers and function (derived from HAE patients) have been performed. However, the Applicant subsequently provided a robust review of the clinical relevance of platelet activation and demonstrated in vitro studies would not be particularly informative as any direct effect on platelet activation would have been apparent after a single dose (both in nonclinical and clinical studies) thus the effect is most likely attributable to the class effect of MOE ASOs.

However, the potential for platelet reduction/thrombocytopenia to occur clinically cannot be discounted as the number of patients in the clinical trials mentioned was low and there is a possibility that any innate immune system effects may be potentiated in the disease state. This is discussed further in Clinical Safety section of the Clinical AR. The NOAEL in this study was 6 mg/kg/wk and the exposure at this dose level provided an exposure-based safety margin to the proposed clinical regimen of ~29 fold (monthly) and ~58-fold (every-8-week). Liver *PKK* mRNA reduction ranged 56-68% at 13 weeks (up to 30 mg/kg) and 31-52% (up to 12 mg/kg) at 39 weeks. Plasma protein *PKK* reduction plateaued at Day 184 (75- 87%, up to 12mg/kg) and was sustained at this level for the remainder of dosing. No toxicities associated with pharmacological reduction of PKK were reported. The toxicokinetic evaluation in monkeys noted a more-than-proportional increase in exposure and the distribution of the compound in kidney and liver indicate a non-linearity in the

pharmacokinetics, which becomes more evident at higher doses, such as 12 and 30 mg/kg/week. However, no unexpected toxicities were observed in the 39-week study and thus in the absence of evidence of accumulation there are no additional concerns. Anti-drug antibodies (ADAs) were measured in the 39-week monkey study. No noticeable changes in target organ tissue concentrations or plasma exposure were observed in animals positive for ADAs and no immune-related toxicities were noted, thus it can be concluded that ADAs did not impact the safety profile of donidalorsen.

Genotoxicity and carcinogenicity

Genotoxicity studies were conducted with donidalorsen using in vitro and in vivo methods as outlined in ICH S2 (R1). The study designs were in line with guidance. In vitro experiments were carried out in the presence and absence of S9-mix with vehicle and positive controls and the in vivo study utilised the subcutaneous route of administration. All studies employed sufficient dose levels to assess genotoxicity. Donidalorsen was negative for genotoxicity across the battery of tests.

Carcinogenicity was evaluated in a 27-week study in Tg.rasH2 mice. There were no increases in the incidences of neoplasms in female mice and no increase in malignant tumours in male mice following 27 weeks of exposure. Hepatocellular adenomas were noted in several male mice at all doses however they were not considered representative of a true carcinogenic effect of donidalorsen and more likely reflect an increased susceptibility of this species to liver tumours exacerbated by ASO uptake and possible accompanying proinflammatory effects. While this may provide an explanation for the observations, there was an absence of plasma exposure data in this study to allow a direct derivation of safety to the clinical exposure. Additionally, the increased sensitivity of male animals to the effects of donidalorsen was not initially addressed. The Applicant provided substantive literature evidence to support the comparability of exposure data in CD-1 and Tg.rasH2 mice including a study comparing the species strains in terms of the class effects of ASOs (Kim et al., 2019) and the carcinogenicity assessment of a recently approved 2-MOE', inotersen (Kim et al., 2025). The former study suggests target organ tissue exposure (in the liver) is essentially similar in both CD-1 and Tg.rasH2 mice for the 2 of the 3 ASOs investigated, however there was greater variability for the 3rd ASO (ISIS 421856) suggesting higher tissue exposure in the CD-1 mice. No plasma exposure was measured in this study thus it cannot be commented on. In the studies with inotersen, similar tissue concentrations were observed in the liver and kidney in CD-1 and Tg.rasH2 mice at comparable dose levels following 26-week treatment. The plasma AUC for the 26-week treatment in CD-1 mice was used to extrapolate estimated exposure at the equivalent dose in Tg.rasH2 (with equivalent dosing duration) providing a 3-fold safety margin to anticipated clinical exposure for inotersen. A comparison of the tissue exposure for donidalorsen suggests it is similar between CD-1 mice and Tg.rasH2 for target organs and thus based on the totality of the information provided on the distribution kinetics of 2-MOE's the extrapolation of the plasma exposure in the 6-week mouse PK study could support the derivation of an extrapolated safety margin for the Tg.rasH2 study. The increased sensitivity in males was noted by the Applicant but no mechanistic reason could be given, however a similar sensitivity was observed in chronic mouse studies for other 2'MOE ASOs and there is evidence of higher male susceptibility in mouse models of severe liver disease. Thus, based on the weight of evidence the carcinogenicity assessment to date is acceptable for the CHMP. The Applicant previously sought CHMP Scientific Advice on a waiver for a 2-year rat carcinogenicity study which was agreed by SAWP based on the weight of evidence presented, however the Applicant decided to conduct this study to fulfil regulatory requirements in other jurisdictions. The in-life phase of this study is ongoing and will be concluded in Q4 2025.

Reproductive and Developmental toxicity

The combined fertility and EFD study did not reveal any effects at most fertility or developmental endpoints, however an increase in percent abnormal sperm was noted and considered non-adverse. The Applicant attributed the statistically significant increased percent abnormal sperm in treated animals to the higher-than-expected baseline levels of abnormal sperm, including historical control animals and pointed to the variability in this finding, driven by a select number of animals. Additionally, the presence of such in various dose groups without a clear trend for either donidalorsen or the surrogate also supports the absence of a pharmacodynamic-related effect or an RNA-independent effect. Additionally, the Applicant reported that a finding of seminiferous tubule degeneration in the testes was identified and subsequently designated as a background finding despite no evidence of such in the control group. The testes findings were generally unilateral observations; bilateral findings were of low incidence and within historical control ranges thus the reviewing pathologists did not consider them related to treatment. This is acceptable. The Applicant reported that there were no test article-related effects on skeletal tissues in foetuses, however the incidence of such effects in the mouse surrogate treatment group was notably higher than control, suggesting a possible on-target effect of *PKK* mRNA inhibition. To date there has been no reports of similar effects for licensed *PKK* inhibitors (berotralstat, landadelumab) and no skeletal abnormalities were noted in mice treated with the mouse surrogate at 24/mg/wk (with 94% *PKK* mRNA inhibition) suggesting the incidence of skeletal malformations was not PD-related and may not be relevant for donidalorsen. Dose-dependent increase in exposure was confirmed in parental liver over the dose range of 1 to 10 mg/kg/wk, and there was minimal exposure in the placenta (0.1 and 0.7% of the maternal liver exposure at 4 and 10 mg/kg/wk), and donidalorsen concentrations in the foetal liver were BLQ. Parental liver concentrations of donidalorsen were comparable to levels observed in the 26-week repeat dose toxicity mouse study, indicating a level of comparability. The plasma exposure-based safety margin in the 26-week study, extrapolated from a 6-week PK study, correspond to an extrapolated exposure-based safety margin of 4.8-fold (adjusted for q2w dosing schedule and monthly clinical dosing) to the clinical exposure. In the absence of plasma exposure data for the FEED/EFD study, we can draw comparison to the available PK data, however the Applicant was asked to justify this in line with the recommendations outlined in ICH S5 (R3). It was maintained that the maternal target organ tissue concentrations in the combined Fertility and EFD mouse study were similar to that observed in mice in the 13- and 26-week repeat dose toxicology studies, and the extrapolation of an exposure-based safety margin was appropriate (based on comparable tissue exposure in the 6-week mouse PK study and the 26-week study). The pharmacokinetic evaluation of donidalorsen did not identify evidence of accumulation in this species thus an extrapolation of exposure data is acceptable.

The Applicant provided a summary table of tissue concentrations in studies with other 2'-MOEs (GalNAc conjugated and non-conjugated) that would suggest that they are not readily distributed to foetal tissues, however there is evidence of some placental distribution. This data is supportive in the safety assessment of donidalorsen, in addressing potential off-target effects or non-PD-related effects. Several points of clarification were raised to provide a comprehensive evaluation of reproductive and developmental toxicity. Based on previously discussed issues regarding the extrapolation of exposure data from the mouse PK study, target organ exposure in the combined fertility and EFD study is considered sufficient to identify pharmacodynamic or off-target effects during each stage of organogenesis as per ICH S5 (R3). An assessment of reproductive parameters from the repeat dose study in NHPs did not reveal any relevant effects at acceptable safety margins to the clinical dose. The Applicant previously sought CHMP Scientific Advice on the necessity for studies in rabbits and it was agreed that studies may not be required on the basis that neither donidalorsen nor the unconjugated PS-only compound, ISIS 546254, were pharmacologically active in the rabbit. The Applicant has justified the absence of a donidalorsen EFD/FEED study on the basis

that the GalNac conjugation (absent for ISIS 546254) does not alter distribution to reproductive tissues. While no supportive evidence has been given for this, it can be accepted that the chemical modification associated with GalNac conjugation is relatively well supported based on the approval of other GalNac-conjugated ASOs e.g. no EFD rabbit studies were conducted for GalNac-conjugated eplontersen (*Wainzua*, EMEA/H/C/006295) based on leveraged assessment of EFD rabbit studies for inotersen (*Tegsedi*, EMEA/H/C/004782)") which is non-GalNac conjugated but harbours the identical sequence. The same rationale can be applied for donidalorsen based on the EFD studies with ISIS 546254, thus there are no findings of concern in relation to EFD. Additionally, the presence of a PS backbone is proposed to be associated with a higher reported level of toxicity and thus the absence of effects with ISIS 546254 in the rabbit EFD study is conservative considering the greater safety profile associated with donidalorsen's mixed PS/PO backbone. This justification is well supported in the literature and is accepted by the CHMP.

Findings in the PPND study were limited to non-adverse liver, kidney and spleen weight changes and liver enzyme elevation in maternal animals. There were no observed effects on fertility and reproductive function in F0 females and behavioural, fertility, and reproductive function in the F1 offspring. This was demonstrated at relevant tissue exposure and extrapolated plasma exposure form the 6-week PK mouse study, as outlined previously.

Statements in section 4.6 and 5.3 of the SmPC adequately reflect the nonclinical data presented for donidalorsen.

Assessment of paediatric data on non-clinical aspects

As described in preceding sections, a juvenile toxicity study was conducted to support a future indication in HAE patients aged 2-12 years. The effects noted were similar to that observed in repeat dose toxicity studies in mice, at similar tissue concentrations. The proposed indication for the current procedure includes adolescents aged 12 and over. The age range of animals in the repeat dose toxicity studies (5-8 weeks (mice) and 2-6 years (monkeys) encompasses the relevant developmental stages to adequately address safety in this adolescent cohort. It is noted from the clinical assessment that in low-body-weight individuals (including adolescents and light adults), AUC values may reach up to 47.5 µg·h/mL, i.e. approximately 9-fold higher than the 5.24 µg·h/mL used for the initial safety margins estimation. This suggests that the safety margins indicated in non-clinical package, based on the 26-week and 39-week repeat dose toxicity studies in mice and monkeys, respectively, may not be representative of the worst-case clinical exposure.

No exposure-based safety margins were presented for the juvenile animal study conducted, nor AUC values, only Cmax at the NOAEL was identified. Oligonucleotide-related toxicities are primarily driven by cumulative exposure over time, rather than by peak concentration (Cmax). AUC better reflects tissue distribution, accumulation, and sustained exposure, all of which are relevant to the known class effects of oligonucleotides, such as hepatic or renal toxicity.

The Applicant was asked to provide the AUC at the NOAEL from the juvenile animal study, relevant safety margins and a justification that this is applicable for paediatric patients ≥12 years of age. They were also asked to provide an assessment of the impact of the highest clinical AUC values (e.g. 47.5 µg·h/mL) on the safety margins or alternatively, justify why the current safety margins indicated in the non-clinical package, based on average AUC are considered sufficient to cover such variability in exposure as seen in low-body-weight adult subjects. The response provided by the Applicant did not fully address the points for clarification. No AUC data was available for the juvenile mouse study. The lack of AUC data from the juvenile mouse study prevents a quantitative assessment of potential increased sensitivity in younger subjects. The

non-clinical AUC data from monkeys are considered representative for adolescents (≥12 years) and provide some reassurance regarding safety margins. However, the calculated margins (3–6-fold) are relatively narrow compared with worst-case clinical exposure. It is agreed that additional study data would not provide further useful information for this procedure, and overall sufficient data are available for adolescents (≥12 years), thus this issue was not pursued from a nonclinical perspective.

ERA

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, donidalorsen is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

Overall, the studies provide adequate evidence that donidalorsen is effective at inhibiting PKK mRNA in vitro and hepatic PKK mRNA and plasma PKK protein in human transgenic mice and cynomolgus monkeys. These results support the concept that inhibition of PKK using an ASO may be an effective treatment for HAE. The pharmacokinetic properties of donidalorsen have been sufficiently characterised in mouse, rats and monkeys and support the dosing frequency in the clinical setting. The metabolism of donidalorsen is similar in human as in nonclinical species. The species employed in the nonclinical toxicology studies are appropriate and target organs of toxicity were clearly identified from these studies

The nonclinical package for donidalorsen is supportive of a marketing authorisation application in the intended indication.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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

- **Tabular overview of clinical studies**

Table 1. Summary of clinical development program of donidalorsen for treatment of HAE

Study Number Phase	Study Title	Study Population	Number of Patients/ Subjects	Treatment Duration	Treatment Groups, Route of Administration, Dosing Regimen(s)	Study Status/ Data Cutoff Date	Module Location
Phase 2/3 Studies							
ISIS 721744-CS5 Phase 3	A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)	Patients aged ≥ 12 years with a documented diagnosis of HAE-1 or HAE-2	91 randomly assigned, 90 treated	24 weeks	Donidalorsen 80 mg SC every 4 weeks or every 8 weeks or matching placebo	Complete	5.3.5.1
ISIS 721744-CS2 Phase 2	A Randomized, Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Clinical Efficacy of ISIS 721744, a Second-Generation Ligand-Conjugated Antisense Inhibitor of Prekallikrein, in Patients with Hereditary Angioedema	Patients aged ≥ 18 years with a documented diagnosis of HAE-1 or HAE-2 (for inclusion in Part A) or nC1-INH-HAE (previously known as HAE Type III) (for inclusion in Part B)	23	16 weeks	Part A Donidalorsen 80 mg SC once every 4 weeks Placebo Part B Donidalorsen 80 mg SC once every 4 weeks	Complete	5.3.5.1

Study Number Phase	Study Title	Study Population	Number of Patients/ Subjects	Treatment Duration	Treatment Groups, Route of Administration, Dosing Regimen(s)	Study Status/ Data Cutoff Date	Module Location
ISIS 721744-CS7 Phase 3 Open-label	An Open-Label, Long Term Safety and Efficacy Study of Donidalorsen in the Prophylactic Treatment of Hereditary Angioedema (HAE)	Patients who had completed the pivotal study ISIS 721744-CS5 (OLE patients) or who were aged ≥ 12 years with a documented diagnosis of HAE-1/HAE-2 and who had been on a stable dose of prophylactic treatment with lanadelumab, berotralstat, or a C1-esterase inhibitor (Switch patients)	83 rollover from ISIS 721744-CS5 + 64 Switch patients treated	156 weeks	OLE patients administered donidalorsen 80 mg once every 4 weeks OLE patients administered donidalorsen 80 mg SC once every 8 weeks Switch patients administered donidalorsen 80 mg SC once every 4 weeks	Ongoing (Data cutoff date for submission: 28 Feb 2024)	5.3.5.2
ISIS 721744-CS3 Phase 2 OLE	An Open-Label Extension Study of ISIS 721744 in Patients with Hereditary Angioedema	Patients who had completed the pivotal study ISIS 721744-CS2 with HAE-1, HAE-2, or nC1-INH- HAE	20 rollover from ISIS 721744-CS2	208 weeks	Fixed Dosing Period [a]: Donidalorsen 80 mg once every 4 weeks Flexible Dosing Period (HAE-1/HAE-2) [b]: Donidalorsen 80 mg once every 4 weeks Donidalorsen 80 mg once every 8 weeks Donidalorsen 100 mg once every 4 weeks	Ongoing (Data cutoff date for submission: 26 Feb 2024)	5.3.5.2

Study Number Phase	Study Title	Study Population	Number of Patients/ Subjects	Treatment Duration	Treatment Groups, Route of Administration, Dosing Regimen(s)	Study Status/ Data Cutoff Date	Module Location
Phase 1 Studies							
ISIS 721744-CS1 Phase 1	A Double-Blind, Placebo-Controlled, Dose-Escalation Phase 1 Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of ISIS 721744, an Antisense Oligonucleotide Inhibitor of PKK, Administered Subcutaneously to Healthy Volunteers	Healthy volunteers	32	13 weeks	Donidalorsen 20, 40, 60, or 80 mg SC every 4 weeks	Complete	5.3.3.1
ISIS 721744-CS9 Phase 1	Single Dose, Randomized, Open-Label, Two-Period Crossover, Bioequivalence Study Comparing Two Subcutaneous Formulations: Manual Injection with Syringe/Vial and Autoinjector (AI) with ISIS 721744 (Donidalorsen) in Healthy Adults	Healthy volunteers	78	2 periods of 15 days each	Donidalorsen 80 mg SC single dose crossover (n = 2 total doses)	Complete	5.3.1.2

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Bioanalytical methods

An overview of the bioanalytical methods that were used to evaluate key pharmacological endpoints in clinical studies are presented in Table 2 below.

Table 2. Bioanalytical methods used in donidalorsen clinical studies

Method ID	Sample Matrix	Testing Sites	Bioanalytical Method	Assay Description	Validation Status	Assay Validation Report	Clinical Studies
Donidalorsen							
ICD 672 – V1.00	Human plasma	PPD Laboratories (Richmond, Virginia)	Hybridization ECL	Quantitation of donidalorsen and total full-length ASO	Validated - PPD	721744-MV04 Addendum 1 Addendum 2	ISIS 721744-CS1 (Phase 1) ISIS 721744-CS2 (Phase 2a) ISIS 721744-CS3 (Phase 2[a]) ISIS 721744-CS5 (Phase 3) ISIS 721744-CS7 (Phase 3[b]) ISIS 721744-CS9 (Phase 3 Bioequivalence)
ICD 672.1-PPD	Human urine	PPD Laboratories (Richmond, Virginia)	Hybridization ELISA	Quantitation of donidalorsen and total full-length ASO	Partially Validated-PPD	721744-MV05	ISIS 721744-CS1 (Phase 1)
Prekallikrein (PKK)							
US-EL-80-S5	Human serum/ plasma	Medpace (Cincinnati, Ohio)	Chromogenic ELISA	Quantification of PKK	Validated	PKK-BRV02	ISIS 721744-CS1 (Phase 1) ISIS 721744-CS2 (Phase 2a)
ICD 934 -PPD Version 1.00 ICD 934 PPD Version 2.00 ICD 934 PPD Version 2.01 ICD 934 PPD Version 2.02	Human plasma	PPD Laboratories (Richmond, Virginia)	Fluorescence ELISA	Quantification of PKK	Validated-PPD	PKK-BRV01, PKK-BRV01-Addendum 1, PKK-BRV01-Addendum 2	ISIS 721744-CS2 (Phase 2a) ISIS 721744-CS1 (Phase 1) ISIS 721744-CS3 (Phase 2[a]) ISIS 721744-CS5 (Phase 3) ISIS 721744-CS9 (Phase 3 Bioequivalence) ISIS 721744-CS7 (Phase 3[b])
Anti-Drug Antibodies Against Donidalorsen							
ICDIM 361 – PPD V1.00 ICDIM 361 – PPD V 1.02	Human plasma	PPD Laboratories (Richmond, Virginia)	ELISA	Detection of anti-drug antibodies against donidalorsen	Validated-PPD	721744-MV06, 721744-MV06 Addendum 1	ISIS 721744-CS1 (Phase 1) ISIS 721744-CS2 (Phase 2) ISIS 721744-CS3 (Phase 2[a]) ISIS 721744-CS5 (Phase 3) ISIS 721744-CS7 (Phase 3[b])

Source: Reports as indicated in the table
Abbreviations: ASO = antisense oligonucleotide; ECL = electrochemiluminescence; ELISA = enzyme-linked immunosorbent assay; HAE = hereditary angioedema; OLE = open-label extension; PKK = prekallikrein
[a] ISIS 721744 CS3 is an OLE study in patients with HAE who rolled over from Study ISIS 721744-CS2.
[b] ISIS 721744-CS7 is an open-label study that includes an OLE part for patients who rolled over from pivotal Study ISIS 721744-CS5 and a switch part for patients who were donidalorsen-naïve and previously maintained on HAE prophylactic therapy with lanadelumab, berotralstat, or a C1-esterase inhibitor.

Population PK Analysis (ISIS 721744-PPK01)

A population PK model was developed to evaluate and quantify covariates on donidalorsen PK and PD [i.e. plasma prekallikrein (PKK)] and to perform simulations to support dose selection. The model was built using data from studies in HVs (Study ISIS 721744-CS1 and Study ISIS 721744-CS9) and patients with HAE (Study ISIS 721744-CS5 and Study ISIS 721744-CS2).

The overall percentage of post-dose PK concentrations below the lower limit of quantification (LLOQ) was <4%. Therefore, PK concentrations below the LLOQ were excluded.

An exploratory graphical analysis of PK stratified by ADA status showed that donidalorsen plasma C_{trough} levels at and beyond onset of ADA positivity were substantially higher compared with concentrations prior to onset

of ADA, while there was no difference in PKK concentrations. As a result, trough concentrations can no longer serve as a reliable surrogate for tissue exposure in PKPD modelling. Therefore, PK samples at and beyond the onset of ADA were excluded from the PK analysis, and Bayesian estimates of these PK concentrations were obtained by the final PK model, while PKK concentrations at and beyond onset of ADA were retained in the population PKPD analysis, to avoid biased parameter estimates.

Covariates were evaluated for inclusion in the model with a systematic stepwise search using forward addition ($p<0.01$) and backward elimination ($p<0.001$). Covariates assessed are presented in Table 3.

Table 3. Covariates assessed in the population PK analysis

Covariate	Code	Value	Parameters
Age at baseline (yrs)	AGE	Continuous	CL/F, V/F
Body weight at baseline (kg)	WTKG	Continuous	Absorption, CL/F, V/F, peripheral parameters
Estimated glomerular filtration rate at baseline (mL/min/1.73m^2) [a]	EGFR	Continuous	CL/F
Alanine aminotransferase at baseline (IU/L)	ALT	Continuous	CL/F
Aspartate aminotransferase at baseline (IU/L)	AST	Continuous	CL/F
Bilirubin at baseline (IU/L)	BILI	Continuous	CL/F
Albumin at baseline (IU/L)	ALB	Continuous	CL/F
PKK at baseline (mg/dL)	BLPKK	Continuous	CL/F
Sex	SEXF	Categorical	CL/F, V/F
Race	RACE	Categorical	CL/F, V/F
Ethnicity	ETHNIC	Categorical	CL/F, V/F
Region (Geographical location)	REGION	Categorical	CL/F, V/F
Disease status (HV, HAE)	DSSTAT	Categorical	CL/F, V/F
HAE Type (Healthy, I, II, III)	HAETYPE	Categorical	CL/F, V/F
Drug presentation (vial, auto-injector)	DEVICE	Categorical	Absorption, F
Site of administration (arm, abdomen, thigh)	ROUTE	Categorical	Absorption, F
Hepatic dysfunction category (NCI-ODWG criteria [8])	HEPATC	Categorical	CL/F
Concomitant medication use [b]	CONMED	Categorical	CL/F

[a] $eGFR_{cr}$ [9] = 141 · min(SCR/κ, 1)$^{\alpha}$ · (max(SCR/κ, 1))$^{-1.209}$ · 0.993$^{Age\ (yrs)}$ · 1.018 [if female] · 1.159 [if Black], where SCR = serum creatinine in mg/dL, κ = 0.7 (if female) or 0.9 (if male), α = -0.329 (if female) or -0.411 (if male), min(SCR/κ, 1) is the minimum of SCR/κ or 1.0, (max(SCR/κ, 1) is the maximum of SCR/κ or 1.0

[b] Depending on the number of participants with concomitant medications, similar concomitant medications belonging to the same class will be categorized accordingly and evaluated as necessary.

CL/F, apparent clearance; F, bioavailability; HAE, hereditary angioedema; HV, healthy volunteer; V/F = apparent volume of distribution

A total of 177 individuals (101 healthy volunteers and 76 HAE patients) contributed 4242 concentrations to the analysis. Table 4 and Table *5* display summaries of continuous and categorical, respectively, baseline clinical and demographic characteristics of the subjects included in the population PK analysis.

Table 4. Summary of continuous clinical and demographic variables

	721744-CS1	721744-CS2	721744-CS5	721744-CS9	Total
N	23	14	62	78	177
Age (y)	59 (32, 64)	33 (21, 60)	37 (12, 68)	43 (20, 64)	43 (12, 68)
Weight (kg)	79 (57.7, 114.9)	89.9 (58.1, 129)	78 (37, 151.9)	77.1 (50.6, 114.8)	78 (37, 151.9)
eGFR (mL/min/1.73m^2)	99.5 (78.4, 135)	109 (67.5, 129)	106 (62.5, 146)	105 (74.4, 159)	104 (62.5, 159)
ALT (U/L)	18 (9, 41)	13 (8, 32)	15 (7, 100)	15 (7, 32)	15 (7, 100)
AST (U/L)	17 (13, 25)	14 (11, 21)	16 (8, 77)	17 (9, 29)	16 (8, 77)
Bilirubin (mg/dL)	0.54 (0.19, 1.1)	0.515 (0.3, 0.94)	0.5 (0.1, 1.29)	0.475 (0.17, 1.12)	0.5 (0.1, 1.29)
ALB (g/dL)	4.3 (3.8, 5.2)	4.35 (3.8, 4.8)	4.4 (3.8, 5.0)	4.2 (3.6, 4.6)	4.3 (3.6, 5.2)
BLPKK (mg/L)	129.07 (81.12, 183.01)	96.55 (69.07, 146.27)	118 (68.6, 321)	142.5 (43.7, 265)	127 (43.7, 321)

ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BLPKK, baseline prekallikrein concentration; eGFR, estimated glomerular filtration rate using CKD-EPI formula [9]

Results presented as median (min - max).

Table 5. Summary of categorical clinical and demographic variables

	721744-CS1	721744-CS2	721744-CS5	721744-CS9	Total
N	23	14	62	78	177
Sex					
Male	11 (47.8)	3 (21.4)	26 (41.9)	39 (50.0)	79 (44.6)
Female	12 (52.2)	11 (78.6)	36 (58.1)	39 (50.0)	98 (55.4)
Race					
White	10 (43.5)	14 (100)	58 (93.5)	39 (50.0)	121 (68.4)
Black or African American	8 (34.8)	0 (0)	1 (1.6)	34 (43.6)	43 (24.3)
Asian	4 (17.4)	0 (0)	1 (1.6)	2 (2.6)	7 (4.0)
American Indian or Alaska Native	0 (0)	0 (0)	1 (1.6)	0 (0)	1 (0.6)
Multiple	1 (4.3)	0 (0)	0 (0)	3 (3.8)	4 (2.3)
Other	0 (0)	0 (0)	1 (1.6)	0 (0)	1 (0.6)
Ethnicity					
Not Hispanic or Latino	23 (100)	13 (92.9)	57 (91.9)	70 (89.7)	163 (92.1)
Hispanic or Latino	0 (0)	1 (7.1)	5 (8.1)	8 (10.3)	14 (7.9)
Region					
North America	23 (100)	12 (85.7)	12 (19.4)	78 (100)	125 (70.6)
Europe	0 (0)	2 (14.3)	33 (53.2)	0 (0)	35 (19.8)
Middle East	0 (0)	0 (0)	17 (27.4)	0 (0)	17 (9.6)
Disease Status					
Healthy	23 (100)	0 (0)	62 (100)	78 (100)	101 (57.1)
HAE	0 (0)	14 (100)	0 (0)	0 (0)	76 (42.9)
HAE Type					
Healthy	23 (100)	0 (0)	0 (0)	78 (100)	101 (57.1)
HAE Type I	0 (0)	10 (71.4)	59 (95.2)	0 (0)	69 (39.0)
HAE Type II	0 (0)	1 (7.1)	3 (4.8)	0 (0)	4 (2.3)
HAE Type III	0 (0)	3 (21.4)	0 (0)	0 (0)	3 (1.7)
Hepatic Dysfunction Category [a]					
Normal liver function	23 (100)	14 (100)	60 (96.8)	78 (100)	175 (98.9)
Mild liver impairment	0 (0)	0 (0)	2 (3.2)	0 (0)	2 (1.1)
Renal Impairment Category [b]					
Normal renal function	20 (87)	13 (92.9)	57 (91.9)	71 (91.0)	161 (91.0)
Mild renal impairment	3 (13)	1 (7.1)	5 (8.1)	7 (9.0)	16 (9.0)

Results presented as N (%).

[a] Hepatic dysfunction category defined as per National Cancer Institute – Organ Dysfunction Working Group [8].

[b] Absolute estimated glomerular filtration rate (eGFR) values were used to define renal function categories, where eGFR estimated by CKD-EPI formula [9] is multiplied by the individual's body surface area (BSA) and divided by 1.73. Normal renal function: 90 mL/min, Mild renal impairment: 60 – 89 mL/min.

The final population PK model was a two-compartment model with first-order absorption and linear elimination. Body weight was included as a covariate on CL/F, Vc/F, Q/F, and Vp/F with exponents estimated for each of these parameters. Site of administration and drug presentation were included as covariates on ka. Disease status was included as a covariate on Vc/F and Q/F.

In addition, the effect of age, eGFR, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin, PKK, sex, race, ethnicity, region, HAE type, and hepatic dysfunction category on PK parameters were explored but were not found to be significant.

Parameter estimates of the final model are provided in Table *6*.

Table 6. Final population PK model parameter estimates

Parameter	Estimate	%RSE
CL/F (L/h) [a]	12.8	2.83
Covariate of Weight on CL/F	1.52	8.37
V_c/F (L) [b]	69.8	6.28
Covariate of Weight on V_c/F	2.34	7.86
Covariate of Disease Status (HAE patient) on V_c/F	0.426	29.8
Q/F (L/h) [c]	2.58	5.25
Covariate of Weight on Q/F	1.79	12.7
Covariate of Disease Status (HAE patient) on Q/F	-0.261	12.3
V_p/F (L) [d]	1840	6.29
Covariate of Weight on V_p/F	1.60	17.6
k_a (1/h) [*]	0.952	8.26
Covariate of Site of Administration of k_a	-0.338	36.8
Covariate of Drug Presentation of k_a	0.262	36.1
BSV% CL/F (Sh%)	19.6 (7.90)	13.9
Correlation between CL/F and V_c/F	0.753	16.0
Correlation between CL/F and Q/F	0.682	19.3
Correlation between CL/F and V_p/F	0.438	27.4
Correlation between CL/F and k_a	0.380	31.2
BSV% V_c/F (Sh%)	59.0 (9.41)	14.0
Correlation between V_c/F and Q/F	0.741	16.1
Correlation between V_c/F and V_p/F	0.564	18.6
Correlation between V_c/F and k_a	0.721	22.2
BSV% Q/F (Sh%)	10.2 (14.8)	15.7
Correlation between Q/F and V_p/F	0.921	15.8
Correlation between Q/F and k_a	0.439	30.0
BSV% V_p/F (Sh%)	42.9 (17.5)	15.2
Correlation between V_p/F and k_a	0.303	40.9
BSV% k_a (Sh%)	42.8 (24.4)	22.1
σ_{logdd}	0.296	2.49

BSV%, between-subject variability expressed as CV%; CL/F, apparent clearance; k_a, first-order absorption rate constant; Q/F, apparent inter-compartmental clearance; RSE, relative standard error; Sh, shrinkage for BSV; V_c/F, apparent volume of distribution for the central compartment; V_p/F, apparent volume of distribution for the peripheral compartment; σ_{logdd}, additive component of the residual error model on log-transformed data.

[a] $CL/F = 12.8 \cdot (WTKG / 70)^{1.52}$

[b] $V_c/F = 69.8 \cdot (WTKG / 70)^{2.34} \cdot 1.426$ [for HAE patient]

[c] $Q/F = 2.58 \cdot (WTKG / 70)^{1.79} \cdot 0.739$ [for HAE patient]

[d] $V_p/F = 1840 \cdot (WTKG / 70)^{1.60}$

[*] $k_a = 0.952 \cdot 0.662$ [for arm injection] $\cdot 1.262$ [for autoinjector]

Body weight was the most influential covariate on PK exposure. Compared to a healthy volunteer, a HAE patient was predicted to have no change in AUCτss, 23% lower Cmax,ss, and 15% lower Ctrough,ss. Injection into arm was predicted to have a 12% lower Cmax,ss compared to injection into abdomen or thigh. The magnitude of the effect of autoinjector on AUCτss, Cmax,ss, and Ctrough,ss was within the 0.80 to 1.25-fold range.

Bioequivalence

The autoinjector is the intended formulation of donidalorsen for commercial use and was introduced in the Phase 3 open-label extension study ISIS 72174-CS7. Vials were used in all other studies including the pivotal Phase 3 study ISIS 721744-CS5.

Study ISIS 721744-CS9

This was a single dose, randomized, open-label, two-period crossover, bioequivalence study comparing two subcutaneous formulations of donidalorsen (autoinjector and vial) in healthy adults. 78 eligible participants were randomized in a 1:1 ratio to 1 of 2 sequences of treatment to receive donidalorsen 80 mg via subcutaneous injection (abdomen only) using an autoinjector or manual injection using a single-use vial. There was a washout period of at least 28 days but no more than 42 days between each dosing in each treatment period.

Bioequivalence analysis indicated that the calculated 90% CI for the ratios of adjusted GM for $AUC_{0\text{-}336h}$ and C_{max}, between autoinjector (Test Drug) and vial (Reference Drug), were contained within the predefined equivalence range of 80% to 125% establishing PK bioequivalence between the 2 formulations.

A substantial reduction in PKK levels was noted following subcutaneous administration of donidalorsen. The reductions in PKK were similar between autoinjector and vial.

Absorption, Distribution, Metabolism and Excretion

Following SC administration in healthy volunteers (HVs) (Study ISIS 721744-CS1), donidalorsen was absorbed rapidly into the systemic circulation with median Tmax ranging from 1.0 to 3.0 hours. After reaching Cmax, plasma concentrations declined in a multiphasic fashion, with an initial, relatively rapid disposition phase, followed by a slower elimination phase with a terminal elimination half-life of approximately 1 month. The low renal clearance observed over the first 24 hours supports the hypothesis that the initial plasma clearance is the result of rapid tissue uptake, not excretion. Similar plasma concentration profiles were observed on Days 1 and Day 85, with little to no increase observed in mean plasma Cmax or AUC following 4 doses of donidalorsen compared to single-dose administration, consistent with minimal plasma accumulation and time-invariant plasma kinetics. Plasma Cmax and AUC increased in a dose-dependent, but greater than dose proportional, manner over the dose range of 20 to 80 mg.

Donidalorsen distributes primarily to the liver and kidney cortex after subcutaneous administration. However, at clinically relevant doses, there is greater distribution to the liver. The improved distribution to the liver is attributed to the GalNAc ligand. The population estimate of apparent volumes of distribution for the central (Vc/F) and peripheral (Vp/F) compartments were 69.8 L and 1840 L, respectively (ISIS 721744-PPK01). Donidalorsen is highly bound to human plasma proteins (>98% bound), with little change over a concentration range of 5 to 150 µg/mL (ISIS 721744-IS03). The high protein binding limits glomerular filtration and urinary excretion.

Oligonucleotides such as donidalorsen are not substrates for the classic CYP metabolic pathways. The primary route of elimination of donidalorsen is initial rapid hydrolysis of the GalNAc conjugate following uptake into

tissues, and the unconjugated donidalorsen (i.e., the ASO-moiety) is slowly metabolized by endo- and exonucleases prior to renal excretion as chain-shortened oligonucleotides.

Metabolite profiling of human plasma peak and trough samples at steady-state showed that there were no circulating donidalorsen metabolites relative to total full-length oligonucleotides. Profiling of length-based oligonucleotide metabolites in urine samples detected numerous chain-shortened oligonucleotide metabolites, in addition to donidalorsen (ISIS 721744-CS1), indicating their rapid excretion to urine once generated.

Minimal linker-related metabolites were detected in human plasma, and when present, the highest levels typically presented at the 2-hour time point. However, concentrations of linker-related metabolites in urine are substantially higher than in plasma. The minimal concentrations of linker-related metabolites observed in plasma over time suggest that linker-related metabolites are minimally released to circulation and subsequently rapidly excreted to urine or faeces (ISIS 721744-IS12).

PK in the target population

In both healthy participants and patients with HAE, similar plasma concentration profiles were observed on Days 1 and 85, with little to no increase observed in mean plasma Cmax or AUC values following 4 doses of donidalorsen versus single-dose administration, consistent with the expected result of little or no plasma accumulation and time invariant plasma kinetics. However, approximately 2-fold accumulation in Ctrough levels was observed with every 4 weeks subcutaneous administration. The population estimate of the terminal elimination half-life in a typical patient with HAE is 31.4 days (ISIS 721744-PPK01), which is consistent with the value observed in HVs.

Based on final population PK model simulations of patients with HAE receiving donidalorsen 80 mg once every 4 weeks with vial as the drug product presentation, the geometric means for PK parameters of AUCtau, ss, Cmax, ss, and Ctrough, ss were 5240 ng.h/mL, 417 ng/mL, and 0.755 ng/mL, respectively. For the patients with HAE receiving donidalorsen 80 mg once every 8 weeks with vial as the drug product presentation, the geometric means for PK parameters of AUCtau, ss, Cmax, ss, and Ctrough, ss were 5210 ng.h/mL, 416 ng/mL, and 0.255 ng/mL, respectively.

The population PK analysis identified disease status (HAE patient) as a statistically significant covariate. Compared to a healthy volunteer (HV) reference, a HAE patient was predicted to have no difference in $AUC_{\tau,ss}$, 23% lower $C_{max,ss}$, and 15% lower $C_{trough,ss}$.

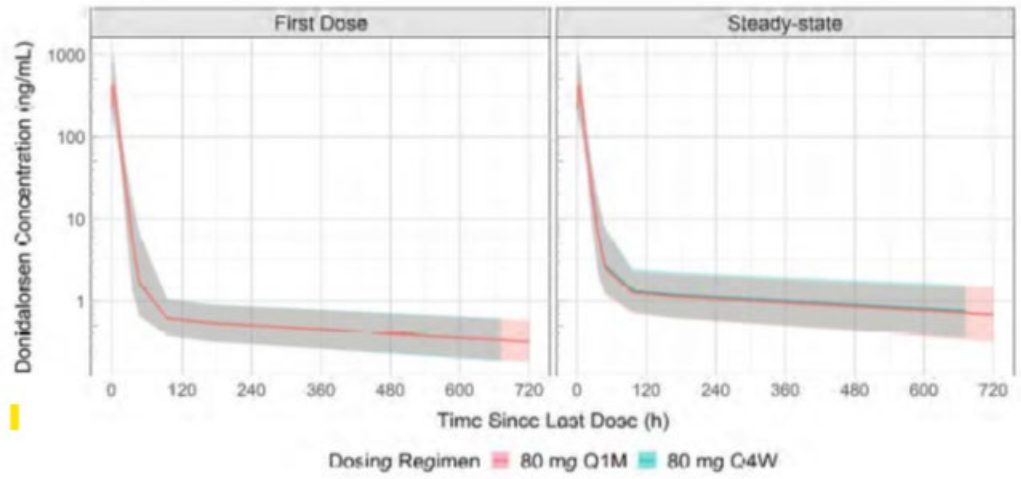
Disease status was also identified as a statistically significant covariate on PD in the population PKPD analysis. Based on population parameter estimates, the typical value of baseline PKK was 139 mg/L and 121 mg/L for HVs and patients with HAE, respectively. Compared to HV reference, patients with HAE were predicted to have 6% smaller maximum PKK percent change from Baseline over the steady-state dosing interval and 7% smaller PKK percent change from Baseline at the PK $C_{trough,ss}$. This effect was not considered clinically meaningful.

Simulations to support dose selection in HAE patients

Simulations were performed using the final population PK and PKPD models.

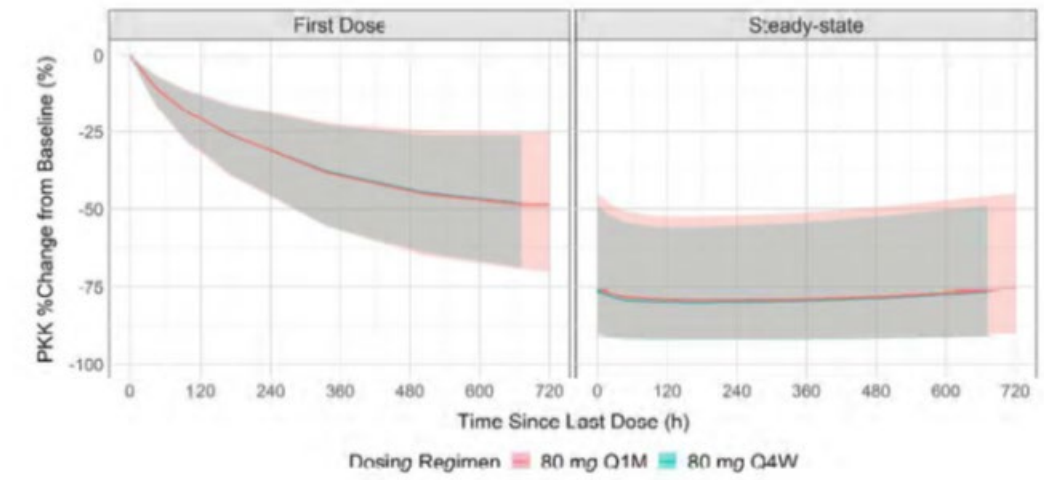
The simulated PK and PD profiles in HAE patients following 80 mg donidalorsen Q4W for 13 doses and 80 mg donidalorsen Q1M for 12 doses are shown in Figure 2 and Figure 3, respectively. Overall, both the PK and PD profiles for 80 mg Q4W and Q1M regimens overlap following the first dose and at steady-state with only marginal difference in median (80% prediction interval (PI)) donidalorsen concentrations and PKK percent change from baseline.

Figure 2. Simulated PK concentration-time profile (80 mg Q4W/Q1M dosing regimen)



Q4W = every four weeks, Q1M = every month.
Solid line shows the median concentration and shaded region is the 80% prediction interval (PI) for the median.

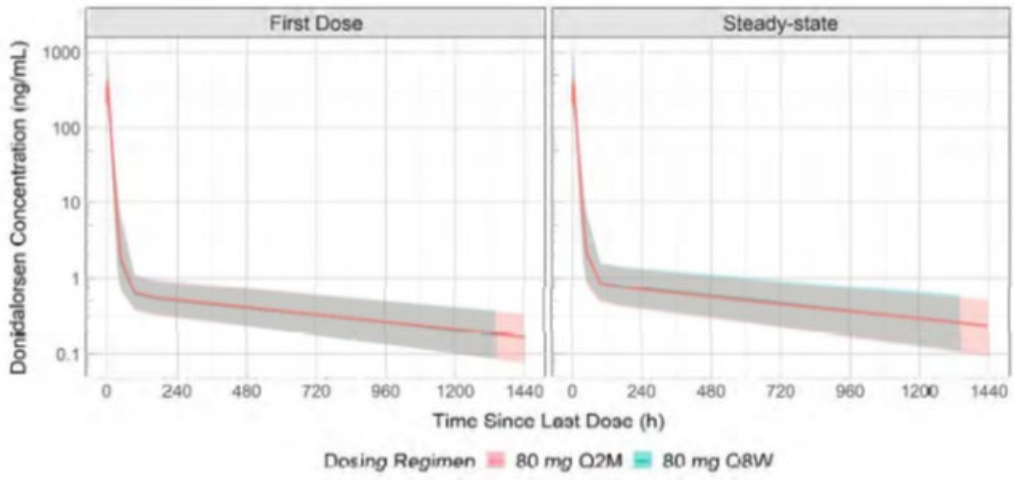
Figure 3. Simulated PKK percent change from baseline over time (80 mg Q4W/Q1M dosing regimen)



Q4W = every four weeks, Q1M = every month.
Solid line shows the median PKK percent change from baseline over time and shaded region is the 80% prediction interval (PI) for the median.

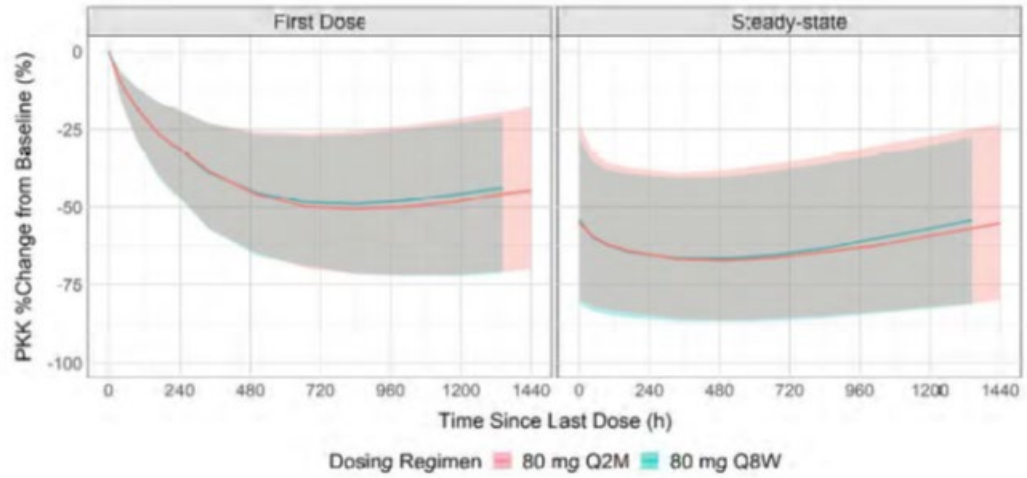
The simulated PK and PD profiles in HAE patients following 80 mg donidalorsen Q8W for 7 doses and 80 mg donidalorsen Q2M for 6 doses are shown in Figure 4 and Figure 5, respectively. Overall, both the PK and PD profiles for 80 mg Q8W and Q2M regimens overlap following the first dose and at steady-state with only marginal difference in median (80% PI) donidalorsen concentrations and PKK change from baseline.

Figure 4. Simulated PK concentration-time profile (80 mg Q8W/Q2M dosing regimen)



Q8W = every eight weeks, Q2M = every two month.
Solid line shows the median concentration and shaded region is the 80% prediction interval (PI) for the median.

Figure 5. Simulated PKK percent change from baseline over time (80 mg Q8W/Q2M dosing regimen)



Q8W = every eight weeks, Q2M = every two month.
Solid line shows the median PKK percent change from baseline over time and shaded region is the 80% prediction interval (PI) for the median.

For the simulated PK exposure metrics at steady-state by dosing frequency for HAE patients, there were only marginal differences (≤5%) in median Cmax,ss and AUCτss exposure between the Q4W and Q1M regimens and between the Q8W and Q2M regimens. The median Ctrough,ss was 9% lower for 80 mg Q1M compared to 80 mg Q4W, and 9% lower for 80 mg Q2M compared to 80 mg Q8W.

In addition, the median Cmax,ss and AUCτss were only marginally different (5%) between 80 mg Q2M and 80 mg Q1M regimens and between 80 mg Q8W and 80 mg Q4W regimens. However, the AUC1440h would be approximately 2-fold higher for Q1M regimens compared to Q2M regimens and the AUC1344h would be

approximately 2-fold higher for Q4W regimens compared to Q8W regimens. The median Ctrough,ss was 66% lower for 80 mg Q2M compared to 80 mg Q1M and 66% lower for 80 mg Q8W compared to 80 mg Q4W.

The median PKK percent change from baseline at the PK Ctrough,ss was similar for 80 mg Q1M compared to 80 mg Q4W (difference <1%) and for 80 mg Q2M compared to 80 mg Q8W (difference <2%). However, the median PKK percent change from baseline at the PK Ctrough,ss was 27% lower in magnitude for 80 mg Q2M compared to 80 mg Q1M and 29% lower in magnitude for 80 mg Q8W compared to 80 mg Q4W.

Special populations

	Age 65-74 (Older subjects number)	**Age 75-84 (Older subjects number)**	**Age 85+ (Older subjects number)**
Study 721744-CS2	1	0	0
Study 721744-CS3	1[a]	0	0
Study 721744-CS5	2	0	0
Study 721744-CS7	4[b]	0	0
Total	6	0	0

OLE= open-label extension

[a]Includes patients rolled over from study 721744-CS2 into the OLE study

[b]Includes 3 newly enrolled switch subjects, and 1 patient rolled over from study 721744-CS5 to the OLE study

Impaired renal function

The population PK analysis did not find Baseline eGFR as a statistically significant covariate impacting donidalorsen PK. Furthermore, the range of post-hoc estimates of steady-state exposure were overlapping between patients with normal renal function (N = 161) and patients with mild renal impairment (N = 16).

Consistent with PK exposure metrics, the range of post-hoc estimates of steady-state percent change-from Baseline for PKK were overlapping between patients with normal renal function (N = 161) and patients with mild (N = 16) renal impairment.

In conclusion, no dose adjustment is necessary in patients with mild renal impairment (estimated eGFR ≥60). Donidalorsen has not been studied in patients with moderate or severe renal impairment, or in patients with ESRD.

Impaired hepatic function

The population PK analysis did not find Baseline AST, ALT, or bilirubin as statistically significant covariates impacting donidalorsen PK. Furthermore, the post-hoc estimates of steady-state exposure were overlapping between patients with normal hepatic function (N = 175) and patients with mild hepatic impairment (N = 2).

Donidalorsen is a GalNAc-conjugated ASO, which facilitates its delivery to the hepatocytes, via ASGPR-mediated uptake. Although it has been reported in the literature that ASGPR expression is altered in various types of liver diseases, the findings were somewhat inconsistent. For example, ASGPR expression was found to be upregulated in cirrhotic liver disease by 1 group (Witzigmann et al. 2016), but it was found to be decreased in patients with increasing progression of chronic viral hepatitis, including cirrhosis, by another group (Sugahara et al. 2003).

Nonetheless, GalNAc-mediated uptake is unlikely to be affected even by greater than 50% reduction in ASGPR levels, due to the high capacity of ASGPR relative to the low pharmacologically relevant dose levels

(Willoughby et al. 2018). Consistent with this, steady-state PKK reduction was similar between patients with normal hepatic function (N = 175) and patients with mild (N = 2) hepatic impairment.

In conclusion, no dose adjustment is recommended for patients with mild hepatic impairment. Donidalorsen has not been studied in patients with moderate or severe hepatic impairment.

Gender

Gender was not identified as a statistically significant covariate in the population PK analysis. The predicted steady-state PK exposures following the administration of donidalorsen 80 mg Q4W were slightly higher in females (N = 98) than males (N = 79). These small differences in PK exposures are attributable to differences in body weight and are not considered clinically meaningful.

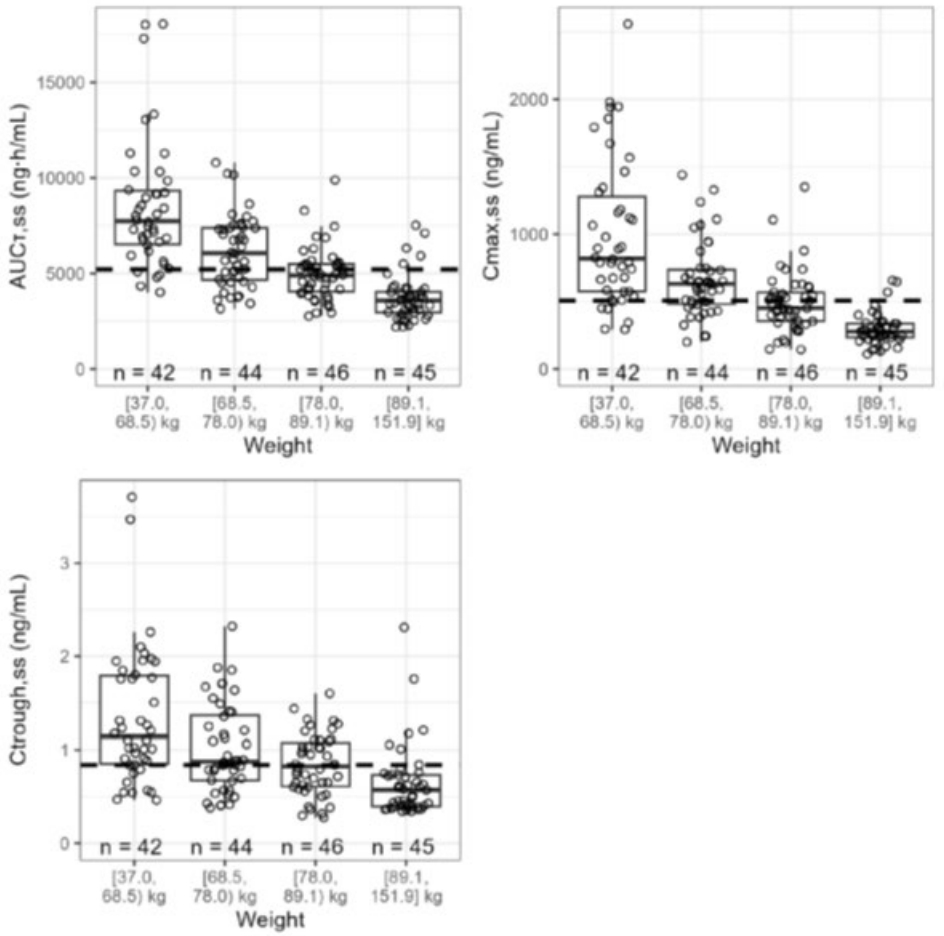
Ethnic factors

Race, ethnicity, or region were not identified as statistically significant covariates in the population PK analysis. Consistent with these findings, there were no clinically meaningful differences in donidalorsen exposure metrics among categories of race, ethnicity, and region. No dose adjustment is warranted on the basis of race, ethnicity, or region.

Weight

The population PK analysis identified body weight as a statistically significant covariate and as the most influential factor on donidalorsen PK. Compared to the reference subject (body weight of 70 kg), a subject with a body weight at the 10^{th} percentile of the weight distribution (i.e., 60 kg) was predicted to have a 26% higher AUCtau,ss, 39% higher Cmax,ss, and 23% higher Ctrough,ss. A subject at the 90th percentile of the weight distribution (i.e., 100 kg) was predicted to have a 42% lower AUCtau,ss, 54% lower Cmax,ss, and 38% lower Ctrough,ss. Post-hoc PK metrics at steady-state versus body weight are presented in Figure 6.

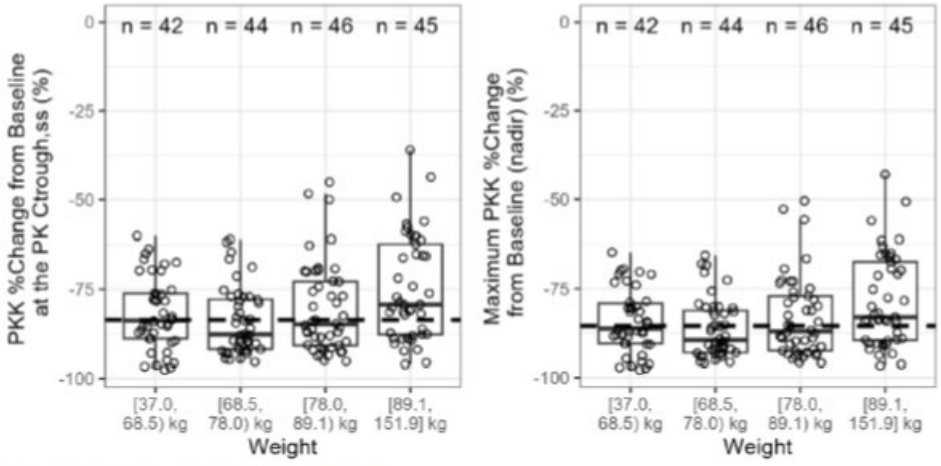
Figure 6. Post-hoc pharmacokinetic metrics at steady-state versus covariates: body weight



Source: Report ISIS 721744-PPK01, Figure 87
Note: The tick horizontal lines (middle of the boxes) represent the median of the data, the hinges (top and bottom of the boxes) represent the 25th and 75th percentiles (i.e., IQR); the top and bottom whiskers extend to the largest and smallest values within 1.5 * IQR of the hinges, respectively. Circles denote individual data; The horizontal dashed lines represent the median of the data across all categories. The data presented here from the Population pharmacokinetic (PopPk) model reflects all clinical trials.
Abbreviations: $AUC_{tau, ss}$ = AUC over a dosing interval (i.e., 4 weeks) at steady-state; $C_{max, ss}$ = maximum plasma concentration at steady-state; $C_{trough, ss}$ = trough concentration at steady-state; IQR = interquartile range

Post-hoc PKK metrics at steady-state were similar across different body weight groups (Figure 7) and the ER analysis (721744-PPK02) further supports that the differences in PK do not translate to clinically meaningful differences in PKK reduction, efficacy, or safety. Therefore, the impact of body weight on donidalorsen PK is not considered clinically meaningful, and no dose adjustment is required on the basis of body weight.

Figure 7. Post-hoc PKK metrics at steady-state versus covariates: body weight



Source: Report ISIS 721744-PPK01, Figure 99
Notes: The tick horizontal lines (middle of the boxes) represent median of the data, the hinges (top and bottom of the boxes) represent the 25th and 75th percentiles (i.e., IQR); the top and bottom whiskers extend to largest and smallest values within 1.5 * IQR of the hinges, respectively. Circles denote individual data; The horizontal dashed lines represent the median of the data across all categories. The data presented here from the Population pharmacokinetic (PopPk) model reflects all clinical trials.
Abbreviations: $C_{trough, ss}$ = trough concentration at steady-state; IQR = interquartile range; PKK = prekallikrein

Age

Age was not identified as a statistically significant covariate in the population PK analysis. Furthermore, the steady-state exposures following donidalorsen 80 mg subcutaneously once every 4 weeks were similar between age groups (Adolescents aged 12 to 17 [N = 6], adults aged < 18 to 39 years [N = 75], 40 to 64 years [N = 94], and ≥ 65 years [N = 2]), with differences between groups being smaller than within-group variability. Therefore, no dose adjustment is required on the basis of age in patients 12 years of age and older. Donidalorsen has not been studied in children <12 years of age.

Pharmacokinetic interaction studies

In vitro studies showed that donidalorsen and its main linker metabolite M8 are not inducers or inhibitors of CYP-mediated oxidative metabolism (ISIS 721744-IS06; ISIS 721744-IS09; ISIS 721744-IS13, ISIS 721744-IS14), nor are they inhibitors or substrates for major drug transporters (ISIS 721744-IS07, ISIS 721744-IS15). No in vitro DDI was observed with regards to plasma protein binding displacement between donidalorsen and other highly plasma protein-bound drugs, namely warfarin and ibuprofen (ISIS 721744-IS05). Therefore, clinical DDI studies were not deemed necessary and were not conducted.

For a detailed assessment of the in vitro DDI studies see the non-clinical section of this report.

2.6.2.2. Pharmacodynamics

Mechanism of action

Donidalorsen is designed to selectively bind to PKK mRNA, consequently degrading it via RNase H1, preventing production of the PKK protein. By decreasing the amount of liver-derived PKK protein circulating in the plasma, donidalorsen treatment will theoretically result in reduction of bradykinin formation and the downstream clinical manifestations of vascular permeability.

Primary pharmacology

In the Phase 1 study **ISIS 721744-CS1** in healthy volunteers, subcutaneous administration of donidalorsen resulted in a dose-dependent reduction of PKK concentration (Figure 8) and plasma proenzyme activation (PPA, maximal capacity to generate bradykinin under the activated conditions) (Figure 9). The nadir was generally reached around Day 71. At Day 99, 2 weeks after the last dose, the reduction with donidalorsen 80 mg Q4W dose was -93.6% for PKK concentration and -98.6% for plasma proenzyme activation. PKK and plasma proenzyme activation levels remained significantly reduced 13 weeks after the last dose of donidalorsen.

Figure 8. Mean PKK results over time

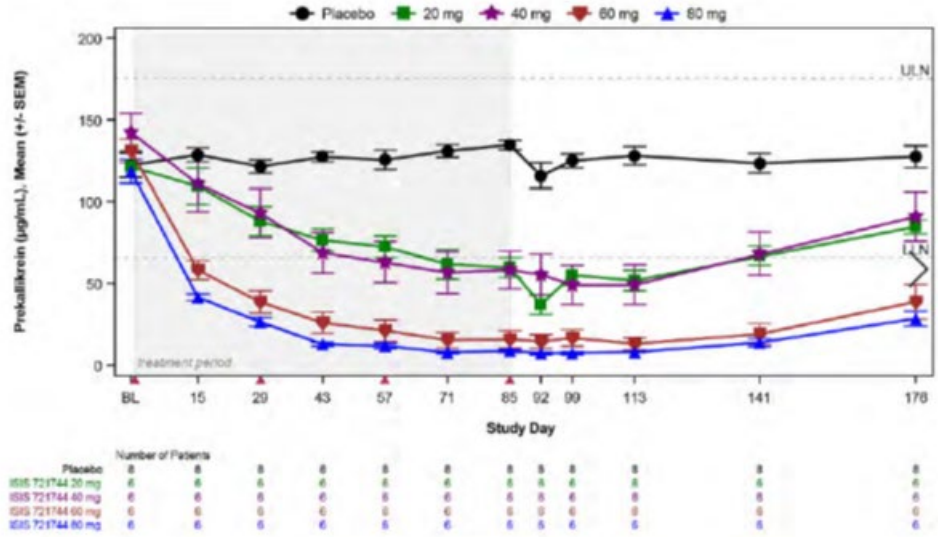
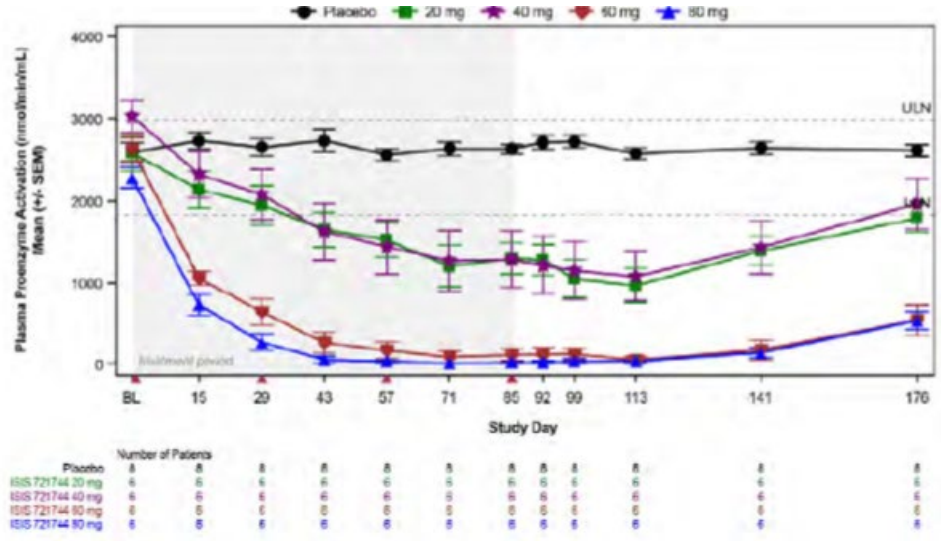


Figure 9. Mean plasma proenzyme activation results over time

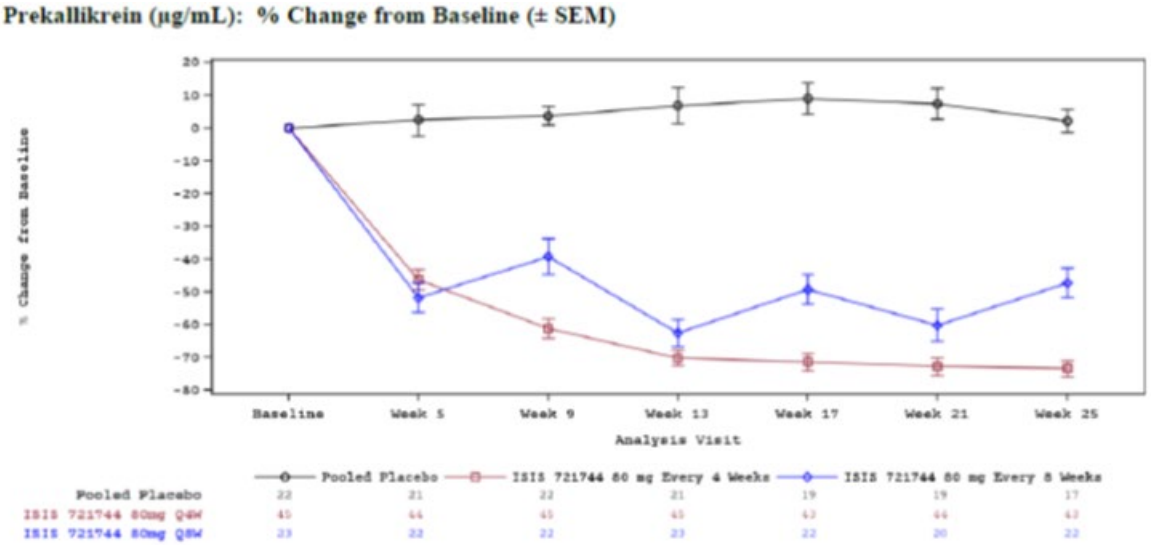


In the Phase 2 study **ISIS 721744-CS2**, in patients with HAE, treatment with donidalorsen 80 mg Q4W resulted in a robust reduction of PKK, PPA and cleaved HMWK (a biomarker for bradykinin release). The nadir for PKK of -64.7% was reached at Day 85 and the nadir for PPA of -70.9% was reached at Day 113.

In the pivotal study **ISIS 721744-CS5**, in patients with HAE, the mean percentage change from Baseline to Week 25 for PKK levels indicated a higher reduction in the donidalorsen-4 group (-73.38%) compared with the donidalorsen-8 group (-47.22%), and minimal change in the placebo group (+2.10%).

The donidalorsen-4 group resulted in a flatter profile of the PKK suppression within the dosing interval compared with the peak/trough profile for the donidalorsen-8 group (Figure 10). PKK suppression at trough upon repeated dosing increased gradually over time in the donidalorsen-4 group up to Week 21 (Day 141), after which the decrease in PKK levels reached a plateau suggestive of near steady-state levels. These data are consistent with time to reach near steady-state levels for the donidalorsen plasma through concentrations in donidalorsen-4 group. PKK suppression at trough in the donidalorsen-8 group also appeared to reach near steady-state during Week 13 to Week 21 (Day 141).

Figure 10. Mean (± SEM) percent change from baseline in prekallikrein assessments over time from baseline to end of on-treatment (full analysis set)



Source: Figure 14.2.9.1.
Baseline is defined as the average of the predose Study Day 1 value and the last predose value prior to Study Day 1.
Note: Error bars indicate the standard error of the mean. ISIS 721744 = donidalorsen.
Abbreviations: Q4W = once every 4 weeks; Q8W = once every 8 weeks; SEM = standard error of mean

Secondary pharmacology - Evaluation of QT Prolongation

The studies that included an assessment of donidalorsen cardiac safety were an in vitro hERG channel study (ISIS 721744-IS08), an in vivo safety pharmacology study (ISIS 721744-AS03), a 13- and 9-month repeat dose toxicity study in cynomolgus monkeys (ISIS 721744-AS02), and a first-in-human Phase 1 clinical study in healthy participants (ISIS 721744-CS1). In addition, cardiac safety ECG data were collected in all clinical studies conducted in patients with HAE.

Overall, the collected data and an integrated clinical and nonclinical risk assessment, along with platform data from the related ASOs within the same chemical class as donidalorsen, support the conclusion of a lack of QTc prolongation effect with donidalorsen treatment:

- Donidalorsen was negative in blocking hERG current at concentrations up to 300 µM (>1000-fold higher than the observed Cmax exposure after 4 monthly doses of donidalorsen 80 mg dose in study ISIS-721744 CS1).
- Donidalorsen demonstrated no significant effect on ECG intervals observed in preclinical safety pharmacology and toxicology studies in animals, including telemetered cynomolgus monkeys following a single administration of 12- and 30 mg/kg doses. The Cmax achieved at the 30 mg/kg single dose in monkeys was 46- to 57-fold higher than the Cmax exposures observed after 4 monthly doses of 80-mg in healthy participants (geometric mean Cmax 0.718 µg/mL).
- Donidalorsen has a large molecular weight and therefore, a low likelihood of direct ion channel interactions.
- No clinically meaningful changes in ECG parameters including QT intervals were observed, as evidenced by a lack of correlation between donidalorsen plasma concentration and change in QT corrected using the Fridericia's formula (ΔΔQTcF) across the dose range (20 to 80 mg subcutaneous injection) tested in the Phase 1 study ISIS 721744-CS1 (detailed below). Although a supratherapeutic dose was not included in this study, significantly higher exposure multiples (46- to 57-fold higher Cmax) were tested in the preclinical safety pharmacology study, which indicated no significant effect on ECG intervals. This model has been shown to be sensitive in detecting threshold QTc prolongation on the order of 10 ms (Chui et al. 2021).
- There were no notable trends or safety concerns observed for ECG findings in the Phase 1, 2, and 3 clinical studies.
- No QTc prolongation was observed for ten 2'-MOE ASOs that had completed Phase 1 studies (Yu et al. 2017). These results support the conclusion that 2'-MOE ASOs, as a chemical class, do not cause QT prolongation at clinically meaningful dose levels.
- The lack of QT effect with mipomersen, a 2'-MOE ASO, was demonstrated in a thorough QT (TQT) study (Report 2010 ISIS). Similarly, volanesorsen does not cause QTc prolongation, as demonstrated by a thorough QT study (ISIS 304801 CS13). These results further support the conclusion that 2'-MOE ASOs, as a chemical class, do not cause QT prolongation at clinically meaningful dose levels.
- Furthermore, custirsen (Rabinovich-Guilatt et al. 2015) a 2'-MOE ASO, and 2'-MOE GalNAc-conjugated ASOs such as AZD8233 (Rekić et al. 2022), olezarsen and eplontersen, showed no effect on QTc intervals based on specified concentration-QT analyses.

A more detailed review of the in vitro and nonclinical studies can be found in the non-clinical section of this report.

Study ISIS 721744-CS1

Electrocardiogram data were available from 6 subjects each in the donidalorsen 20, 40, 60, and 80 mg once every 4 weeks multiple-dose groups, and from 8 placebo-treated subjects. Continuous 12-lead ECG recordings (holters) were performed from 30 minutes prior to dosing up to 24-hours following the dose on Day 1, including the 24-hour time point.

Overall, there were no clinically significant changes in ECG parameters (HR, PR, QRS intervals, QTcF). Mean change-from-baseline QTcF (ΔQTcF) after administration of donidalorsen followed the placebo pattern across post-dose time points, and mean placebo-corrected change-from-baseline QTcF (ΔΔQTcF) ranged from -6.3

ms at 1.5 hours in the 20 mg dose group to 6.0 ms at 12 hours in the 60 mg dose group, without indication of dose-dependency.

A linear mixed-effect model with a treatment effect-specific intercept was fitted to ΔQTcF versus donidalorsen plasma concentrations and provided a reasonable fit to the data. Neither the slope for the donidalorsen concentrations nor the treatment effect-specific intercept were statistically significant from zero at 10% significance level.

Using concentration-QTc analysis, a QTcF effect above 10 ms can be excluded within the observed range of donidalorsen plasma concentration up to 780 ng/mL.

Immunogenicity

Treatment-emergent ADA were characterized by a late onset and low peak titers. The overall median onset of treatment-emergent ADA ranged from 29 to 169 days, with median peak ADA titer ranging from 50 to 400 following donidalorsen 80 mg once every 4 weeks or once every 8 weeks for 16 to 208 weeks across studies.

In the pivotal Phase 3 Study ISIS 721744-CS5, the incidence rate of treatment-emergent ADA was 20.0% in the donidalorsen-4 group and 21.7% in the donidalorsen-8 group following treatment for 25 weeks. In Study ISIS 721744-CS7, the incidence rate of treatment-emergent ADA was 36.2% and 21.4% for the OLE donidalorsen-4 and OLE donidalorsen-8 groups following the administration of donidalorsen for a median exposure duration of 227 days (32.4 weeks), and 42.2% for the Switch Patient group following the administration of donidalorsen 80 mg for a median exposure duration of 253 days (36.1 weeks), as of the data cutoff date of 28 Feb 2024. In Study ISIS 721744-CS3, the incidence rate of treatment-emergent ADA was 64.3% to 75.0% following donidalorsen 80 mg once every 4 weeks or every 8 weeks for up to 208 weeks.

Impact of Immunogenicity on PK

Donidalorsen plasma PK parameters, including Cmax, Tmax, and AUC, were generally similar between ADA-negative and ADA-positive patients in patients with HAE (ISIS 721744-CS2, ISIS 721744-CS5). However, higher plasma donidalorsen Ctrough levels were observed in ADA-positive patients compared with ADA-negative patients across donidalorsen clinical studies. The increase in trough concentrations in ADA-positive patients is consistent with observations in other ASOs (Henry et al. 2022; Yu et al. 2020).

Impact of immunogenicity on pharmacodynamics

The impact of immunogenicity on plasma PKK reductions over time was evaluated across clinical studies. The presence of ADA did not have any effect on PKK reduction in donidalorsen-treated patients with HAE across the clinical studies (ISIS 72144-CS5, ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS7).

Pharmacokinetic-Pharmacodynamic and Exposure-Response Analyses

Population PKPD analysis (721744-PPK01)

The population PKPD model was built using data from studies in healthy volunteers (Study ISIS 721744-CS1 and Study ISIS 721744-CS9) and patients with HAE (Study ISIS 721744-CS5 and Study ISIS 721744-CS2). Covariates were evaluated for inclusion in the model using a stepwise forward addition ($p<0.01$) and backward elimination ($p<0.001$) approach. Covariates assessed are presented in Table 7.

Table 7. Covariates assessed in the population PKPD analysis

Covariate	Code	Value	Parameters
Age at baseline (yrs)	AGE	Continuous	k_{out}, DE, BL
Body weight at baseline (kg)	WTKG	Continuous	k_{out}, DE, BL
PKK at baseline (mg/L)	BLPKK	Continuous	k_{out}, DE
Sex	SEXF	Categorical	k_{out}, DE, BL
Race	RACE	Categorical	k_{out}, DE, BL
Ethnicity	ETHNIC	Categorical	k_{out}, DE, BL
Region (Geographical location)	REGION	Categorical	k_{out}, DE, BL
Disease status (HV, HAE)	DSSTAT	Categorical	k_{out}, DE, BL
HAE Type (Healthy, I, II, III)	HAETYPE	Categorical	k_{out}, DE, BL
Hepatic dysfunction category (NCI-ODWG criteria [8])	HEPATC	Categorical	k_{out}, DE, BL

BL, baseline; DE, drug effect parameters; HAE, hereditary angioedema; HV, healthy volunteer; k_{out} = first-order elimination rate

A total of 177 individuals (101 HVs and 76 HAE patients) contributed 1159 PKK concentrations to the analysis. No trends in PKK over time were observed for placebo subjects, hence, placebo subjects were excluded from the analysis.

The final population PKPD model was an indirect response model with inhibition on the rate of PKK production by donidalorsen. Disease status was included as a covariate on baseline (BL) and IC50, where BL and IC50 were 13% lower and 77% higher, respectively, for HAE patients compared to HVs. Parameter estimates of the final model are provided in Table 8. Diagnostic plots for the final model are shown in Figure 11 and a VPC is shown in Figure 12.

Table 8. Final population PKPD model parameter estimates

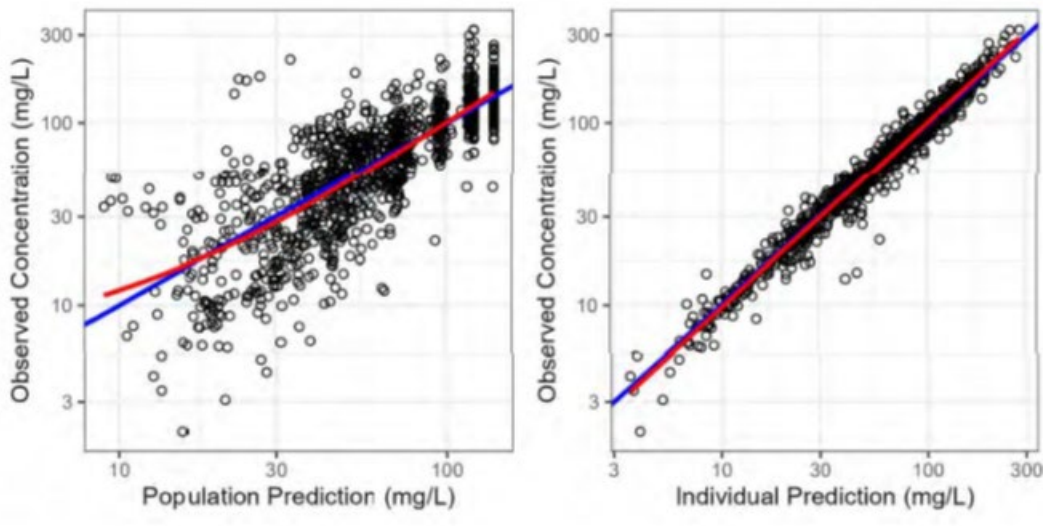
Parameter	Estimate	%RSE
BL (mg/L) [a]	139	2.52
Covariate of Disease Status (HAE patient) on BL	-0.132	24.4
k_{out} (h^{-1})	0.00266	4.70
I_{max}	0.992	1.19
IC_{50} (ng/mL) [b]	0.158	11.0
Covariate of Disease Status (HAE patient) on IC_{50}	0.770	28.5
BSV% BL (Sh%)	25.9 (10.0)	16.4
BSV% k_{out} (Sh%)	36.6 (28.6)	21.0
BSV% IC_{50} (Sh%)	83.1 (11.7)	16.4
σ_{prop}	0.159	6.13

BL, baseline; BSV%, between-subject variability expressed as CV%; IC_{50}, concentration yielding half of the maximum inhibitory effect; I_{max}, maximal fractional inhibitory capacity; k_{out}, first-order loss rate constant; RSE, relative standard error; Sh, shrinkage for BSV; σ_{prop}, proportional component of the residual error model.

[a] BL = 139 · 0.868 [for HAE patient]

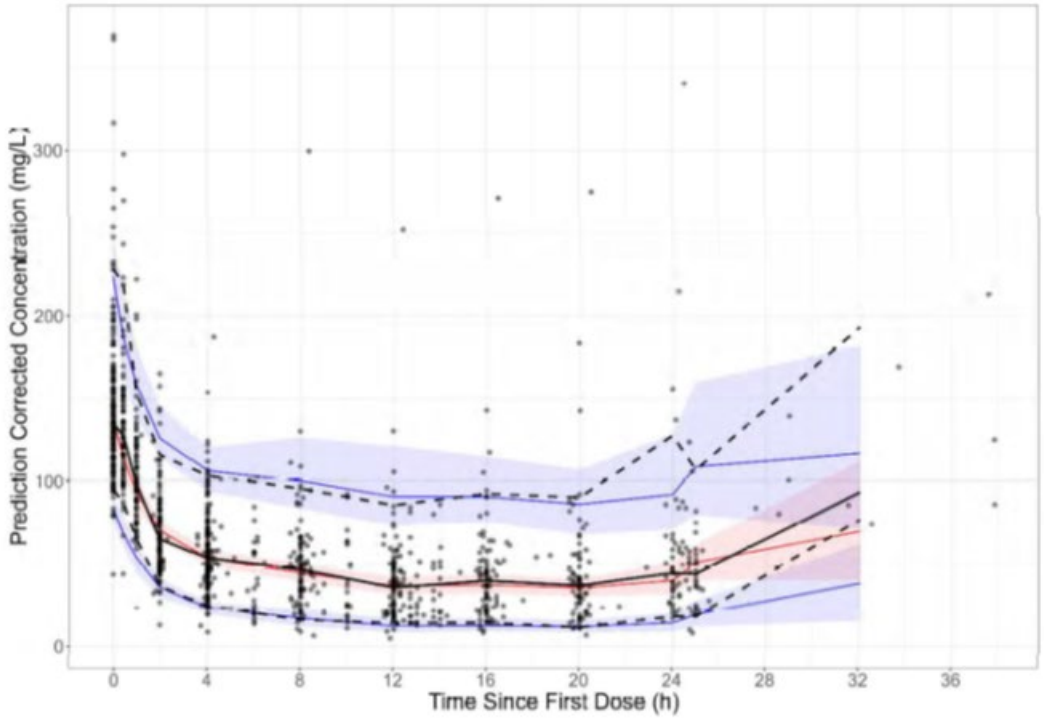
[b] IC_{50} = 0.158 · 1.770 [for HAE patient]

Figure 11. Observed vs. Predicted concentration from the final population PKPD model



Blue line, line of unity; red line, Loess smooth trend line

Figure 12. pcVPC of the final population PKPD model (overall)



Circles, individual observed data; dashed lines, observed 5th & 95th percentiles of the observed data; solid black line, observed median concentration. Colored lines and shaded areas, model predicted 5th, 50th, & 95th percentiles and 95% confidence interval around the model predicted 5th, 50th, & 95th percentiles.

Note: Data from studies and treatment regimens are pooled, including differing duration of treatment and follow-up times.

The effect of disease status on the maximum PKK percent change from baseline (nadir) over the steady-state dosing interval and PKK percent change from baseline at the PK Ctrough,ss were within the 80% to 125%

range. Compared to healthy volunteers, HAE patients were predicted to have 6% smaller maximum PKK percent change from baseline and 7% smaller PKK percent change from baseline at the PK Ctrough,ss.

Exposure-response relationships for PD biomarker (PKK), select efficacy and safety endpoints (721744-PPK02)

The E-R analyses evaluated the relationship between model-predicted donidalorsen exposure metrics and plasma PKK, efficacy, and safety endpoints, using data from 84 patients with HAE from pivotal study ISIS 721744-CS5.

Exposure-PD/Efficacy endpoints

Mean percentage reductions of PKK from baseline at Week 25 were >50% across all donidalorsen exposure tertiles. Higher Ctrough,ss and AUC1344h,ss trended towards greater reductions of PKK from baseline. These results are consistent with the greater reductions in PKK observed for the donidalorsen 80 mg every 4 weeks regimen compared with the every 8 weeks regimen, since the more frequent dosing regimen is associated with an approximately 2-fold higher Ctrough,ss and AUC1344h,ss.

Similarly, mean percentage reductions from baseline in HAE attack rate from Week 1 to Week 25 were >60% across all donidalorsen exposure tertiles. The mid- and highest tertiles of Ctrough,ss and AUC1344h,ss had greater mean percent reductions from baseline in HAE attack rate compared to the lowest tertiles of Ctrough,ss and AUC1344h,ss.

Exposure-Safety endpoints

No substantial differences were observed across donidalorsen exposure tertiles for any safety endpoint including maximum values of ALT, AST, TB, and UPCR, percentage maximal decrease from baseline in eGFR, maximum decrease from baseline in platelet count, percentage of subjects with moderate or severe TEAEs, percentage of subjects with AESI, and percentage of subjects with AE leading to dose modification and/or discontinuation. Overall, these results suggest lack of exposure-safety relationships within the exposure range associated with the dose regimens studied in the study.

In addition, no substantial differences were observed between patients receiving donidalorsen or placebo, with the exception of maximum ALT and maximum AST. The mean difference in maximum ALT between donidalorsen and placebo favoured placebo for the mid-tertiles of Ctrough,ss and AUC1344h,ss; however, there were no substantial differences between placebo and donidalorsen at the lowest and highest tertiles of Ctrough,ss and AUC1344h,ss. The mean difference in maximum AST between donidalorsen and placebo favoured placebo for the highest tertile of Ctrough,ss and mid-tertile of AUC1344h,ss. Trends towards favouring placebo were observed for other tertiles of donidalorsen exposure, however, their respective 95% CIs included the null value. The mean differences in maximum ALT and AST between donidalorsen and placebo did not appear clinically meaningful at any tertile of donidalorsen exposure.

Exposure response analysis (721744-PPK03)

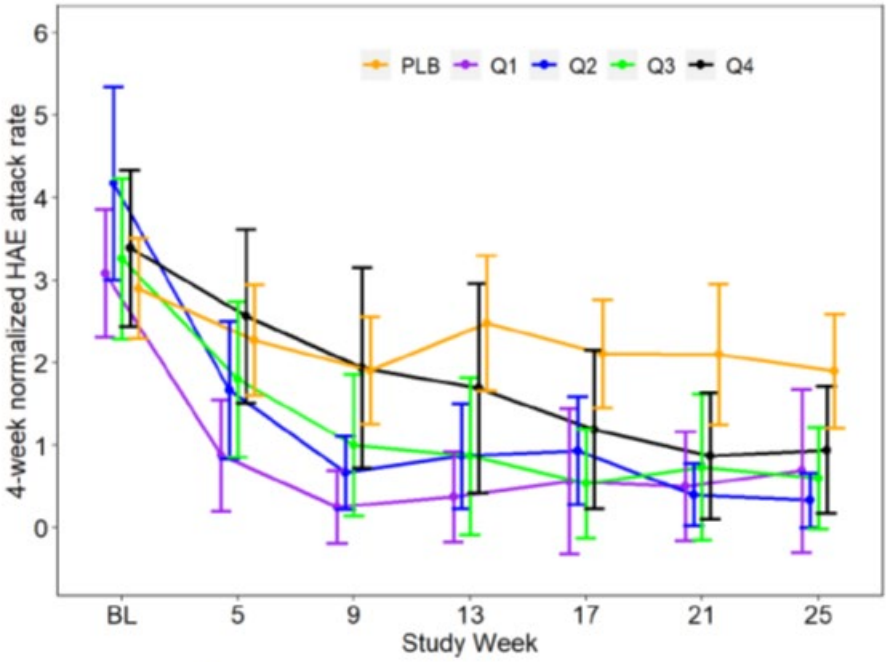
The ER analysis evaluated the relationship between plasma PKK concentration and normalized per 4 weeks HAE attack rate using data from the pivotal study ISIS 721744-CS5.

A longitudinal model was developed with per 4-week normalized HAE attack rate as a dependent variable and per 4-week average PKK concentrations as an independent variable. The population PKPD model was used to derive PKK concentration metrics for each donidalorsen-treated patient. Covariates were evaluated for inclusion in the model using a stepwise forward inclusion ($p<0.01$) and backward elimination ($p<0.001$)

strategy. The following covariates were evaluated: body weight, age, sex, baseline PKK concentration, baseline per 4-week normalized HAE attack rate, and ADA status.

A total of 84 patients, including 22 randomized to placebo, were included in the analysis. Exploratory analyses indicated that there was a clear and PKK-dependent effect of donidalorsen on the mean per 4-week normalized HAE attack rate. Lower PKK concentrations were correlated with the largest reduction in per 4-week normalized HAE attack rate (Figure 13).

Figure 13. Per 4-week normalised HAE attack rate stratified by $PKK_{avg,4W}$ quartiles, with placebo as a separate group in study ISIS 721744-CS5



Source: pkk-attack-rate-exploratory.html
Notes: The dots and error bars at Week 5 represent the mean and 90% CI of the number of attacks from Day 1 (start treatment) to Day 28 in each of the $PKK_{avg,4W}$ exposure quartiles and placebo, those at Week 9 the number of attacks from Day 29 to Day 56, at Week 13 from Day 57 to Day 84, etc.
Abbreviations: BL=baseline; CI=confidence interval; HAE=hereditary angioedema; PKK=prekallikrein; $PKK_{avg,4W}$=per 4-week average PKK concentration; PLB=placebo; Q1=first quartile; Q2=second quartile; Q3=third quartile; Q4=fourth quartile

The final model was a direct-effect model in which the effect of PKK concentration on the per 4-week normalized HAE attack rate was modelled with a sigmoid Emax relation. The model included the effect of baseline per 4-week normalized HAE attack rate on Emax and the effect of baseline PKK concentration on EC50. Parameters of the final model are provided in Table 9. A VPC for the final model is presented in Figure 14.

Table 9. Parameter estimates of the final per 4-week normalised HAE attack rate model

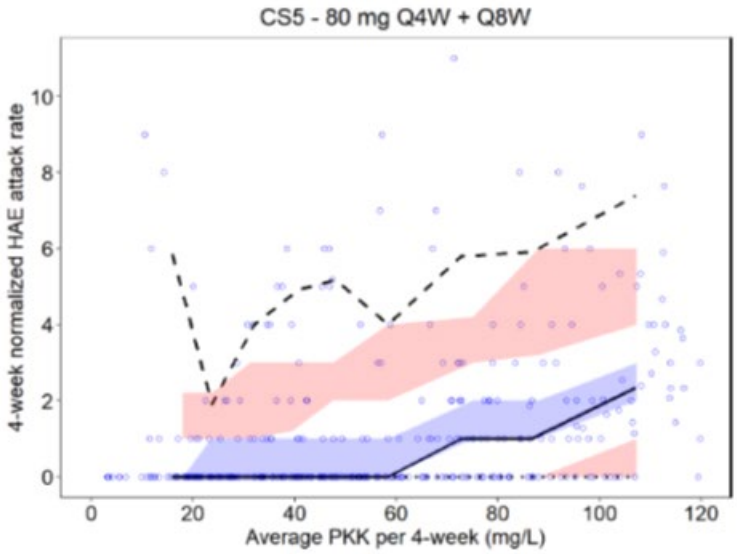
Parameter	Estimate (RSE%)	Bootstrap Statistics*		Shrinkage
		Median	95% CI	
E_{max}	4.50 (9.6%)	4.54	[3.31, 5.78]	-
EC_{50} (mg/L)	110 (0.8%)	111	[85.7, 124]	-
Hill	2.60 (16%)	2.61	[1.84, 4.20]	-
b_{HAE}	1.03 (7.9%)	1.03	[0.85, 1.19]	-
b_{PKK}	0.13 (14.3%)	0.13	[0.04, 0.18]	-
Interindividual Variability				
IIV on E_{max} (CV%)	27.9 (28.8%)	26.5	[7.0, 42.2]	42.7
IIV on Hill (CV%)	131 (14.6%)	129	[82.2, 255]	27.1
Secondary Parameters				
EC_{10}	47.1	-	-	-
EC_1	18.7	-	-	-

Source: pkk-attack-rate-modeling.html

* Based on 909 (out of 1000) replicates that minimized successfully. Condition number = 25.5; Residual variability is not constant; therefore, the residual error depends on the mean rate.

Abbreviations: b_{HAE}=coefficient for effect of baseline per 4-week normalized HAE attack rate; b_{PKK}=coefficient for the effect of baseline PKK concentration; CI=confidence interval; CV%=percent coefficient of variation; EC_{50}=effective $PKK_{avg,4W}$ associated with 50% reduction from the maximum attack rate; EC_{10}=effective $PKK_{avg,4W}$ associated with 90% reduction from the maximum attack rate; EC_1=effective $PKK_{avg,4W}$ associated with 99% reduction from the maximum attack rate; E_{max}=per 4-week HAE attack rate at infinite PKK concentration; IIV=interindividual variability; PKK=prekallikrein; RSE%=percent relative standard error

Figure 14. Visual predictive check per 4-week normalised HAE attack rate versus $PKK_{avg,4W}$ for the final model



Source: pkk-attack-rate-modeling.html

Notes: Model predictions were rounded to the nearest integer to mimic the nature of the observed data before calculating summary statistics presented in the VPC. Open blue circles represent the individual data points. The solid black line is the median observed per 4-week normalized HAE attack rate. The dotted and dashed black lines are the observed 5th and 95th percentiles, respectively. The blue area represents the 95% CI of the simulated median, and the red areas are the 95% CI of the simulated 5th and 95th percentiles, respectively, based on 1000 replicates.

Abbreviations: BL=baseline; CI=confidence interval; HAE=hereditary angioedema; PKK=prekallikrein; $PKK_{avg,4W}$=per 4-week average concentration; Q4W=every 4 weeks; Q8W=every 8 weeks; VPC=visual predictive check

The Hill coefficient was estimated to be 2.6, which suggests a steep relationship between average PKK concentrations and per 4-week normalized HAE attack rate. The Emax showed a strong positive correlation with the baseline per 4-week normalized HAE attack rate with the power exponent (bHAE) estimated at 1.03. Exponent for the effect of baseline PKK level (bPKK) was estimated to be 0.13, suggesting a weak dependency on the baseline PKK level on the effect of donidalorsen treatment on efficacy endpoint. Taken together, these results indicate that the per 4-week normalized HAE attack rate over time normalized by the baseline rate (i.e, % reduction from the baseline) is largely independent of the baseline per 4-week normalized HAE attack rate or starting PKK levels prior to donidalorsen treatment, suggesting a generally comparable and clinically meaningful response across the patient population.

2.6.3. Discussion on clinical pharmacology

Bioanalytical methods

Quantification of donidalorsen concentrations in human plasma (721744-MV04)

Validation

It is noted that the validation was developed based on the May 2001 FDA Guidance for Industry – *Bioanalytical Method Validation*. The most recent and relevant guideline for this method validation for this

application would be the ICH guideline M10 on bioanalytical method validation and study sample analysis, coming into effect in January 2023 for EMA applications.

The hybridization-based ECL to quantify donidalorsen in human plasma has been adequately described. The assay was appropriately validated with respect to quantitation range, dilutional linearity precision and accuracy, hook effect, stability, selectivity/specificity, potential interference, and cross-reactivity to selected putative metabolites, haemolysis effect, quantitation of unconjugated donidalorsen (ISIS 780124) and total full-length ASO, and potential anti-drug antibody against donidalorsen interference. The validation of these methods are in line with the ICH M10 guideline.

The established calibration curve and limits of quantification are considered acceptable. Dilutional linearity is considered demonstrated. No apparent hook effect was shown up to 5000nM concentrations. Stability is considered to have been demonstrated in line with the ICH M10 guidance, freeze-thaw and sufficient bench-top stability (up to 24 hours) are considered acceptable, and stability in frozen matrix for up to 2480 days at -80°C was demonstrated in Addendum 2 to the validation report. Selectivity and specificity were shown adequately. The metabolite cross reactivity assay demonstrated the 19-mer metabolite could interfere with the assay, however as the presence of this metabolite is considered minimal it is accepted that this is not significant in practice. No interference was shown from the presence of anti-drug antibody.

The assessment of accuracy and precision in the validation was questioned as method validation was completed prior to implementation of ICH M10 guidance. Instead, intraassay accuracy and precision were demonstrated by evaluating calibration standards and quality control samples during sample analysis, and assay robustness was confirmed. Specifically, a minimum of 552 quality control (QC) samples (low QC, medium QC, high QC) across 6 clinical studies (ISIS 721744-CS1, -CS2, -CS3, -CS5, -CS7, and -CS9) were assessed for intra-assay precision and accuracy, with results showing % coefficient of variation (CV) less than 10.1% and % bias within +/- 6.77%. Additionally, intra-assay precision and accuracy at the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) calibrators were evaluated in at least 272 samples, with %CV less than 9.42% and % bias within +/- 4.75%. In this case, it is considered that the method demonstrates adequate intraassay accuracy and precision.

Assay performance

Assay performance in ISIS 721744-CS1, ISIS 721744-CS9, ISIS 721744-CS2, and ISIS 721744-CS5 are considered acceptable, and are in line with the requirements set in the ICH M10 guideline. Calibration curves are described as in the validation and met the acceptance criteria. Sufficient QCs were run and meet the acceptance criteria. In each study approximately 10% of samples were selected for incurred sample reanalysis. This number of samples is considered sufficient (10% for 1000 samples, and 5% of samples >1000) and the incurred sample reanalysis showed reproducibility (>2/3 within 20% of original measurement) of the method.

Quantitation of Prekallikrein Concentrations in Human Plasma by Fluorescence ELISA Method (721744-BRV01)

Validation

It is noted that the validation was developed based on two white papers and the EMA Guideline on Bioanalytical Method Validation, Feb 2012 and FDA Guidance for Industry – Bioanalytical Method Validation, May 2018. The most recent and relevant guideline for this method validation for this application would be the ICH guideline M10 on bioanalytical method validation and study sample analysis, coming into effect in

January 2023 for EMA applications. However, as more up to date guidance has been used in comparison to method 721744-MV04, most of the method has been validated in line with ICH M10 guidance.

The fluorescence ELISA method to quantify PKK in human plasma has been adequately described. The assay was adequately validated for calibration curve performance, establishment of each and buffer quality control (QC) preparation and concentration determination, precision and relative accuracy, sensitivity, matrix effects, haemolysis, parallelism, specificity, stability, and robustness.

The run acceptance criteria were well established and the method to determine the calibration curve and limits of quantification were sufficiently detailed and are considered acceptable. The relative accuracy and precision results met the criteria outlined in ICH M10. No haemolysis effect was detected. Selectivity and dilutional integrity were investigated by parallelism. Parallelism was determined in healthy and disease state individuals over 5 serial dilutions (8,000-fold to 128,000-fold dilutions). Stability was demonstrated in the original validation report and was extended further in Addendum 2 to the report. In Addendum 2, freeze-thaw stability was demonstrated for up to 8-cycles, bench-top stability for up to 25.44 hours, and long-term storage for 371 days at -80°C.

Specificity for the assay was also validated for donidalorsen, high molecular weight kininogen (HMWK), and cleaved kallikrein. The Applicant has concluded that there is no interference from donidalorsen, HMWK or cleaved kallikrein on other assays.

Assay performance

The standard curve and QC performance in ISIS 721744-CS1*,* ISIS 721744-CS9, ISIS 721744-CS2, and ISIS 721744-CS5 are considered acceptable, and are in line with the requirements set in the ICH M10 guideline. Calibration curves are described as in the validation and met the acceptance criteria. Sufficient QCs were run and meet the acceptance criteria.

Incurred sample reanalysis was performed for PKK in ISIS 721744-CS5 for approximately 10% with 90% of samples meeting the criteria for acceptance. <10% of samples were reanalysed, however as the number reanalysed was sufficiently close to 10% this can still be considered acceptable.

The samples from study ISIS 721744-CS9 were analysed within the validated long-term stability. The applicant has provided an adendum to the method validation report (PKK-BRV01 Final Report Addensum 3) which has demonstrated long-term stability up to 24 months which covers the time taken to analyse the samples from ISIS 721744-CS1, ISIS 721744-CS2, and ISIS 721744-CS5.

Detection of Anti-ISIS 721744 Antibodies in Human Plasma (721744-MV06)

Validation

It is noted that this validation was based on guidelines set forth in the April 2016 FDA Draft Guidance for Industry – *Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products* and the April 2008 EMEA *Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins*. The most relevant guidelines would be the 2017 EMA *Guideline on Immunogenicity assessment of therapeutic proteins* and the ICH M10 guideline bioanalytical method validation and study sample analysis.

The validation report for the method to detect donidalorsen ADAs is well described. The report details the negative controls, positive controls, and reference standards well. The screening, titration, and confirmatory assay cut-points are overall well described. The inter-assay precision has been validated and meets the criteria set in the ICH M10 guidelines. The sensitivity of the assay is adequately described. Titer precision is sufficiently well validated. No apparent hook effect was demonstrated at concentrations up to 56,000 ng/mL.

There was no apparent haemolysis or lipemic effects on detection of ADAs. No matrix interference was detected. These results can be accepted in both healthy and disease state (HAE) individuals.

Intra-assay precision was not demonstrated in line with the ICH M10 guidance. The intra-assay precision of the assay was subsequently evaluated during sample analysis. A minimum of 758 quality control samples (NC, SLPC, HPC) across 5 clinical studies (ISIS 721744-CS1, ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, and ISIS 721744-CS7) were assessed for intra-assay precision, with results demonstrating a %CV of less than 15.5%.

Epitope specificity was demonstrated using donidalorsen and ION-942898 (same sequence as donidalorsen but contains uniform phosphodiester backbone and deoxyribose sugars on bases) and ION-942-899 (different sequence from donidalorsen but same chemistry and gamer). The assay met the specificity criteria but the observed % inhibition for ION-942898 and ION-942899 indicated that the antibodies in the positive control are likely recognizing shared epitopes. This is not expected to have an impact on interpreting data from clinical trials.

Freeze-thaw stability was demonstrated over 6 freeze/thaw cycles and bench-top stability was shown over 26 hours; however, both the stability and reference control samples were inadvertently not diluted to concentrations that resulted in OD readings below the titer cut point. Additional stability assessments were performed to to support the requirements for ISIS 721744-CS2 ADA analysis, during which 7 and 9 freeze/thaw cycles were evaluated as outlined in the original validation plan. These additional stability data are consistent with and supportive of the original validation results. It is agreed that despite the original evaluation not crossing the titer cut point (TCP), that there is no impact on the integrity or reliability of the sample analysis.

No specific method for the detection of neutralising antibodies has been developed to correctly characterise their neutralising capacity in accordance with EMA guideline. The applicant states that, since donidalorsen exerts its biological activity at the intracellular level any ADA cannot directly neutralize the binding to the human plasma prekallikrein mRNA. For this reason, the neutralizing potential of ADA has evaluated from the combined ADA, PK and PD data. These data showed that the presence of ADA had no impact on peak plasma exposure although higher Ctrough was observed in positive patients.

Assay performance

The overall performance of the assay is considered acceptable in ISIS 721744-CS1, ISIS 721744-CS2, and ISIS 721744-CS5. QC precision performance was considered acceptable for each study.

Pharmacokinetics

Population PK analysis

The methods used to develop the population PK model are acceptable. Data handling methods were adequately justified and considered acceptable.

The final population PK model was a standard two-compartment disposition model with first-order absorption and linear elimination. The statistically significant covariates included in the model were body weight on CL/F, Vc/F, Q/F, and Vp/F, site of administration (arm compared to abdomen or thigh) and drug presentation (autoinjector compared to vial) on ka, and disease status (HAE patient compared to HV) on Vc/F and Q/F. Inter-individual variability of key PK parameters was moderate: apparent clearance 19.6%, apparent volume of the central compartment 59.0%, and absorption rate constant 42.8%.

Parameters of the final model were estimated with good precision and IIV shrinkage was acceptably low. GOF plots and VPCs support an adequate fit of the final model. Overall, the model is deemed fit for performing simulations and for subsequent PKPD and exposure-response analyses.

Bioequivalence between autoinjector and vial (Study ISIS 721744-CS9)

The primary endpoint of bioequivalence was achieved for autoinjector compared to vial as the ratio of adjusted GMs 90% CI values for AUC0-336h and Cmax were contained within the standard acceptance criteria of 80% to 125%. Similar reductions in serum assay concentrations between the two formulations provides further support for comparable efficacy. Overall, the results support use of the autoinjector.

PK in healthy subjects (Study ISIS 721744-CS1)

The PK of donidalorsen was consistent with the expected and previously reported PK for compounds within this chemical class. Donidalorsen was absorbed rapidly into the systemic circulation after SC injection, reaching Cmax after ~1 to 3 hours. Plasma exposures (Cmax and AUC) were dose dependent but greater than dose-proportional over the dose range of 20 to 80 mg. This is adequately reflected in section 5.2 of the SmPC.

There was little or no increase in Cmax or AUC following 4 doses of donidalorsen (every 4 weeks), consistent with minimal plasma accumulation and time-invariant plasma kinetics.

After reaching Cmax, donidalorsen concentrations decline in a biphasic manner over time, with an initial rapid distribution phase dominating the plasma clearance, followed by a much slower elimination phase (likely reflecting slow metabolism elimination from the tissues), with a terminal elimination half-life of 4-5 weeks. Plasma trough concentrations of donidalorsen increased with increasing dose, consistent with trough plasma concentrations reflecting drug exposure in the major tissues of distribution (i.e. the liver).

The mean percent doses excreted in urine values (for intact donidalorsen) within the first 24 hours post-dose were very low (< 1%). The low renal clearance of donidalorsen over the first 24 hours is consistent with the expectation that the initial plasma clearance is mainly due to distribution to tissues and not due to excretion.

PK in the target population

PK parameters of donidalorsen are comparable between healthy volunteers and patients with HAE. Although disease status (HAE patient) was identified as a statistically significant covariate in the population PK and PKPD models, the impact on PK exposure and PKK levels was small and not clinically relevant.

Simulations based on the population PK and PKPD models provide support for 80 mg donidalorsen administered Q4W or Q1M and Q8W or Q2M to achieve sufficient PKK reductions in HAE patients. There does not appear to be any clinically relevant difference in PK or PKK between Q4W and Q1M regimens and between Q8W and Q2M regimens. Greater reductions in PKK were observed for the 80 mg every 4 weeks compared to every 8 weeks regimen, as the former regimen is associated with higher donidalorsen exposures.

Special populations

- ***Renal impairment***

Only a small fraction of the donidalorsen dose is excreted renally. Therefore, mild renal impairment is not expected to impact the PK or PD of donidalorsen, which is consistent with findings of the population PK and PKPD analyses. However, no patients with moderate or severe renal impairment or ESRD were included in

these analyses. Therefore, use of donidalorsen in patients with moderate or severe renal impairment should only be used in these patients if the anticipated clinical benefit outweighs the risk (section 4.2 of the SmPC).

- ***Hepatic impairment***

Differences in donidalorsen PK are not expected in patients with liver impairment because donidalorsen is primarily eliminated by nuclease-mediated degradation. This is consistent with similar donidalorsen PK metrics found in patients with normal liver function (N=175) and patients with mild hepatic impairment (N=2) in the population PK analysis.

The impact of liver impairment on donidalorsen PD is less clear because it has been reported in the literature that ASGPR expression may be altered in liver disease. By contrast, altered GalNAc-mediated uptake is considered unlikely due to the high capacity of ASGPR relative to the low pharmacologically relevant dose levels (Willoughby et al. 2018), which is consistent with similar PKK metrics found in patients with normal liver function (N=175) and patients with mild hepatic impairment (N=2) in the PKPD analysis.

Overall, despite a limited number of patients included in the population PK and PKPD analyses, a dose adjustment is not considered necessary in patients with mild hepatic impairment. There are no clinical data in patients with moderate or severe hepatic impairment. Therefore, donidalorsen should only be used in these patients if the anticipated clinical benefit outweighs the risk (section 4.2 of SmPC).

- ***Gender***

There were no notable influences of gender on donidalorsen PK or PD. A dose adjustment on the basis of gender is not warranted.

- ***Ethnic factors***

There were no notable influences of race or ethnicity or region on donidalorsen PK or PD. A dose adjustment on the basis of ethnic factors is not warranted.

- ***Weight***

Body weight was the most influential covariate on donidalorsen PK. However, the PKPD analyses showed that the impact of body weight on donidalorsen PK does not translate into clinically relevant changes in PKK reduction or efficacy. Whilst the ER analysis showed no relationship between donidalorsen plasma concentrations and safety events, this finding is based on a limited number of patients of low body weight. In fact, there were only 3 patients with a body weight <50 kg and only one patient with a body weight <40 kg (see Safety Section for further discussion).

- ***Age***

A total of 6 elderly patients were studied in the donidalorsen clinical trials and 5 patients over 65 years were included in the population PK and PKPD analyses. Despite these low numbers, age was not identified as having an impact on donidalorsen PK or PD. Furthermore, the available data indicate a comparable safety profile of donidalorsen between the <65 years and elderly population. Overall, it is agreed that a dose adjustment in elderly patients is not necessary.

Donidalorsen is indicated for adult and adolescent patients (≥12 years of age). In the population PK and PKPD analyses, age was not identified as a significant covariate on donidalorsen PK or PD, although only 6 adolescents were included in the dataset. Nevertheless, it is considered unlikely that donidalorsen PK would differ between adolescents and adults provided body weight is accounted for. Overall, it is agreed that adolescent patients can receive the same dose as adult patients with HAE.

Pharmacokinetic interaction studies

In vitro DDI studies indicated that donidalorsen has a very low potential for involvement in plasma protein binding, CYP-mediated, or transporter-mediated DDIs. In vitro studies also indicated that the THA-linker metabolite M8 has a very low potential for involvement in CYP- and transporter-mediated DDIs. The conducted in vitro DDI studies are sufficient to conclude that clinical DDI studies are not needed.

Pharmacodynamics

Primary pharmacology

Confirmation of the mechanism of action was provided by demonstrating the primary pharmacodynamic effect of donidalorsen in the clinical studies, i.e. reduction in plasma PKK.

Across the clinical studies, donidalorsen showed dose-dependent and sustained reductions in PKK from Baseline in healthy participants (ISIS 721744-CS1) and in patients with HAE (ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, and ISIS 721744-CS7). The long-lasting pharmacologic effect is consistent with the long elimination half-life of donidalorsen.

Secondary pharmacology – Evaluation of QT prolongation

A lack of QT effect has been demonstrated based on in vitro, nonclinical and clinical data with donidalorsen.

Donidalorsen was negative in blocking hERG current at concentrations up to 300 µM (>1,000 times higher than the anticipated human exposure) and no effects of donidalorsen on ECG intervals were seen in preclinical toxicology and safety pharmacology studies in animals, including telemetered cynomolgus monkeys at doses up to 30 mg/kg. In all clinical studies, there were no clinically significant changes in ECG parameters in subjects who received donidalorsen treatment. Further, data for multiple 2'-MOE ASOs support the conclusion that this drug class is unlikely to cause QTc prolongation.

In Study ISIS 721744-CS1, concentration-QTc analysis indicated that donidalorsen has no effect on QT intervals, with exclusion of QTcF effect above 10 ms at dose levels of 20-80 mg. However, a supratherapeutic dose was not evaluated.

Based on in vitro studies, donidalorsen has a very low potential for drug-drug interactions and organ impairment is unlikely to impact donidalorsen exposure because donidalorsen is primarily eliminated by nuclease-mediated degradation. Consistent with this, mild renal and hepatic impairment were not identified as significant covariates in the population PK analysis. Therefore, higher donidalorsen exposure resulting from DDIs or organ impairment can be excluded. Body weight was the most influential covariate impacting donidalorsen PK. A subject at the 10th percentile of the body weight distribution (i.e. 60 kg) is predicted to have a 39% higher Cmax,ss compared to a reference subject of 70 kg. However, in the pivotal study ISIS 721744-CS5, the minimum weight of study patients was 37 kg and there were no notable trends or safety concerns observed for ECG findings.

Overall, the totality of data supports a lack of QT prolongation effect with donidalorsen at therapeutic doses. Lack of conducting a Thorough QTc study is accepted.

Immunogenicity

ADAs against donidalorsen had no impact on peak (Cmax) and total (AUC) plasma exposures, although higher Ctrough was observed in ADA-positive patients. The increase in trough concentrations in ADA-positive patients is consistent with observations in other ASOs. There was no clinically meaningful impact of ADA

positivity on pharmacodynamics (PKK concentrations). These findings suggest that ADAs against donidalorsen are not neutralizing and do not affect pharmacological response.

Pharmacokinetic-Pharmacodynamic and Exposure-Response Analyses

- ***Population PKPD analysis (721744-PPK01)***

The methods used for model development are acceptable. The final model was an indirect response model, where the effect of donidalorsen was modelled as an Imax model inhibiting the production of PKK. GOF plots and VPCs support an adequate fit of the model. The statistically significant covariates included in the model were disease status (HAE patient compared to HV) at baseline and IC50. However, the magnitude of covariate effects is not considered clinically significant. The model is deemed fit for performing simulations and for subsequent use in ER analyses.

- ***Exposure-response relationships for PD biomarker (PKK), select efficacy and safety endpoints (721744-PPK02)***

PD/Efficacy endpoints

Mean percentage reductions of PKK from Baseline at Week 25 were >50% across all donidalorsen exposure tertiles in Study ISIS 721744-CS5. Higher Ctrough,ss and AUC1344h,ss exposures trended towards greater reductions of PKK from Baseline at Week 25. This finding is consistent with the greater reductions in PKK observed for the 80 mg Q4W compared to Q8W regimen, since the former is associated with approximately 2-fold higher Ctrough,ss and AUC1344h,ss.

Similarly, mean percentage reductions from Baseline in HAE attack rate from Week 1 to Week 25 were >60% across all donidalorsen exposure tertiles. Higher Ctrough, ss and AUC1344h,ss exposures trended towards greater reductions in HAE attack rate from Baseline.

Safety endpoints

No substantial differences in safety endpoints were observed across donidalorsen exposure tertiles. No clinically meaningful differences in safety endpoints were observed across exposure tertiles for donidalorsen for moderate or severe TEAEs and clinically laboratory evaluations (liver chemistry, renal function, and platelet count).

- ***Exposure response analysis (721744-PPK03)***

In this analysis, an ER model was developed to evaluate the relationship between plasma PKK and the clinical efficacy endpoint of normalized per 4-week HAE attack rate, based on data from Study ISIS 721744-CS5. A sigmoid Emax model best fitted the data. Baseline per 4-week normalized HAE attack rate and baseline PKK were included as covariates with predictive value for Emax and EC50, respectively. Age, body weight, sex and treatment-emergent ADA status were not identified as significant covariates on the model parameters.

Parameters of the final model were estimated with good precision. GOF plots of the final model showed that IPRED and PRED had good correlation with the overall observed data. VPCs indicated that the predictive performance of the model was not adequate for performing simulations. This is not a major concern since the model was only intended for supportive purposes.

Model parameters indicated that the per 4-week normalized HAE attack rate over time normalized by the baseline rate (i.e. % reduction from the baseline) is largely independent of the baseline per 4-week normalized HAE attack rate or starting PKK levels prior to donidalorsen treatment. This suggests a generally comparable and clinically meaningful response across the patient population.

Dose justification

The proposed starting dose regimen for donidalorsen is 80 mg administered subcutaneously once monthly, with an option of 80 mg once every 2 months for patients who are attack-free for at least 3 months on monthly dosing of donidalorsen. The commercial drug product presentation is donidalorsen 80 mg in a prefilled syringe with an autoinjector, which is supported by the results of the bioequivalence study (ISIS 721744-CS9).

The dosing regimen is supported by the efficacy and safety data in patients with HAE (ISIS 721744-CS5 and ISIS 721744-CS2), the population PK and PK/PD modelling and simulation, and the ER analyses. The desired and favourable ER relationships for pharmacodynamic biomarker (PKK), efficacy, and safety in patients with HAE compared with placebo, together with the modelling and simulation results, support that no dose adjustment is needed with respect to intrinsic or extrinsic factors, including body weight, sex, age (including adolescents), race, ethnicity, injection site, mild renal impairment, or mild hepatic impairment. Available clinical data, together with the lack of age as a significant covariate across the population PK, PD, and ER analyses of HAE attack rate confirms similarity of PK/PD and characteristics across adolescent and adult patient population, justifying the proposed indication and the dose regimen.

A comparison of the once every 4 weeks versus once-monthly and once every 8 week versus once every 2 month dosing regimens showed no meaningful differences in the simulated PK exposure (Cmax, ss, Ctrough, ss, and AUCtau, ss) or PD biomarker (PKK). Therefore, a once-monthly dosing regimen is expected to result in the same safety and efficacy profile as a once every 4 weeks dosing regimen, which supports the dosing regimen proposed in the SmPC (section 4.2).

2.6.4. Conclusions on clinical pharmacology

The clinical pharmacology of donidalorsen is considered sufficiently characterised.

2.6.5. Clinical efficacy

2.6.5.1. Dose response studies

The proposed recommended dosing regimen for donidalorsen for routine prevention of recurrent attacks of HAE in adults and adolescents 12 years of age and older is 80 mg administered by subcutaneous injection once monthly, with an option of changing to 80 mg once every 2 months for patients who are attack free for at least 3 months on monthly dosing of donidalorsen.

The dose level of 80 mg once every 4 weeks was selected based on the safety, tolerability, PK, pharmacodynamic, and immunogenicity data from the ISIS 721744-CS1 study in healthy volunteers, and on the clinical efficacy data of the ISIS 721744-CS2 and ISIS 721744-CS5 study in patients with HAE.

- ISIS 721744-CS1 evaluated doses of donidalorsen at 20, 40, 60, and 80 mg that were administered to healthy volunteers once every 4 weeks for a total of 12 weeks (treatment period). All dose levels were generally well tolerated and induced a dose-and exposure- dependent reduction in plasma PKK. The highest dose level evaluated of 80 mg once every 4 weeks produced a mean decrease in plasma PKK levels of 93.6% from Baseline on Day 99, which was 2 weeks after the last administration.

- ISIS 721744-CS2 evaluated doses of donidalorsen at 80 mg that were administered to patients with HAE-1, HAE-2, and nC1-INH-HAE (previously referred to as type III HAE) once every 4 weeks for a total of 16 weeks. A dose of 80 mg donidalorsen once every 4 weeks resulted in a mean plasma PKK reduction of approximately 60% from Baseline on Day 113. The HAE attack rate was reduced relative to placebo by 90% from Week 1 to Week 17, and by 97% from Week 5 to Week 17. These results support that the dosage of 80 mg administered once every 4 weeks results in a robust reduction of plasma PKK concentration, plasma proenzyme activation (PPA, maximal capacity to generate bradykinin under the activated conditions), and cleaved HMWK (a biomarker for bradykinin release).
- ISIS 721744-CS5 study evaluated doses of donidalorsen at 80 mg that were administered to patients with HAE-1, HAE-2, once every 4 weeks over a 24-week Treatment Period. A dose of 80 mg donidalorsen once every 4 weeks resulted in an 81% reduction in HAE attack rate for the donidalorsen-4 group in comparison to the placebo group.
- In the ISIS 721744-CS2 OLE study, ISIS 721744-CS3, an alternative administration schedule of donidalorsen 80 mg once every 8 weeks was also used to evaluate the efficacy and safety of a reduced administration frequency. Patients treated at a dose of donidalorsen 80 mg once every 8 weeks had a reduction of HAE attack rate by over 80% compared with the ISIS 721744-CS2 Baseline (Run-in Period). Therefore, patients could potentially benefit from the alternative administration regimen that has a reduced administration frequency.
- A second dosing regimen of 80 mg once every 8 weeks was also included in the pivotal study (ISIS 721744-CS5) to evaluate the efficacy and safety of a reduced dosing frequency.
- Both administration schedules (80 mg every 4 weeks and 80 mg every 8 weeks) have been generally well tolerated by patients. Minimal to no correlation was observed between donidalorsen clearance and exposure with body weight when data were combined from studies in healthy volunteers and patients with HAE. These data indicate a fixed dose of 80 mg was appropriate across the entire population enrolled in this current study (Study ISIS 721744-CS5).
- The dosing regimen was also supported by sub-chronic and chronic toxicity studies in mice and monkeys of up to 6- and 9-months administration duration, respectively. For a dosage of 80 mg once every 4 weeks in adult humans (70 kg), the safety margin was approximately 21-fold and 32-fold based on the dose and area under the curve (AUC), respectively, at no-observed adverse- effect level (NOAEL) in the 9-month monkey study.

2.6.5.2. Main study

ISIS 721744-CS5: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)

Methods

This Phase 3 pivotal study was a double-blind, placebo-controlled, randomized study evaluating donidalorsen 80 mg administered as a subcutaneous injection every 4 weeks or every 8 weeks in patients with HAE over a 24-week Treatment Period.

Patients with HAE-1 or HAE-2 were randomly assigned in a 2:1 ratio to Cohort A (donidalorsen 80 mg once every 4 or matched placebo) or Cohort B (donidalorsen 80 mg once every 8 weeks or matched placebo), respectively. Within each cohort, patients were randomly assigned in a 3:1 ratio to receive donidalorsen or a

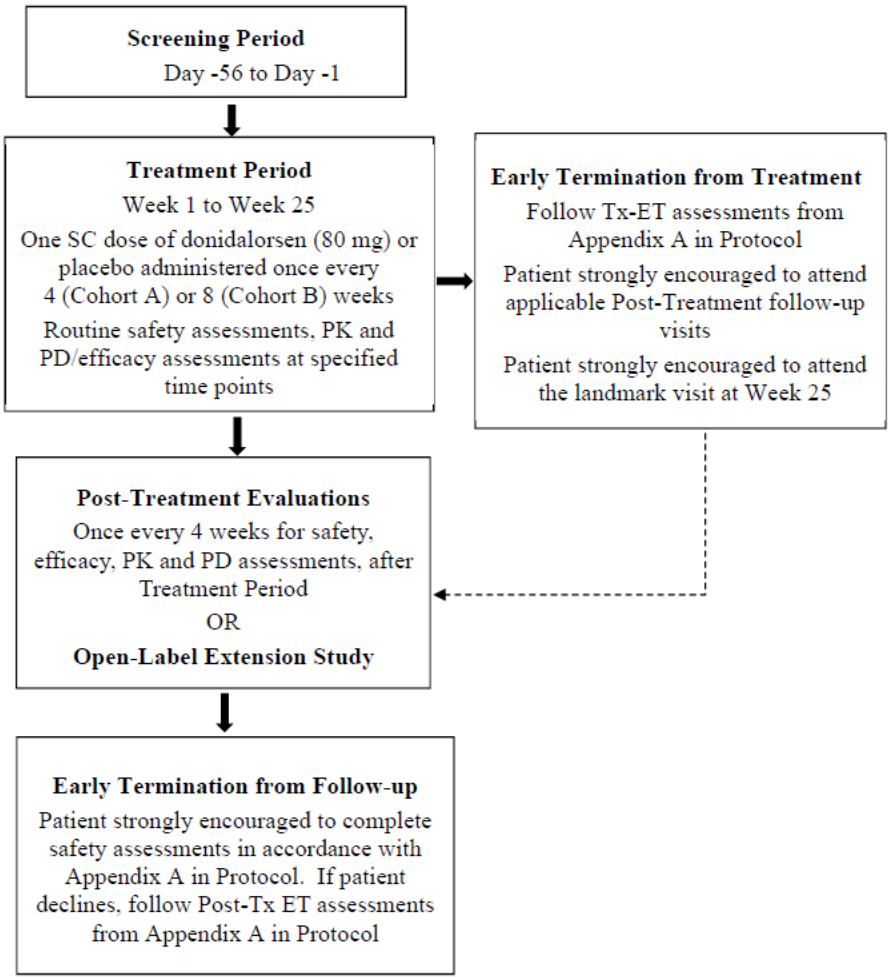
matching volume of placebo. Data from patients receiving placebo in Cohort A and Cohort B were pooled for analyses, this pooled placebo group is hereafter referred to as the placebo group.

The study consisted of 3 periods, including an up to 8-week (56-day) Screening Period (also known as the Run-in Period for HAE attack rate Baseline), a 24-week Treatment Period (also known as the On-Treatment Period), and an up to 13-week Post-Treatment Period. The length of study participation for each patient was up to 11 months. A patient was randomly assigned to treatment as soon as the patient experienced ≥ 2 HAE attacks during the up to 8-week and met all other eligibility requirements.

Patients who experienced at least 5 attacks/month for 2 consecutive months after Week 5 (considered lack of efficacy) had the opportunity to discontinue from treatment in the current study and either progress to the Post-Treatment Period or enroll in the OLE study. Patients not participating in the OLE study entered an up to 13-week Post-treatment Period. Patients who completed Study Visit Week 25, and met eligibility requirements, could enroll in the OLE study any time after the Week 25 visit. If a patient chose to enroll in the OLE study.

During the study, patients were instructed to report details of any HAE attack to the study site within 72 hours of the onset of the attack. Throughout the Screening (Run-in Period), Treatment, and Post-Treatment Periods, site personnel contacted the patients approximately weekly in order to inquire about any attack that may have occurred.

Figure 15. Study schema



- **Study Participants**

Main inclusion criteria:

- Patients must have been aged ≥ 12 years at the time of informed consent and, as applicable, assent.
- Patients must have had a documented diagnosis of HAE-1/HAE-2 based upon ALL of the following:

  a. Documented clinical history consistent with HAE (subcutaneous or mucosal, non-pruritic swelling episodes without accompanying urticaria) (Maurer et al. 2022).

  b. Diagnostic testing results that confirmed HAE-1/HAE-2: C1-INH functional level < 40% normal level. Patients with a functional level of 40% to 50% of normal could be enrolled if their complement factor C4 level was below the lower limit of normal (LLN) or when a known pathogenic mutation in the SERPING1 gene had been demonstrated.

  c. At least 1 of the following: age at reported HAE onset ≤ 30 years; a family history consistent with HAE-1/HAE-2; or complement component 1q within the normal range.

- Patients must experience a minimum of 2 HAE attacks (confirmed by the Investigator) during the Screening Period.
- Had access to, and the ability to use, ≥ 1 acute medication(s) (e.g., plasma derived, or recombinant C1-INH concentrate or a bradykinin 2-receptor antagonist) to treat angioedema attacks.

Main exclusion criteria:

- Concurrent diagnosis of any other type of recurrent angioedema, including acquired, idiopathic angioedema or HAE with normal C1-INH (also known as HAE Type 3).
- Any clinically significant abnormalities in screening laboratory values that would render a patient unsuitable for inclusion in the study. The following laboratory values were exclusionary:

    a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 3 × upper

    limit of normal (ULN).

    b. Total bilirubin > 1.5 × ULN OR, if due to Gilbert's syndrome, > 5 mg/dL.

    c. Platelet count < 130 K/mm3.

    d. For patients ≥ 18 years old: Estimated glomerular filtration rate (eGFR) < 45 mL/min

    e. For patients 12 and < 18 years old: eGFR < 60 mL/min
- Patients with a history of acquired coagulopathies or bleeding diathesis (e.g., thrombocytopenia, disseminated intravascular coagulation, coagulopathy of liver disease, drug-induced platelet dysfunction, hyperfibrinolysis, acquired clotting factor inhibitors) and inherited bleeding disorders (e.g., hemophilia A, hemophilia B, other clotting factor deficiencies, qualitative platelet disorders, inherited thrombocytopenia, vascular abnormalities) and hypercoagulability.
- Any clinically significant renal or hepatic diseases.
- Exposure to any of the following medications:

a. Angiotensin-converting enzyme (ACE) inhibitors or any estrogen containing medications with systemic absorption (such as oral contraceptive or hormonal replacement therapy) within 4 weeks prior to Screening.

b. Chronic prophylaxis with Takhzyro (lanadelumab), Haegarda® (C1-esterase inhibitor SQ), Cinryze and Ruconest (C1 esterase inhibitor) or Orladeyo (berotralstat) within 5 half-lives prior to Screening (i.e., Takhzyro within 10 weeks prior to Screening, Haegarda/Berinert 2000/3000® subcutaneously/Cinryze/Ruconest within 2 weeks prior to screening, Orladeyo within 3 weeks prior to Screening).

c. Oligonucleotides (including small interfering ribonucleic acid [siRNA]) within 4 months of Screening if single administration received, or within 12 months of Screening if multiple doses received. This exclusion did not apply to vaccines.

- **Treatments**

Description of trial intervention

The study drug was to be administered as a single subcutaneous injection every 4 weeks (Cohort A) or every 8 weeks (Cohort B) by blinded study staff during on-site visits at the Study Centers or by a Home Healthcare professional. Self-administration of donidalorsen or placebo was allowed after administration instructions and

training were provided by qualified site personnel and a home healthcare nurse was available to complete all predose procedures.

The study drug was contained in 2 mL stoppered glass vials. Vials of study drug were for single use only. Batch CP721744-002 (donidalorsen) and batch CPPLAC-033 (placebo) were used in the study.

Concomitant and rescue therapies

Prohibited concomitant medications

- Chronic prophylaxis for angioedema attacks, except for a stable dose of androgens or tranexamic acid. Any use of other HAE prophylactic agents such as lanadelumab or berotralstat was not permitted during the Treatment Period.

- ACE inhibitors or any estrogen-containing medications with systemic absorption (such as oral contraceptive or hormonal replacement therapy)

- Any oligonucleotides (including siRNA) other than donidalorsen

- Any other investigational drug or device

Permitted concomitant medications - rescue therapies

During the course of the study, the use of acute HAE medications (plasma-derived or recombinant C1-INH concentrates, bradykinin 2-receptor antagonists, or kallikrein inhibitors) to treat angioedema attacks was allowed, as medically indicated. Patients were allowed to be treated with on-demand therapy as determined by their treating physician.

All other stable medications (if not excluded above) were allowed, so long as the dose and type was not expected to change during the study.

- **Objectives**

Primary Objective

Evaluate the clinical efficacy of donidalorsen in patients with HAE

Secondary Objective

Evaluate the effects of donidalorsen (donidalorsen 80 mg every 4 weeks or every 8 weeks) on the quality and pattern of HAE attacks and their impact on QoL

- **Outcomes/endpoints**

Note: SmPC section 5.1 presents results from week 0 to week 24, corresponding to week 1 to week 25 in this report

Primary endpoint

The primary endpoint was the time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25 compared to placebo for the donidalorsen 80 mg once every 4 weeks group.

Secondary endpoints

1. Comparison of the time-normalized number of Investigator-confirmed HAE attacks (per every-4-week) from Week 5 to Week 25 between ISIS 721744 80 mg every-4-weeks group and pooled-placebo group

2. Comparison of the number of patients with a ≥ 70% reduction from baseline in Investigator-confirmed HAE attacks (per every-4-week) from Week 5 and Week 25 between ISIS 721744 80 mg every-4-weeks group and pooled-placebo group
3. Comparison of the percentage of Investigator-confirmed HAE attack-free patients from Week 5 to Week 25 between ISIS 721744 80 mg every-4-weeks group and pooled-placebo group
4. Comparison of the time-normalized number of moderate or severe Investigator-confirmed HAE attacks (per every-4-week) from Week 5 to Week 25 between ISIS 721744 80 mg every-4-weeks group and pooled-placebo group
5. Comparison of the number of Investigator-confirmed HAE attacks requiring acute therapy (per every-4-week) from Week 5 to Week 25 between ISIS 721744 80 mg every-4-weeks group and pooled-placebo group
6. Comparison of the change in AE-QoL total score at Week 25 between ISIS 721744 80 mg every-4-weeks group and pooled-placebo group
7. Comparison of the time-normalized number of Investigator-confirmed HAE attacks (per every-4-week) from Week 1 to Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group
8. Comparison of the time-normalized number of Investigator-confirmed HAE attacks (per every-4-week) from Week 5 to Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group
9. Comparison of the number of patients with a ≥ 70% reduction from baseline in Investigator-confirmed HAE attacks (per every-4-week) from Week 5 and Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group
10. Comparison of the percentage Investigator-confirmed HAE attack-free patients from Week 5 to Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group
11. Comparison of the time-normalized number of moderate or severe Investigator-confirmed HAE attacks (per every-4-week) from Week 5 to Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group
12. Comparison of the number of Investigator-confirmed HAE attacks requiring acute therapy (per every-4-week) from Week 5 to Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group
13. Comparison of the change in AE-QoL total score at Week 25 between ISIS 721744 80 mg every-8-weeks group and pooled-placebo group

- **Sample size**

The power and sample size estimations were calculated using simulations based on a generalized linear model for count data assuming a Poisson distribution. The primary endpoint was the time-normalized number of Investigator-confirmed HAE attacks (per 4 week) from Week 1 to Week 25. Assuming an HAE attack rate of 13.26 attacks per 6-month period in the placebo group and an HAE attack rate of 1.38 attacks per 6-month period in the donidalorsen-4 group, the sample size of 54 patients (2:1 ratio) would provide at least 90% power for the primary endpoint, with a 0.05 significance level. A total of approximately 84 patients (42 per 4 weeks group, 21 in the placebo group, and 21 in the donidalorsen-8 group) were planned to be enrolled in this study to account for potential early dropouts and to facilitate some general safety evaluations.

- **Randomisation and Blinding (masking)**

Patients were randomly assigned via the Interactive Response Technology (IRT) system to subcutaneous injections of 80 mg donidalorsen or placebo. Approximately 56 patients were planned to be randomly assigned to once-every-4-week administration (Cohort A) and 28 patients were planned to be randomly assigned to once-every-8-week administration (Cohort B). Within each cohort, patients were randomized in a 3:1 ratio to receive 80 mg donidalorsen or identical volume and appearance of placebo. For purposes of planned analyses, the placebo patients from each cohort were for the comparison with donidalorsen-treated patients.

The Sponsor and all patients, monitors, and Study Center personnel related to the study (except for the DSMB and the unblinded statistical/programming support staff) remained blinded throughout the study until all patients had completed the study and the database had been locked.

If a patient had suffered an SAE, and/or when knowledge of the treatment assignment would have impacted the clinical management of the patient, the Investigator had the ability to unblind the treatment assignment for that patient via the IRT system.

Specific employees of the Sponsor were unblinded after the last patient had completed the end of the Treatment Period (Week 25/Treatment Early Termination) as described in the Unblinding Plan. Any Sponsor representatives who were unblinded during the study were not involved in the conduct of the study after becoming unblinded.

- **Statistical methods**

Planned analyses

The following hypothesis was tested for the primary efficacy endpoint:

H_0: $\lambda_{\text{ISIS 721744}} / \lambda_{\text{placebo}} = 1$ versus H_1: $\lambda_{\text{ISIS 721744}} / \lambda_{\text{placebo}} \neq 1$

$\lambda_{\text{ISIS 721744}}$ refers to the Investigator-confirmed HAE attack rate from Week 1 to Week 25 in the ISIS 721744 80 mg every 4 weeks group and λ_{placebo} refers to the Investigator-confirmed HAE attack rate during the treatment period in the placebo group.

The null hypothesis is that the Investigator-confirmed HAE attack rate ratio from Week 1 to Week 25 is 1 (no difference between treatment groups) versus the alternative hypothesis that the Investigator-confirmed HAE attack rate ratio is not 1. Estimated attack rate ratios less than 1 would indicate that patients treated with ISIS 721744 80 mg every 4 weeks, on average, have a lower incidence of Investigator-confirmed HAE attacks from Week 1 to Week 25. The hypothesis was tested using a Poisson regression model.

The primary analysis was performed using a Poisson regression model and Pearson chi-square scaling of standard errors to account for potential overdispersion on the FAS. The model included fixed effect for treatment group (categorical), baseline (the time-normalized run-in period attack rate, continuous), and the treatment-by-baseline interaction as covariates, and the logarithm of time in every-4-week that each patient was observed from Week 1 to Week 25 was used as an offset variable.

All patients who discontinued the Treatment Period early and started OLE, and patients who discontinued the Treatment Period early and were lost to follow-up before Week 25, had their time-adjusted attack rate included in the analysis.

From this model, the least squares mean rate, the standard error and the corresponding 95% confidence interval for each treatment group as well as, the model adjusted mean rate ratios of ISIS721744 80 mg

every 4 weeks relative to the pooled placebo group and the corresponding 95% confidence interval were estimated. These estimates were reported as mean event rates per every-4-week by transforming the estimates using the exponential function. The p-value of the Wald-based chi-square test was reported for testing the hypothesis.

The percentage difference in mean Investigator-confirmed HAE attack rate between ISIS 721744 80 mg every 4 weeks and the pooled placebo was calculated as 100% × (model adjusted mean rate ratio –1). Similarly, the estimated upper and lower confidence limits for the model adjusted mean rate ratio can be transformed by subtracting 1 and multiplying by 100% to calculate 95% confidence intervals for the percentage change.

The comparison between ISIS 721744 80 mg every-8-weeks group and the pooled-placebo group in the time-normalized number of Investigator-confirmed HAE attacks (per every-4-week) from Week 1 to Week 25 was analysed in the same way including sensitivity analyses and supportive analyses discussed below.

Sensitivity Analysis 1 – Negative Binomial (NB) Regression

A sensitivity analysis was performed by fitting a NB regression (Negative Binomial regression) model, which allows the variance in a different function of the mean. The model included fixed effects for treatment group (categorical), baseline (the time normalized run-in period attack rate, continuous), and the treatment-by-baseline interaction as covariates, and the logarithm of time in every-4-week that each patient was observed will be used as an offset variable.

All patients who discontinued the Treatment Period early and started OLE, and patients who discontinued the Treatment Period early and were lost to follow-up before Week 25, had the time-adjusted attack rate included in the analysis.

Supportive Analysis – Per-Protocol Set (PPS)

Primary analysis and sensitivity analysis 1 described above were repeated on the PPS.

Sensitivity Analysis 3 – Multiple Imputation Incorporating Pattern Mixture Model

For patients who discontinued the Treatment Period early and started OLE, and patients who discontinued the Treatment Period early and lost to follow-up before Week 25, the primary analysis assumed that the missing data is MAR. To evaluate the robustness of inferences made under MAR, a sensitivity analysis on FAS was performed based on imputed data by using MI method assuming J2R. For each imputed data set, the Poisson regression model used for the primary analysis was performed. Results were combined using Rubin's rules.

Sensitivity Analysis 4 – Tipping-Point Analysis

A tipping-point analysis was conducted to measure the potential effect of missing data on the reliability of the primary efficacy analysis.

In this analysis, a range of progressively more conservative assumptions about the number of events occurring in the post-withdrawal period was explored in order to find the assumption which reversed the conclusion (i.e., yield a non-significant p-value) of the primary analysis.

The assumption that reverses the conclusion is referred to as the tipping point. Once the tipping point is identified, the clinical plausibility of the assumption was assessed.

Missing event rate was imputed by using the MI method described below. In each run, the Poisson regression model used for the primary analysis will be performed. Results were combined using Rubin's rules.

The range of assumptions was to be determined after treatment unblinding to ensure an appropriate range is explored based on the magnitude of treatment effect and pattern of missing data.

Handling of missing data

Missing primary and secondary endpoints data were handled as following:

• For time-normalized Investigator-confirmed HAE attack endpoints, the primary analysis for the primary endpoint and the main analyses for secondary endpoints had the time-adjusted attack rate included in the analysis; sensitivity analyses were performed based on imputed data by using multiple imputation (MI) methods assuming Jump-to-Reference (J2R).

• For number of patients with a clinical response on HAE and HAE attack-free, responder was derived based on time-adjusted Investigator-confirmed HAE attack rate per every-4-week. The main analysis considered the HAE attack rate per every-4- week are the same before and after withdrawal; sensitivity analysis was performed based on imputed data by using the MI method assuming J2R.

• For the AECT total score, the main analysis was performed based on imputed data by last observation carried forward (LOCF). Additionally, sensitivity analyses were performed based on imputed data by using MI methods assuming J2R.

• For the AE-QoL total score, main analysis was performed by using MMRM model without missing data imputation; sensitivity analyses were performed based on imputed data by using MI methods assuming J2R.

Multiple Imputation – Investigator-confirmed HAE attack

Patients with the study termination date earlier than 25 weeks since the Study Day 1 had two portions of treatment period, the pre-withdrawal part and the post-withdrawal part.

Investigator-confirmed HAE attacks observed in the study were combined with the imputed data to estimate the event rates for each treatment group. Missing data during the post-withdrawal part were imputed by the following methods assuming J2R.

First, the Poisson regression model used for the primary analysis were fitted to the data using a Bayesian approach, with non-informative priors for the mean and variance-covariance matrix to provide a joint posterior for the parameters in this model.

Second, independent samples were then drawn from the posterior distributions of model parameters fit using the Bayesian analysis. For each patient with missing data during the post-withdrawal part, these sampled values of the model parameters were then used to generate a set of values for the expected unobserved attack.

Last, the expected number of attacks for the post-withdrawal part is added to the number of attacks in the pre-withdrawal part to get the total number of attacks over treatment period for patients who have missing data.

The post-withdrawal part of each pattern-specific distribution may be modelled using approaches discussed below:

Jump-to-Reference (J2R)

The J2R approach is a conservative imputation approach that assumes that a patient receiving active Study Drug (ISIS 721744) does not sustain benefit after discontinuing study treatment. In J2R, missing event rate

in both the ISIS 721744 treatment group and the pooled placebo group will be imputed by setting to the event rate of the pooled placebo group during the pre-withdrawal part.

Tipping Point

The tipping point approach progressively shifts the imputed analysis results towards a point where the analysis conclusion is reversed (i.e., the analysis yields a nonsignificant p-value). The study adopted a two-way tipping point approach where a series of shift (denoted as δ) is applied to both active Study Drug (ISIS 721744) and placebo treatment arm. As the underlying distribution of primary endpoint is a Poisson distribution, the parameter of Poisson distribution (denoted as λ) is adjusted by δ as

follows:

$$\lambda_{\text{(ISIS 721744, tipping point)}} = \lambda_{\text{(ISIS 721744, MAR-imputed)}} \times \delta_{\text{(ISIS 721744)}}$$

$$\lambda_{\text{(Placebo, tipping point)}} = \lambda_{\text{(Placebo, MAR-imputed)}} \times \delta_{\text{(ISIS 721744)}}$$

The sequences of both λ starts at 1, where $\lambda_{\text{(ISIS 721744, tipping point)}}$ is increased by 0.25 upwards and $\lambda_{\text{(Placebo, tipping point)}}$ goes backwards to 0. The increment of 0.25 may be subject to adjustment in case the tipping points are not yet to be discovered. The upper limit may expand further in case the 'tipping-point' that yields non-significant conclusion has not appeared yet. Under each combination of $\lambda_{\text{(ISIS 721744, tipping point)}}$ and $\lambda_{\text{(Placebo, tipping point)}}$, the same analysis method as Missing at Random (MAR) is applied to acquire the p-value of the analysis. For example, if there are 6 numbers (from 1 to 2.25 by 0.25) set as δ, there should be in total 36 corresponding p-values in the whole tipping point analysis.

For each imputation method used, at least 100 imputed datasets will be generated.

Multiple Imputation Jump-to-Reference (J2R) – AECT Total Score and AE-QoL Total Score

The Markov Chain Monte Carlo (MCMC) method was used under the multivariate normality assumption to impute the missing AE-QoL total score and AECT total score by treatment group. The variable list for imputations included the baseline score, as well as all available post-baseline scores within the treatment period, in the order of protocol defined visits. The SAS procedure PROC MI was used in the multiple simulation. EM algorithm was used to derive a set of initial parameter estimates for MCMC method. A non-informative prior (Jeffreys prior) was used to derive the posterior distribution of the parameters. In case there were missing baselines, it was to be imputed first by the average of available baselines across all patients in the FAS. In the case of non-monotone missing pattern, MCMC method will be used first so that each imputed data set has a monotone missing pattern based on the order of the variable list.

The J2R approach is detailed in (Carpenter *et al.* 2013) and is a conservative imputation approach that assumes that a patient receiving active Study Drug (ISIS 721744) does not sustain benefit after discontinuing study treatment. In J2R, missing data in the pooled placebo group will be imputed under a within treatment arm MAR assumption. For a patient with missing data in the ISIS 721744 treatment group, their AE-QoL total score or AECT total score distribution is set to equal that of the pooled placebo group. For each imputation method used, at least 100 imputed datasets will be generated.

Last Observation Carried Forward (LOCF) – AECT Total Score

For each subject, the missing AECT total score at Week 25 will be imputed by the available value at the nearest previous post-baseline visit. For example, if a subject early withdraws at Week 13, the subject's Week 25 will be imputed by the available total score at Week 13.

Missing Dates for Investigator-confirmed HAE attacks

Missing start or end date and time for Investigator-confirmed HAE attacks were imputed. In general, missing start time were to be imputed as 0:00 and missing end time were to be imputed as 23:59. However, the following rules were to be applied for the attacks satisfying the corresponding conditions, in order to conservatively classify the attacks as separate, distinct attacks with at least 24 hours in between:

• For Investigator-confirmed HAE attacks with a missing start time and a non-missing start date one calendar day after the end date of the previous attack, the start time was to be imputed using the end time of the previous Investigator-confirmed HAE attack to ensure there are 24 hours in between the 2 attacks.

• For Investigator-confirmed HAE attacks with a missing start time and a non-missing end date/time within 24 hours from the end date/time of the previous attack, the attack should be considered as one attack with the previous attack

• For Investigator-confirmed HAE attacks with missing end time and a non-missing end date one calendar day before the start date of the next attack, the end time was to be imputed as the start time of the next Investigator-confirmed HAE attack to ensure there are 24 hours in between the 2 attacks.

• For Investigator-confirmed HAE attacks with a missing end time and non-missing start date/time within 24 hours from the start date/time of the next attack, the attack should be considered as one attack with the next attack.

Planned subgroup analyses

Exploratory subgroup analyses were to be performed on the primary efficacy endpoint for age, sex, race, regions, and if the patient had taken any predefined prophylactic therapy.

The subgroups include the following:

- Age Category (12 to 17, 18 to 39, 40-64, ≥ 65)
- Sex (Male, Female)
- Race (Caucasian, Other)
- Regions (North America, Europe, Middle East)
- Taken any predefined prophylactic therapy, including lanadelumab, berotralstat, C1- esterase inhibitor? (Yes, No)

In addition, exploratory subgroup analyses were to be conducted on the primary endpoint and other preselected efficacy endpoints if any patient was considered to have a clinical history and/or screening period/Baseline HAE attack rate that is atypical for patients with HAE-1 or HAE-2 and that may compromise the interpretation of the efficacy data ("atypical patients"); "atypical patients" were to be determined by the Medical Monitor and/or Investigators. Any "atypical patients" were to be identified prior to unblinding the data. The exploratory subgroup analyses would be based on "typical patients" (i.e., excluding "atypical patients") using the same methods as described for the efficacy endpoint for the Full Analysis Set.

Error probabilities, adjustment for multiplicity and interim analyses

Multiple endpoints

Multiplicity due to multiple endpoints was controlled by using a hierarchical ranking strategy in the order presented above. Should the null hypothesis for the primary efficacy endpoint be rejected, other endpoints

were to be tested in order. If any test is not statistically significant, the test(s) at the lower rank were considered exploratory. Other statistical analyses planned in the SAP but not listed below were considered as exploratory analyses with no adjustments for multiplicity. All tests were conducted at a two-sided alpha level of 0.05.

Interim Analyses

No interim analysis was planned for this study. Primary analyses were conducted after the End of Treatment period when all patients had completed Week 25 visit, stayed in the study for at least 166 days since the Study Day 1 or withdrawn from the study before Week 25 visit. Study results with continuing collected post-treatment data was updated at the End of Study.

Results

- **Participant flow**

Of the 116 patients who were screened, 91 patients met the eligibility criteria and were randomly assigned to treatment (46 patients in the donidalorsen-4 group, 23 patients in the donidalorsen-8 group, and 22 patients in the placebo group). One (1) patient in the donidalorsen-4 group withdrew consent prior to receiving study drug.

A summary of patient disposition is provided in Table 10 below.

Table 10. Patient disposition (All screened patients)

Patient Disposition	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 weeks (N = 46) n (%)	Donidalorsen 80 mg Every 8 weeks (N = 23) n (%)	All Patients (N = 116) n (%)
Patients Randomized[a]	22	46	23	91
Patients Dosed[b]	22 (100)	45 (97.8)	23 (100)	90 (98.9)
Patients Who Completed the Study Treatment	18 (81.8)	44 (95.7)	21 (91.3)	83 (91.2)
Patients Who Terminated Early from the Study Treatment	4 (18.2)	2 (4.3)[b]	2 (8.7)	8 (8.8)
Primary Reason for Early Termination of Study Treatment				
Voluntary Withdrawal	0	1 (2.2)[b]	0	1 (1.1)
Pregnancy	1 (4.5)	0	0	1 (1.1)
Adverse Event or SAE	0	0	1 (4.3)[d]	1 (1.1)
Lack of Efficacy[c]	3 (13.6)	1 (2.2)	1 (4.3)	5 (5.5)
Patients Who Completed Post-Treatment Follow-up	0	1 (2.2)	3 (13.0)	4 (4.4)
Patients Who Terminated Early from Post-Treatment Follow-up	22 (100)	45 (97.8)	20 (87.0)	87 (95.6)

Patient Disposition	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 weeks (N = 46) n (%)	Donidalorsen 80 mg Every 8 weeks (N = 23) n (%)	All Patients (N = 116) n (%)
Primary Reason for Early Termination from Post-Treatment Follow-up				
Voluntary Withdrawal	2 (9.1)	1 (2.2)	0	3 (3.3)
Pregnancy	1 (4.5)	0	0	1 (1.1)
Rolled over to OLE arm of ISIS 721744-CS7	19 (86.4)	44 (95.7)	20 (87.0)	83 (91.2)

Source: Table 14.1.2

[a] The denominator of the percentages in the table are the number of randomized patients.

[b] One patient in the donidalorsen-4 group withdrew consent prior to receiving study drug.

[c] Lack of efficacy was defined per protocol as patients who experienced at least 5 HAE Investigator-confirmed attacks per month for 2 consecutive months after Week 5. These patients terminated treatment early in the current study and enrolled into the OLE arm of Study ISIS 721744-CS7.

[d] One patient [redacted] in the donidalorsen-8 group discontinued due to an AE of ALT increased. Although the stopping rule was not met by [redacted] in the donidalorsen-8 group, study drug was permanently discontinued due to the AE of ALT increased per Investigator's decision.

Abbreviations: AE = adverse event; ALT = alanine aminotransferase; HAE = hereditary angioedema; OLE = open-label extension; SAE = serious adverse event(s)

- **Recruitment**

First Patient Screened: 25 January 2022

First Patient Dosed: 10 March 2022

Last Patient Enrolled: 07 June 2023

Last Patient, Last Dose: 17 October 2023

Last Patient, Last Visit: 09 November 2023

Database Lock Date: 18 December 2023

- **Conduct of the study**

Amendments

The protocol was amended twice.

Table 11. Changes by protocol amendment

Protocol Version (Date) • Change/Rationale for Amendments
Amendment 1 (01 Oct 2021) • Secondary and exploratory objectives were summarized into more cohesive objectives because details were already provided in the endpoints. • Added the inclusion and rationale for of a second dosing Cohort with a dose frequency of per 8 weeks (donidalorsen 80 mg once per 8 weeks). • Additional patients were added to the study with a new dosing cohort (donidalorsen 80 mg every 8 weeks). • Updated exclusion criteria regarding total bilirubin: Total bilirubin > 1.5 × ULN OR, unless if due to Gilbert's syndrome, (> 5 mg/dL). • A specific threshold (< 130,000/mm^3) for platelet exclusion was added. • The exclusion criteria related to estimated glomerular filtration rate (eGFR) was updated to include appropriate age-dependent formulas and to ensure exclusionary eGFR values were appropriate for each age group. • A clarification was added to the collection of HAE attack details that confirmed HAE attacks were based on symptoms and Investigator diagnosis, not on presence of symptoms alone. • Safety stopping rules for platelet count and actions in patients with confirmed low platelet count were updated to increase monitoring frequency for platelets for counts between 100,000 /mm^3 and 125,000/mm^3 and added requirement for Sponsor approval for continued dosing when platelets were ≥ 75,000/mm^3 to ≤ 100,000/mm^3. • The definition of clinically relevant non-major bleeding events was updated to the most current version (Kaatz et al., 2015). • Added a safety monitoring plan.

Protocol Version (Date) • Change/Rationale for Amendments
• Updated the Safety and PK population definitions to include randomized patients. • The primary efficacy analysis was updated to reflect that the primary analysis was to compare between the 80 mg donidalorsen-4 group versus placebo group. • The recall period for the WPAI questionnaire was 7 days. Therefore, this assessment was completed by the patient weekly throughout the entire study rather than intermittently as specified in the original protocol. Appropriate changes were reflected in Appendix A Schedule of Procedures. • Added directions to follow if urine pregnancy test was positive in Schedule of Procedures. • ECG-related assessment changes were added to the Schedule of Procedures including predose and 2 hours after dose. • Updated Schedule of Procedures by adding a physical exam at every visit to examine for reactions due to study drug administration. • Updated Schedule of Procedures and PK Sampling Schedule to reflect the new dosing groups. • The time points for PK sampling were accidentally inverted in the original protocol. Study Drug administration did not occur at Week 25 and therefore, the time points for PK sampling at Weeks 21 and 25 were switched. Also added 2-hours post-dosing at Week 21 only for Cohort A and at Week 17 only for Cohort B. • Added the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Sept. 2007 to indicate how AEs were graded.
Amendment 2 (12 Jul 2022) • Added a clarification on how many attacks were required for a patient to be randomized i.e., ≥ 2 HAE attacks. • Changed inclusion criteria 6 and 7 and contraception requirements to include the use of acceptable instead of highly effective contraceptive methods. • Added a clarification to the exclusion criteria to exclude patients with alcohol or drug abuse. • Added a clarification to contraception requirements to note that the exclusion of combined estrogen and progesterone hormonal contraception was intended for oral hormonal therapy, and not for intrauterine or intravaginal estrogens. • Updated the UPCR to a more accurate lab measure: UPCR > 1000 mg/g confirmed a quantitative total urine protein measurement of > 1.0 g/24 hours. • Added a clarification that the final analysis was to be performed before the follow-up period was completed. • Deleted reference that dose reductions were allowed from the protocol.

Abbreviations: AE = adverse event(s); ECG = electrocardiogram; HAE = hereditary angioedema; IRT = Interactive Response Technology; PK = pharmacokinetics; SAP = statistical analysis plan; UPCR = urine protein-creatinine ratio; WPAI = Work Productivity and Impairment

- **Baseline data**

Demographic and Baseline characteristics were generally similar across the 3 treatment groups and the study population was representative of the target population.

Across all treatment groups, the majority of patients were White (93.3%, 95.7%, and 81.8% for the donidalorsen-4, donidalorsen-8, and placebo groups, respectively).

Mean body mass index (BMI) was high across all treatment groups (28 and 27 kg/m2 for the donidalorsen-4 and donidalorsen-8 groups, respectively, and 29 kg/m2 for the placebo group).

Table 12. Summary of patient demographics and baseline characteristics (Full analysis set)

Demographics/Baseline Characteristics Category/Statistic	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 45) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	All Patients (N = 90) n (%)
Age (years)				
Mean (SD, SEM)	35.4 (11.03, 2.35)	39.6 (15.23, 2.27)	34.1 (13.22, 2.76)	37.2 (13.88, 1.46)
Median (P25, P75)	33.0 (27.0, 44.0)	39.0 (29.0, 52.0)	33.0 (24.0, 45.0)	35.5 (27.0, 48.0)
Min, Max	21, 59	12, 68	12, 66	12, 68
Mean 95% CI	30.52, 40.30	34.98, 44.13	28.41, 39.85	34.25, 40.06
Age Group (years)				
12 to 17	0	4 (8.9)	3 (13.0)	7 (7.8)
18 to 39	15 (68.2)	19 (42.2)	12 (52.2)	46 (51.1)
40 to 64	7 (31.8)	21 (46.7)	7 (30.4)	35 (38.9)
≥ 65	0	1 (2.2)	1 (4.3)	2 (2.2)
Sex				
Male	14 (63.6)	17 (37.8)	11 (47.8)	42 (46.7)
Female	8 (36.4)	28 (62.2)	12 (52.2)	48 (53.3)

HAE History

Overall, the HAE history was generally similar across the 3 treatment groups with the exception of an imbalance in the historical number of HAE attacks over the prior 12-month period between the placebo and donidalorsen treatment groups.

A history of laryngeal angioedema attacks experienced was reported in 52.2% of patients; however, the majority of patients experienced peripheral (94.4%) or abdominal (94.4%) attacks.

Similar to what was observed for the historical number of HAE attacks, the mean (SD; median) HAE attack rate was lower for the placebo group during the Run-in Period per 4 weeks (see Table 13 below). This disparity may indicate that patients who were randomly assigned to placebo had more mild disease as determined by HAE attack rate/frequency of attacks than those assigned to donidalorsen treatment groups.

Table 13. HAE history (Full analysis set)

	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 45) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	All Patients (N = 90) n (%)
Family History of HAE (n [%])				
Yes	17 (77.3)	41 (91.1)	20 (87.0)	78 (86.7)
No	5 (22.7)	4 (8.9)	3 (13.0)	12 (13.3)
The Number of Attacks in the Past 12 Months				
Mean (SD, SEM)	29.1 (21.13, 4.50)	45.7 (43.04, 6.42)	33.3 (21.95, 4.58)	38.5 (34.57, 3.64)
Median (P25, P75)	24.0 (12.0, 47.0)	36.0 (15.0, 57.0)	36.0 (14.0, 40.0)	35.0 (14.0, 48.0)
Min, Max	1, 75	0, 208	1, 80	0, 208
Mean 95% CI	19.77, 38.50	32.74, 58.60	23.77, 42.75	31.22, 45.70
Historical C1-INH Activity (%)				
N	19	26	12	57
Mean (SD, SEM)	26.57 (16.065, 3.686)	25.42 (12.816, 2.513)	24.67 (10.120, 2.922)	25.65 (13.301, 1.762)
Median (P25, P75)	28.00 (17.00, 39.00)	27.00 (17.00, 33.00)	24.50 (16.00, 32.50)	26.00 (17.00, 33.00)
Min, Max	2.9, 53.0	4.0, 56.0	9.0, 40.0	2.9, 56.0
Mean 95% CI	18.831, 34.317	20.247, 30.600	18.236, 31.097	22.118, 29.177
Years from Diagnosis to Informed Consent				
Mean (SD, SEM)	18.2 (10.18, 2.17)	18.9 (13.15, 1.96)	21.5 (12.43, 2.59)	19.4 (12.24, 1.29)
Median (P25, P75)	18.0 (13.0, 22.0)	17.0 (10.0, 25.0)	19.0 (10.0, 35.0)	17.0 (10.0, 26.0)
Min, Max	2, 36	0, 52	0, 40	0, 52

	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 45) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	All Patients (N = 90) n (%)
Mean 95% CI	13.67, 22.70	14.94, 22.84	16.10, 26.85	16.81, 21.94
Age at Onset (Years)				
N	21	45	23	89
Mean (SD, SEM)	12.9 (7.19, 1.57)	11.6 (9.85, 1.47)	10.9 (7.73, 1.61)	11.7 (8.70, 0.92)
Median (P25, P75)	10.0 (7.0, 18.0)	10.0 (5.0, 15.0)	9.0 (6.0, 14.0)	10.0 (6.0, 15.0)
Min, Max	2, 26	1, 58	2, 33	1, 58
Mean 95% CI	9.59, 16.13	8.64, 14.56	7.53, 14.21	9.87, 13.54
HAE Type (n [%])				
HAE-1	20 (90.9)	42 (93.3)	22 (95.7)	84 (93.3)
HAE-2	2 (9.1)	3 (6.7)	1 (4.3)	6 (6.7)
Types of Attacks Experienced (n [%])				
Peripheral	20 (90.9)	43 (95.6)	22 (95.7)	85 (94.4)
Abdominal	20 (90.9)	43 (95.6)	22 (95.7)	85 (94.4)
Laryngeal	12 (54.5)	22 (48.9)	13 (56.5)	47 (52.2)
Any Predefined Prophylactic Therapy Use[b] (n [%])				
Yes	5 (22.7)	6 (13.3)	5 (21.7)	16 (17.8)
No	17 (77.3)	39 (86.7)	18 (78.3)	74 (82.2)
Run-In Period HAE Attack Rate (attacks/4-weeks)[a]				
Mean (SD, SEM)	2.90 (1.657, 0.353)	3.61 (2.236, 0.333)	3.18 (2.147, 0.448)	3.33 (2.086, 0.220)
Median (P25, P75)	2.50 (1.71, 3.20)	3.29 (1.91, 4.67)	2.71 (1.30, 4.20)	2.85 (1.71, 4.31)
Min, Max	1.0, 8.1	0.5, 10.0	0.7, 8.4	0.5, 10.0
Mean 95% CI	2.164, 3.633	2.943, 4.287	2.254, 4.111	2.892, 3.766
Run-In Period HAE Attack Rate (n [%])				
≤ 2 attacks/4-weeks	6 (27.3)	12 (26.7)	10 (43.5)	28 (31.1)
2 to 5 attacks/4-weeks	14 (63.6)	25 (55.6)	9 (39.1)	48 (53.3)
> 5 attacks/4-weeks	2 (9.1)	8 (17.8)	4 (17.4)	14 (15.6)

Source: Table 14.1.4.1

[a] The Run-in Period (Screening) HAE attack rate for each patient was calculated as the number of Investigator-confirmed HAE attacks occurring during the Run-in Period divided by the number of days the patient contributed to the Run-in Period and then multiplied by 28.

[b] First-line prophylactic agents and excluded attenuated androgens and tranexamic acid.

Prior and Concomitant Medications

Overall, prior and concomitant medication use was similar across the 3 treatment groups.

Prior medication

The majority of patients received at least 1 prior medication, including 44 (97.8%) patients in the donidalorsen-4 group, 23 (100%) in the donidalorsen-8 group, and 21 [95.5%] in the placebo group. The most common prior medications taken by ≥ 20% of patients in at least 1 treatment group by preferred term included medications for use in HAE such as complement C1 esterase inhibitors (48.9%, 56.5%, and 31.8% in the donidalorsen-4, donidalorsen-8, and placebo groups, respectively), icatibant acetate (46.7%, 39.1%,

and 50.0%, respectively), and icatibant (13.3%, 21.7%, and 22.7%, respectively), as well as paracetamol (20.0%, 13.0%, and 4.5%, respectively).

Concomitant medication

The majority of patients in the study received at least 1 concomitant medication (41 [91.1%] in the donidalorsen-4, 21 [91.3%] in the donidalorsen-8, and 22 [100%] in the placebo groups).

The most common concomitant medications taken by ≥ 20% of patients in at least 1 treatment group during the study included the short-term use of permitted rescue medications such as complement C1 esterase inhibitors (37.8%, **52.2%,** and 50.0% in the donidalorsen-4, donidalorsen--8, and placebo groups, respectively), icatibant acetate (35.6%, 39.1%, and 50.0%, respectively), and icatibant (22.2%, 13.0%, and 27.3%, respectively). Other concomitant medications included paracetamol (24.4%, **34.8%,** and 31.8%, respectively) and ibuprofen (22.2%, **34.8%**, and 22.7%, respectively).

Measurements of Treatment Compliance

The mean treatment compliance was similar across the 3 treatment groups (99.44%, 98.55%, and 101.52% for the donidalorsen-4, donidalorsen-8, and placebo groups, respectively).

- **Numbers analysed**

The Full Analysis Set, which was used for efficacy analysis, consisted of 90 patients who were randomly assigned and treated with at least 1 dose of donidalorsen or placebo. The Safety Set, Per Protocol Set, and PK Set consisted of 90 (98.9%), 84 (92.3%), and 68 (74.7%) patients across treatment groups, respectively.

In the Per Protocol Set, 6 patients from the Full Analysis Set (2 patients in the donidalorsen-4 group, 2 patients in the donidalorsen-8 group, and 2 patients in the placebo group) were excluded due to major protocol deviations that could have compromised interpretation of efficacy.

The "typical patient" subgroup was used only for exploratory subgroup analysis. Three (3) patients in the donidalorsen-4 group and 1 patient in the donidalorsen-8 group were determined not to be typical patients with HAE by the Medical Monitor due to the number and type of HAE attacks and were therefore excluded from the "typical patient" subgroup. The 3 excluded patients in the donidalorsen-4 group were sisters enrolled at the same site; the 1 excluded patient in the donidalorsen-8 group was the daughter of one of the 3 sisters enrolled in the donidalorsen-4 group. These 4 patients had unusually high HAE attack rates at Baseline (Run-in Period) all attacks (with a few exceptions) for these patients were abdominal.

Table 14. Analysis population (All screened patients)

Analysis Sets	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 46) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)
Safety Set[a]	22 (100)	45 (97.8)	23 (100)
Full Analysis Set[b]	22 (100)	45 (97.8)	23 (100)
Per-Protocol Set[c]	20 (90.9)	43 (93.5)	21 (91.3)
PK Set[d]	0	45 (97.8)	23 (100)

Source: Table 14.1.2

[a] The Safety Set included all randomized patients who received at least 1 dose of Study Drug (donidalorsen or placebo).

[b] The Full Analysis Set (FAS) included all randomized patients who received at least 1 dose of Study Drug (donidalorsen or placebo).

[c] The Per-Protocol Set included all patients in the FAS who were treated according to the protocol without any major deviations that could have compromised the interpretation of efficacy

[d] The PK Set included all patients who were randomized and received at least 1 dose of donidalorsen and had at least 1 evaluable PK sample.

Abbreviations: FAS = full analysis set, PK = pharmacokinetic(s)

- **Outcomes and estimation**

Primary endpoint

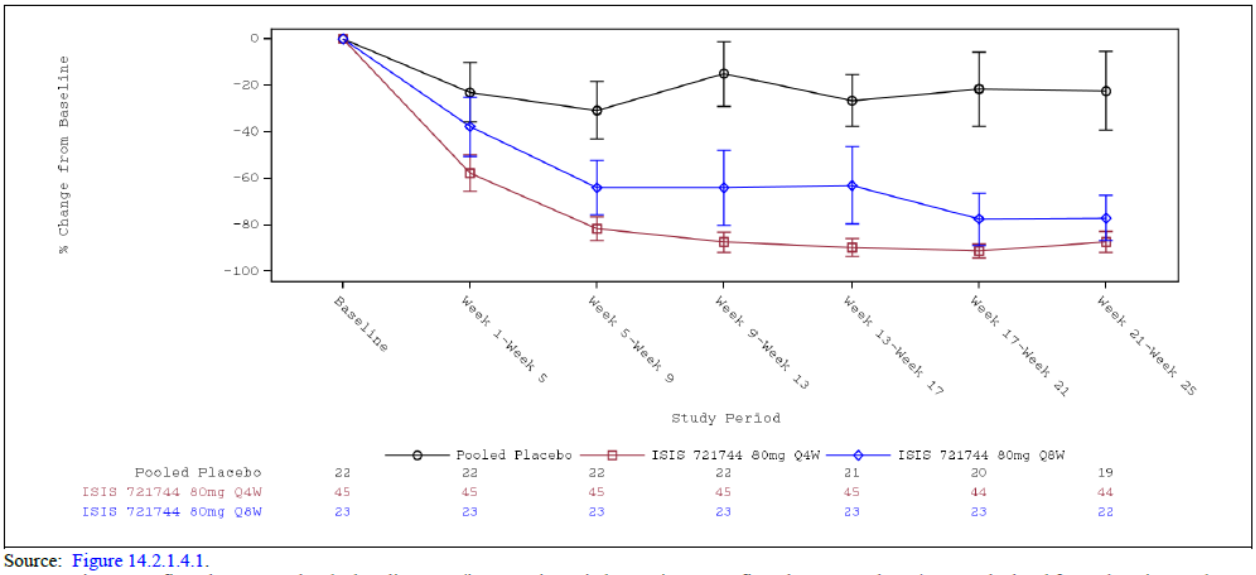
Time-Normalized Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) from Week 1 to Week 25 in the Donidalorsen-4 Group

The primary efficacy endpoint (Endpoint 1), the comparison of the time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25 between the donidalorsen-4 group and the placebo group, was met in this study.

The mean (SD, SEM) time-normalized HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 0.89 (1.851, 0.276) for the donidalorsen-4 group compared with 2.29 (1.807, 0.385) for the placebo group. The LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 0.44 (95% CI: 0.265, 0.727) for the donidalorsen-4 group and 2.26 (95% CI: 1.657, 3.085) for the placebo group, representing an 81% (95% CI: (-89.3%, -64.9%) reduction relative to placebo for the donidalorsen-4 group (statistically significant, $p < 0.001$).

Analysis of time-normalized, Investigator-confirmed HAE attack rates (per 4 weeks) over successive 4-week periods for the donidalorsen-4 group showed a reduction in attack rate already during Weeks 1 to 5, which was sustained over the 25-week treatment period (Figure 16 below).

Figure 16. Percent change from baseline mean (± SEM) of investigator-confirmed HAE attack rate (per 4 weeks) over successive 4-week periods from week 1 to week 25 (Full analysis set)



Source: Figure 14.2.1.4.1.
For Investigator-confirmed HAE attacks, the baseline rate, (i.e., Run-in Period Investigator-confirmed HAE attack rate), was calculated for each patient as the number of Investigator-confirmed HAE attacks that occurred during the Run-in Period divided by the number of days contributed to the Run-in Period multiplied by 28 days.
Note: The per 4-week HAE attack rate was calculated for each patient as number of HAE attacks occurring during each 4-week period divided by the number of days the patient contributed to the period multiplied by 28. Week 1 to Week 5 starts from Day 1 to Day 28; Week 5 to Week 9 starts from Day 29 to Day 56; Week 9 to Week 13 starts from Day 57 to Day 84; Week 13 to Week 17 starts from Day 85 to Day 112, and so on. Error bars indicate the standard error of the mean. ISIS 721744 = donidalorsen.
Abbreviations: HAE = hereditary angioedema; Q4W = once per 4 weeks; Q8W = once 8 weeks; SEM = standard error of mean

Sensitivity Analyses: Time-Normalized Investigator-confirmed HAE Attack Rate (per 4 Weeks) from Week 1 to Week 25

Four sensitivity analyses were conducted on the time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 for all treatment groups including:

- Sensitivity Analysis 1 – Negative Binomial (NB) Regression
- Supportive Analysis – Per-Protocol Set (PPS)
- Sensitivity Analysis 3 – Multiple Imputation Incorporating Pattern Mixture Model
- Sensitivity Analysis 4 – Tipping-Point Analysis

The results of these sensitivity analyses were consistent with the primary analysis results.

Secondary Endpoints

Table 15. Summary of efficacy analyses in hierarchical testing sequence (Full analysis set)

No	Endpoint	Assessment Least Square Mean (Unless Otherwise Noted)	Placebo (N = 22)	Donidalorsen 80 mg Per 4 Weeks (N = 45)	Donidalorsen 80 mg Per 8 Weeks (N = 23)	P-Value/ Nominal P-Value
1	Primary	**Donidalorsen-4 Group**: Time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25	2.26	0.44	--	< 0.001[a]
2	Secondary	**Donidalorsen-4 Group**: Time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25	2.25	0.30	--	< 0.001[a]
3	Secondary	**Donidalorsen-4 Group**: Patients with ≥ 70% reduction from Baseline in Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25, %	18.2	82.2	--	< 0.001[a]
4	Secondary	**Donidalorsen-4 Group**: Percentage of Investigator-confirmed HAE attack-free patients from Week 5 to Week 25, %	9.1	53.3	--	0.003[a]
5	Secondary	**Donidalorsen-4 Group**: Time-normalized number of moderate or severe Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25	1.15	0.12	--	< 0.001[a]
6	Secondary	**Donidalorsen-4 Group**: Number of Investigator-confirmed HAE attacks requiring acute therapy (per 4 weeks) from Week 5 to Week 25	1.80	0.15	--	< 0.001[a]
7	Secondary	**Donidalorsen-4 Group**: Change in AE-QoL total score at Week 25	-6.19	-24.76	--	< 0.001[a]
8	Secondary	**Donidalorsen-8 Group**: Time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25	2.26	--	1.02	0.004[a]
9	Secondary	**Donidalorsen-8 Group**: Time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25	2.25	--	0.90	0.004[a]
10	Secondary	**Donidalorsen-8 Group**: Patients with ≥ 70% reduction from Baseline in Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25, %	18.2	--	65.2	0.004[a]
11	Secondary	**Donidalorsen-8 Group**: Percentage of Investigator-confirmed HAE attack-free patients from Week 5 to Week 25, %	9.1	--	34.8	0.240

No	Endpoint	Assessment Least Square Mean (Unless Otherwise Noted)	Placebo (N = 22)	Donidalorsen 80 mg Per 4 Weeks (N = 45)	Donidalorsen 80 mg Per 8 Weeks (N = 23)	P-Value/ Nominal P-Value
Note: Due to lack of statistical significance for Endpoint 11, subsequent hierarchical endpoints were statistically considered exploratory. All p values < 0.05 are nominally significant.						
12	Secondary	**Donidalorsen-8 Group**: Time-normalized number of moderate or severe Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25	1.15	--	0.68	0.173
13	Secondary	**Donidalorsen-8 Group**: Number of Investigator-confirmed HAE attacks requiring acute therapy (per 4 weeks) from Week 5 to Week 25	1.80	--	0.59	0.004[b]
14	Secondary	**Donidalorsen-8 Group**: Change in AE-QoL total score at Week 25	-6.19	--	-19.85	0.010[b]
15	Exploratory	**Donidalorsen-4 Group**: Change in AE-QoL domain score of Functioning at Week 25	-12.21	-36.67	--	< 0.001[b]
16	Exploratory	**Donidalorsen-4 Group**: Change in AE-QoL domain score of Fears/Shame at Week 25	-5.90	-29.82	--	< 0.001[b]
17	Exploratory	**Donidalorsen-4 Group**: Change in AE-QoL domain score of Fatigue/Mood at Week 25	-3.48	-11.56	--	0.147
18	Exploratory	**Donidalorsen-4 Group**: Change in AE-QoL domain score of Nutrition at Week 25	-5.75	-21.40	--	0.001[b]
19	Exploratory	**Donidalorsen-8 Group**: Change in AE-QoL domain score of Functioning at Week 25	-12.21	--	-28.29	0.022[b]
20	Exploratory	**Donidalorsen-8 Group**: Change in AE-QoL domain score of Fears/Shame at Week 25	-5.90	--	-26.04	0.004[b]
21	Exploratory	**Donidalorsen-8 Group**: Change in AE-QoL domain score of Fatigue/Mood at Week 25	-3.48	--	-7.11	0.561
22	Exploratory	**Donidalorsen-8 Group**: Change in AE-QoL domain score of Nutrition at Week 25	-5.75	--	-16.48	0.046[b]
23	Secondary	**Donidalorsen-4 Group**: Percentage of patients who are well controlled on the AECT at Week 25, %	40.9	91.1	--	< 0.001[b]

No	Endpoint	Assessment Least Square Mean (Unless Otherwise Noted)	Placebo (N = 22)	Donidalorsen 80 mg Per 4 Weeks (N = 45)	Donidalorsen 80 mg Per 8 Weeks (N = 23)	P-Value/ Nominal P-Value
24	Secondary	Donidalorsen-8 Group: Percentage of patients who are well controlled on the AECT at Week 25, %	40.9	--	73.9	0.028[b]

Sources: Table 14.2.1.1-1, Table 14.2.2.1-1, Table 14.2.3.1, Table 14.2.4.1-1, Table 14.2.5.1, Table 14.2.6.1, Table 14.2.7.1, Table 14.2.7.4, and Table 14.2.8.1
Note: Assessments were conducted in hierarchical sequence as described in the ISIS 721744-CS5 Statistical Analysis Plan (v 2.0, 13 Dec 2023). All assessments were compared with the placebo group.
Note: Donidalorsen-4 group = patients who received donidalorsen 80 mg every 4 weeks; donidalorsen-8 group = patients who received donidalorsen 80 mg every 8 weeks.
[a]Statistical significance ($p < 0.05$).
[b]Nominal statistical significance ($p < 0.05$).
Abbreviations: AECT = Angioedema Control Test; AE-QOL = Angioedema Quality of Life; HAE = hereditary angioedema

Time-normalized Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) from Week 1 to Week 25 for the Donidalorsen-8 Group

The mean (SD, SEM) time-normalized HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 1.19 (1.629, 0.340) for the donidalorsen-8 group, compared with 2.29 (1.807, 0.385) for the placebo group,

For the donidalorsen-8 group (Endpoint 8), the LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 1.02 (95% CI: 0.651, 1.594) and 2.26 (95% CI: 1.657, 3.085) for the placebo group, representing a 55% reduction (95% CI: -73.9%, -22.3%) relative to placebo for the donidalorsen-8 group (statistically significant, p = 0.004).

In an analysis of time-normalized, Investigator-confirmed HAE attack rate (per 4 weeks) over successive 4-week periods for the donidalorsen-8 group, the reduction in HAE attacks progressively became larger over time and approached that of the donidalorsen-4 group by Week 25. This delay to peak effect may be due to the longer time necessary for the drug to achieve steady state for the longer time between doses (8 weeks) compared with every 4 weeks.

Time-Normalized Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) from Week 5 to Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group

The mean (SD, SEM) time-normalized HAE attack rate from Week 5 to Week 25 was 0.75 (1.898, 0.283) and 1.09 (1.714, 0.357) for the donidalorsen-4 and donidalorsen-8 groups, respectively, compared with 2.32 (1.926, 0.411) for the placebo group.

The LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 5 to Week 25 was 0.30 (95% CI: 0.151, 0.581) for the donidalorsen-4 group and 2.25 (95% CI: 1.594, 3.183) for the placebo group (Endpoint 2), representing an 87% (95% CI: -93.8%, -71.9%) reduction relative to the placebo group for the donidalorsen-4 group (statistically significant, p < 0.001).

For the donidalorsen-8 group (Endpoint 9), the LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 5 to Week 25 was 0.90 (95% CI: 0.529, 1.520) for the donidalorsen-8 group and 2.25 (95% CI: 1.594, 3.183) for the placebo group representing a 60% (95% CI: -78.8%, -25.2%) reduction relative to the placebo group for the donidalorsen-8 group (statistically significant, p = 0.004).**Time-Normalized Investigator-Confirmed Moderate or Severe HAE Attack Rate (Per 4 Weeks) from Week 5 to Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group**

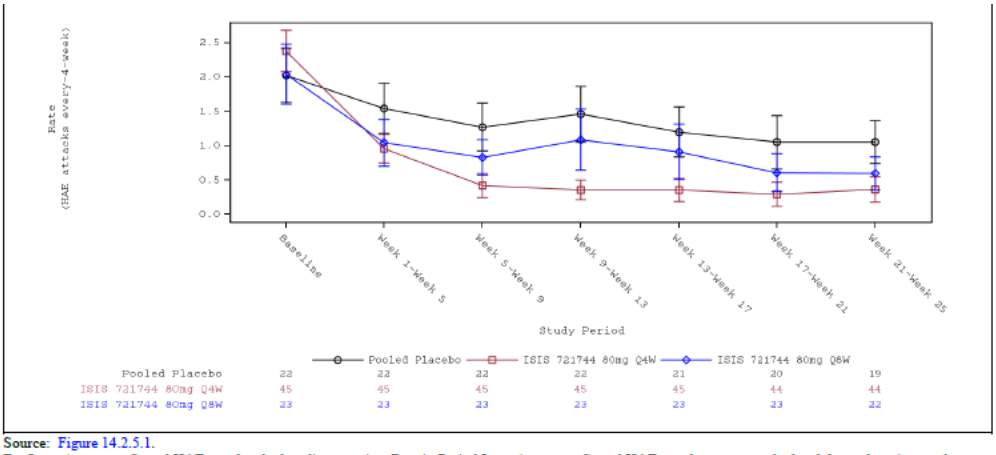
The mean (SD, SEM) time-normalized Investigator-confirmed moderate or severe HAE attack rate (per 4 weeks) from Week 5 to Week 25 was 0.37 (1.026, 0.153) and 0.90 (1.617, 0.337) for the donidalorsen-4 and donidalorsen-8 groups, respectively, compared with 1.35 (1.707, 0.364) for the placebo group.

The LSM time-normalized Investigator-confirmed moderate or severe HAE attack rate (per 4 weeks) from Week 5 to Week 25 was 0.12 (95% CI: 0.044, 0.351) for the donidalorsen-4 group and 1.15 (95% CI: 0.718,

1.831) for the placebo group (Endpoint 5), representing a reduction of 89% (95% CI: -96.5%, -66.1%) in the moderate or severe HAE attack rate (per 4 weeks) relative to placebo for the donidalorsen-4 group (statistically significant, $p < 0.001$).

For the donidalorsen-8 group (Endpoint 12), the LSM time-normalized Investigator-confirmed moderate or severe HAE attack rate (per 4 weeks) from Week 5 to Week 25 was 0.68 (95% CI: 0.372, 1.229). While the percent difference relative to the placebo group in the moderate or severe HAE attack rate (per 4 weeks) for the donidalorsen-8 group was a reduction of 41% (95% CI: -72.4%, 26.0%), (nominal $p = 0.173$).

Figure 17. Absolute value mean (± SEM) of time-normalised investigator-confirmed moderate or severe HAE attack rate (per 4 weeks) over successive 4-week periods from week 1 to week 25 (Full analysis set)



Source: Figure 14.2.5.1.
For Investigator-confirmed HAE attacks, the baseline rate, i.e., Run-in Period Investigator-confirmed HAE attack rate, was calculated for each patient as the number of Investigator-confirmed HAE attacks that occurred during the Run-in Period divided by the number of days contributed to the Run-in Period multiplied by 28 days.
Note: The per 4-week HAE attack rate was calculated for each patient as number of HAE attacks occurring during each 4-week period divided by the number of days the patient contributed to the period multiplied by 28. Week 1 to Week 5 starts from Day 1 to Day 28; Week 5 to Week 9 starts from Day 29 to Day 56; Week 9 to Week 13 starts from Day 57 to Day 84; Week 13 to Week 17 starts from Day 85 to Day 112, and so on. Error bars indicate the standard error of the mean. ISIS 721744 = donidalorsen.
Abbreviations: HAE = hereditary angioedema; Q4W = once every 4 weeks; Q8W = once every 8 weeks; SEM = standard error of mean

Time-Normalized Investigator-Confirmed HAE Attack Rate Requiring Acute Therapy from Week 5 to Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group

Table 16. Analyses of time-normalized investigator-confirmed HAE attacks requiring acute therapy (per 4 Weeks) from week 5 to week 25 (Full analysis set)

	Placebo (N = 22)	Donidalorsen 80 mg Every 4 Weeks (N = 45)	Donidalorsen 80 mg Every 8 Weeks (N = 23)
Run-In Period HAE Attacks Requiring Acute Therapy[a]			
Mean (SD, SEM)	2.01 (1.975, 0.421)	2.53 (2.179, 0.325)	2.79 (2.296, 0.479)
Time-Normalized HAE Attacks Requiring Acute Therapy[b]			
Mean (SD, SEM)	1.88 (1.834, 0.391)	0.46 (1.290, 0.192)	0.94 (1.688, 0.352)
Poisson Regression[c]			
Least Square Mean Rate (95% CI)	1.80 (1.232, 2.616)	0.15 (0.057, 0.391)	0.59 (0.308, 1.146)
Model Adjusted Mean Rate Ratio (95% CI)	--	0.08 (0.030, 0.234)	0.33 (0.155, 0.706)
Wald Chi-Square P-Value	--	< 0.001	0.004
Percentage Difference Relative to Placebo (95% CI)[d]	--	-92% (-97.0%, -76.6%)	-67% (-84.5%, -29.4%)

Source: Table 14.2.6.1

[a] The run-in period (Screening) HAE attack rate for each patient is calculated as number of Investigator-confirmed HAE attacks requiring acute therapy occurring during the Run-in Period divided by the number of days the patient contributed to the Run-in Period multiplied by 28.

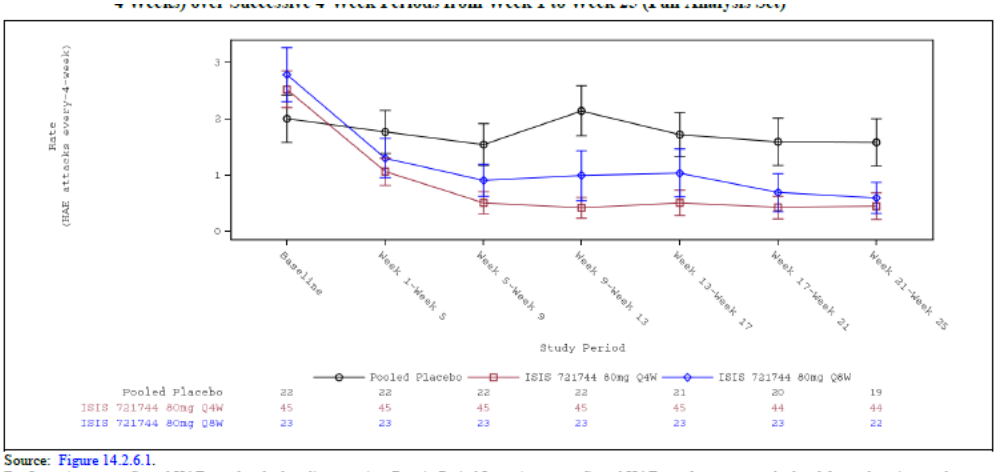
[b] The time-normalized HAE attack rate is calculated as number of Investigator-confirmed HAE attacks requiring acute therapy occurring from Week 5 to Week 25, divided by the number of days the patient contributed to the period, multiplied by 28.

[c] The Poisson regression model and the Negative Binomial regression model includes treatment groups, baseline (the Run-in Period HAE attack rate), the treatment-by-baseline interaction as a covariate, and the logarithm of time in every-4-week that each patient was observed from Week 5 to Week 25 used as an offset variable (e.g., log (6) if the patient stayed in the study for 24 weeks). Pearson chi-square scaling of standard errors was used in the Poisson regression model to account for potential overdispersion.

[d] The percentage difference in mean Investigator-confirmed HAE attack rate between donidalorsen 80 mg and placebo is calculated as 100% * (mean rate ratio -1). Similarly, the estimated upper and lower confidence limits for the mean rate ratio can be transformed by subtracting 1 and multiplying by 100% to calculate 95% confidence intervals for the percentage change.

Abbreviations: CI = confidence interval; HAE = hereditary angioedema; SD = standard deviation; SEM = standard error of mean

Figure 18. Absolute value mean (± SEM) of investigator-confirmed rate of HAE attacks requiring acute therapy (per 4 weeks) over successive 4-week periods from week 1 to week 25 (Full analysis set)



Source: Figure 14.2.6.1.
For Investigator-confirmed HAE attacks, the baseline rate, i.e., Run-in Period Investigator-confirmed HAE attack rate, was calculated for each patient as the number of Investigator-confirmed HAE attacks that occurred during the Run-in Period divided by the number of days contributed to the Run-in Period multiplied by 28 days.
Note: The per 4-week HAE attack rate is calculated for each patient as number of HAE attacks occurring during each 4-week period divided by the number of days the patient contributed to the period multiplied by 28. Week 1 to Week 5 starts from Day 1 to Day 28; Week 5 to Week 9 starts from Day 29 to Day 56; Week 9 to Week 13 starts from Day 57 to Day 84; Week 13 to Week 17 starts from Day 85 to Day 112, and so on. Error bars indicate the standard error of the mean. ISIS 721744 = donidalorsen.
Abbreviations: HAE = hereditary angioedema; Q4W = once every 4 weeks; Q8W = once every 8 weeks; SEM = standard error of mean

Percent Reduction by Thresholds from Baseline in Time-Normalized Investigator-Confirmed HAE Attacks from Week 5 to Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group

Clinical response rate was analysed by the number of patients with a ≥ 50%, ≥ 70%, or ≥ 90% reduction from Baseline in Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25 in each treatment group. Note, although all thresholds were considered secondary endpoints, only ≥ 70% was included in the efficacy hierarchical testing; therefore, the ≥ 50% and ≥ 90% reduction analyses were considered exploratory and the p-values were considered nominal.

The percentage of patients with ≥ 50% reduction from Baseline in Investigator-confirmed HAE attacks from Week 5 and Week 25 was 93.3% and 82.6% for the donidalorsen-4, and donidalorsen-8 groups, respectively, compared with 27.3% in the placebo groups (nominally significant, $p < 0.001$ for both donidalorsen groups).

The percentage of patients with a ≥ 70% reduction from Baseline in Investigator-confirmed HAE attacks from Week 5 and Week 25 was 82.2% and 65.2% for the donidalorsen-4 group (Endpoint 3) and the donidalorsen-8 group (Endpoint 10), respectively, compared with 18.2% in the placebo group. These differences were statistically significant ($p < 0.001$ and $p = 0.004$, respectively).

The percentage of patients ≥ 90% reduction from Baseline in Investigator-confirmed HAE attacks from Week 5 and Week 25 was 62.2% and 47.8% for the donidalorsen-4 and donidalorsen-8 groups, respectively, compared with 9.1% in the placebo group (nominally significant, $p = < 0.001$ and $p = 0.014$, respectively).

Percentage of Investigator-Confirmed HAE Attack-Free Patients from Week 5 to Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group

Of the 45 patients in the donidalorsen-4 group, 24 (53.3%) were HAE attack-free from Week 5 to Week 25, compared with 2 of 22 (9.1%) patients in the placebo group (Endpoint 4); this difference was statistically significant ($p = 0.003$).

Although a larger percentage of patients (8 of 23 [34.8%]) in the donidalorsen-8 group were HAE attack-free from Week 5 to Week 25 compared with the placebo group (Endpoint 11), the difference between these groups did not reach statistical significance (p = 0.240).

Change in AE-QoL Questionnaire Total Score at Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group

The LSM reduction (improvement) from Baseline in the AE-QoL total score at Week 25 was 24.8 points for the donidalorsen-4 group and 19.9 points for the donidalorsen-8 group, compared with 6.2 points for the placebo group (p < 0.001 and nominal p = 0.010, respectively). The reduction observed in the donidalorsen treatment groups was clinically meaningful (i.e., ≥ 6 points).

Percentage of Well-Controlled Patients on the AECT at Week 25 in the Donidalorsen-4 Group and Donidalorsen-8 Group

For the donidalorsen-4 and donidalorsen-8 groups, 91.1% and 73.9% of patients, respectively, had well controlled angioedema activity (per an AECT score ≥ 10 at Week 25) compared with 40.9% of the placebo group (nominal p < 0.001 and nominal p = 0.028, respectively).

- **Ancillary analyses**

Subgroup Analyses: Time-Normalized Investigator-Confirmed HAE Attack Rate from Week 1 to Week 25 (study ISIS 721744-CS5)

Exploratory subgroup analyses were conducted on the primary efficacy endpoint (comparison of the time-normalized number of Investigator-confirmed HAE attacks [per 4 weeks] from Week 1 to Week 25 for the donidalorsen-4 group) by the following:

- Age (12 to 17 years, 18 to 39 years, and 40 to 64 years, and ≥ 65 years)
- Sex (male and female)
- Race (Caucasian and other/multiple)
- Region (North America, Europe, Middle East)
- Predefined prophylactic therapy

In addition, an analysis was conducted on the "typical patient" subgroup.

Age

For adolescent patients (aged 12 to 17 years), a 97.1% decrease (95% CI: -106.26%, -88.01%) from Baseline (Run-in Period) in the time-normalized HAE attack rate (per 4 weeks) from Week 1 to Week 25 was observed, which was similar to the 88.5% (95% CI: -94.75%, -82.30%) decrease for patients aged 18 to 39 years; patients aged 40 to 64 years had a numerically lower percent change from Baseline, although the 95% CI overlap with younger adults (72.6% decrease [95% CI: -86.56%, -58.66%]). Only 1 patient was aged ≥ 65 years in the donidalorsen-4 group.

Sex

The mean percent decrease in Investigator-confirmed HAE attack rate (per 4 weeks) from Baseline to Week 1 to Week 25 were similar between male (82.2% decrease [95% CI: -91.03%, -73.36%]) and female (81.0% decrease [95% CI: -91.80%, -70.27%]) patients.

Region

The mean percent decrease in Investigator-confirmed HAE attack rate (per 4 weeks) from Baseline to Week 1 to Week 25 were generally similar for patients across regions, including North America (73.0% decrease [95% CI: -98.14%, -47.87%]), Europe (85.8% decrease [95% CI: -92.37%, -79.12%]) and the Middle East (78.0% decrease [95% CI: -100.65%, -55.32%]).

Race

The subgroup analysis for race showed small differences in mean percent decrease in Investigator-confirmed HAE attack rate (per 4 weeks) from Baseline to Week 1 to Week 25 between Caucasian (83.1% decrease [95% CI: -89.71%, -76.54%] and other/multiple races patients (58.3% decrease [95% CI: -188.77%, 72.20%]; however, the 95% CI are overlapping, so these differences likely represent chance findings in small patient subgroups (< 10 patients).

Predefined Prophylactic Use

The subgroup analysis for predefined prophylactic use showed small differences in mean percent decrease in Investigator-confirmed HAE attack rate (per 4 weeks) from Baseline to Week 1 to 25 between patients who received predefined prophylactic use (71.0% decrease [95% CI: -92.68%, -49.14%] compared with no predefined prophylactic therapy use (83.1% decrease [95% CI: -91.01%, -75.19%]); however, the 95% CI are overlapping, so these difference likely represent chance findings in small patient subgroups (< 10 patients).

"Typical Patient"

For the primary endpoint, the LSM time-normalized Investigator confirmed attack rate (per 4 weeks) from Week 1 to Week 25 for the "typical patient" donidalorsen-4 group was 0.35 and the "typical patient" placebo group was 2.14, representing an 84% reduction (95% CI: -91.5%, -69.4%]) relative to the placebo, which was similar to the reduction in HAE attack rate for the overall donidalorsen-4 group; therefore, the 3 "atypical patients" did not appear to affect the overall assessment of the time-normalized number of Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 for the donidalorsen-4 group.

Of note, three (3) patients in the donidalorsen-4 group and 1 patient in the donidalorsen-8 group were determined not to be typical patients with HAE by the Medical Monitor due to the number and type of HAE attacks and were therefore excluded from the "typical patient" subgroup.

- **Summary of main efficacy results**

The following Table 17 summarises the efficacy results from the main study supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 17. Summary of efficacy for trial ISIS 721744-CS5

Title: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)	
Study identifier	ISIS 721744-CS5 Donidalorsen (ISIS 721744) for treatment of Prophylactic treatment of HAE EudraCT Number: 2021-002571-19 ClinicalTrials.gov Identifier: NCT05139810

Title: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)		
Design	A Phase 3 pivotal study (ISIS 7217144-CS5), double-blind, placebo-controlled, randomized study evaluating donidalorsen 80 mg administered as a subcutaneous injection every 4 weeks or every 8 weeks in patients with HAE over a 24-week Treatment Period. Patients with HAE type I (HAE-1) or type II (HAE-2) were randomly assigned in a 2:1 ratio to Cohort A (donidalorsen-4 group or matched placebo) or Cohort B (donidalorsen-8 group or matched placebo), respectively. Within each cohort, patients were randomly assigned in a 3:1 ratio to receive donidalorsen or a matching volume of placebo. Data from patients receiving placebo in both Cohort A and Cohort B were pooled for analyses; this pooled placebo group is hereafter referred to as the placebo group. The study consisted of 3 periods including an up to 8-week (56-day) Screening Period (also known as the Run-in Period for HAE attack rate Baseline), a 24-week Treatment Period (also known as the On-Treatment Period), and an up to 13-week Post-Treatment Period; patients could elect to enrol in the open-label extension arm of Study ISIS 721744-CS7 rather than complete the Post-Treatment Period. A patient was randomly assigned to treatment as soon as the patient experienced ≥ 2 HAE attacks during the up to 8-week Screening Period, had completed all other screening activities, and met all other eligibility requirements. Patients who experienced at least 5 attacks/month for 2 consecutive months after Week 5 (considered lack of efficacy) had the opportunity to discontinue from treatment in the current study and either progress to the Post-Treatment Period or enroll in the OLE study. If a patient chose to enroll in the OLE study, the patient was to be discontinued from the current study.	
	Duration of main phase: Screening Period:	24 weeks Day -56 to Day -1
Hypothesis	Superiority	
Treatment groups	Placebo	Single subcutaneous (SC) injection of matching volume placebo 0 mg/mL; 0.8 mL solution per vial
		every 4 weeks (Cohort A) or every 8 weeks (Cohort B) over a 24-week Treatment Period (also known as the On-Treatment Period), and an up to 13-week Post-Treatment Period (N=22)
	Donidalorsen 80 mg	80 mg administered as a single subcutaneous injection every 4 weeks or every 8 week over a 24-week Treatment Period (also known as the On-Treatment Period), and an up to 13-week Post-Treatment Period (N=69)
Endpoints and definitions		
Primary	From Week 1 to Week 25 compared to placebo	The primary endpoint was the time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25 compared to placebo for the donidalorsen 80 mg once every 4 weeks group (hereafter referred to as the donidalorsen-4 group).

Title: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)		
Secondary	From Week 1 to Week 25	The secondary objective was to evaluate the effects of donidalorsen (donidalorsen 80 mg every 4 weeks or every 8 weeks) on the quality and pattern of HAE attacks and their impact on QoL.
	From Week 1 to Week 25	The time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) compared to placebo for the donidalorsen 80 mg once every 8 weeks group (hereafter referred to as the donidalorsen-8 group).
	From Week 5 to Week 25	The time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) compared to placebo.
	From Week 5 to Week 25	The percentage of Investigator-confirmed HAE attack-free patients compared to placebo.
	From Week 5 to Week 25	The time normalized number of moderate or severe Investigator-confirmed HAE attacks (per 4 weeks) compared to placebo
	Between Week 5 to Week 25	The number of patients with a clinical response defined as a ≥ 50%, ≥ 70%, or ≥ 90% reduction from Baseline (ie, screening rate) in Investigator-confirmed HAE attack rate compared to placebo.
	at Week 25	Percent of patients who are well controlled on the AECT.
	at Week 25	Change in Angioedema Quality of Life (AE-QoL) questionnaire total score.
	From Week 5 to Week 25	The number of Investigator-confirmed HAE attacks requiring acute HAE therapy compared with placebo.
Exploratory endpoints		Change or percent change from Baseline compared to placebo: PKK level, GAD-7 questionnaire score, EQ-5D-5L, PGIS, WPAI, Change in each AE-QoL questionnaire domain score, Time-normalized number of Investigator-confirmed HAE attacks (per every-4-week) from Week 17 to Week 25, Investigator-confirmed HAE attacks that involves the larynx, Incidence of ER visits, all cause hospitalization, and total inpatient days, and PGIC. Other exploratory endpoints were assessment on PK , as appropriate.
Last Patient Last Visit Database lock	09 November 2023 18 December 2023	
Results and Analysis		
Analysis description	**Primary Analysis: Time-Normalized Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) from Week 1 to Week 25 for the Donidalorsen-4 Group**	

Title: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)					
Analysis population	**Full Analysis Set (FAS)** All randomized patients who received at least 1 administration of study drug. Analyses or summaries on the Full Analysis Set were performed by randomized treatment group. **Per-Protocol Set (PPS)** All patients in the Full Analysis Set who were treated according to the protocol without any major deviations that could have compromised the interpretation of efficacy. Major deviations that could have compromised the interpretation of efficacy were determined prior to unblinding for statistical analyses. Analyses or summaries on the Per Protocol Set were performed by randomized treatment group.				
Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) from Week 1 to Week 25 (FAS)	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group	P-Value/
	Number of subjects	22	45	23	
	Least square mean rate (95% CI)	2.26 (1.657, 3.085)	0.44 (0.265, 0.727)	--	
	Percentage difference relative to placebo (95% CI)[b]	--	-81% (-89.3%, -64.9%)	--	< 0.001[a]
Analysis description	**Secondary analyses: Donidalorsen-4 and Donidalorsen-8 Groups: Time-normalized number of Investigator confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25**				
Number of Investigator confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25 (FAS)	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group	
	Number of subjects	22	45	23	
	Least square mean rate (95% CI)	2.25 (1.594, 3.183)	0.30 (0.151, 0.581)	0.90 (0.529, 1.520)	
	Wald Chi-Square P-value		< 0.001[a]	0.004[a]	
	Percentage difference relative to placebo (95% CI)[b]		-87% (-93.8%, -71.9%)	-60% (-78.8%, -25.2%)	
Patients with ≥ 70% reduction from Baseline in Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25, % (FAS)	**Secondary analysis: Donidalorsen-4 and Donidalorsen-8 Groups: Patients with ≥ 70% reduction from Baseline in Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25, %**				
	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group	
	Number of subjects	22	45	23	
	Reduction ⩾ 70%, n (%)	4 (18.2)	37 (82.2)	15 (65.2)	
	Reduction < 70%, n (%)	18 (81.8)	8 (17.8)	8 (34.8)	
	Odds Ratio (95% CI)[c]	--	34.74 (7.32, 164.87)	9.17 (2.05, 41.09)	
	Nominal P-value	--	< 0.001[a]	0.004[a]	

Title: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)				
Percentage of Investigator-confirmed HAE attack-free patients from Week 5 to Week 25, % (FAS) Patients with Attack Rate Reduction ≥ 90%	**Secondary analysis: Donidalorsen-4 and Donidalorsen-8 Groups: Percentage of Investigator-confirmed HAE attack-free results (100% reduction) from Week 5 to Week 25, %**			
	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group
	Number of subjects	22	45	23
	Reduction ⩾ 90%, n (%)	2 (9.1)	28 (62.2)	11 (47.8)
	Reduction < 90%, n (%)	20 (90.9)	17 (37.8)	12 (52.2)
	Odds Ratio (95% CI)[c]		17.04 (3.36, 86.42)	8.70 (1.56, 48.52)
	Nominal P-value		< 0.001[a]	0.014[a]
Number of moderate or severe Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25 (FAS)	**Secondary analysis: Donidalorsen-4 and Donidalorsen-8 Groups: Time-normalized number of moderate or severe Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25**			
	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group
	Number of subjects	22	45	23
	Least square mean rate (95% CI)	1.15 (0.718, 1.831)	0.12 (0.044, 0.351)	0.68 (0.372, 1.229)
	P-Value/Nominal P-Value		< 0.001[a]	0.173
	Percentage difference relative to placebo (95% CI)[b]		-89% (-96.5%, -66.1%)	-41% (-72.4%, 26.0%)
Number of Investigator-confirmed HAE attacks requiring acute therapy (per 4 weeks) from Week 5 to Week 25 (FAS)	**Secondary analysis: Donidalorsen-4 and Donidalorsen-8 Groups:** Number of Investigator-confirmed HAE attacks requiring acute therapy (per 4 weeks) from Week 5 to Week 25			
	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group
	Number of subjects	22	45	23
	Least square mean rate (95% CI)	1.80 (1.232, 2.616)	0.15 (0.057, 0.391)	0.59 (0.308, 1.146)
	Wald Chi-Square P-Value	--	< 0.001[a]	0.004[d]
	Percentage difference relative to placebo (95% CI)[b]	--	-92% (-97.0%, -76.6%)	-67% (-84.5%, -29.4%)
Change in AE-QoL total score at Week 25 (FAS)	**Secondary analysis: Donidalorsen-4 and Donidalorsen-8 Groups: Change in AE-QoL total score at Week 25**			
	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group
	Number of subjects	22	45	23
	Least square mean rate (95% CI)	-6.19 (-13.737, 1.353)	-24.76 (-29.860, -19.652)	-19.85 (-26.960, -12.734)
	Treatment difference in LSM (95% CI)	--	-18.56 (-27.673, -9.454)	-13.65 (-24.024, -3.286)
	Nominal P-Value	--	< 0.001[a]	0.010[a]
Number of Investigator confirmed	**Secondary analysis: Donidalorsen-8 Group: Time-normalized number of Investigator confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25**			

Title: A Phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema (HAE)					
HAE attacks (per 4 weeks) from Week 1 to Week 25 (FAS)	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group	P-Value/ Nominal P-Value
	Number of subjects	22	45	23	
	Least square mean rate (95% CI)	2.26 (1.657, 3.085)	--	1.02 (0.651, 1.594)	
	Percentage difference relative to placebo (95% CI)[b]	--	--	-55% (-73.9%, -22.3%)	0.004[a]
Number of Investigator confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25 (FAS)	**Secondary analysis: Donidalorsen-4 and Donidalorsen-8 Groups: Time-normalized number of Investigator confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25**				
	Treatment group	Placebo	donidalorsen-4 group	donidalorsen-8 group	
	Number of subjects	22	45	23	
	Least square mean rate (95% CI)	2.25 (1.594, 3.183)	0.30 (0.151, 0.581)	0.90 (0.529, 1.520)	
	Model adjusted mean rate ratio (95% CI)	--	0.13 (0.062, 0.281)	0.40 (0.212, 0.748)	
	Wald Chi-Square P-value	--	< 0.001[a]	0.004[a]	

Donidalorsen-4 group = donidalorsen-4 group 80 mg Every 4 weeks; donidalorsen-8 group = Donidalorsen 80 mg Every 8 weeks; SC = subcutaneous; CI = confidence interval; HAE = hereditary angioedema; QoL = quality of life; AE-QoL = Angioedema Quality of Life; LSM = least square mean; AECT = Angioedema Control Test; PKK = Prekallikrein; GAD-7 = Generalized anxiety disorder-7 questionnaire score; EQ-5D-5L = EuroQoL-5-Dimensions quality of life questionnaire; PPS = Per-Protocol Set; PGIS = Patient Global Impression of Severity; WPAI = Work productivity and impairment questionnaire score; ER = emergency room; PK = pharmacokinetic.

[a] Statistical significance ($p < 0.05$) and is based on Poisson regression.

[b] The percentage difference in mean Investigator-confirmed HAE attack rate between donidalorsen 80 mg and placebo groups were calculated as 100% * (mean rate ratio -1). Similarly, the estimated upper and lower confidence limits for the mean rate ratio can be transformed by subtracting 1 and multiplying by 100% to calculate 95% confidence intervals for the percentage change.

[c] The odds ratio, its 95% confidence interval and p-value were calculated based on a logistic regression with baseline (the time-normalized Run-in Period attack rate) and the Treatment-by-Baseline interaction as covariates.

[d] Nominal statistical significance ($p < 0.05$).

2.6.5.3. Clinical studies in special populations

Adolescents ≥ 12-year-old

The study ISIS 721744-CS5 enrolled 7 adolescents. This is in line with PIP agreement (at least 6 adolescents). A further 4 adolescents were enrolled to the switch part of the ISIS 721744-CS7 study.

Elderly population

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
Study 721744-CS2	1	0	0
Study 721744-CS3	1[a]	0	0
Study 721744-CS5	2	0	0
Study 721744-CS7	4[b]	0	0
Total	6	0	0

OLE= open-label extension

[a]Includes patients rolled over from study 721744-CS2 into the OLE study

[b]Includes 3 newly enrolled switch subjects, and 1 patient rolled over from study 721744-CS5 to the OLE study

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable

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

Not applicable

2.6.5.6. Supportive studies

Study ISIS 721744-CS2

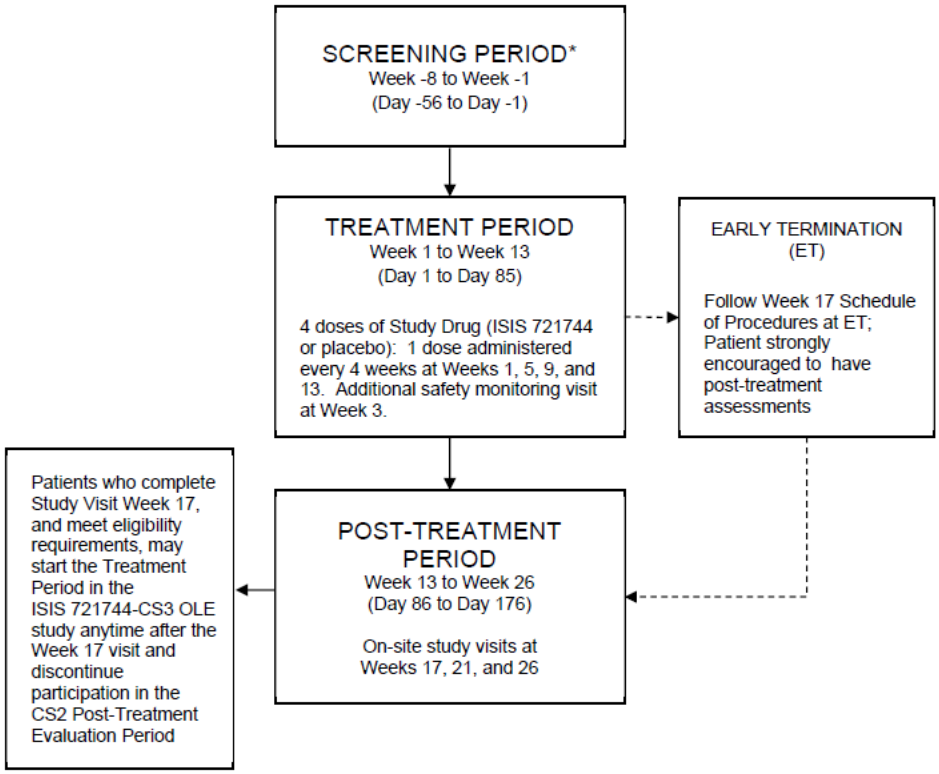
Trial title: A Randomized, Double-Blind, Placebo-Controlled, Phase 2a Study to Assess the Clinical Efficacy of ISIS 721744, a Second-Generation Ligand-Conjugated Antisense Inhibitor of Prekallikrein, in Patients with Hereditary Angioedema

Methods

The study was conducted in 2 parts concurrently with different designs under a master protocol: Part A was randomized, double-blind, and placebo-controlled and Part B was open-label. Patients were allocated into Part A or Part B according to type of HAE, either HAE-1/HAE-2 in Part A (C1-INH-HAE) or nC1-INH-HAE (referred to in the tables and figures as Type 3 HAE) in Part B. The study visit schedule and procedures were nearly identical in Parts A and B. In Part A, approximately 18 patients with HAE-1/HAE-2 were to be randomized to subcutaneous (SC) injections of ISIS 721744 80 mg or placebo in a 2:1 ratio (ISIS 721744:placebo). In Part B, approximately 6 patients with nC1-INH-HAE were to be administered open-label SC injections of ISIS 721744 80 mg. Due to the rarity of nC1-INH-HAE, enrollment in Part B may have ended early as Study Centers were unable to enroll sufficient patients; this did not affect the completion of Part A.

The study consisted of Screening Period of up to 8 weeks, a 12-week Treatment Period when patients received fixed SC doses of Study Drug every 4 weeks during 4 on-site study visits, and a 4- to 13-week Post-Treatment Period, which was determined by whether a patient enrolled in the ISIS 721744-CS3 open-label extension (OLE) study.

Figure 19. Study schema



Study Participants

Patients must have been aged ≥ 18 years at the time of informed consent.

Patients must have had a documented diagnosis of HAE-1/HAE-2 (for inclusion in Part A) or nC1-INH-HAE (for inclusion in Part B) as defined below:

Documented diagnosis of HAE-1/HAE-2 based upon ALL of the following:

- Documented clinical history consistent with HAE (SC or mucosal, non-pruritic swelling episodes without accompanying urticaria) (Maurer et al. 2018).
- Diagnostic testing results that confirmed HAE-1/HAE-2: C1-INH functional level < 40% normal level. Patients with a functional level of 40% to 50% of normal could be enrolled if their complement factor C4 (C4) level was below the lower limit of normal (LLN) or if a known pathogenic mutation in the *SERPING1* gene had been demonstrated.
- At least 1 of the following: age at reported HAE onset ≤ 30 years; a family history consistent with HAE-1/HAE-2; or complement component 1q within the normal range.

Documented diagnosis of nC1-INH-HAE based upon documented clinical history consistent with HAE (SC or mucosal, non-pruritic swelling episodes without accompanying urticaria) (Maurer et al. 2018) AND any 1 of the following:

- A clinical diagnosis of BK-mediated angioedema as confirmed with threshold-stimulated kallikrein activity and Investigator-confirmed response to acute use of a BK targeted treatment (icatibant or ecallantide).
- One (1) of the established mutations (c.1032C>A, Thr309Lys; c.1032C>G, Thr309Arg; c.971_1018+24del72*; or c.892_909dup) in the factor XII gene.
- The established mutation in the plasminogen gene (c.988A>G, p.Lys330Glu).
- The established mutation in the angiopoietin-1 gene (c.355G>T, p.A119S).

Patients must have:

- Experienced a minimum of 2 HAE attacks (assessed by the Angioedema Activity Score [AAS] and confirmed by the Investigator) during the Screening Period.
- Completed the AAS questionnaire on a daily basis (minimum of 4 daily assessments per week) for the duration of the Screening Period.
- Patients must have had access to, and the ability to use, ≥ 1 acute medication(s) (e.g., plasma-derived or recombinant C1-INH concentrate or a BK2-receptor antagonist) to treat angioedema attacks.

Exclusion criteria were the same as for ISIS 721744-CS5 study.

Treatments

In Part A, patients with HAE-1/HAE-2 were randomized via the interactive response technology (IRT) system to SC injections of ISIS 721744 80 mg or placebo in a 2:1 ratio (ISIS 721744:placebo).

In Part B, patients with nC1-INH-HAE were enrolled via the IRT system to receive open-label SC injections of ISIS 721744 80 mg.

Allowed Concomitant Therapy

During the course of the study, the use of acute medications (plasma-derived or recombinant C1-INH concentrate, BK2-receptor antagonist, or kallikrein inhibitor) to treat angioedema attacks was allowed as medically indicated. Patients could be treated with on-demand therapy as determined by their treating physician.

All other stable medications (if not excluded below) were allowed as long as the dose and type were not expected to change during the study.

Disallowed Concomitant Therapy

Chronic prophylaxis for angioedema attacks, except for a stable dose of androgens. Any use of lanadelumab was not permitted. As noted above, the use of acute medications to treat angioedema attacks was allowed as medically indicated.

Objectives

Primary Objective

The primary objective of this study was to evaluate the clinical efficacy of antisense inhibitor of PKK (ISIS 721744) in patients with HAE-1, HAE-2, or nC1-INH-HAE.

Secondary Objective

The secondary objectives were to evaluate safety and tolerability of ISIS 721744 in patients with HAE-1/HAE-2 or nC1-INH-HAE and to evaluate the effect of ISIS 721744 on plasma PKK and other relevant biomarkers.

Outcomes/endpoints

Primary Endpoint

The primary endpoint was the time-normalized number of HAE attacks (per month) from Week 1 to Week 17.

Secondary endpoints

- The time-normalized number of HAE attacks (per month) from Week 5 to Week 17
- The time-normalized number of moderate or severe HAE attacks (per month) from Week 5 to Week 17
- The number of patients with a clinical response (defined as a ≥ 50%, ≥ 70%, or ≥ 90% reduction from Baseline in HAE attack rate) by Week 17
- The number of HAE attacks requiring acute therapy from Week 5 to Week 17
- Cleaved high molecular weight kininogen (cHK) levels at Weeks 9 and 17
- PKK activity at Weeks 9 and 17
- Consumption of on-demand medication at Weeks 9 and 17
- Angioedema quality of life (AE-QoL) questionnaire score at Weeks 9 and 17

Sample size

The primary endpoint is the time-normalized number of HAE attacks (per month) from Week 1 to Week 17. From historical data, the placebo group is estimated to have 6.8 HAE attacks per 4-month period. If the ISIS 721744 80 mg group is assumed to have 2.8 HAE attacks per 4-month period, then with a 0.05 significance level and using Poisson model, the sample size of 18 patients (12 patients administered SC injections of ISIS 721744 80 mg and 6 patients administered placebo) will provide at least 90% power for the primary endpoint, considering a 10% missing data or dropout rate for both active treatment and placebo. The sample size of 18 patients with HAE-1/HAE-2 is also considered sufficient for safety and tolerability evaluation. There is no statistical rationale for the sample size of 6 patients with HAE-nC1-INH.

Randomisation and blinding (masking)

Patients were allocated into Part A or Part B according to type of HAE: HAE-1/HAE-2 in Part A and nC1-INH-HAE (Type 3 HAE) in Part B. Only Part A was randomized and placebo controlled. Patients were enrolled and/or randomized at the Week 1 Visit, after all screening assessments had been completed and after the Investigator had verified that they were eligible.

For Part A, the Sponsor and all patients, monitors, and Study Center personnel related to the study were blinded throughout the study. However, if a patient experienced an SAE, and/or when knowledge of the treatment assignment would have affected the clinical management of the patient, the Investigator could unblind the treatment assignment for that patient via the IRT system.

Both ISIS 721744 and placebo were provided as injections for SC administration and were identical in appearance.

Statistical methods

Planned analyses

Descriptive summary statistics including number of subjects, mean, median, standard deviation, standard error, interquartile range (25th percentile, 75th percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables were used to summarize most data. All statistical tests were conducted using 2- sided tests with 5% Type I error rate unless otherwise stated. In view of the exploratory nature of this study, adjustments for multiplicity of testing were not generally used. Both central and local lab data were used in the analyses, including by visit summaries, figures and abnormality summary.

Analysis of Primary Endpoint

The primary efficacy endpoint, the time-normalized number of investigator-confirmed HAE attacks per month (defined as 28 days) during the on-treatment period from Week 1 to Week 17 (28 days after last dose administration), was compared between ISIS 721744 and placebo groups in Part A using a Poisson regression model and Pearson chi-square scaling of standard errors to account for potential overdispersion. The model included fixed effect for treatment group (categorical), the time normalized Run-in Period attack rate (continuous) as a covariate, and the logarithm of time in month (days from first dose date to 28 days after last dose administration divided by 28) that each patient was observed during the period was used as an offset variable.

From this model, the least squares mean rate and standard error for each treatment group, as well as the mean rate ratios relative to the placebo group and corresponding 95% confidence intervals (CIs), were estimated. The p-value of Wald-based chi-square test were also reported. The percentage difference in mean investigator-confirmed HAE attack rate between ISIS 721744 and placebo was calculated as 100% * (mean rate ratio –1). Similarly, the estimated upper and lower confidence limits for the mean rate ratio were transformed by subtracting 1 and multiplying by 100% to calculate 95% CIs for the percentage change.

The following sensitivity analyses were performed on the primary efficacy endpoint to evaluate the robustness of the results.

- The primary analysis described above was repeated in the PP population as a sensitivity analysis.
- The primary efficacy endpoint was compared between ISIS 721744 and placebo in Part A using a Negative Binomial model. The model included fixed effects for treatment group (categorical), the time normalized Run in Period attack rate (continuous) as a covariate, and the logarithm of time in month (days from first dose date to 28 days after last dose administration divided by 28) that each patient was observed during the on-treatment period was used as an offset variable. The analysis was conducted in the ITT population.

Part B data were summarized using descriptive statistics. The exact 95% CI for mean based on the gamma distribution was reported.

Analysis of Secondary Endpoints

Time-normalized number of Investigator-confirmed HAE attacks (per month) from Week 5 to Week 17

This endpoint was analysed using the same method as described for the primary efficacy endpoint and was conducted in both ITT and PP populations. Only patients who completed Week 5 (29 days) HAE assessments

were included in the analysis. The logarithm of time in month from Week 5 to Week 17 for each patient was used as an offset variable in the model.

Time-normalized number of moderate or severe Investigator-confirmed HAE attacks (per month) from Week 5 to Week 17

This endpoint was analysed using the same method as described for the primary efficacy endpoint and was conducted in both ITT and PP populations. Only patients who completed Week 5 (29 days) of HAE assessments were included in the analysis. The logarithm of time in months from Week 5 to Week 17 for each patient was used as an offset variable in the model.

The number of patients with a clinical response (defined as a ≥ 50%, ≥ 70%, or ≥ 90% reduction from Baseline in HAE attack rate) by Week 17

For each patient, a Treatment Period HAE attack rate from Week 5 to Week 17 and Run-in Period HAE attack rate were calculated. The percentage reduction was calculated as the Treatment Period HAE attack rate from Week 5 to Week 17 minus the Run-in Period HAE attack rate divided by the Run-in Period HAE attack rate. Patients who discontinued treatment early due to lack of efficacy or AEs were considered as non-responders and included in the denominator for the calculation of the proportion in the ITT population. Patients who discontinued due to other reason were excluded from analysis.

Risk difference comparing ISIS 721744 80 mg group to the placebo group in Part A and corresponding exact 95% CI (Santner and Snell 1980) were provided. The Fisher's Exact test p-value was also reported. The analysis was conducted in both ITT and PP populations.

The number of Investigator-confirmed HAE attacks requiring acute therapy from Week 5 to Week 17

This endpoint was analyzed using the same method as described for the primary efficacy endpoint and was conducted in both ITT and PP populations. HAE attacks requiring acute therapy included attacks with medical intervention or hospitalization marked on the eCRFs.

Change and percent change in cHK and PKK levels at Week 5 to Week 17

The change and percent change from Baseline in cHK and PKK levels at each visit during the treatment period were compared between ISIS 721744 and placebo in Part A using the mixed effects model with repeated measures (MMRM). The response variable was the change or percent change from Baseline at post-Baseline visit up to Week 17. For patients who terminated treatment early, only the data collected up to 42 days after last dose administration were included. The MMRM included effects of treatment (ISIS 721744 or placebo), time (categorical), treatment-by-time interaction, and Baseline value. The analysis was conducted in both ITT and PP populations. The unstructured covariance model was used to model the withinpatient errors, shared across treatments and small sample adjustments to standard errors and tests were made following the Kenward- Roger approach (Kenward and Roger 1997). Additional details were provided in the SAP.

CHK and PKK levels as well as the change and percent changes from Baseline over time were summarized using descriptive statistics by visit, treatment group, and part. The change and percent change in plasma proenzyme activation levels were analysed.

Consumption of on-demand medication by Weeks 9 and 17

The number and percentage of patients who used on-demand medication by Week 9 (Day 57) and by Week 17 were tabulated by treatment group and part. On-demand medications were identified based on manual review.

AE-QoL score at Weeks 9 and 17

AE-QoL was evaluated by determining its 4 individual domain scores and a total score. Each item answered by the patient scored between 0 and 4 points depending on the answer option chosen by the patient. The first answer option received 0 points, the second option 1 point, the third option 2 points, etc. Details of the calculation of the AE-QoL total score and domain scores are provided in the SAP. The AE-QoL total score and domain scores were analysed using the same method as described for cHK and were conducted in both ITT and PP populations.

Error probabilities, adjustment for multiplicity and interim analyses

No multiplicity adjustment was planned for this study.

No interim analysis was planned for this study.

Changes from protocol-specified analyses

There were no changes to the analyses described in the SAP, version 1.0

Results

Participant flow

20 patients were randomized (6 to placebo and 14 to ISIS 721744) and received Study Drug. Nineteen (19) randomized patients completed study treatment. One patient (ISIS 721744) voluntarily withdrew in Week 13 due to travel time to the site and work schedule.

Two (2) patients completed the Post-Treatment Period. Of the 18 patients who did not complete the Post-Treatment Period, 17 rolled over into OLE Study ISIS 721744-CS3 and one patient terminated due to withdrawal of consent.

In Part B, 6 patients were screened, and 3 patients were dosed and completed the study treatment. The reasons for the screen failures of the 3 patients include the following: 2 patients due to not meeting inclusion criterion 3b (negative threshold stimulated assay or no confirmation of pathogenic mutation) and 1 patient for not meeting inclusion criterion 4b (did not complete Angioedema Activity Score [AAS] per protocol).

Recruitment

First Patient Screened: 13 November 2019

First Patient Enrolled (Randomized): 7 January 2020

Last Patient, Last Visit: 15 March 2021

Conduct of the study

Amendments

The original protocol (3 May 2019) was amended 3 times.

The main amendments to the study protocol are described below:

Amendment 1 (21 October 2019):

- Specified a minimal compliance level in the inclusion criteria for completion of the Angioedema Activity Score, during the screening period, as a requirement prior to randomization to treatment in Part A, or initiation of treatment in Part B, of the study.
- Broadened the exclusion criteria to include elevated PTT, history of coagulopathy or bleeding diathesis, and renal and hepatic diseases.
- Modified the exclusion criteria to allow for participants who tested positive for hepatitis B or C enzyme but were non-reactive.
- Provided the definition of an HAE attack and delineated how discrete attacks would be counted.
- Designated adverse events of special interest (AESIs).
- In the Schedule of Procedures, added anti-drug antibody testing at Day 15 and Day 29 visits and removed the requirement for a physical examination from the day 15 visit.

Amendment 2 (29 January 2020):

- Updated the established mutations in the plasminogen and angiopoietin genes from the legacy description to the Human Genome Variation Society description in the diagnostic report. The plasminogen gene was changed from c.9886A>G to 988A>G and the angiopoietin-1 gene from c.807G>T to 355G>T.

Amendment 3 (5 May 2020):

- Adjusted the length of time patients must not have received lanadelumab prior to screening for ISIS 721744-CS2 from 6 months to 10 weeks (i.e., 5 times the ~14-day half-life for lanadelumab).

Baseline data

Demographics/Baseline Characteristics

In Part A, demographic characteristics were similar between patients who received placebo and those who received ISIS 721744. Overall, 65% were female, the mean age was 38.5 years (range 21 to 66), 95.0% were white, and the mean BMI was 28.6 kg/m2 (range 19.6 to 48.1).

In Part B, all 3 patients were female, the mean age was 34.0 years (range 25 to 40), all were white, and the mean BMI was 38.4 kg/m2 (range 35.5 to 42).

HAE History

In Part A, the HAE history was generally similar between the patients who received placebo and those who received ISIS 721744. Overall, 90% had HAE-1, at least 95% had experienced peripheral and abdominal HAE attacks, and none had used lanadelumab, The mean number of attacks in the previous 12 months was 23.8 (range 4 to 70), the mean HAE attack rate in the Run-in Period was 2.70 (range 1 to 5.6), and the patients were about evenly divided among the 3 categories of attack rate.

In Part B, all 3 patients had HAE-3 and had experienced peripheral and abdominal HAE attacks, and 1 patient had used lanadelumab and discontinued because it was ineffective. The mean number of attacks in the previous 12 months was 61.3 (range 36 to 100), the mean HAE attack rate in the Run-in Period was 4.23 (range 2.3 to 7.4), and 1 patient had 1 to < 2 attacks/4 weeks and 2 patients had ≥ 3 attacks/4 weeks.

Prior medications for HAE were complement C1 esterase inhibitor for 10 patients (50%) in Part A and 1 patient (33.3%) in Part B, icatibant acetate (or icatibant) for 10 patients (50%) in Part A and 3 patients (100%) in Part B, and conestat alfa for 1 patient (33.3%) in Part B.

In Part A, 19 patients (95.0%) used concomitant medications. All 6 placebo-treated patients (100%) and 5 ISIS 721744-treated patients (35.7%) used drugs for HAE attacks: complement C1 esterase inhibitor (5 placebo, 83.3%; 3 ISIS 721744, 21.4%) or icatibant (3 placebo, 50.0%; 2 ISIS 721744, 14.3%). In Part B, all 3 patients used concomitant medications. One patient used drugs for HAE attacks (both icatibant and complement C1 esterase inhibitor).

Numbers analysed

In Part A, all 20 randomized patients were included in the safety, ITT, and PP populations. In Part B, all 3 patients were included in the safety, ITT, and PP populations

Outcomes and estimation

Primary endpoint – part A

During the on-treatment period from Week 1 to Week 17, the monthly mean HAE attack rate was 0.23 for the ISIS 721744 group and 2.21 for the placebo group. The percentage difference was -90% (95% CI, -76% to -96%; $p < 0.001$).

Secondary endpoints – part A

- From Week 5 to Week 17, the monthly mean HAE attack rate was 0.07 for the ISIS 721744 group and 2.06 for the placebo group. The percentage difference was -97% (95% CI, -69% to -100%; $p = 0.003$).
- From Week 5 to Week 17, the monthly mean moderate or severe HAE attack rate was 0.05 for the ISIS 721744 group and 1.25 for the placebo group. The percentage difference was -96% (95% CI, -65% to -100%; $p = 0.004$).
- The proportions of patients with a ≥ 50%, ≥ 70%, or ≥ 90% reduction from Baseline in HAE attack rate from Week 5 to Week 17 were 100%, 92.3%, and 92.3%, respectively, in the ISIS 721744 group vs 33.3%, 16.7%, and 0% in the placebo group ($p \leq 0.004$).
- From Week 5 to Week 17, the mean number of Investigator-confirmed HAE attacks requiring acute therapy was 0.07 in the ISIS 721744 group and 1.40 in the placebo group. The percentage difference was -95% (95% CI, -52% to -99%; $p = 0.009$).
- On-demand medication was used by 85.7% of patients in the ISIS 721744 group and 100% of patients in the placebo group by Week 9.
- Greater improvement was seen with ISIS 721744 vs. placebo for the AE-QoL total score and the 4 domain scores. For the total score at Week 17, the mean change from Baseline was -26.85 with ISIS 721744 vs -6.15 with placebo ($p = 0.002$).
- Between Week 5 and Week 17, the percentage of attack-free patients was 92.3% in the ISIS 721744 group vs 0% in the placebo group.

Efficacy results for part B

In Part B for the 3 nC1-INH-HAE patients, the monthly mean attack rate was 4.23 (range 2.3 to 7.4) during the Run-in Period and 1.52 (range 0 to 4.1) from Week 1 to Week 17.

In Part B for the 3 nC1-INH-HAE patients, the monthly mean moderate or severe attack rate from Week 5 to Week 17 was 0.89 (range 0 to 2.7).

In Part B the proportions of patients with a ≥ 50%, ≥ 70%, or ≥ 90% reduction from Baseline in HAE attack rate from Week 5 to Week 17 were 2/3, 2/3 and 1/3 respectively

In Part B for the 3 nC1-INH-HAE patients, the mean number of attacks requiring acute therapy from Week 5 to Week 17 was 0.89 (range 0 to 2.7).

Table 18. Attack rates per month of 3 patients with HAE-nC1-INH

Attack rate per month	Patient 1	Patient 2	Patient 3
Baseline			
All attacks	2.3	7.4	3.0
Moderate or severe attacks	1.6	5.9	3.0
Attacks requiring acute therapy	0.8	5.9	0
Week 1-17			
All attacks	0	4.1	0.5
Moderate or severe attacks	0	2.3	0
Attacks requiring acute therapy	0	2.3	0
Week 5-17			
All attacks	0	5.0	0.3
Moderate or severe attacks	0	2.7	0
Attacks requiring acute therapy	0	2.7	0

Study ISIS 721744-CS7

Study title: An Open-Label, Long Term Safety and Efficacy Study of Donidalorsen in the Prophylactic Treatment of Hereditary Angioedema (HAE)

Study design

This study was a Phase 3, ongoing, global open-label, 2-arm study (ISIS 721744-CS7) conducted in parallel at multiple centers to evaluate the long-term safety and efficacy of donidalorsen in preventing angioedema attacks in patients with HAE-1 and HAE-2. Two (2) treatment arms were evaluated in this study as follows:

• OLE patients – Patients who rolled-over from ISIS 721744-CS5 (hereafter referred to as the Index Study).

• Switch patients – Patients who did not roll over from another donidalorsen study (donidalorsen-naïve) and were previously maintained on HAE prophylactic therapy with lanadelumab, berotralstat, or a C1-esterase inhibitor.

The study comprised 4 periods, including a Qualification Period for OLE patients (Weeks -4 to -1) or a Screening Period for Switch patients (Weeks -10 to -1), a Treatment Period (Weeks 1 to 53) for all patients (OLE and Switch), an Extended Treatment Period (Weeks 54 to 157), and a Post-Treatment Period (end of treatment + 13 weeks).

Following the Week 53 visit, patients were to receive donidalorsen for up to an additional 104 weeks in the Extended Treatment Period. In this Extended Treatment Period, patients could either continue to receive donidalorsen 80 mg per the dosing schedule prior to Week 53 (every 4 or 8 weeks) or, as determined by clinical symptoms (per Investigator), change dose frequency from donidalorsen 80 mg every 4 weeks to every 8 weeks or every 8 weeks to every 4 weeks. Change in dose frequency could occur at any time during the Extended Treatment Period.

Patients were to enter the Post-Treatment Period after completion of, or early termination from, the Treatment Period or Extended Treatment Period for final safety evaluations,

OLE patients who were being treated with donidalorsen during the Index Study could continue their treatment on the ISSI 721744-CS7 Study without interruption. Patients treated with donidalorsen 80 mg or placebo once every 4 weeks in the Index Study (Index Study Cohort A) received donidalorsen 80 mg every 4 weeks beginning 28 ± 3 days after the last dose in the Index Study.

Patients treated with donidalorsen 80 mg or placebo once every 8 weeks in the Index Study (Index Study Cohort B) were to receive donidalorsen 80 mg every 8 weeks in the OLE part of the current study beginning 56 ± 3 days after the last dose in the Index Study unless they were not attack-free for ≥ 8 weeks (Weeks 17-25 during the Index Study), in which case they were to receive donidalorsen 80 mg every 4 weeks.

Switch Patients

During the Screening Period for Switch patients, eligibility was to be confirmed up to 70 days prior to administration of donidalorsen. Switch patients had to be on a stable dose of prophylaxis treatment with lanadelumab, berotralstat, or a C1 esterase inhibitor for at least 12 weeks prior to the Screening Period and were to continue to take their HAE prophylactic treatment during the Screening Period. The schedule for the Switch patients to take their final dose of the prior prophylactic therapy is summarized below:

Lanadelumab: Patient was to take the last dose of lanadelumab 14 days ± 3 days prior to Day 1

Berotralstat: Patient was to continue taking a stable dose of berotralstat for 14 days ± 3 days after Day 1

C1-esterase inhibitor: Patient was to continue taking a stable dose of C1-esterase inhibitor for 14 days ± 3 days after Day 1

Switch patients were to be treated with donidalorsen 80 mg once every 4 weeks for the Treatment Period (Week 1 to 53). If a Switch patient participated in the Extended Treatment Period, they were to continue to receive donidalorsen 80 mg once every 4 weeks for an additional 104 weeks, or as determined by clinical symptoms (per Investigator), they could change dosages to donidalorsen 80 mg every 8 weeks, and/or back to donidalorsen 80 mg every 4 weeks. Change in dose frequency could occur at any time in the Extended Treatment period.

For Switch patients, the timing of the switch from the previous prophylactic HAE drug to donidalorsen was based on the half-life of the individual drugs. Given the relatively long half-life of lanadelumab, donidalorsen dosing was initiated at the end of lanadelumab dosing interval while berotralstat and C1-esterase inhibitor were administered concurrently with donidalorsen for 14 days given their short half-life to prevent any loss of efficacy. Given that ASOs such as donidalorsen are not substrates, inducers and/or inhibitors of general metabolic enzymes (cytochrome P450s) and transporters, donidalorsen is anticipated to have no drug-drug interaction with any switch therapies from a PK perspective. Therefore, donidalorsen administration was not anticipated to affect the exposure and thereby efficacy or safety of the switch therapies.

Study population

Main inclusion criteria

Open-Label Extension Patients ONLY

Satisfactory completion of ISIS 721744-CS5 (randomized placebo-controlled Index Study) through Week 25 or patients who were allowed to exit ISIS 721744-CS5 study per protocol with an acceptable safety and tolerability profile.

Switch Patients ONLY

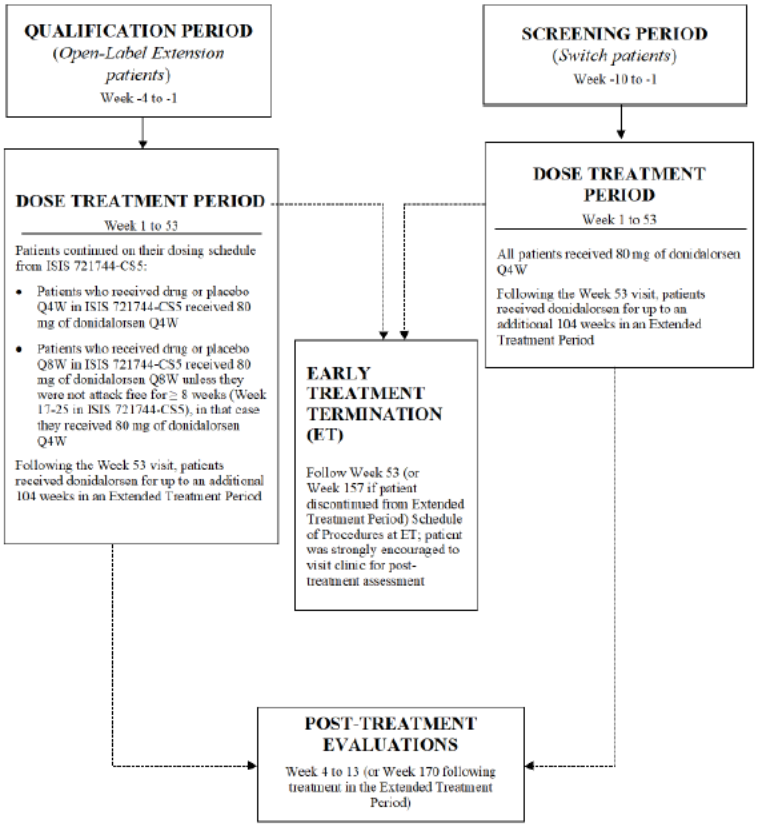
Patients were to be aged ≥ 12 years at the time of informed consent and, as applicable, assent.

Patients were to have a documented diagnosis of HAE-1/HAE-2 based upon all of the following:

- Documented clinical history consistent with HAE (subcutaneous or mucosal, non-pruritic swelling episodes without accompanying urticaria) (Maurer et al. 2018).
- Diagnostic testing results that confirmed HAE-1/HAE-2: C1-INH functional level < 40% normal level. Patients with a functional level of 40% to 50% of normal were to be enrolled if their complement factor C4 level was below the lower limit of normal or if a known pathogenic mutation in the SERPING1 gene had been demonstrated.

At least 1 of the following: age at reported HAE onset ≤ 30 years; a family history consistent with HAE-1/HAE-2; or complement component 1q within the normal range. Patients were to be on a stable dose (≥ 12 weeks) of prophylaxis treatment with lanadelumab or berotralstat or a C1-esterase inhibitor prior to the Screening Period.

Figure 20. Design and treatment schema-ISIS 721744-CS7



Allowed Concomitant Therapy

During the course of the study, the use of HAE acute medications (plasma-derived or recombinant C1-INH concentrate, BK2-receptor antagonists, or kallikrein inhibitors) to treat angioedema attacks was allowed, as medically indicated. Patients could be treated with on-demand therapy as determined by their treating physician.

Consumption of on-demand medications (to treat acute attacks) was to be determined as part of the secondary efficacy endpoints. On-demand medications include the following concomitant medications:

• C1 esterase inhibitors (human), also known as BERINERT, CINRYZE

• C1 esterase inhibitor (recombinant) also known as RUCONEST

• Plasma Kallikrein Inhibitor (human) ecallantide, also known as KALBITOR

• Bradykinin antagonist icatibant, also known as FIRAZYR

Disallowed Concomitant Therapy

- Chronic prophylaxis for angioedema attacks, except for a stable dose of androgens or tranexamic acid. Any chronic use of other prophylactic agents such as lanadelumab, berotralstat, or C1-esterase inhibitors were not permitted during the Treatment Period.
- Angiotensin-converting enzyme inhibitors or any estrogen-containing medications with systemic absorption (such as oral contraceptive or hormonal replacement therapy). The use of intrauterine and

intravaginal estrogen was allowed. Chronic estrogen use for gender reassignment was allowed as long as the dose was stable for at least 12 weeks prior to Screening and throughout the Treatment Period.

- Any oligonucleotides (including small interfering RNA) other than donidalorsen. This exclusion did not apply to vaccines

Objective and endpoints

The primary objective of this study was to further investigate the safety profile of the product

Secondary objective

To evaluate the long-term efficacy and the effects of donidalorsen on the number of HAE attacks and their impact on the quality of life (QoL) of patients with HAE

Secondary endpoints

- The time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 53
- The time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 53
- The percentage of Investigator-confirmed HAE attack-free patients from Week 5 to Week 53
- The time-normalized number of moderate or severe Investigator confirmed HAE attacks (per 4 weeks) from Week 5 to Week 53
- The number of Investigator-confirmed HAE attacks (per 4 weeks) requiring acute therapy from Week 5 to Week 53
- Angioedema Quality of Life (AE-QoL) questionnaire total score over 52 weeks

Statistical Methods

Planned analyses were defined in the Statistical Analysis Plan (SAP), version 1.0, dated 03 Apr 2024. Due to the absence of a control group in this study, no formal statistical testing was conducted, and all efficacy data were presented as descriptive statistics.

The following analysis sets were used for the analysis of data as described within each analysis set.

- The Safety Set was to include all enrolled patients who received at least 1 dose of donidalorsen.
- The Full Analysis Set was to include all enrolled patients who received at least 1 dose of donidalorsen.
- The Per Protocol Set was to include all patients in the Full Analysis Population who were treated according to the protocol with no major protocol deviations that could compromise the interpretation of efficacy. Major protocol deviations that could compromise the interpretation of efficacy were to be determined prior to the data cutoff date.
- The PK Set was to include all patients who enrolled and received at least 1 dose of donidalorsen and had at least 1 evaluable PK sample.

Results

Participants flow

A total of 83 OLE patients rolled over from the Index study (ISIS 72144-CS5) into the current study. Of these 83 patients, 69 were treated with donidalorsen 80 mg once every 4 weeks in the current study (and constitute the OLE donidalorsen-4 group), including 44 patients who received donidalorsen 80 mg every 4 weeks during both the Index Study and the current study (Index/OLE donidalorsen-4 group), 6 patients who received donidalorsen 80 mg every 8 weeks in the Index study and every 4 weeks in the current study (Index donidalorsen-8/OLE donidalorsen-4 group), and 19 patients who received placebo in the Index study and donidalorsen 80 mg every 4 weeks during the current study (Index placebo/OLE donidalorsen-4 group).

Fourteen patients who were treated with donidalorsen 80 mg every 8 weeks during the Index Study remained on the same dose regimen during the current study (Index/OLE donidalorsen-8 group).

All patients who received placebo during the Index Study received donidalorsen 80 mg every 4 weeks in the current study. After Week 53 (i.e., during the Extended Treatment Period), patients could change the frequency of dosing from every 4 weeks to every 8 weeks based on clinical symptoms (per Investigator decision), no patients had changed dosing frequency by the time of the data cutoff date for this CSR.

This study was ongoing at the data cutoff date for this CSR, and the majority of OLE patients had not completed Week 53 (52-week Treatment Period). Seven (8.4%) patients had completed this period and entered the Extended Treatment Period, including 3 (6.8%) patients in the ex/OLE donidalorsen-4 group, 2 (10.5%) in the Index placebo/OLE donidalorsen-4 group, and 2 (14.3%) in the Index/OLE donidalorsen-8 group.

Switch Patients

A total of 65 patients who were previously treated with another HAE prophylactic treatment (lanadelumab, berotralstat, or C1-esterase inhibitor) were enrolled into the current study (Switch patients) and continued to receive their prior prophylactic HAE therapy throughout the screening period; 64 (98.5%) Switch patients received at least 1 dose of donidalorsen 80 mg once every 4 weeks. Of these 64 Switch patients, 31 patients had previously received lanadelumab (prior lanadelumab group), 11 patients had previously received berotralstat (prior berotralstat group), and 22 patients had previously received C1-esterase inhibitor (prior C1-esterase group).

This study was ongoing at the interim data cutoff date. At the time of the data cutoff date, the majority of Switch patients (58 [89.2%]) had completed at least 17 weeks of study treatment. Overall, 12 (18.5%) Switch patients completed 1 year of study treatment (52-Week Treatment Period) and had entered into the Extended Treatment Period. No patients had completed the 3-year Extended Treatment Period.

Outcome and estimation

The mean (SD, SEM) time-normalized Investigator confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 53 of the current study for the OLE patients was 0.22 (0.41, 0.05); Index Run-in Baseline rate was 3.42 (2.12, 0.23).

For the OLE patients, the mean percent change in time-normalized, Investigator-confirmed HAE attack rate (per 4 weeks) for Week 1 to Week 53 of the current study was a reduction of 93.11% (95% CI: -95.87%, -90.34%) from the Index Run-in Baseline. For the OLE donidalorsen-4 group, the mean percent change in time-normalized, Investigator-confirmed HAE attack rate (per 4 weeks) was a reduction of 93.33% (95% CI:

-96.26%, -90.40%). The mean percent change in time-normalized, Investigator-confirmed HAE attack rate (per 4 weeks) for the OLE donidalorsen-8 group was a reduction of 92.01% (-100.81%, -83.20%)

Other efficacy endpoints

- Reductions from Index Run-in Baseline of over 90% were seen in the time-normalized Investigator confirmed moderate or severe HAE attack rate (per 4 weeks) from Week 5 to Week 53.
- The reduction in moderate or severe HAE attacks was similar between the OLE donidalorsen-4 group (94.01% [95% CI: -96.88%, -91.13%]) and OLE donidalorsen-8 group (88.18% [95% CI: -97.97%, -78.40%]).
- Reductions from Index Run-in Baseline of over 90% were seen in the time-normalized Investigator confirmed HAE attack rate (per 4 weeks) of Investigator-confirmed HAE attacks that required acute-HAE therapy from Week 5 to Week 53.
- Clinically meaningful (i.e., ≥ 6 points) reductions from Index Run-in Baseline in AE-QoL score were seen in OLE patients, indicating improvement in QoL. The overall mean (standard deviation [SD], SEM) change from Index Run-in Baseline for the total AE-QoL score at Week 13 (n = 78) and Week 25 (n = 56) were reductions of 26.4 (16.73, 1.89) and 27.0 (15.99, 2.14) points, respectively.

Switch patients

For the Switch patients, the mean (SD, SEM) time-normalized Investigator confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 53 was 0.30 (0.46, 0.06); Switch Baseline rate was 0.85 (1.28, 0.16). The mean percent change from the Switch Baseline was a reduction of 66.12% (95% CI: -79.69, -52.55).

The mean (95% CI) percent change in the HAE attack rate were reductions of 51.05% (-78.77%, -23.32%), 76.47% (-93.38%, -59.56%), and 78.20% (-97.31%, -59.09%) for the prior lanadelumab, prior berotralstat, and prior C1-esterase inhibitors groups, respectively.

Other efficacy endpoints

- An 81.92% (95% CI: -95.60%, -68.24%) reduction from Switch Baseline in the time-normalized Investigator-confirmed moderate to severe HAE attack rate (per 4 weeks) was seen in Switch patients from Week 5 to Week 53.
- Mean (SD, SEM) reductions (improvements) in AE-QoL total score of 10.4 (12.6, 1.75) and 11.9 (16.8, 2.56) points were observed at Week 17 (n = 52) and Week 25 (n = 43), respectively. Both of these values exceed the threshold of a 6-point reduction, indicating clinically-meaningful improvements.
- The percentage of patients with well-controlled disease (AECT ≥ 10) increased from 66.7% at Baseline to 93.0% at Week 17.

Study ISIS 721744-CS3

Study tittle: An Open-Label Extension Study of ISIS 721744 in Patients with Hereditary Angioedema, ISIS 721744-CS3

Study design

The study consisted of 4 periods including a Qualification Period, a Treatment Period, an Extended Treatment Period, and a Post-Treatment Period. The Treatment Period was composed of a Fixed Dosing Period

(Treatment Period 1) and a Flexible Dosing Period during which the donidalorsen dosage for each patient could be changed (Treatment Period 2).

All eligible patients were to receive donidalorsen 80 mg once every 4 weeks from Week 1 to Week 13 (Fixed Dosing Period). Following completion of the first 12-week period, patients (based on the Investigator and Sponsor Medical Monitor recommendation) had 3 different dosing options starting at Week 17 (Flexible Dosing Period):

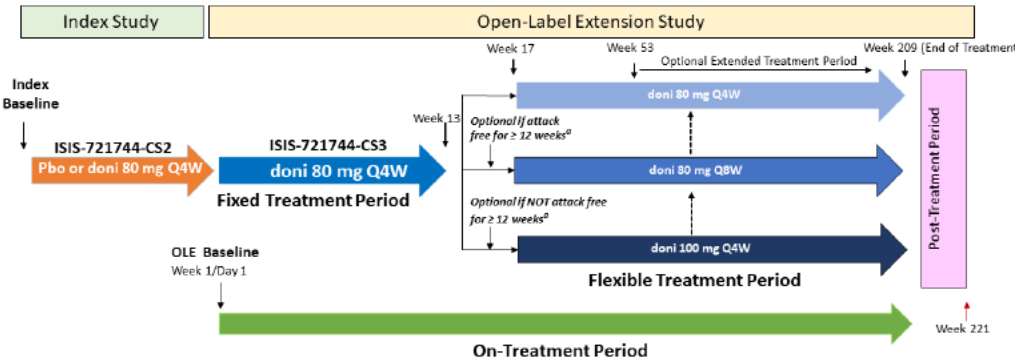
• Patients could continue receiving donidalorsen 80 mg every 4 weeks.

• For patients who were attack free for ≥ 12 weeks after entering this OLE Study (the Fixed Dosing Period): The Investigator could initiate a dose at a reduced frequency of donidalorsen 80 mg every 8 weeks. The reduced frequency dosing could begin at any study visit starting at or after Week 17. If a patient's disease was not adequately controlled on donidalorsen 80 mg every 8 weeks, then dosing could return to donidalorsen 80 mg every 4 weeks.

• For patients who were **not** attack free for ≥ 12 weeks after entering this OLE Study (the Fixed Dosing Period): The Investigator could initiate a higher dose of donidalorsen at 100 mg every 4 weeks. The higher dose could begin at any study visit starting at or after Week 17. If patients developed any tolerability or safety issue during treatment, the dose could be reduced back to donidalorsen 80 mg every 4 weeks.

At the end of the 52-week Treatment Period (Fixed Dosing + Flexible Dosing), patients had the option of receiving donidalorsen in an Extended Treatment Period (during which flexible dosing was maintained) for up to an additional 156 weeks (approximately 3 years), for a Total Treatment Period (Treatment + Extended Treatment) of up to approximately 209 weeks (approximately 4 years) or enter the 12-week Post-Treatment Period.

Figure 21. Trial design



Note: All doses were administered subcutaneously
[a]Optional dosing determined by the Investigator. For patients who were attack-free for ≥ 12 weeks, after entering this OLE Study, the Investigator could initiate a switch to donidalorsen 80 mg every 8 weeks. The switch could begin at any visit starting at Week 17. If patients were not adequately controlled on 80 mg every 8 weeks, then dosing could return to 80 mg every 4 weeks. For patients who were not attack-free for ≥ 12 weeks, after entering this OLE Study, the Investigator could initiate a switch to donidalorsen 100 mg every 4 weeks. The switch could begin at any visit starting at Week 17. If patients developed any tolerability or safety issue, the dose could be reduced back to 80 mg every 4 weeks.
Abbreviations: doni = donidalorsen; OLE = open-label extension; Pbo = placebo; Q4W = every 4 weeks; Q8W = every 8 weeks.

An initial interim analysis was conducted once all patients enrolled in this OLE Study and had completed 1 year of study drug administration. A second interim analysis was conducted after all patients had completed 2 years of study drug administration. The CSR provided is based on the third interim analysis following the database lock on 26 Mar 2024 (data were collected through the data cut date of 26 Feb 2024).

Study population

The main inclusion criteria

Satisfactory completion of ISIS 721744-CS2 (Index Study) through Week 17 with an acceptable safety and tolerability profile, per Sponsor and Investigator judgement.

Objective and endpoints

The primary objective of this study was the safety profile of the product

Secondary objective

To evaluate the efficacy of extended dosing, and alternative dosing and/or dose frequency with donidalorsen in patients with HAE

Secondary endpoints

• The time-normalized HAE attacks (per 4 weeks) by treatment

• Consumption of on-demand medications

Statistical Methods

Planned analyses were defined in the SAP, version 3.0, dated 14 Mar 2024. Due to the absence of a control group in this study, no formal statistical testing was conducted, and all efficacy data were presented as descriptive statistics.

Pharmacokinetic (PK) parameters were summarized using number of patients, mean, standard deviation, coefficient of variation (CV), geometric mean, geometric %CV, median, minimum, and maximum.

The following analysis populations were to be used for the analysis of data as described within each analysis population:

- The Safety Population: All enrolled patients who received at least 1 dose in this OLE Study.
- Intent-to-Treat (ITT) Population: All enrolled patients in this OLE Study.
- Per Protocol Population: All patients in the ITT Population who were treated according to the protocol with no major protocol deviations that could compromise the interpretation of efficacy. Major protocol deviations that could have compromised the interpretation of efficacy were determined prior to the database lock.
- PK Population: All patients who were enrolled and received at least 1 dose of study drug (donidalorsen) and had at least 1 evaluable PK sample

Results

Participants flow

In this OLE Study, 20 patients received study drug, including 17 patients in the HAE-1/HAE-2 group and 3 patients in the nC1-INH-HAE group. Of the 17 patients in the HAE-1/HAE-2 group, 12 patients were in the HAE-1/HAE-2 Index donidalorsen-4 group and the remaining 5 patients were in the HAE-1/HAE-2 Index placebo group.

At the time of this third interim analysis (data cut on 26 Feb 2024), 15 patients had completed ≥ 2 years of study drug and 14 patients had completed ≥ 3 years of study drug at the time of the data cut off.

A total of 6 patients discontinued early from study treatment including 4 (23.5%) patients in the HAE-1/HAE-2 group and 2 (66.7%) in the nC1-INH-HAE group. In the HAE-1/HAE-2 Index placebo group, 1 (20.0%) patient discontinued due to Investigator judgment. In the HAE-1/HAE-2 Index dondidalorsen-4 group, 1 (8.3%) patient discontinued due to a TEAE (6 events of injection site discolouration), and 2 (16.7%) patients discontinued due to voluntary withdrawal. In the nC1-INH-HAE group, 1 (33.3%) patient discontinued due to voluntary withdrawal and 1 (33.3%) patient discontinued due to pregnancy. No patient withdrew due to meeting a study stopping rule.

Doses used in the study

During the Flexible Dosing Period, 8 patients HAE-1/HAE2 group changed from the treatment regimen with donidalorsen every 4 weeks to the treatment regimen with donidalorsen every 8 weeks. Of these 8 patients, 5 patients continued to receive treatment every 8 weeks for a mean duration of 703 days (range [values rounded]: 33.99 to 1285.99 days) as of the data cut date for this third interim analysis. The remaining 3 patients switched back to donidalorsen every 4 weeks.

Three patients were treated with donidalorsen 100 mg every 4 weeks during this OLE Study including one patient in the HAE-1/HAE-2 group and 2 patients in the nC1-INH-HAE group.

The ITT Population was used for the efficacy analysis and consisted of 17 patients in the HAE-1/HAE-2 group and 3 patients in the nC1-INH-HAE group. The Per Protocol, PK, and Safety Populations also included all 20 patients enrolled in the study.

Note: The HAE-1/HAE-2 group and nC1-INH-HAE group were analyzed separately.

Concomitant Medications

One or more concomitant medication was taken by all patients in this study and use was similar across treatment groups. Concomitant medications across all treatment groups included those used for the acute treatment of HAE attacks, which were allowed per protocol. The most common of these acute treatments (≥ 3 patients in either the HAE-1/HAE-2 group or the nC1-INH-HAE group) included complement C1 esterase inhibitors (7 [41.2%] patients in the HAE-1/HAE-2 group and 2 [66.7%] patients in the nC1-INH-HAE group), icatibant acetate (4 [23.5%] and 2 [66.7%]), and icatibant (1 [5.9%] and 3 [100%]).

Outcome and estimation

HAE-1/HAE-2 Group

Overall, for all patients in the HAE-1/HAE-2 group, the mean (SD, SEM) time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) for the On-Treatment Period was 0.06 (0.066, 0.016). The mean percent change from the Index Run-in Baseline was a reduction of 96.42% (95% CI: -99.121%, -93.711%).

For the HAE-1/HAE-2 Index donidalorsen-4 group, the data indicate that the effect of donidalorsen observed in the Index Study was sustained through this OLE Study to the data cut date for this interim analysis. For the HAE-1/HAE-2 Index placebo group, data show that, on receiving donidalorsen treatment in this OLE Study, these patients achieved a reduction in HAE attack rate similar to that observed in the HAE-1/HAE-2 Index donidalorsen-4 group during the Index Study.

nC1-INH-HAE Group

For the 3 patients in the nC1-INH-HAE group, the mean (SD, SEM) time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) at the Index Run-in Baseline was 4.23 (2.735, 1.579). The mean (SD, SEM) time-normalized Investigator-confirmed rate of HAE attacks (per 4 weeks) for the On-Treatment Period was

1.76 (2.209, 1.275), which represents a reduction of 68.40% (95% CI: -126.204%, -10.602%) from the Index Run-in Baseline.

Flexible Dosing Period: Time-Normalized Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) HAE-1/HAE-2 Group

Eight patients in the HAE-1/HAE-2 group changed dosing frequency from donidalorsen 80 mg every 4 weeks during the Fixed Dosing Period to donidalorsen 80 mg every 8 weeks during the Flexible Dosing Period (OLE donidalorsen-8 group); 5 of these patients remained at this dose and frequency at the time of the data cut for this CSR, and 3 patients returned to the more frequent dosing of donidalorsen 80 mg every 4 weeks dosing due to experiencing HAE attacks. During the Flexible Dosing Period, the 8 patients in the OLE donidalorsen-8 group had a mean (SD, SEM) time-normalized Investigator- confirmed HAE attack rate (per 4 weeks) of 0.30 (0.571, 0.202) and a mean reduction in the time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Index Run-in Baseline of 82.58% (95% CI: -112.296%, -52.859%). Three of these patients receiving donidalorsen 80 mg every 8 weeks remained attack free over the duration of the study.

For the 1 patient in the OLE donidalorsen-100 mg-4 group, the mean time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) for the Flexible Dosing Period was 0.11 with a mean percent change from the Index Run-in Baseline of a 95.16% reduction.

Out of 3 patients with nC1-INH-HAE, 1 patient was treated with donidalorsen 80 mg every 4 weeks and 2 patients were treated with donidalorsen-100 mg every 4 weeks during this OLE Study

Other efficacy endpoints

• For patients in the HAE-1/HAE-2 group, reductions of over 90% were also seen in the time-normalized Investigator-confirmed moderate or severe HAE attack rate and in the time-normalized Investigator-confirmed HAE attack rate for HAE attacks requiring acute HAE therapy.

• Angioedema Quality of Life (AE-QoL) scores were improved from the Index Run-in Baseline with donidalorsen treatment in this study, and the change from the Index Run-in Baseline over time was clinically meaningful and generally similar between patients in the donidalorsen-4 group and patients in the HAE-1/HAE-2 Index placebo group indicating that once they switched to donidalorsen treatment in the OLE, patients treated with placebo in the Index Study were able to achieve similar improvements in QoL to those that received donidalorsen throughout the Index and OLE studies.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The pivotal study (ISIS 721744-CS5) had a double-blind, placebo-controlled design. The study design was overall acceptable. Choosing a placebo control to study the prophylactic effect of donidalorsen on recurrent attacks of HAE was endorsed by the CHMP Scientific Advice.

The study consisted of 3 periods including an 8-week screening period, a 24-week treatment period, and 13-week post-treatment period.

In the study, two dose levels were tested. In Cohort A patients received donidalorsen 80 mg once every 4 whereas in Cohort B the treatment was given less frequently, i.e. donidalorsen 80 mg once every 8 weeks. In

both cohorts, matching placebo was used as a control. Data from patients receiving placebo in Cohort A or Cohort B were pooled for the analyses.

Therefore, the recommended dose 80mg SC once monthly and the frequency of dosing, which could be extended to 80 mg SC once every two months once a patient is well controlled (e.g., attack-free) for at least 3 months while receiving Dawnzera, are considered acceptable and supported by the clinical data.

Patients experiencing a significant number of angioedema attacks (at least 5 attacks/month for 2 consecutive months after Week 5) could discontinue the treatment period and be transferred directly to the open label study to ensure that they received an active treatment for their condition.

The randomisation was not stratified.

Study population

The pivotal study enrolled only patients with HAE-1 or HAE-2 whereas patients with HAE with normal C1-INH level and function (also known as HAE Type III) were excluded. The study enrolled adult patients and adolescents.

A diagnosis was made based on both clinical presentation (a clinical history consistent with HAE) and diagnostic test results. In relation to diagnostic testing, C1-INH functional level < 40% normal was considered as sufficient to make a diagnosis. Patients with a functional level of 40% to 50% of normal could be as well enrolled if their complement factor C4 level was below the normal limit or when a known pathogenic mutation in the SERPING1 gene had been demonstrated. Although the inclusion criteria are similar to those used in other developments, they differ slightly from the criteria recommended by the international WAO/EAACI guideline (2022). Nevertheless, they are considered acceptable.

In relation to the severity of the disease, all patients eligible for enrolment had to experience a minimum of 2 HAE attacks (confirmed by the Investigator) during the Screening Period, in which other prophylactic treatments for HAE were prohibited. This requirement limits the study population only to patients with recurrent attacks which is reflected in the wording of the indication.

The use of acute HAE medications (plasma-derived or recombinant C1-INH concentrates, bradykinin 2-receptor antagonists, or kallikrein inhibitors) to treat angioedema attacks was allowed in the study while chronic prophylactic therapies for angioedema attacks (except for a stable dose of androgens or tranexamic acid) were prohibited.

Study endpoints

The primary endpoint for the study was the time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25 compared to placebo for the donidalorsen 80 mg once every 4 weeks group (abbreviated as donidalorsen-4 group). This outcome measure is considered acceptable. The treatment period of 25 weeks is acceptable for the evaluation of the frequency of attacks.

Additional outcome measures used in the study were the Angioedema Quality of Life (AE-QoL) Questionnaire and Angioedema Control Test (AECT) Questionnaire which are validated tools to assess symptom-specific health-related QOL impairment and disease activity in patients with recurrent angioedema.

The primary endpoint analysed the efficacy from the beginning of treatment (from Week 1 to Week 25) whereas almost all of the secondary endpoints investigated the period from Week 5 to Week 25 in which the efficacy of the product is likely to be established (considering the delay in onset of action). For AE-QoL and AECT questionnaires assessments were made at Week 25. In relation to the hierarchical order the efficacy of

the more frequent dosing regimen (i.e. donidalorsen 80 mg every 4 week) was investigated first followed by endpoints investigating less frequent dosing (i.e. donidalorsen 80 mg every 8 weeks).

The choice of secondary efficacy endpoints is accepted. The interpretation of secondary endpoints defined for the period from week 5 to 25 could be complicated by patients discontinuing their assigned treatment or withdrawing from the study prior to week 5. However, as no patients who received study drug discontinued their assigned treatment or withdrew from study ISIS 721744-CS5 prior to Week 5, the secondary endpoints for the period from week 5 to 25 can be accepted as supportive.

Sample size

Sample size estimation was based on a generalized linear model for count data assuming a Poisson distribution which is considered appropriate. Assuming an HAE attack rate of 13.26 attacks per 6-month period in the placebo group and an HAE attack rate of 1.38 attacks per 6-month period in the donidalorsen 80 mg every 4 weeks group, the sample size of 54 patients (2:1 ratio donidalorsen:placebo) provided at least 90% power with a 0.05 significance level. A total of 84 patients (42 in the donidalorsen every-4-weeks group, 21 in the pooled placebo group, and 21 in the donidalorsen every-8-weeks group) were planned to take into account potential early dropouts.

The limited sample size is acceptable considering that HAE is a rare condition and is anyway larger than the samples size proposed at the time of EMA SA (at the time of SA only one donidalorsen dosage (80 mg Q4W) was proposed for the pivotal trial).

Statistical methods

The primary endpoint was the time-normalized number of Investigator-confirmed HAE attacks per month from Week 1 to Week 25 using the treatment policy strategy, hence irrespective of intercurrent events (ICEs) such as early treatment discontinuation for any reasons and use of not allowed concomitant therapy. This is supported since it reflects the treatment effect expected in clinical practice.

To assess the robustness of the primary analysis, 4 sensitivity analyses were performed:

1. Negative Binomial Regression, 2. Poisson Regression on PPS, 3. Multiple Imputation Incorporating Pattern Mixture Model with assumption of Jump to Reference (Placebo), 4. Tipping Point analysis.

Overall, the proposal for missing data imputation using different assumptions for missingness, missing at random (MAR) and missing not at random (MNAR), including also a tipping point analysis, is considered appropriate.

The primary analysis was performed using a Poisson regression model including treatment group, baseline time-normalized run-in period attack rate, and treatment-by-baseline interaction; in addition, the logarithm of time that each patient was observed from Week 1 to Week 25 was used as an offset variable. The estimates were reported as mean event rates per every-4-week by transforming the estimates using the exponential function. The p-value of the Wald-based chi-square test was reported for testing the hypothesis. Moreover, the percentage difference in mean Investigator-confirmed HAE attack rate between donidalorsen 80 mg and the pooled placebo was calculated as 100% × (model adjusted mean rate ratio –1). The Poisson regression model applied for the primary and secondary endpoints based on the attack rates is considered appropriate.

The treatment policy strategy used for secondary endpoints was similarly as for the primary endpoint except for the period from Week 5 to Week 25 applied to almost all secondary endpoints.

Multiplicity was controlled by using a hierarchical ranking strategy in the testing sequence.

For each clinical response (defined as a ≥ 50%, ≥ 70%, or ≥ 90% reduction from baseline in Investigator-Confirmed HAE attack rate) and the Percentage of Investigator-confirmed HAE attack-free patients from Week 5 to Week 25 the Odds ratio was estimated using a Logistic regression model with the baseline, and the treatment-by-baseline interaction as covariates.

The Angioedema Quality of Life (AE-QoL) questionnaire was included among the secondary endpoints. This is a validated tool to assess symptom specific health-related QoL impairment in patients suffering from recurrent angioedema. Change from Baseline in AE-QoL total score at Week 25 was analysed using the mixed model with repeated measures (MMRM). The MMRM model included effects of treatment, time, Treatment-by-Time interaction, Baseline, and Treatment-by-Baseline interaction. This is appropriate.

Efficacy data and additional analyses

Participant flow

91 patients met the eligibility criteria and were randomly assigned to treatment (46 patients in the donidalorsen-4 group, 23 patients in the donidalorsen-8 group, and 22 patients in the placebo group). The number of patients recruited to the study was in line with those planned in the sample size calculation.

The majority of patients (91%) completed the study treatment period. The most common (> 1 patient in any treatment group) reason for early treatment termination was lack of efficacy (1 [2.2%] patient in the donidalorsen-4 group and 1 [4.3%] in the donidalorsen-8 group compared with 3 [13.6%] in the placebo group).

Patients who are not responding to the treatment

The applicant was requested to clarify the approach to patients who are not responding to the treatment. It is agreed that there are no major differences in the patient demographics and baseline characteristics of donidalorsen patients who had < 50% reduction in HAE attack rate compared to those who had ≥ 50% reduction in HAE attack rate, although the numbers of non-responders are very low and no firm conclusion can be drawn. The CHMP agreed that the decision to stop treatment will be made by the treating physicians based on their clinical judgement. No specific recommendations are required in the SmPC.

Baseline characteristics

The mean age of enrolled patients was 37.2 years and ranged from 12 to 68. Seven patients were aged between 12 and 17 years. Of these 7 (7.8%) patients, 4 (8.9%) were in the donidalorsen-4 group, and 3 (13.0%) were in the donidalorsen-8 group. This was in line with PIP agreement (as at least 6 adolescents were requested to be enrolled). No adolescent patients were included in the placebo group. Only 2 patients aged >65 years were enrolled.

The arms were not fully balanced with respect to gender. While 36% of patients in the placebo arm were female, in both treatment arms the percentage of female patients was more than 50%. As some publications indicate a more severe disease in women (e.g. Caballero 2012, Steiner 2016), this imbalance could advantage the placebo arm.

The majority of patients were White (91%), while other races were represented by single subjects.

On average, enrolled patients experienced 38 attacks of angioedema in the 12 months prior to recruitment, although the range of reported attacks was wide (from 0 to 208 attacks per 12 months). A corresponding

frequency of attacks was observed during the run-in period i.e., 3.3 attacks per 4 weeks (range from 0.5 to 10).

There was an imbalance in the number of attacks between the treatment groups with the lowest number observed in the placebo group (2.9/week at screening) and the highest in the donidalorsen-4 group (3.6/week at screening). This could indicate that a less severe population of patients was recruited to the placebo group, and this imbalance again could advantage the placebo arm.

Most patients (93%) had Type I C1-INH HAE whereas 6 (6.7%) patients had Type II C1-INH HAE, which is lower than seen in a general population of HAE patients. In line with the inclusion criteria no patient with Type III HAE was recruited to the study.

Concomitant medication

The majority of patients (>91% in any treatment group) received concomitant treatment while in the study including medications used as a rescue therapy such as complement C1 esterase inhibitors, icatibant acetate and icatibant.

The stable dose of androgens or tranexamic acid was allowed during the study, these treatments are generally used for the prophylaxis of the HAE attacks, although with modest/uncertain efficacy.

Efficacy of donidalorsen 80 mg every 4 weeks- donidalorsen-4 group

The primary endpoint in the study was the time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25 in the donidalorsen 80 mg once every 4 weeks group (abbreviated as donidalorsen-4 group) as compared to the placebo group. This primary endpoint was met.

The LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 0.44 (95% CI: 0.265, 0.727) for the donidalorsen-4 group and 2.26 (95% CI: 1.657, 3.085) for the placebo group, representing an 81% (95% CI: (-89.3%, -64.9%) reduction relative to placebo for the donidalorsen-4 group (statistically significant, $p < 0.001$).The primary endpoint result is considered as not only statistically significant but also clinically relevant.

The results of the sensitivity analyses were consistent with the primary analysis result with treatment effects of similar magnitudes.

Results of sensitivity analyses were consistent with the main analysis although slightly lower: Negative Binomial Regression, -81% (-89.2%, -65.7%); Per Protocol Population, -81% (-90.1%, -63.7%); Multiple Imputation assuming J2R, -81% (-89.2%, -65.8%).

The primary endpoint was supported by the secondary endpoints results although it is important to emphasise that most secondary endpoints for the donidalorsen-4 group analysed the period from Week 5 to Week 25.

As expected, due to the mechanism of action, the relative reduction of Investigator-confirmed HAE attacks (from Week 5 to Week 25) in the donidalorsen-4 group as compared to placebo was higher (87%) than those reported for the whole treatment period (from Week 1 to Week 25).

Better results for the donidalorsen every 4 weeks regimen were shown both in terms of the rate of responders (defined as reduction of at least ≥ 70% reduction from baseline) and in the rate of attack-free patients from Week 5 to Week 25. The percentage of patients with a ≥ 70% reduction from Baseline in Investigator-confirmed HAE attacks from Week 5 and Week 25 was 82.2%, compared with 18.2% in the placebo group. These differences were statistically significant ($p < 0.001$). Of the 45 patients in the

donidalorsen-4 group, 24 (53.3%) were HAE attack-free from Week 5 to Week 25, compared with 2 of 22 (9.1%) patients in the placebo group. Further, the time-normalized number of attacks requiring acute therapy from Week 5 to Week 25 was much lower in the donidalorsen-4 group than in the placebo arm (mean: 0.46 versus 1.88, respectively).

Finally, better results for donidalorsen 80 mg every 4 weeks regimen over placebo were also reported for patient reported outcomes (including changes in AE-QoL Questionnaire).

The data from patients receiving placebo in Cohort A or Cohort B were pooled for analyses. This is acceptable.

As emphasised by the applicant, due to the mechanism of action, the efficacy of donidalorsen would be expected towards the end of the first 4-week dosing period. Therefore, to better understand the efficacy of the product at the beginning of treatment the applicant was requested provide the number of attacks during first two weeks and separately during the first month of treatment. The analysis of the data provided showed no meaningful clinical response during first week of treatment in particular in the less frequent dosing group. The mean percent change from run-in period (SD, SEM) was -12.72 (105.761, 22.548) for the placebo group, -33.33 (100.281, 14.949) for the Donidalorsen 80 mg Every 4 Weeks group and 9.37 (148.031, 30.867) for the Donidalorsen 80 mg every 8 Weeks group.

In the following week of treatment (week 2 to 3) both treatment groups achieved more than 50 % reduction in the rate of HAE attacks. The mean percent change from run-in period (SD, SEM) was -28.63 (108.383, 23.107) for the placebo group, -55.29 (82.218, 12.256) for the Donidalorsen 80 mg Every 4 Weeks group and -67.13 (65.235, 13.603) for the Donidalorsen 80 mg Every 8 Weeks group.

Considering the data as presented above and the mechanism of action of the product, the following statement was provided in section 4.2 of the SmPC: *Based on clinical data, a gradual reduction in attack rate is seen as early as Week 1 after the initial dose of donidalorsen with an expected maximum effect after 1 month.*

In addition, in order to provide further insight on the efficacy on the product the applicant was requested to clarify the number of laryngeal angioedema cases while on treatment in comparison to placebo. Laryngeal angioedema attacks were infrequent in the study. During the run-in period, the mean (SD, SEM) laryngeal HAE attack rate was 0.11 (0.354, 0.053) for the donidalorsen every 4 weeks group and 0.05 (0.181, 0.038) for the donidalorsen every 8 weeks group compared with 0.26 (0.672, 0.143) for the placebo group. The frequency of laryngeal angioedema attacks decreased with treatment. From Week 1 to Week 25, 78% reduction (95% CI: -96.3%, 31.3%) for the donidalorsen every 4 weeks group and 75% reduction (95% CI: -97.6%, 164.9%) for the donidalorsen every 8 weeks group relative to placebo was observed for the least squares means (LSM) time-normalized Investigator-confirmed laryngeal HAE attack rate (per 4 weeks).

The primary endpoint was supported by the results recorded in the second double-blind study (ISIS 721744-CS2).

Subgroup analyses

Exploratory subgroup analyses (by age, sex, race, region, predefined prophylactic therapy) were conducted on the primary efficacy endpoint. The presented subgroup analyses do not suggest substantial differences in efficacy for the subgroups investigated.

A lower treatment effect (71.0% decrease from baseline) was reported in patients receiving prophylactic prior to enrolment as compared to those with no history of prophylaxis (83.1% decrease from baseline).

Patients receiving other prophylactic treatment before enrolment theoretically could potentially experience rebound effect during the run-in period. However, a potential rebound effect from this prior prophylactic therapy could not be assessed based on the data collected during the study run-in period.

Efficacy of donidalorsen 80 mg every 8 weeks- donidalorsen-8 group

The efficacy of donidalorsen 80 mg every 8 weeks was investigated in the ISIS 721744-CS5 and ISIS 721744-CS7 studies.

As shown in the ISIS 721744-CS5 study, the efficacy of donidalorsen every 8 weeks was inferior as compared to donidalorsen every 4 weeks. In this study only a 55% reduction relative to placebo for Time-Normalized Investigator-Confirmed HAE Attack Rate from Week 1 to Week 25 was reported in the donidalorsen-8 group as compared to 81% reduction relative to placebo for the donidalorsen-4 group. Therefore, some patients (not attack-free for ≥ 8 weeks) had to be transferred to more frequent dosing in the extension study ISIS 721744-CS7.

On the other hand, fourteen patients (with lower frequency of HAE attacks at screening) who were treated with donidalorsen 80 mg every 8 weeks in the ISIS 721744-CS5 study could remain on the same dose during the extension study. This subgroup of patients showed a very good overall response (92 % reduction relative to placebo) after one year of treatment.

Therefore, a less frequent dosing regimen could be a valuable option for some (but not all) patients with HAE. It is agreed that it should be introduced after more intensive treatment (i.e. 80mg every 4 weeks) and as proposed, only if patients responded to this initial treatment well (i.e. are attack free) (please see further discussion below).

No notable differences in baseline characteristics could be identified for responses versus non-responders to the less frequent dosage regimen.

Patients with nC1-INH-HAE

In the ISIS 721744-CS2 study which could enroll HAE-nC1-INH patients, a clinical diagnosis was to be confirmed by either threshold-stimulated kallikrein activity results and Investigator-confirmed response to acute use of a BK targeted treatment (icatibant or ecallantide) or the presence of one of the established mutations associated with HAE (i.e. mutation in factor XII gene, plasminogen gene or angiopoietin-1 gene.). The SmPC (section 4.4) includes recommendation to perform genetic testing to confirm diagnosis.

None of enrolled HAE-nC1-INH patients had an established mutation and these patients were enrolled based on the medical history and the results of the kallikrein activity assay. The lack of identified mutation does not preclude the use of the product in patients with HAE-nC1-INH provided that bradykinin overproduction is confirmed. An appropriate diagnosis including the selection of relevant diagnostic tests is at the discretion of the treating physician. Accordingly, a recommendation is included in the SmPC (section 4.4).

As HAE-nC1-INH is known to be caused by mutations leading to bradykinin overproduction, these mutations are potential targets for donidalorsen. Therefore, a statement is added in section 4.4 of the SmPC to highlight that p*atients with HAE-nC1-INH having mutations that are not associated with the kallikrein-kinin system (KKS) pathway are not expected to respond to Dawnzera.*

Further, the response to treatment should be monitored in these patients and the treatment should be discontinued if clinical response is not observed after 4 months of treatment which is included in the SmPC (section 4.2). The mutation status of nC1-INH HAE patients who are not responding to treatment will be collected post-marketing and discussed in the lack of efficacy section of the PSUR.

3 patients with nC1-INH HAE were recruited to Part B of ISIS 721744-CS2 study, received donidalorsen 80 mg every 4 weeks as an open label treatment. Subsequently, they were transferred to ISIS 721744-CS3 study. The SmPC highlights that the data on the use of donidalorsen in HAE patients with HAE-nC1-INH is limited.

These 3 patients experienced frequent attacks prior to the randomization (mean: 61.3 (range 36 to 100) in previous 12 months and 4.23 during the screening period) and they responded to treatment, although the response in one patient was suboptimal.

The number of attacks decreased from a monthly mean attack rate 4.23 (during the Run-in Period) to 1.52 from Week 1 to Week 17 (76% reduction). One patient was attack-free from Week 1 to end of treatment.

No differences in baseline characteristics could be identified which would explain this inconsistent response.

Although data on the use in patients with nC1-INH-HAE is limited, a broad indication as proposed by the applicant is supported. The available data for nC1-INH-HAE patients are clearly presented in section 5.1 of the SmPC**.**

Long term efficacy

In the pivotal study (ISIS 721744-CS5) patients were treated for 24 weeks. Subsequently patients could continue their treatment in the treatment period (for 52 weeks) of the open label, long-term study (ISIS 721744-CS7) and for an additional two years in the Extended Treatment Period of this study.

As the ISIS 721744-CS7 study is still ongoing, and most patients had not completed Week 53 (52-week Treatment Period), the long-term efficacy data from this study is incomplete.

Nevertheless, the data presented so far seems to indicate that efficacy is maintained or even slightly improved with a longer treatment as 93.11% reduction in the time-normalized, Investigator-confirmed HAE attack was reported for 83 patients who receive their treatment in the treatment period of this extension study.

Post-treatment

In light of the non-clinical data and theoretical risk of rebound after stopping donidalorsen, the applicant was requested to present the attack rates (vs baseline) after discontinuation of donidalorsen in HAE patients included in studies from entire development program. The Applicant presented individual post-treatment period data available from all phase 2 and 3 studies.

In the ISIS 721744-CS5 study 3 patients completed post-treatment FU (two completed the treatment and one discontinues the treatment early). In the ISIS 721744-CS2 study there was 1 patient who completed treatment and entered post-treatment follow-up. In the ISIS 721744-CS3 one patient completed the treatment and 6 discontinued the treatment early. In the ISIS 721744-CS7 study (10 patients (previously on donidalorsen or placebo) and 12 patients who switched from other LTP).

Although the data are limited, it is agreed that there was no increase in the attack rates (versus baseline) after discontinuation of donidalorsen.

Switching to less frequent dosing regimens

The Open-Label Extension Study, ISIS 721744-CS3 also provided data supporting the recommendation for switching of patients to less frequent dosing regimens for patients who were attack free for ≥ 12 weeks on treatment with donidalorsen 80 mg once every 4 weeks.

During the Flexible Dosing Period in this study, for 8 out of 20 patients the frequency of dosing could be reduced. Of these 8 patients, 5 patients continued to receive treatment every 8 weeks for a mean duration of 703 days. The remaining 3 patients switched back to donidalorsen every 4 weeks.

Although the approach of switching patients to a less frequent dosing regimen was investigated in a limited number of participants, this could be a valuable option for some patients.

Finally, although initially the efficacy of the donidalorsen every 8 weeks group dose was inferior as compared to the donidalorsen every 4 weeks group, the efficacy of the less frequent regimen appears to improve with a longer treatment period. Therefore, the following recommendation has been added in section 4.2 the SmPC: *A dosing interval of 80 mg once every 2 months may be considered if the patient is well controlled (e.g., attack free) for at least 3 months while receiving Dawnzera.*

Switching from other prophylactic treatments

A total of 64 patients who were previously treated with another HAE prophylactic treatment (lanadelumab, berotralstat, or C1-esterase inhibitor) were enrolled into the ISIS 721744-CS7 study and received donidalorsen 80 mg once every 4 weeks. Based on the provided efficacy data it can be agreed that there was no deterioration effect due to changing the prophylactic treatment. Superiority as compared to the previous prophylactic therapy cannot be determined due to limitations of the design of this study (the study was observational, no formal statistical testing was conducted, and the efficacy data was presented descriptively).

For these patients, the timing of the switch from the previous prophylactic HAE drug to donidalorsen was based on the half-life of the individual drugs. Given the relatively long half-life of lanadelumab, donidalorsen dosing was initiated at the end of lanadelumab dosing interval while berotralstat and C1-esterase inhibitor were administered concurrently with donidalorsen for 14 days given their short half-life to prevent any loss of efficacy. SmPC section 4.2 includes a table with recommendations for patients that are changing their HAE prophylactic therapy from berotralstat, a C1 esterase inhibitor, or lanadelumab to Dawnzera.

Assessment of paediatric data on clinical efficacy

Only a limited number of adolescent subjects were included in the provided donidalorsen studies: 7 adolescents in the pivotal study (4 in the donidalorsen-4 group, and 3 in the donidalorsen-8 group) and 4 newly enrolled adolescents in the OLE study CS7 who switched from other prophylactic treatments (1 lanadelumab, 3 C1-esterase inhibitor). Nine adolescents were treated with donidalorsen Q4W while two adolescents from the pivotal study continued with Q8W dosage.

For adolescent patients in the pivotal study, a 97.1% decrease (95% CI: -106.26%, -88.01%) from baseline in the HAE attack rate from Week 1 to Week 25 was observed, which was similar to the 88.5% (95% CI: -94.75%, -82.30%) decrease for patients aged 18 to 39 years; patients aged 40 to 64 years had a numerically lower percent change from baseline (72.6% decrease [95% CI: -86.56%, -58.66%]).

Although based on limited data available, it is agreed that the efficacy results for adolescent OLE and Switch patients in study ISIS 721744-CS7 is in line with those observed in the overall study population.

ADA effect on efficacy outcomes

Donidalorsen seems to have significant immunogenicity potential. Although the provided data suggests that ADA are characterised by low titres and do not adversely impact efficacy, small sample size and timeframe limit the interpretation of the data. The Applicant was requested to provide and discuss updated data from two ongoing studies, CS3 and CS7 regarding the effect of ADA on efficacy outcomes.

Although in ISIS 721744-CS3 study the clinical response was not significantly different between the ADA positive and negative patients, in ISIS 721744-CS7 study, the HAE attack rate reduction was numerically lower in the ADA positive as compared to ADA negative Switch patients.

Indication

The proposed indication is broad as it covers all subgroups of hereditary angioedema including Type I HAE (which presents with a deficiency of C1-INH), Type II HAE (which presents with a dysfunctional C1-INH), and Type III HAE with normal C1-INH activity. This supported by clinical evidence in all 3 HAE sub-types.

Donidalorsen is proposed to be used as a prophylactic treatment (i.e. for prevention of recurrent attacks) and not as a treatment for acute HAE attacks, which is supported by the mechanism of action of the product. Appropriate wording is included in section 4.2 and 4.4 of the SmPC

2.6.7. Conclusions on the clinical efficacy

The pivotal study ISIS 721744-CS5 met its primary endpoint, and the effect of the treatment is clinically relevant for patients with C1-INH HAE. The efficacy is also supported by the results from secondary endpoints and the second double-blind study (ISIS 721744-CS2) which also included a limited number of patients with nC1-INH HAE. Moreover, the results from the longer-term open label trials support the maintenance of efficacy over time.

Therefore, the CHMP considers that the clinical efficacy of donidalorsen in the routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older therapeutic indication has been demonstrated.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

The clinical studies of donidalorsen in patients with HAE (CS5, SC2, CS3 and CS7) and in HV (CS1 and CS9) are listed below.

At the time of submission, 2 of the studies were ongoing, OLE studies, CS3 and CS7- the data cut-off date for these ongoing studies was February 2024.

In updates received during the procedure, upon request, the applicant has informed that study CS3 is now closed (CSR under completion). CS3 duration was up to 4 years- an initial year followed by up to 3 additional years. The new data cut-off date for CS3 was 25 Mar 2025, so the new safety update for CS3 covers an additional 15 months.

In updates received during the procedure, upon request, the applicant has informed that study CS7 is still ongoing. It is intended to run CS7 for 3 years- but this may be extended further- with a view to transition

subjects to commercial donidalorsen when it will be available. The new data cut-off date for CS7 was 27 Jan 2025, so the new safety update for CS7 covers an additional 13 months.

The primary conclusions are derived from pivotal study CS5. Study CS2 is supportive for safety and is included in an integrated pool comprising placebo-controlled studies of CS5 and CS2 (Pool 1). Additional supportive evidence for establishing the safety profile of donidalorsen was generated by pooling safety data from CS5, CS2, CS3, and CS7 (Pool 2).

Pooling has been applied in the presentation of the safety data as follows:

Pool 1	Patients in randomized, double-blind, placebo-controlled studies (Phase 2 study CS2 and Phase 3 Study CS5).
Pool 2	Patients in randomized, double-blind, placebo-controlled studies and OLE studies (CS2, CS3, and OLE CS5 and OLE CS7).
Pool 3	All patients in Pool 2 plus healthy volunteers (CS1, CS2, CS3, CS5, CS7, and CS9). Studies CS1 and CS9 are Phase 1 healthy volunteer studies. Only exposure data are presented for this pool, which represents the totality of donidalorsen exposure included within this marketing authorisation application.

The latest exposure tables, as provided during the procedure, upon request are provided below in Table 19 and Table 20.

Table 19. Summary of donidalorsen exposure – Pool 3 (CS1, CS2, CS3, CS5, CS7, and CS9)

Study	Exposure Duration (Person-year)[a]	Dosed	Number of Patients	
			24 Weeks	52 Weeks
Phase 1 CS1	7.4	24	0	0
Phase 2 CS2 + CS3	67.9	22	20	18
Phase 3 CS5 + CS7	249.9	155	146	136
Phase 1 CS9	11.8	78	0	0
Total	337.0	279	166	154

Note: Safety Set – Pool 3 includes patients who received at least 1 dose of the study drug (donidalorsen or placebo) in randomized, double-blind, placebo-controlled studies and open-label studies (ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, and ISIS 721744-CS7), and healthy volunteers who received any dose of donidalorsen in ISIS 721744-CS1 and ISIS 721744-CS9.

[a]Total donidalorsen exposure in person-year is calculated as sum of duration of donidalorsen exposure (days) across all patients of the specific study / 365.25.

Table 20. Summary of donidalorsen exposure by dosing regimen – Pool 3 (CS1, CS2, CS3, CS5, CS7, and CS9)

Dosing Regimen/Study	Exposure Duration (Person-year)[a]	Dosed	Number of Patients	
			24 Weeks	52 Weeks
Donidolorsen 80 mg Every 4 Weeks				
Phase 1 CS1	1.8	6	0	0
Phase 2 CS2 + CS3	42.6	22	19	10
Phase 3 CS5 + CS7	214.7	141	132	120
Phase 1 CS9	NA	NA	NA	NA
Total	259.1	169	151	130
Donidolorsen 80 mg Every 8 Weeks				
Phase 1 CS1	NA	NA	NA	NA
Phase 2 CS2 + CS3	18.6	8	6	5
Phase 3 CS5 + CS7	35.2	35	21	14
Phase 1 CS9	NA	NA	NA	NA
Total	53.8	43	27	19

NA = not applicable.
Note: Safety Set – Pool 3 includes patients who received at least 1 dose of the study drug (donidalorsen or placebo) in randomized, double-blind, placebo-controlled studies and open-label studies (ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, and ISIS 721744-CS7), and healthy volunteers who received any dose of donidalorsen in ISIS 721744-CS1 and ISIS 721744-CS9.

[a]Total donidalorsen exposure in person-year is calculated as sum of duration of donidalorsen exposure (days) across all patients of the specific study / 365.25.

Based on the updated data provided, at any dose, 166 have been dosed for more than 24 weeks and 154 have been dosed for more than 52 weeks. Specifically, for the proposed 80mg 4-weekly dose, 151 have been dosed for more than 24 weeks, and 130 have been dosed for more than 52 weeks.

In relation to the pivotal study, CS5, the median duration of exposure was 168 days in the donidalorsen 4-weekly group, and 167.96 in the donidalorsen 8-weekly group.

In CS5, of the 91 patients randomised the majority completed treatment, with a higher proportion in the donidalorsen groups completing versus placebo; donidalorsen-4 group (95.7% completion rate) and donidalorsen-8 group (91.3% completion rate). 83 subjects then rolled into the OLE CS7, of which only 2 had left the trial as of the data lock date at time of MAA submission. There was a similar retention rate in CS2 and rollover rate into OLE CS3.

2.6.8.2. Adverse events

An overview of Safety in the Phase 1 (HV) studies is outlined below; the rest of the Safety section focuses on the 4 studies that involved HAE patients- CS5, CS2, CS3 and CS7.

Phase 1 studies

In CS1, 32 healthy adult volunteers were enrolled in a double-blind, randomized, placebo-controlled, dose-escalation study conducted at a single centre. The study included 4 multiple-dose cohorts, with individual doses of 20, 40, 60, or 80 mg, respectively. Of the 24 subjects who received ISIS 721744, 15 (62.5%) reported 39 TEAEs and 3 (37.5%) of the 8 subjects who received placebo reported 7 TEAEs. Most TEAEs with ISIS 721744 (97.4%) were mild in severity. All TEAEs were reported as mild except for Proteinuria(severe) for Subject 1714-1308, who received ISIS 721744 at 80 mg. The proteinuria was reported as a severe TEAE from Study Day 85(100 mg/dL) to Study Day 99 (30 mg/dL) and a moderate TEAE from Study Day 99 to 141 (negative). The subject had a high level of exercise during that time and the event resolved without treatment and was considered unlikely related to Study Drug. The only TEAE reported for more than 2 subjects receiving multiple doses of ISIS 721744 that was not at the injection site was Cough (3 subjects, 12.5%), which was not reported for any placebo-treated subjects. The only TEAEs at the injection site reported for more than 2 subjects receiving multiple doses of ISIS 721744 were Injection site erythema and

Injection site pruritus (each 2 subjects, 8.3%). No local cutaneous reactions at the injection site or flu-like reactions were reported. No deaths or serious adverse events (SAEs) were reported and no subject discontinued study participation. The overall incidence rate of immunogenicity for healthy volunteer subjects in this study was low.

Study CS9 was a bioequivalence study, where 78 healthy volunteer adults were randomized in a 1:1 ratio to 1 of 2 sequences of treatment to receive donidalorsen 80 mg via subcutaneous injection (abdomen only) using an autoinjector or a manual injection using a single-use vial (syringe with manual injection)- so each subject received 2 doses, with a minimum of 28 days between doses. One (1.3%) participant experienced 2 serious AEs (SAEs) (cholecystitis and pancreatitis acute) and 13 (16.7%) participants experienced 22 treatment-emergent AEs (TEAEs). The majority of TEAEs by maximum severity were mild (9 [11.5%] participants). There were 4 (5.1%) participants with moderate TEAEs. The most common TEAEs were headache, constipation and nausea. One participant experienced 7 Study Drug-related mild and moderate TEAEs. There were more Study Drug-related TEAEs experienced by this participant after administration of the vial (Reference Drug) formulation than after administration of autoinjector (Test Drug) formulation. There were no Study Drug-related TEAEs related to autoinjector recorded. No treatment-emergent SAEs (TESAEs), TEAEs leading to discontinuation of Study Drug, TEAEs leading to discontinuation of the study, TEAEs related to autoinjector, TEAEs leading to death, TEAEs related to local cutaneous reactions at the injection site (LCRIS), TEAEs related to flu-like symptoms, or TEAEs related to bleeding were reported.

Overview of AEs:

Phase 3 pivotal study- CS5

There was a high rate of TEAEs in pivotal study CS5 and pivotal study CS2. In the phase 3 pivotal study the highest rate of AEs was in the placebo group (81.8%), followed by the donidalorsen- 4 group (73.3%), and followed by the donidalorsen- 8 group (60.9%).

Pool 1 (CS2 +CS5)

In Pool 1 (CS2 and CS5), again the highest rate of TEAEs was again in the placebo group (82.1%), followed by the donidalorsen-4 group (74.2%) with the lowest rate in the donidalorsen-8 group (60.9%).

Pool 2 (CS2 + CS3 + CS5 +CS7)- **updated**

In Pool 2, with additional long-term exposure to donidalorsen (but no additional exposure to placebo), the proportion of patients who experienced TEAEs was 93.3%in the donidalorsen-4 group, 69.8% in the donidalorsen-8 group, and 82.1% in the placebo group.

Severity of TEAEs:

Phase 3 pivotal study- CS5

Table 21. Summary of treatment-emergent adverse events by severity and severe treatment-emergent adverse events by preferred term (Safety set)

Preferred Term Severity[a, b]	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 45) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)
Patients with at least 1 TEAE[c]			
Mild	**7 (31.8)**	**17 (37.8)**	**5 (21.7)**
Moderate	**9 (40.9)**	**15 (33.3)**	**7 (30.4)**
Severe	**2 (9.1)**	**1 (2.2)**	**2 (8.7)**
Severe Treatment-Emergent Adverse Events			
Headache	1 (4.5)	1 (2.2)	0
Blood creatine phosphokinase increased	0	0	1 (4.3)
Epicondylitis	0	0	1 (4.3)
Limb injury	1 (4.5)	0	0

Pool 2 (CS2 + CS3 + CS5 +CS7)

The majority of patients in Pool 2 experienced TEAEs that were mild or moderate in severity.

The proportion of patients who experienced severe TEAEs in Pool 2 was similar across the dose arms: 9.2% in the donidalorsen-4 group, 7%% in the donidalorsen-8 group, and 7.1% in the placebo group.

Mild TEAEs had a higher incidence in placebo arm than in the donidalorsen treated arms. Moderate TEAEs occurred more frequently in the donidalorsen arms (59.5%% of donidalorsen-4 patients, and 27.9%% of donidalorsen -8 subjects had a moderate TEAE v 32.1% of placebo patients) than in the placebo arm.

In the donidalorsen-4 group, 12 (9.2%) patients experienced severe TEAEs, including headache (2 patients), nausea (2 patients), and cardiac failure acute, toothache, constipation, pyrexia, COVID-19, gastroenteritis, pneumonia, mastoiditis, otitis media chronic, neck pain, torticollis, meningioma, pulmonary oedema, pruritus, and deep vein thrombosis (all in 1 patient each).

In the donidalorsen-8 group, 3 (7.0%) patients experienced severe TEAEs, including epicondylitis, blood creatine phosphokinase increased, and spinal pain. In the donidalorsen-100 mg-4 group, 1 (33.3%) patient experienced a severe TEAE of dermatitis.

Related TEAEs

In the pivotal study CS5, a greater proportion of patients experienced related TEAEs in the donidalorsen-4 group (42.2%) compared with the donidalorsen-8 group (17.4%) and placebo group (27.3%).

In Pool 2, a greater proportion of patients experienced a related TEAE in donidalorsen-4 group (41.7%) compared with the donidalorsen-8 group (18.6%), and placebo arms (35.7%). The highest rate of related TEAE was in the donidalorsen 100mg arm. However, this arm provides very limited information given that it concerns only 3 subjects (and this is also not a dose under review). Mild related TEAEs were more frequent in the placebo treated patients, but moderate and severe TEAEs (even though much less common) were more frequent in donidalorsen treated patients. 11.3% of donidalorsen (all doses) treated patients had moderate related TEAEs and 2.3% of donidalorsen (all doses) treated subjects had severe related TEAEs.

A breakdown of the most common related TEAEs reported in CS5 and Pool 1 is displayed in Table 22 below.

Table 22. Treatment-emergent adverse events related to study drug in >5% of patients in any treatment group by preferred term – CS5 + Pool 1 (CS2 and CS5)

	Study CS5			Pool 1 (Study CS2 and Study CS5)			
Preferred Term	Placebo (N = 22)	Donidalorsen 80 mg Every 4 Weeks (N = 45)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Placebo (N = 28)	Donidalorsen 80 mg Every 4 Weeks (N = 62)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Total Donidalorsen 80 mg (N = 85)
Patients with at least one TEAE, n (%)	**6 (27.3)**	**19 (42.2)**	**4 (17.4)**	**10 (35.7)**	**24 (38.7)**	**4 (17.4)**	**28 (32.9)**
Injection site erythema	0	6 (13.3)	1 (4.3)	0	7 (11.3)	1 (4.3)	8 (9.4)
Headache	3 (13.6)	3 (6.7)	0	5 (17.9)	4 (6.5)	0	4 (4.7)
Injection site discolouration	0	3 (6.7)	1 (4.3)	0	4 (6.5)	1 (4.3)	5 (5.9)
Injection site pain	0	3 (6.7)	0	0	3 (4.8)	0	3 (3.5)
Injection site pruritus	0	3 (6.7)	0	0	3 (4.8)	0	3 (3.5)

There were 4 subjects with severe TEAEs (3 subjects) considered related across all of the studies, 3 of the subjects were on donidalorsen- 4 treatment, and the third on donidalorsen 100mg 4 weekly. All 4 events were considered non serious and are summarized in the Table 23 below. This table has been updated with the additional safety data provided.

Table 23. Table of severe related TEAEs across all studies

Patient, study	**Details of severe related TEAE**
Donidalorsen 80mg 4 weekly- CS7 Switch **subject 1**	Severe, non-serious, headache onset D86, duration 2 days, resolved Severe, non-serious, torticollis onset D171- 10 days, resolved 2 separate events, same patient
Donidalorsen 80mg 4 weekly- CS5 **Subject 2**	Severe, non-serious, headache onset D140, duration 1 day, resolved
Donidalorsen 100mg 4 weekly- CS3 **Subject 3**	Severe, non-serious dermatitis onset D286, ongoing Further information was sought on this case during the procedure. *Additional detail as follows:* *no reported medical history at trial enrolment; however, the list of concomitant medication would suggest otherwise. It is particularly noted that this subject was concomitantly taking diphenhydramine (an anti-histamine) as well as a number of medications that might cause itch such as oxycodone and dihydromorphone. A number of the*

	concomitant medication might also cause dermatitis- or worsen dermatitis. *There is no clear indication of the affected site- the anatomical distribution is unspecified. Overall, while the AE was graded as severe and related, the subject was able to continue in the trial. There is no indication that it was an ISR, albeit that detail regarding the anatomical distribution is lacking.*
Donidalorsen 80 mg Q4W, OLE patient in CS7 **Subject 4**	Severe retching, resolved in less than one hour.

Pivotal Study CS5

TEAEs occurring in > 5% of patients in any patient group in CS5 are displayed in Table 24 below.

Table 24. Treatment-emergent adverse events in >5% of patients in any treatment group in CS5 or pool 1 by preferred term – CS5 + Pool 1 (CS2 and CS5)

	Study CS5			Pool 1 (Study CS2 and Study CS5)			
Preferred Term	Placebo (N = 22)	Donidalorsen 80 mg Every 4 Weeks (N = 45)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Placebo (N = 28)	Donidalorsen 80 mg Every 4 Weeks (N = 62)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Total Donidalorsen 80 mg (N = 85)
Patients with any TEAEs, n (%)	**18 (81.8)**	**33 (73.3)**	**14 (60.9)**	**23 (82.1)**	**46 (74.2)**	**14 (60.9)**	**60 (70.6)**
Headache	4 (18.2)	6 (13.3)	2 (8.7)	6 (21.4)	8 (12.9)	2 (8.7)	10 (11.8)
Nasopharyngitis	4 (18.2)	5 (11.1)	3 (13.0)	4 (14.3)	6 (9.7)	3 (13.0)	9 (10.6)
Injection site erythema	0	6 (13.3)	1 (4.3)	0	7 (11.3)	1 (4.3)	8 (9.4)
Urinary tract infection	0	4 (8.9)	2 (8.7)	1 (3.6)	5 (8.1)	2 (8.7)	7 (8.2)
Influenza	2 (9.1)	2 (4.4)	4 (17.4)	2 (7.1)	2 (3.2)	4 (17.4)	6 (7.1)
Upper respiratory tract infection	1 (4.5)	4 (8.9)	2 (8.7)	1 (3.6)	4 (6.5)	2 (8.7)	6 (7.1)
Injection site discolouration	0	3 (6.7)	1 (4.3)	0	4 (6.5)	1 (4.3)	5 (5.9)
Abdominal discomfort	0	3 (6.7)	0	0	3 (4.8)	0	3 (3.5)
Cough	1 (4.5)	2 (4.4)	1 (4.3)	2 (7.1)	2 (3.2)	1 (4.3)	3 (3.5)
Injection site pain	0	3 (6.7)	0	0	3 (4.8)	0	3 (3.5)
Injection site pruritus	0	3 (6.7)	0	0	3 (4.8)	0	3 (3.5)
Vomiting	1 (4.5)	0	2 (8.7)	1 (3.6)	1 (1.6)	2 (8.7)	3 (3.5)
Gastroenteritis	2 (9.1)	1 (2.2)	1 (4.3)	2 (7.1)	1 (1.6)	1 (4.3)	2 (2.4)
Nausea	1 (4.5)	1 (2.2)	0	2 (7.1)	2 (3.2)	0	2 (2.4)
Oral herpes	0	0	2 (8.7)	0	0	2 (8.7)	2 (2.4)
Oropharyngeal pain	1 (4.5)	0	1 (4.3)	2 (7.1)	1 (1.6)	1 (4.3)	2 (2.4)

	Study CS5			Pool 1 (Study CS2 and Study CS5)			
Preferred Term	Placebo (N = 22)	Donidalorsen 80 mg Every 4 Weeks (N = 45)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Placebo (N = 28)	Donidalorsen 80 mg Every 4 Weeks (N = 62)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Total Donidalorsen 80 mg (N = 85)
Abdominal pain	2 (9.1)	1 (2.2)	0	3 (10.7)	1 (1.6)	0	1 (1.2)
COVID-19	2 (9.1)	1 (2.2)	0	2 (7.1)	1 (1.6)	0	1 (1.2)
Dyspepsia	2 (9.1)	0	0	2 (7.1)	1 (1.6)	0	1 (1.2)
Limb injury	4 (18.2)	0	1 (4.3)	4 (14.3)	0	1 (4.3)	1 (1.2)
Sinusitis	2 (9.1)	0	0	2 (7.1)	0	0	0

Adverse drug reactions:

The applicant describes the reasoning and approach to determining ADRs as follows:

Safety data for patients with HAE who received any dose of donidalorsen (pivotal study CS5, Pool 1 [CS2 + CS5], and Pool 2 [CS2 +CS3 +CS5 + CS7]) was evaluated for potential adverse drug reactions (ADRs). All TEAEs were evaluated. Furthermore, clinical laboratory evaluations, vital signs, electrocardiograms, exposure-response relationship (study CS5 only), and immunogenicity were also considered in the evaluation for ADRs.

To determine ADRs, a thorough medical evaluation of available data was conducted to assess the causal relationship of a TEAE to the IMP. Assessments included:

- Temporal relationship between TEAE and exposure to IMP

- Evaluation of the extent to which the AE was consistent with the pharmacology of donidalorsen
- Consistency of the pattern of symptoms across other studies/indications.
- Biologic plausibility based on donidalorsen mechanism of action and class effect
- Event or laboratory test abnormality, with plausible time relationship to IMP intake
- De-challenge/re-challenge information: evidenced based and frequently occurring with reasonable causality and likelihood related to IMP

Injection Site Reactions:

Phase 3 pivotal study- CS5

A greater proportion of patients in the donidalorsen-4 group (24.4%) experienced injection site reaction compared with the donidalorsen-8 (4.3%) group and the placebo group (4.5%). All injection site reactions were nonserious, mild in severity, and did not result in any action taken with study drug. Most injection site reactions resolved spontaneously without sequelae or any treatment.

Pool 1 (CS2 +CS5)

In Pool 1, donidalorsen-4 group (21.0%) v donidalorsen-8 (4.3%) group v placebo group (7.1%) had ISRs. All injection site reactions were nonserious, mild in severity, and did not result in any action taken with study drug.

Pool 2 (CS2 + CS3 + CS5 +CS7)

In Pool 2, with additional long-term exposure to donidalorsen 32.5% of the donidalorsen 80 mg 4 weekly treated subjects, and 4.7% of the 80mg 8 weekly treated subjects had ISRs. All injection site reactions were nonserious, the majority were mild in severity. The most common injection site reactions observed were injection site, erythema, injection site discolouration, injection site pain, injection site pruritus, and injection site bruising.

One patient in the donidalorsen-100 mg-4 group from study CS3 experienced recurrent injection site reaction/Local cutaneous reaction at the injection site, leading to permanent study drug discontinuation. This patient experienced multiple instances of moderate injection site discolouration, see further discussion of this subject in the LCRIS discussion, and in the Discontinuation due to AEs section.

2.6.8.3. Serious adverse event/deaths/other significant events

Serious adverse events

Phase 3 pivotal study- CS5

No patients in the donidalorsen-4 or donidalorsen-8 groups experienced a serious TEAE in the CS5 study (one severe SAE of limb injury in the placebo arm but was considered unrelated)

Pool 1(CS2 +CS5)

No additional SAEs reported.

Pool 2(CS2 + CS3 + CS5 +CS7)

12 patients with SAEs were reported in the OLE studies. Most of these events were assessed as unrelated to study drug per the Investigator and Sponsor.

From Study CS3:

A patient in the nC1-INH-HAE group experienced a serious TEAE of **abortion spontaneous (moderate)** that was considered not related by the Investigator. The patient was confirmed positive for pregnancy prior to the event and was permanently discontinued from study drug.

From Study CS7

A patient in the OLE donidalorsen-4 group experienced 2 serious TEAEs of **meningioma (severe) and pulmonary oedema (severe).** The patient had a medical history of dyslipidaemia, hypertension, metabolic syndrome, obesity, chronic pyelonephritis and hepatic steatosis. On Study Day 210, the patient was hospitalized for the serious TEAE of meningioma. Craniotomy and excision of a left-brain tumor Study Day 211. The event was considered not related by the Investigator; however, study drug was interrupted due to this event (last dose administered on Study Day 208). Following discharge from the hospital (Study Day 243), the event was downgraded to nonserious and was ongoing at the time of database lock. During hospitalization (Study Day 214), the patient experienced another serious TEAE of pulmonary oedema requiring intubation; the event was considered **not related** by the Investigator and study drug was interrupted at the time of event; the event was considered resolved.

A patient in the OLE donidalorsen-4 group experienced a **serious TEAE of cardiac valve disease (moderate**). The patient had a medical history of acute rheumatic fever. On Study Day 50, an echocardiography revealed moderate mitral regurgitation, moderate mitral stenosis, and moderate aortic stenosis; the patient was clinically asymptomatic. The event was considered **not related** by the Investigator and no action was taken with study drug; the event was ongoing at the time of database lock.

A patient in the OLE donidalorsen-4 group experienced 2 serious TEAEs of **mastoiditis (severe) and otitis media chronic (severe).** The patient had a medical history of rhinitis allergic and rhinorrhoea. On Study Day 150, the patient was hospitalized for both serious TEAEs; the patient underwent tympanoplasty the next day (Study Day 151) and was discharged on Study Day 154. Both events were considered **not related** by the Investigator and no action was taken with the study drug; the events were considered resolved.

A patient in the OLE donidalorsen-4 group experienced a serious TEAE of **uterine leiomyoma (moderate).** The patient had a medical history of uterine leiomyoma. On Study Day 50, the patient was noted to have multiple new growths requiring hospitalization; the patient subsequently underwent hysterectomy on Study Day 89. The event was considered **not related** by the Investigator and no action was taken with the study drug; the event was considered resolved.

A patient whose female partner (nonparticipant) experienced a serious TEAE of spontaneous abortion (moderate severity). The event was assessed as **not related** to study treatment; no changes were made to study treatment.

A patient experienced a serious TEAE of epistaxis (moderate severity), Study Day 351 on 80mg Q4W, hospitalised. The event was considered **not related** to study treatment, and no changes were made to treatment. Medical history included arterial hypertension, high blood pressure at time of event. Resolved after with treatment did not recur.

A patient experienced a serious TEAE of foot deformity. The event was assessed as **not related** to the study treatment; no action was taken with the study treatment.

A patient experienced a serious TEAE of completed suicide, resulting in permanent discontinuation of study treatment. The event was assessed by the investigator as **not related** to the study treatment. This was already discussed at time of submission as a late breaking event.

A patient who received lanadelumab prior to enrolment in the study. The patient experienced 2 serious TEAEs of cardiac failure acute (severe) and renal disorder (moderate) on Study Days 61 and 62, respectively. The patient had a medical history of hypertension, cardiac failure congestive, asthma-chronic obstructive pulmonary disease overlap syndrome, and pulmonary hypertension. On Study Day 61, the patient was hospitalised for the serious **TEAE of cardiac failure acute** due to worsening congestive heart failure. During hospitalisation (Study Day 62), the patient experienced **another serious TEAE of renal disorder** (verbatim: T2 hypointense lesion within right kidney); associated laboratory abnormality included severe blood urea nitrogen (BUN) elevation (37mg/dL [reference range 5 to 22 mg/dL). Both events were considered **not related** by the Investigator; the patient was permanently discontinued from study drug on Study Day 79 (last dose administered on Study Day 57). BUN elevations remained elevated (29 mg/dL [Study Day 66]) throughout the study. On Study Day 72, the patient was permanently discontinued from the study. Both events were ongoing at the time of database lock (persists as AE).

A patient experienced a serious TEAE of cellulitis (moderate severity). The event was deemed **unrelated** to study treatment; no action was taken with the study treatment.

Since initial submission of this application, there has been a hypersensitivity SAE (anaphylaxis) in a patient which fulfilled Sampon's criteria, and was considered **related**- see specific details under Hypersensitivity TEAE section.

Deaths:

Phase 3 pivotal study- CS5

There were no deaths in CS5.

Pool 1 (CS2 +CS5)

There were no deaths in Pool 1.

Pool 2 (CS2 + CS3 + CS5 +CS7)

There has been one death in Pool 2, details as follows:

Late breaking event of death since data lock date of February 2024. A patient died by suicide, with no known previous diagnosis of anxiety or depression. It was reported that the subject was living a socially withdrawn life. No autopsy was performed. The event was investigated by the police; the family chose not to share the report. The event was assessed as not related by the Investigator and Sponsor.

AEs of special interest

Two AESIs related to low platelets were defined as the following in all 4 studies:

1. Severe reductions in platelet counts < 50,000/mm^3 accompanied by a MB (Major Bleeding) event or CRNMB (Clinically relevant non-major bleeding) event; or

2. Platelet count of < 25,000/mm^3 independent of a MB event or CRNMB event

In studies CS2 and CS3 only (i.e. not in CS5 or CS7) thrombotic events were also defined as AESIs.

-No patient in any of the 4 HAE trials had a platelet related AESI (Pool 2).

-There were no thrombotic event AESIs in studies CS2 or CS3.

Platelet TEAEs: updated

In CS5, the majority of patients in all treatment groups remained within the normal reference range for platelet counts (≥ 140,000/mm^3) throughout the study: 97.8% in the donidalorsen-4 group, 95.7% in the donidalorsen-8 group, and 90.9% in the placebo group. No patient in any treatment group experienced a severe reduction (to < 100,000/mm^3) in platelet counts. One patient (in the donidalorsen-8 group experienced an isolated platelet count reduction of 13,000/mm3 on Study Day 29. The abnormal value was due to sample handling error. 3 days later, platelet levels were within normal reference range and throughout the study. Five (5.6%) patients experienced a platelet count decrease of ≥ 30% from Baseline (1 [2.2%] patient in the donidalorsen-4 group, 2 [8.7%] patients in the donidalorsen-8 group, and 2 [9.1%] patients in the placebo group). These decreases in platelet counts from Baseline were not sustained and platelet counts were all between > 100,000 to < 140,000/mm^3.

One CS7 patient experienced 2 new nonserious TEAEs of platelet count decreased of moderate severity. Both TEAEs were assessed as related by Investigator and no action was taken with the study treatment. Platelets reduced to 114 x 10^9, moderate severity, but resolved in 2 weeks. Considered possibly related. A second similar episode also moderate happened- resolved within 1 week.

Transient reductions of platelet counts were observed in Pool 2. No patient met the protocol-defined stopping rules for platelet counts. No treatment discontinuations associated with platelet reductions were reported. No MB or CRNMB associated with platelet reductions was observed. Mean platelet counts remained within the reference range and mean percent changes from baseline were not clinically meaningful.

Bleeding TEAEs

Phase 3 pivotal study- CS5

8.9% of the donidalorsen-4 treated subjects, and 4.3% of the donidalorsen-8 treated subjects, and 4.5% of the placebo subjects experienced any bleeding TEAE. None of the events were SAEs, and a majority were mild, none were severe.

There was one moderate bleeding TEAE in a subject treated with donidalorsen- 8- intermenstrual bleeding, normal platelets- considered unrelated, no study drug interruption.

There was a second moderate bleeding TEAE in a subject treated with donidalorsen- 4. Of note this patient did not experience bleeding per se, but had by a rise in PT time, initially noted on Study Day56 (14.9s) and graded mild- changed to moderate on Study Day 110. Also, a mild rise in INR. The event was considered related. Of note this patient had a concurrent condition of inherited blood disorder. Platelets were normal.

Pool 1 (CS2 +CS5)

In Pool 1, the proportion of patients who experienced bleeding TEAEs was 9.7% in the donidalorsen-4 group, 4.3% in the donidalorsen-8 group, and 10.7% in the placebo group- so a similar rate to that seen in CS5 alone. There were 3 additional bleeding AEs in this pool- all related to the injection site. 2 subjects on donidalorsen- 4 and one on placebo experienced haematoma/bruising of the injection site.

Pool 2 (CS2 + CS3 + CS5 +CS7)

17.8% of patient treated with donidalorsen 80mg 4 weekly had a bleeding TEAE, with bleeding/bruising at the injection site accounting for a majority of these bleeding events. All of the bleeding events were mild or moderate in severity. There was just one serious bleeding SAE which was assessed as not related.

Other Safety Topics of Interest

Based on the identified and potential risks associated with donidalorsen and its ASO portion, and the class experience with ASO drugs, hypersensitivity, renal impairment, abnormal liver function and LCRIS were evaluated, together with relevant associated laboratory parameters.

- **Local Cutaneous Reactions at Injection Site- LCRIS**

See also discussion of ISRs under Section 2.6.8.2 above.

Local cutaneous reactions at the injection site were defined by the applicant to try and identify localised skin inflammatory reactions that were longer lasting than a typical injection site reaction> 2 days), and that have been reported following subcutaneous injection of other oligonucleotides. LCRIS was defined as per 2 possible definitions, as follows:

Definition A:	moderate or severe AEs with the PTs Injection site erythema, Injection site swelling, Injection site pruritus, or Injection site pain that started on the day of injection, persisted **for at least 2 days** or ongoing
Definition B:	any AE at the Study Drug injection site, regardless of severity, that led to discontinuation of study drug, where AE at the Study Drug injection site was the principal reason for discontinuation

No patients in any treatment group experienced LCRIS in the CS5 study or in Pool 1.

In Pool 2 (CS2 + CS3 + CS5 +CS7), 4 (2.3%) patients experienced 19 LCRIS events (definition B x 6 events- 1 patient, definition A x13 events- 3 patients) across all treatment groups: 3 (1.9%) patients in the donidalorsen-4 group, all from the CS7 study, experienced 13 events, and 1 (33.3%) patient in the donidalorsen-100 mg-4 group in CS3 experienced 6 events. All were moderate, and none were serious or severe. No patients in the donidalorsen-8 or placebo groups experienced LCRIS events during the study.

The applicant has provided the additional detail requested with regard to a patient who had 6 LCRIS (injection site discolouration/depigmentation) TEAEs, moderate in severity- which eventually led to treatment discontinuation. All were considered related. See more detail under Discontinuation due to adverse events section.

- **Flu like reactions**

Flu-like reactions were defined as AEs with PTs including either (A) Influenza like illness or (B) Pyrexia or Feeling hot or Body temperature increased, plus at least 2 of the following symptoms with the PTs: Chills, Myalgia, or Arthralgia, starting on day of injection or the next day.

No patient in any treatment group in any study or pool experienced a flu-like reaction.

- **Hypersensitivity Adverse Events**

<u>Phase 3 pivotal study- CS5</u>

The highest rate of hypersensitivity reaction was in the placebo group- 22.7% in the placebo group v 11.1% in the donidalorsen- 4 group v 4.3% in the donidalorsen – 8 group experienced at least 1 hypersensitivity TEAE. No patient experienced a serious hypersensitivity TEAE or was discontinued from study drug due to hypersensitivity. All were mild in severity and did not result in any action taken with study drug. The majority of events were not related to study drug and resolved without sequelae.

Pool 1 (CS2 +CS5)

In Pool 1, the highest rate of hypersensitivity reaction was in the placebo group: placebo group (21.4%) experienced at least 1 hypersensitivity TEAE compared with the donidalorsen-4 (14.5%) and donidalorsen-8 (4.3%) groups. No patient experienced a serious hypersensitivity TEAE or was discontinued from study drug due to these events. The majority of hypersensitivity TEAEs in Pool 1 were unrelated to study drug.

Pool 2 (CS2 + CS3 + CS5 +CS7)

Hypersensitivity TEAEs were common in the donidalorsen trials. In Pool 2, 27.6% of the donidorsen 80 mg 4 weekly subjects had any hypersensitivity reaction, versus 7% in the 8-weekly group, 66.7% (2 of the 3 subjects) in the 100 mg group, and 21.4% in the placebo group. Overall, 6 TEAEs (1 serious and 5 nonserious) in 3 (1.6%) patients who received donidalorsen have been assessed by the applicant as reactions, including 1 event in one subject detailed below that met Sampson's criteria for anaphylaxis reaction.

Event 1: A patient with no history of allergies experienced a nonserious event of rash (moderate) approximately 3 hours after receiving donidalorsen on Day 756 and recovered 2 hours later after treatment with a dose of oral diphenhydramine. No action was taken with the investigational product.

Event 2: On Day 816, after receiving donidalorsen, she experienced a severe and serious hypersensitivity (reported term: allergic reaction) event, which started 5 minutes after the injection. The patient then developed an extensive red rash over most of her body, complained of dyspnoea, peri-oral swelling, and chest pain. The Investigator clinically diagnosed the event as a Type I Hypersensitivity reaction, and the patient was treated with diphenhydramine orally and epinephrine subcutaneously. The heart rate at the time of the event was 145 bpm, but blood pressure was not measured. Additional blood tests for further investigation were not performed at that time. The event was resolved on the same day. No concomitant medications were taken apart from ibuprofen before the injection. The Investigator assessed the event as related to donidalorsen and discontinued treatment on the same day due to the event. This case fulfils Sampson's criteria for the diagnosis of anaphylaxis. Tryptase levels or immunoglobulin E (IgE) levels were not available.

It is noted that at the time of both reactions there was no ADA measurement taken- but the last ADA measurement, at 25 days and 85 days before each respective reaction was high at 102400.

- **Renal impairment Adverse Events**

In the pivotal study CS5 there was no donidalorsen treated patient that experienced a renal impairment TEAE.

Table 25 below, provides a listing of the 6 renal TEAEs reported in Pool 2, and Table *26* below, gives a summary of abnormal renal function laboratory parameters for Pool 2, all of which were mild in severity.

Table 25. Renal effect treatment emergent adverse events by system organ class and preferred term safety set – Pool 2 (CS2, CS3, CS5, and CS7)

System Organ Class/ Preferred Term	Placebo (N=28) n (%)	e	Total Donidalorsen (N=177) n (%)	e	Donidalorsen 80 mg Q4W (N=163) n (%)	e	Donidalorsen 80 mg Q8W (N=43) n (%)	e	Donidalorsen 100 mg Q4W (N=3) n (%)	e	Total (N=181) n (%)	e
Patients with any Renal Effect TEAEs	0	0	6 (3.4)	6	4 (2.5)	4	2 (4.7)	2	0	0	6 (3.3)	6
Investigations	0	0	5 (2.8)	5	4 (2.5)	4	1 (2.3)	1	0	0	5 (2.8)	5
Blood creatinine increased	0	0	2 (1.1)	2	2 (1.2)	2	0	0	0	0	2 (1.1)	2
Protein urine present	0	0	2 (1.1)	2	2 (1.2)	2	0	0	0	0	2 (1.1)	2
Blood urea increased	0	0	1 (0.6)	1	0	0	1 (2.3)	1	0	0	1 (0.6)	1
Renal and urinary disorders	0	0	1 (0.6)	1	0	0	1 (2.3)	1	0	0	1 (0.6)	1
Proteinuria	0	0	1 (0.6)	1	0	0	1 (2.3)	1	0	0	1 (0.6)	1

Table 26 below, provides a summary of abnormal renal function reported in Pool 2.

Table 26. Summary of abnormal renal function safety set – Pool 2 (CS2, CS3, CS5, and CS7)

Parameter/ Severity	Placebo (N=28) n (%)	Total Donidalorsen (N=177) n (%)	Donidalorsen 80 mg Q4W (N=163) n (%)	Donidalorsen 80 mg Q8W (N=43) n (%)	Donidalorsen 100 mg Q4W (N=3) n (%)	Total (N=181) n (%)
Creatinine						
Mild	0	3 (1.7)	3 (1.8)	0	0	3 (1.7)
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
Urine Protein/Creatinine Ratio (UPCR)						
< 500 mg/g	27 (96.4)	168 (94.9)	156 (95.7)	34 (79.1)	3 (100)	172 (95.0)
500-1000 mg/g	0	6 (3.4)	3 (1.8)	3 (7.0)	0	6 (3.3)
> 1000 mg/g	0	1 (0.6)	1 (0.6)	0	0	1 (0.6)
Blood Urea Nitrogen (BUN)						
Mild	0	27 (15.3)	23 (14.1)	5 (11.6)	0	27 (14.9)
Moderate	0	3 (1.7)	2 (1.2)	1 (2.3)	0	3 (1.7)
Severe	0	1 (0.6)	1 (0.6)	0	0	1 (0.6)

Safety Set = Pool 2 includes patients who received at least one dose of study drug (donidalorsen or placebo) in randomized, double-blind, placebo-controlled trials and open-label extension trials (ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, or ISIS 721744-CS7 study).
% = 100 x n/N.
Abbreviations: Q4W = every 4 weeks, Q8W = every 8 weeks.
The severity grades are based on the grading scale shown as below.
Creatinine: Normal: <1.8 mg/dL ; Mild: 1.8 - 1.7 mg/dL; Moderate: 1.8 - 2.0 mg/dL; Severe: >=2.1 mg/dL.
BUN: Normal: <23 mg/dL; Mild: 23 - 26 mg/dL; Moderate: 27 - 31 mg/dL; Severe: >31 mg/dL.

The Table 27 below gives an overview of renal impairment parameters and TEAEs for CS5 and Pool 2; Pool 1 is not included as any cases therein are listed under CS5 or Pool 2. For simplicity, individual narratives/subjects listed under CS5 are not listed again under Pool 2.

Table 27. Overview of renal impairment parameters and TEAEs for CS5 and Pool 2

	CS5	***Pool 2 (CS2 + CS3 + CS5 +CS7)-<u>updated</u>** ***individual narratives/subjects listed under CS5 are not listed again under Pool 2***
<u>Renal TEAE</u>	None	2 x Creatinine incr(mild) 1 x Urea incr(mild) 3 x Protein Urine present(mild)
<u>Occurrence of renal stopping rules</u>	None	None
<u>UPCR</u>:	One patient in Donidalorsen-4 group had an isolated elevation in UPCR > 1000 mg/g on Study Day 138 which returned to within reference range by the next visit at week 25	
<u>Creatinine</u>	No raised creatinine	Three (1.9%) patients in the donidalorsen-4 group experienced abnormal serum creatinine levels of 1.5 to 1.7 mg/dL (mild severity).
<u>Blood urea nitrogen (BUN):</u>	No elevations in BUN≥ 26 mg/dL.	One patient (0.6%) **(CS7)** in the donidalorsen-4 group had a high Baseline BUN > 31 mg/dL which remained elevated-peak level of 44 mg/dL on D113 and was still elevated but below baseline level on D285 (29 mg/dL). 3 patients experienced <u>moderate</u> elevations in BUN (27 to 31 mg/dL)
eGFR:	One (1.6%) patient in the donidalorsen-4 group experienced a ≥ 25% reduction from Baseline for eGFR	A ≥ 50% isolated reduction from Baseline for eGFR was experienced by 3 (1.9%) patients in the donidalorsen-4 group. None were recorded as a TEAE by the Investigator.

		A ≥ 25% isolated reduction from Baseline for eGFR was experienced by 17 (10.4%) patients in the donidalorsen-4 group and 4 (9.3%) patients in the donidalorsen-8 group. None were recorded as a TEAE by the Investigator.

- **Liver function**

There were no SAEs concerning liver AEs or liver function AEs in any of the 4 studies as per cut-off date. There were no cases of Hy's law in any in any of the 4 studies as per cut-off date.

There was one treatment discontinuation due to a liver event (moderate ALT rise) at time of first submission, and during this procedure a second case has been reported (see below). In both cases the investigators considered the LFT rises to be related or possibly related.

- **Study CS5, (Donidalorsen- 8 weeks) –** A patient who had ALT increased (initially mild) on Day 58 to 97 from normal range (10-53 g/dL) at baseline that was considered related, led to permanent study drug discontinuation on that same day, even though not meeting stopping criteria. Peak ALT was 230(>3 to< 5 ULN- moderate) on D92; it then slowly declined. Last available ALT was 70 at week 25, patient did not complete LFT follow up. A single abnormal AST elevation was also noted. Mild GGT elevations noted. Bilirubin normal. Prior use of danazol and concomitant use of icatibant acetate. Diagnosed by ultrasound with mild hepatic steatosis (separate TEAE) at D204 (mild severity) that was considered not related to study drug. BMI 26.

- A patient, with no medical history, completed CS5 on the 80 mg 8 weekly regime and had 3 doses without issue. On D112 the patient joined CS7 on an 80mg 4 weekly regime. 9 months later stopped due to a Liver function AE. Bloods at start of CS7 were essentially normal but for GGT 39 (upper limit was 38). First abnormality was on D197 of CS7 when approx 1.5 ULN for ALT, AST, GGT. One month later further increases in ALT and AST to approx 3ULN but GGT not reported. Progressed to moderate. Progression to a peak of 7 ULN for ALT. AST peak was 4ULN. GGT also peaked at 3ULN. Serology for viruses was negative. Total period with raised LFTs was approx 5 month. LFTs normalised without intervention after 5 months- in May 2024 Drug was stopped approx 2 weeks after LFT normalisation as stopping criteria were deemed to have been met even though the LFTs had since normalised. Stopped contraceptive in March 2024 (started Dec 2022 at the time of CS5 entry)

Table 28. Summary of abnormal liver function treatment-emergent adverse events by preferred term – study CS5 and Pool 1 (CS2 and CS5)

	Study CS5			Pool 1 (Study CS2 and Study CS5)			
Preferred Term, n (%)	Placebo (N = 22)	Donidalorsen 80 mg Every 4 Weeks (N = 45)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Placebo (N = 28)	Donidalorsen 80 mg Every 4 Weeks (N = 62)	Donidalorsen 80 mg Every 8 Weeks (N = 23)	Total Donidalorsen 80 mg (N = 85)
Patients with at least one relevant event from drug related hepatic disorders – comprehensive search (SMQ)	**1 (4.5)**	**1 (2.2)**	**1 (4.3)**	**1 (3.6)**	**1 (1.6)**	**1 (4.3)**	**2 (2.4)**
Prothrombin time prolonged	0	1 (2.2)	0	0	1 (1.6)	0	1 (1.2)
Blood bilirubin increased	0	1 (2.2)	0	0	1 (1.6)	0	1 (1.2)
International normalized ratio increased	0	1 (2.2)	0	0	1 (1.6)	0	1 (1.2)
Alanine aminotransferase increased	0	0	1 (4.3)	0	0	1 (4.3)	1 (1.2)
Blood bilirubin unconjugated increased	1 (4.5)	0	0	1 (3.6)	0	0	0
Hepatic steatosis	0	0	1 (4.3)	0	0	1 (4.3)	1 (1.2)
Patients with at least one relevant event form Cholestasis and jaundice of hepatic origin (SMQ)	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Patients with at least one relevant event from Drug related hepatic disorders - severe events only (SMQ)	**0**	**0**	**1 (4.3)**	**0**	**0**	**1 (4.3)**	**1 (1.2)**
Hepatic Steatosis	0	0	1 (4.3)	0	0	1 (4.3)	1 (1.2)

Table 29 lists all of the abnormal liver function TEAEs by PT for Pool 2- so including the OLE studies (CS2, CS3, CS5 and CS7). With the exception of hepatic steatosis TEAEs and one case of steatohepatitis - all other TEAEs relate to liver enzyme laboratory parameters.

Table 29. Abnormal liver function treatment emergent adverse events by system organ class and preferred term safety set – Pool 2 (CS2, CS3, CS5, and CS7)

System Organ Class/ Preferred Term	Placebo (N=28) n (%)	e	Total Donidalorsen (N=177) n (%)	e	Donidalorsen 80 mg Q4W (N=163) n (%)	e	Donidalorsen 80 mg Q8W (N=43) n (%)	e	Donidalorsen 100 mg Q4W (N=3) n (%)	e	Total (N=181) n (%)	e
Patients with at least one relevant event from Drug related hepatic disorders - comprehensive search (SMQ)	1 (3.6)	1	26 (14.7)	56	21 (12.9)	48	5 (11.6)	8	0	0	26 (14.4)	57
Hepatobiliary disorders	0	0	7 (4.0)	8	5 (3.1)	6	2 (4.7)	2	0	0	7 (3.9)	8
Hepatic steatosis	0	0	5 (2.8)	5	3 (1.8)	3	2 (4.7)	2	0	0	5 (2.8)	5
Hyperbilirubinaemia	0	0	1 (0.6)	1	1 (0.6)	1	0	0	0	0	1 (0.6)	1
Hypertransaminasaemia	0	0	1 (0.6)	1	1 (0.6)	1	0	0	0	0	1 (0.6)	1
Steatohepatitis	0	0	1 (0.6)	1	1 (0.6)	1	0	0	0	0	1 (0.6)	1
Investigations	1 (3.6)	1	23 (13.0)	48	19 (11.7)	42	4 (9.3)	6	0	0	24 (13.3)	49
Alanine aminotransferase increased	0	0	12 (6.8)	16	9 (5.5)	11	3 (7.0)	5	0	0	12 (6.6)	16
Hepatic enzyme increased	0	0	7 (4.0)	9	7 (4.3)	9	0	0	0	0	7 (3.9)	9
Aspartate aminotransferase increased	0	0	6 (3.4)	8	6 (3.7)	8	0	0	0	0	6 (3.3)	8
Gamma-glutamyltransferase increased	0	0	5 (2.8)	7	5 (3.1)	7	0	0	0	0	5 (2.8)	7
Blood bilirubin increased	0	0	2 (1.1)	2	1 (0.6)	1	1 (2.3)	1	0	0	2 (1.1)	2
Blood bilirubin unconjugated increased	1 (3.6)	1	0	0	0	0	0	0	0	0	1 (0.6)	1
International normalised ratio increased	0	0	1 (0.6)	1	1 (0.6)	1	0	0	0	0	1 (0.6)	1
Prothrombin time prolonged	0	0	1 (0.6)	5	1 (0.6)	5	0	0	0	0	1 (0.6)	5

Safety Set - Pool 2 includes patients who received at least one dose of study drug (donidalorsen or placebo) in randomized, double-blind, placebo-controlled trials and open-label extension trials (ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, or ISIS 721744-

A summary of abnormal liver function parameters for **CS5** and **Pool 1** is provided below: Table 30.

Table 30. Summary of abnormal liver function laboratory parameters – CS5 and Pool 1 (CS2 and CS5)

Parameter Severity	Study CS5			Pool 1 (Study CS2 and Study CS5)			
	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 45) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	Placebo (N = 28) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 62) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	Total Donidalorsen (N = 85) n (%)
Alanine Aminotransferase (ALT)							
Mild (> ULN to 3.0 × ULN)	0	7 (15.6)	2 (8.7)	1 (3.6)	9 (14.5)	2 (8.7)	11 (12.9)
Moderate (> 3.0 to 5.0 × ULN)	0	0	2 (8.7)	0	0	2 (8.7)	2 (2.4)
Severe (> 5.0 × ULN)	0	0	0	0	0	0	0
Aspartate Aminotransferase (AST)							
Mild (> ULN to 3.0 × ULN)	0	2 (4.4)	3 (13.0)	0	2 (3.2)	3 (13.0)	5 (5.9)
Moderate (>3.0 to 5.0 × ULN)	0	0	0	0	0	0	0
Severe (> 5.0 × ULN)	0	0	0	0	0	0	0
Total Bilirubin[a]							
Mild	1 (4.5)	1 (2.2)	1 (4.3)	1 (3.6)	3 (4.8)	1 (4.3)	4 (4.7)
Moderate	1 (4.5)	0	0	1 (3.6)	0	0	0
Severe	0	1 (2.2)	0	0	1 (1.6)	0	1 (1.2)

Parameter Severity	Study CS5			Pool 1 (Study CS2 and Study CS5)			
	Placebo (N = 22) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 45) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	Placebo (N = 28) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 62) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 23) n (%)	Total Donidalorsen (N = 85) n (%)
Alkaline Phosphate (ALP)							
Mild (1.1 to 2.0 × ULN)	0	0	0	0	0	0	0
Moderate (> 2.0 to 3.0 × ULN)	0	0	0	0	0	0	0
Severe (> 3.0 × ULN)	0	0	0	0	0	0	0
Gamma-Glutamyl Transpeptidase (GGT)							
Mild (> ULN to 2.5 × ULN)	1 (4.5)	7 (15.6)	6 (26.1)	2 (7.1)	8 (12.9)	6 (26.1)	14 (16.5)
Moderate (> 2.5 to 5.0 × ULN)	0	0	0	0	1 (1.6)	0	1 (1.2)
Severe (> 5.0 × ULN)	0	0	0	0	0	0	0
Prothrombin Intl. Normalized Ratio (INR)[b]							
Mild	2 (9.1)	8 (17.8)	4 (17.4)	3 (10.7)	10 (16.1)	4 (17.4)	14 (16.5)
Moderate	0	0	0	1 (3.6)	0	0	0
Severe	0	0	0	0	0	0	0

Source: ISIS 721744-CS5 Ad-hoc Table 14.3.4.2.7; ISS Ad-hoc Table 14.3.4.1.4.1

Given the difference in exposure between the donidalorsen treatment groups and placebo in Pool 2, an exposure-adjusted analysis of abnormal liver function laboratory parameters was performed and is provided in Table 31 below. For ALT and AST mild grade is up to 3 × ULN, moderate is > 3 to 5 × ULN, and severe is > 5 × ULN.

Table 31. Incidence rate (per 100 patient years of exposure) of abnormal liver function – safety set (Pool 2 (CS2, CS3, CS5, and CS7))

	Placebo (N = 28) n (PYE)	Total Donidalorsen (N = 177) n (PYE)	Donidalorsen 80 mg Q4W (N = 163) n (PYE)	Donidalorsen 80 mg Q8W (N = 43) n (PYE)	Donidalorsen 100 mg Q4W (N = 3) n (PYE)	Total (N = 181) n (PYE)
ALT						
Mild	1 (8.7)	80 (25.2)	71 (27.6)	8 (14.9)	2 (30.0)	81 (24.6)
Moderate	0	4 (1.3)	1 (0.4)	3 (5.6)	0	4 (1.2)
Severe	0	4 (1.3)	4 (1.6)	0	0	4 (1.2)
AST						
Mild	0	54 (17.0)	48 (18.7)	6 (11.2)	0	54 (16.4)
Moderate	0	3 (0.9)	3 (1.2)	0	0	3 (0.9)
Severe	0	2 (0.6)	2 (0.8)	0	0	2 (0.6)
Total bilirubin						
Mild	1 (8.7)	12 (3.8)	10 (3.9)	4 (7.4)	0	12 (3.6)
Moderate	1 (8.7)	6 (1.9)	5 (1.9)	1 (1.9)	0	7 (2.1)
Severe	0	2 (0.6)	2 (0.8)	0	0	2 (0.6)
ALP						
Mild	0	4 (1.3)	4 (1.6)	0	0	4 (1.2)
Moderate	0	0	0	0	0	0
Severe	0	0	0	0	0	0
GGT						
Mild	2 (17.5)	54 (17.0)	46 (17.9)	10 (18.6)	0	54 (16.4)
Moderate	0	9 (2.8)	9 (3.5)	0	0	9 (2.7)
Severe	0	2 (0.6)	2 (0.8)	0	0	2 (0.6)
Prothrombin INR						
Mild	2 (17.5)	50 (15.7)	42 (16.3)	8 (14.9)	1 (15.0)	51 (15.5)
Moderate	1 (8.7)	1 (0.3)	1 (0.4)	0	0	2 (0.6)
Severe	0	1 (0.3)	1 (0.4)	0	0	1 (0.3)

Mean absolute values over time for LFTs:

Mean absolute values over time for hepatic transaminases were within reference range at all time points throughout CS5, CS7; and CS3.

2.6.8.4. Mean values over time for other liver chemistry tests were also within reference range throughout the studies. Laboratory findings

Cardiac safety:

A formal QTc study was not conducted.

There were no notable trends or safety concerns observed for ECG findings in any of the 4 studies or integrated pools. No patient experienced a QTcF interval > 500 msec in any treatment group.

The following are the AE/cardiac findings of most significance that occurred- Pool 2:

Patient 1 (in CS5 study)	abnormal, clinically significant ECG at Week 25 which was associated with a TEAE of supraventricular extrasystoles.	Graded mild, not related, and no action was taken with study drug. Ongoing
Patient 2 (from the prior lanadelumab group in CS7) Donidalorsen- 4	abnormal, clinically significant ECG at Week 9;	Medical history of hypertension, cardiac failure congestive, asthma-chronic obstructive pulmonary disease overlap syndrome, and pulmonary hypertension. Experienced a serious TEAE of cardiac failure acute on Study Day 61, and soon after a serious TEAE of renal disorder, which consequently resulted in permanent discontinuation from study drug on D79. See SAE section.
Patient 3 (from the Index placebo/OLE donidalorsen-4 in CS7)	abnormal, clinically significant ECG of sinus bradycardia at Week 25	Medical history of sinus bradycardia which was ongoing during the study.
Patient 4 in the donidalorsen-8 group from CS5	Isolated QTcF interval > 480 msec (measured as 488msec) which was also an increase from Baseline in QTcF > 60 msec, D201	Returned to near normal by D266
Patient 5 in the donidalorsen-4 group from CS5	Increase > 60 msec in QTcF on D88 (323msec to 388msec). Considered not clinically significant.	The QTcF interval remained elevated on Study Day 141 and 169. Medical history of hypercholesterolaemia

Table 40: Summary of Abnormal 12-Lead ECG Categories – Pool 2 (CS2, CS3, CS5, and CS7)

Parameter Category	Placebo (N = 28) n (%)	Donidalorsen 80 mg Every 4 Weeks (N = 156) n (%)	Donidalorsen 80 mg Every 8 Weeks (N = 31) n (%)	Donidalorsen 100 mg Every 4 Weeks (N = 3) n (%)	Total Donidalorsen (N = 173) n (%)
QTcF Interval					
> 450 msec	1 (3.6)	6 (3.8)	2 (6.5)	0	8 (4.6)
> 480 msec	0	0[b]	1 (3.2)	0	1 (0.6)
> 500 msec	0	0[b]	0	0	0
Increase from Baseline[a] in QTcF					
> 30 msec	3 (10.7)	6 (3.8)	4 (12.9)	0	10 (5.8)
> 60 msec	0	1 (0.6)	1 (3.2)	0	2 (1.2)

Source: ISS Ad-hoc Table 14.3.5.4.1.2

Coagulation

Overall, across pivotal study CS5 and the integrated analysis pools, no pattern or clinically meaningful trends were identified at any dose of donidalorsen for coagulation laboratory parameters (which included partial thromboplastin time, D-Dimer, prothrombin INR, and prothrombin Time).

Complement

Overall, across pivotal study CS5 and the integrated analysis pools, there were small fluctuations over time in all treatment arms, no pattern or clinically meaningful trends were identified at any dose of donidalorsen for complement C5a. C5a was used to assess any impact on complement systems in the donidalorsen programme, a well-established biomarker indicative of terminal complement activation as it reflects activity across both the classical and alternative pathways.

Haematology

Overall, across pivotal study CS5 and the integrated analysis pools, no pattern or clinically meaningful trends were identified at any dose of donidalorsen for haematology laboratory parameters (basophils, eosinophils, leukocytes, erythrocyte Mean corpuscular hemoglobin, erythrocytes, hematocrit, hemoglobin, lymphocytes, monocytes, neutrophils, and platelets)

Changes over time in platelets, as well as summaries of TEAEs corresponding to changes in platelets, are discussed in earlier sections.

Chemistry parameters

Overall, across pivotal study CS5 and the integrated analysis pools, no pattern or clinically meaningful trends were identified at any dose of donidalorsen for chemistry laboratory parameters (included creatinine, urea nitrogen, bilirubin (direct and indirect), albumin, ALT, AST, ALP, bicarbonate, calcium, chloride, cholesterol, creatine kinase, GGT, glucose, magnesium, phosphate, potassium, protein, sodium, and urate).

Changes over time in renal function and liver function, as well as summaries of TEAEs corresponding to changes in these laboratory parameters, are discussed in earlier sections.

Vitals

CS5: Overall, no notable trends or safety concerns were observed for vital signs measurements, including blood pressure. No patient experienced a TEAE of hypertension during the study. Transient fluctuations from Baseline were observed in all treatment groups for blood pressure, heart rate, and respiratory rate; none of which were sustained during the study.

Across Pool 2, no notable trends or safety concerns were observed for vital sign measurements.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

Pregnancy

There are very limited data from the use of donidalorsen in pregnant women. Across all clinical studies, 6 pregnancies or partner pregnancies were observed.

- A patient reported a positive serum and urine pregnancy test result. The patient experienced a spontaneous miscarriage, which was reported as a serious, not related TEAE by both the investigator and the Applicant.
- A patient was found to be pregnant via review of medical records for confirmation of a contraceptive method. On Day 126, it was noted that the investigator requested medical records for details regarding her method of birth control. Upon review of her medical records, it was noted the patient was pregnant and had an elective nonsurgical abortion on Day 124.
- A patient reported paternal exposure during pregnancy. The delivery was complicated at full-term (38 weeks) via caesarean section due to an unknown reason. The neonate was born alive and both infant and mother were healthy.
- A patient reported a positive serum and urine pregnancy test result on Day 233. The patient is continuing in the post-treatment follow-up period and the pregnancy is ongoing.
- A patient reported paternal exposure during pregnancy. At the time of the Safety Update data cut-off, the partner’s pregnancy was ongoing.
- A patient experienced exposure during pregnancy. The patient had a positive serum and urine pregnancy test result on Day 533. The patient received her last dose of investigational product on Day 507 (Week 73); at the time of exposure to the investigational product, she was in the first trimester and was using a condom and spermicide for contraception. The patient discontinued the study on Day 533 due to pregnancy and completed an Early Termination Visit. The patient underwent a therapeutic abortion on Day 549.

Breastfeeding

There are no data from donidalorsen breastfeeding women, hence the presence of donidalorsen and/or metabolites in human milk or on their effects on the breastfed child or on milk production has not evaluated.

Renal impairment

Small numbers of patients with mild renal impairment were recruited into the donidalorsen studies. However, no patients with moderate or severe renal disease, or end stage renal disease have received donidalorsen.

Hepatic impairment

Small numbers of patients with mild hepatic impairment were recruited into the donidalorsen studies. However, no patients with moderate or severe hepatic disease have received donidalorsen.

Race

In Pool 1, the incidence of TEAEs overall was lower in White (56 [69.1%]) patients compared to Other race (4 [100.0%]) patients for the total donidalorsen group.

Ethnicity

In Pool 1, the incidence of TEAEs overall was greater in Hispanic or Latino (5 [83.3%]) patients compared to non-Hispanic and Latino (55 [69.6%]) patients for the total donidalorsen group.

Gender

In Pool 1, the incidence of TEAEs overall was similar in female (38 [73.1%]) and male (22 [66.7%]) patients for the total donidalorsen group. Only the PT injection site erythema was noted to have a ≥ 10% absolute difference in incidence between genders, females> males.

Region

In Pool 1, the incidence of TEAEs overall was similar in patients from North America (19 [73.1%] and Europe (32 [78.0%]) and lower in patients from the Middle East (9 [50.0%]) for the total donidalorsen group. Although there are differences in some SOCs between geographic regions, there are only 2 PTs where a ≥ 10% difference between geographic region groups was seen; injection site discolouration and nasopharngitis- on both cases more frequent in the Europe region. The overall data are not suggestive of any meaningful trends or differences in safety profile between geographic regions.

Elderly

Only 3 subjects over the age of 65 years were treated in the controlled trials, all 3 received active, and of 2 of these 3 subjects continued into the OLE studies. A further 2 elderly subjects were recruited as Switch patients into open label study CS7. A comparison of AEs in the overall population versus in the elderly cohort has been provided by the applicant- See Table 32 below. The small number of patients over the age of 65 years significantly impacts the interpretation of such data. The overall rate of AE in the 6 elderly subjects is similar to the general population at 83.3%. Of note there were no subjects treated at > 74 years old.

Table 32. AEs by age range

MedDRA Terms	Age <65 (Total Number = 171) n (%) e	Age 65-74 (Total number = 6) n (%) e	Age 75-84 (Total number = 0) n (%) e	Age 85+ (Total number = 0) n (%) e
Total AEs	159 (93.0) 1777	5 (83.3) 72	0	0
Serious AEs – Total	11 (6.4) 14	0	0	0
- Fatal	1 (0.6) 1	0	0	0
- Hospitalization/prolong existing hospitalization	7 (4.1) 10	0	0	0
- Life-threatening	1 (0.6) 2	0	0	0
- Disability/incapacity	0	0	0	0
- Other (medically significant)	6 (3.5) 7	0	0	0
AE leading to drop-out	7 (4.1) 21	0	0	0
Psychiatric disorders	26 (15.2) 41	1 (16.7) 1	0	0
Nervous system disorders	47 (27.5) 133	1 (16.7) 26	0	0
Accidents and injuries	39 (22.8) 60	3 (50.0) 3	0	0
Cardiac disorders	7 (4.1) 12	0	0	0
Vascular disorders	7 (4.1) 7	1 (16.7) 1	0	0
Cerebrovascular disorders	0	0	0	0
Infections and infestations	128 (74.9) 406	4 (66.7) 12	0	0
Anticholinergic syndrome	0	0	0	0
Quality of life decreased	0	0	0	0
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	15 (8.8) 20	0	0	0
Gastrointestinal disorders	65 (38.0) 217	4 (66.7) 10	0	0
General disorders and administration site conditions	79 (46.2) 404	3 (50.0) 3	0	0
Musculoskeletal and connective tissue disorders	62 (36.3) 117	2 (33.3) 9	0	0
Eye disorders	8 (4.7) 10	1 (16.7) 1	0	0
Investigations	41 (24.0) 96	1 (16.7) 2	0	0
Neoplasm benign, malignant, and unspecified (incl cysts and polyps)	7 (4.1) 8	1 (16.7) 1	0	0
Renal and urinary disorders	11 (6.4) 15	1 (16.7) 1	0	0
Respiratory, thoracic, and mediastinal disorders	42 (24.6) 89	1 (16.7) 2	0	0
Skin and subcutaneous tissue disorders	40 (23.4) 66	0	0	0
Metabolism and nutrition disorders	16 (9.4) 19	0	0	0
Reproductive system and breast disorders	14 (8.2) 19	0	0	0
Ear and labyrinth disorders	13 (7.6) 17	0	0	0
Blood and lymphatic system disorders	9 (5.3) 11	0	0	0
Hepatobiliary disorders	9 (5.3) 10	0	0	0
Immune system disorders	8 (4.7) 14	0	0	0
Pregnancy, puerperium and perinatal conditions	2 (1.2) 2	0	0	0
Endocrine disorders	2 (1.2) 2	0	0	0
Congenital, familial and genetic disorders	1 (0.6) 1	0	0	0

2.6.8.7. Immunological events

In CS5, the incidence rate of treatment-emergent ADA was 20.0% in the donidalorsen 80 mg every 4 weeks and 21.7% in the donidalorsen 80 mg every 8 weeks in patients with HAE following treatment for 25 weeks.

Treatment-emergent ADA were characterized by a late onset and low peak titers. The overall median onset of treatment-emergent ADA ranged from 29 to 169 days, with a median peak ADA titer ranging from 50 to 400 following donidalorsen 80 mg once every 4 weeks or once every 8 weeks for 16 to 208 weeks across studies.

Based on the updated safety data from CS3 (safety population [N = 20], HAE-1/HAE-2 and HAE-nC1-INH combined), 75.0% (15/20) of patients were ADA positive, and all except one patient was treatment-emergent ADA positive. In the CS3 study, the proportion of patients who experienced TEAEs was 100% in the ADA-negative subgroup, and 92.9% in the treatment-emergent ADA group.

Based on the updated safety data from CS7, 56.8% (84/148) of patients were ADA positive with the majority of them with treatment-emergent ADA (91.7% [77/84]). The overall rate of any TEAE was high and similar in the ADA + and ADA- subjects, at 92.2% and 93.8% respectively.

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

No clinical drug-drug interaction studies have been performed with donidalorsen. *In vitro* studies show that donidalorsen is not a substrate or inhibitor of transporters, does not interact with highly plasma protein bound active substances, and is not a substrate or inhibitor/inducer of cytochrome P450 (CYP) enzymes. Donidalorsen is not expected to cause or be affected by drug-drug interactions mediated through drug transporters, plasma protein binding, or CYP enzymes.

2.6.8.9. Discontinuation due to adverse events

Phase 3 pivotal study- CS5

No patients in the donidalorsen-4 group or placebo group permanently discontinued study drug due to TEAEs. One (4.3%) patient in the donidalorsen-8 group discontinued study drug due to a TEAE. A patient experienced a TEAE of ALT increased (moderate severity) that was considered related to the study drug by the Investigator. The patient did not meet the protocol-defined stopping rules for liver chemistry evaluations.

Pool 1 (CS2 +CS5)

The only discontinuation due to AE was as described above for CS5- no additional AE discontinuations in Pool 1.

Pool 2 (CS2 + CS3 + CS5 +CS7) - updated

In Pool 2, 8 subjects on donidalorsen discontinued study drug to TEAE;

Donidalorsen 80mg-8 CS5	**Patient 1**	ALT increased (moderate severity) Patient who had ALT increased (initially mild) on Day 58 to 97 from normal range(10-53g/dL) at baseline that was considered related, led to permanent study drug discontinuation on that same day, even though not meeting stopping criteria. Peak ALT was 230(>3	**Considered related**

		to< 5 ULN- moderate) on D92; it then slowly declined. Last available ALT was 70 at week 25, patient did not complete LFT follow up. A single abnormal AST elevation was also noted. Mild GGT elevations noted. Bilirubin normal. Prior use of a pituitary gonadotropin inhibitor and concomitant use of icatibant acetate. Diagnosed by ultrasound with mild hepatic steatosis (separate TEAE) at D204 (mild severity) that was considered not related to study drug. BMI 26. ***See also specific section of this report discussing LFT AEs.***	
Donidalorsen 80mg-4 (switch patiuent from lanadelumab) CS7	**Patient 2**	2 SAEs resulted in study discontinuation, D72 Acute cardiac failure, renal disorder on Study Days 61 and 62, respectively. Patient had a medical history of hypertension, cardiac failure congestive, asthma-chronic obstructive pulmonary disease overlap syndrome, and pulmonary hypertension. Hospitalized for the serious TEAE of cardiac failure acute due to worsening congestive heart failure. During hospitalization (Study Day 62), the patient experienced another serious TEAE of renal disorder (verbatim: T2 hypointense lesion within right kidney); associated laboratory abnormality included severe blood urea nitrogen (BUN) elevation (37mg/dL [reference range 5 to 22 mg/dL).Both events were considered not related by the Investigator; the patient was permanently discontinued from study drug on Study Day 79 (last dose administered on Study Day 57). BUN elevations remained elevated (29 mg/dL [Study Day 66]) throughout the study. Both events were ongoing at the time of database lock (persists as AE). ***See also LFT derangements in the same patient in a similar timeframe in LFT AE discussion. Also see SAE section.***	Considered Not related
Donidalorsen 100mg-4 CS3	**Patient 3**	Injection site discolouration(moderate)/LCRIS-recurrent Patient who experienced 6 TEAEs of injection site discolouration (moderate) during the Flexible Dosing Period that led to permanent study drug discontinuation On Study Day 693, the patient experienced 3 TEAEs of injection site discolouration (moderate) in the abdomen	**Considered related**

		following administration of donidalorsen 100 mg treatment (every 4 weeks). On Study Day 755, the patient experienced an additional 3 TEAEs of injection site discolouration (moderate) in the same region following administration of donidalorsen 100 mg treatment. All events were considered related to study drug by the Investigator; 5 of the 6 events were ongoing at time of database lock. The patient received the last dose of donidalorsen 100 mg on Study Day 844 and was permanently discontinued from study treatment on Day 890 due to these events. ***Additional detail upon request:*** *The applicant clarifies that three simultaneous separate LCRIS events of injection site discolouration (lower left abdomen, lower right abdomen, and upper right abdomen) were reported on 2 separate occasions, in each case following abdominal injection. The LCRIS events occurred while on 100mg 4 weekly (a higher dose than the proposed posology). On both occasions the investigational product was administered as scheduled in the abdomen. The TTO for the first 3 events was 19 days and the latter 3 events were 27 days from the most recent injections.* *The first 3 LCRIS events occurred around D700 and were reported as verbatim term injection site reaction discolouration, and the later 3 LCRIS events were reported as verbatim term injection site reaction skin depigmentation. No other symptoms such as pain, redness, swelling, itching, or induration at the injection sites were reported. The patient received the last dose of investigational product on Day 844 and was permanently discontinued from study treatment on Day 890 due to these events.* *At the time of the last available report, 5 of the 6 events were ongoing.* *It is noted that patient was anti-drug antibody (ADA) positive at baseline and throughout the treatment period with titres in the low range.* *It is also notable that the latency from time of last injection is quite long for these particular events in this subject; for the other LCRIS events (in other patients) the latency was 1-2 days.*	

		The patient received a total of 31 doses of donidalorsen in the CS3 study.	
Donidalorsen 80 mg Q4W CS7	**Patient 4**	Serious hypersensitivity reaction within 5 minutes of dosing (extensive rash, dyspnoea, perioral swelling, chest pain) that met Sampson's criteria, D816. Discussed in more detail under Hypersensitivity reactions.	**Considered related.**
Donidalorsen 80 mg Q4W CS7	**Patient 5**	Liver enzyme increases, peak ALT/AST rise to > 4ULN (7ULN in the case of ALT). No obvious confounding factors. Discussed in more detail under Liver enzyme/liver events.	**Considered related.**
Donidalorsen 80 mg Q4W CS7	**Patient 6**	Patient receiving donidalorsen 80 mg Q4W experienced a **moderate hypersensitivity reaction**, not classified as serious. Donidalorsen was withdrawn, and the patient subsequently recovered	**Considered related.**
Donidalorsen 80 mg Q4W CS7	**Patient 7**	Patient receiving donidalorsen 80 mg Q4W e**xperienced moderate back pain, mild chest discomfort, and mild dyspnoea;** none were classified as serious. Donidalorsen was withdrawn, and the patient recovered.	**Considered related.**
Donidalorsen 80 mg Q4W CS7	**Patient 8**	Patient receiving donidalorsen 80 mg Q4W experienced a completed **suicide**. This event was assessed as severe and serious with a fatal outcome and was determined to be **unrelated** to the study treatment.	Considered unrelated

2.6.8.10. Post marketing experience

2.6.9. Discussion on clinical safety

Overview of safety dossier

The safety data is derived from one Phase 3 pivotal study (CS5), one Phase 2a study (CS2), 1 open label extension study (CS3), one OLE/Switch study (CS7) and 2 Phase 1 studies.

Additional safety analyses relevant to donidalorsen focused on organ systems of preferential distribution of ASO (hepatic and renal), and AEs known to be associated with subcutaneous ASO delivery (injection site events and flu-like reactions).

Exposure

In terms of total number of subjects dosed with donidalorsen, the number is low - 275 subjects have been dosed altogether, including phase 1. However, the size of the safety set needs to be considered in the context of HAE being a rare disease with an estimated prevalence of approximately 1 in 50,000 individuals worldwide (Sinnathamby et al. 2023).

173 patients have been dosed in the 4 key clinical studies in patients (CS5, CS2 and their respective OLE studies, CS7 and CS3). At the time of submission, most of the patients were recorded at the 24-52 week point of therapy - only 72/173 subjects as of the data cut off have been dosed for more than a year. Based on the updated data provided, at any dose, 166 have been dosed for more than 24 weeks and 154 have been dosed for more than 52 weeks. Specifically, for the 80mg 4 weekly dose, 151 have been dosed for more than 24 weeks, and 130 have been dosed for more than 52 weeks. The exposure in terms of duration of therapy now meets minimum requirements in terms of duration on therapy - as outlined in ICH E1.

There were no notable differences between the treatment arms that might influence the safety findings in terms of demographic factors/age, medical history, HAE severity/characteristics, or concomitant medications.

There has been a low dropout rate in the 2 controlled studies and also in the 2 OLE studies. In CS5, of the 91 patients randomised the majority completed treatment, with a higher proportion in the donidalorsen groups completing versus placebo; donidalorsen-4 group (95.7% completion rate) and donidalorsen-8 group (91.3% completion rate). 83 subjects then rolled into the OLE CS7, of which only 2 had left the trial at the time of MAA submission. There was a similarly high retention rate in CS2 and rollover rate into OLE CS3.

Adverse events

There was a high rate of TEAEs in pivotal study CS5 and in study CS2. In CS5 the highest rate of AEs was in the placebo group (81.8%), followed by the donidalorsen-4 group (73.3%), and followed by the donidalorsen-8 group (60.9%), with a similar pattern seen in Pool 1. In Pool 2, with additional long-term exposure to donidalorsen (but no additional exposure to placebo), the proportion of patients who experienced TEAEs was 93.3% in the donidalorsen-4 group, 69.8% in the donidalorsen-8 group, and 82.1% in the placebo group.

In terms of TEAE severity, in CS5, the majority of TEAEs across all treatment groups were mild or moderate. A greater proportion of patients in the donidalorsen-8 group (8.7%) and the placebo group (9.1%) had severe TEAEs compared with the donidalorsen-4 group (2.2%). Of the 5 severe TEAEs, only 1 event was serious, and 4 TEAEs were unrelated to study drug. Only 3 severe events were in donidalorsen treated subjects. In Pool 2 the majority of TEAEs were mild or moderate and rates of severe TEAEs were similar across the donidalorsen and placebo arms, despite longer exposure in the donidalorsen arms. In the donidalorsen-4 group, 9.2% experienced severe TEAEs, including headache (2 patients), nausea (2 patients), and cardiac failure acute, toothache, constipation, pyrexia, COVID-19, gastroenteritis, pneumonia, mastoiditis, otitis media chronic, neck pain, torticollis, meningioma, pulmonary oedema, pruritus, and deep vein thrombosis (all in 1 patient each). In the donidalorsen-8 group, 7.0% had severe TEAEs, including epicondylitis, blood creatine phosphokinase increased, and spinal pain. In the 3 subjects that received donidalorsen-100 mg every 4 weeks, 33.3% (one patient) experienced a severe TEAE of dermatitis.

In terms relatedness, in CS5, considerably more (42.2%) of the donidalorsen-4 group had related TEAEs compared with the donidalorsen-8 (17.4%) and placebo groups (27.3%). The majority of those AEs in donidalorsen groups were injection site reactions. Mild related TEAEs were more frequent in the placebo treated patients, but moderate and severe (even though much less common) were more frequent in

donidalorsen treated patients. Across all 4 studies there were 4 donidalorsen treated subjects with severe related TEAEs.

In terms of most frequently occurring TEAEs, in pivotal study CS5, for the donidalorsen–4 group the most commonly reported AEs were headache (13.3%), injection site erythema (13.3%), followed by nasopharyngitis (11.1%), UTI (8.9%), URTI (8.9%), and a number of other injection site AEs (injection site discolouration, injection site pain and injection site pruritis), each at 6.7%. In the donidalorsen–8 group the most commonly reported AEs were influenza (17.4%), nasopharyngitis (13%), headache (8.7%), URTI (8.7%), UTI (8.7%), oral herpes (8.7%), vomiting (8.7%) and injection site discoloration (4.3%).

ADRs and Section 4.8 of SmPC

The applicant considered as potential ADRs donidalorsen those treatment events (across all studies) assessed as being causally related to donidalorsen based on the known mechanism of action of donidalorsen, nonclinical evidence, temporal relationship to donidalorsen treatment, response to dose modification including dose interruption, and consideration of the underlying or concomitant conditions of the individual patients.

The applicant specifically examined the AEs that occurred in ≥ 5% and more frequently in the Q4W and Q8W groups than in the placebo treatment group in the CS5 study for consideration as ADRs. The applicant's justification to not consider URTI, UTI and abdominal discomfort identified in this way for Study CS5 as ADRs is acceptable.

Influenza was not identified as an ADR, even though the incidence of influenza was 17.4% for 80 mg Q8W, 4.4% for 80 mg Q4W, and 9.1% for placebo, (meaning that the difference from placebo in the 80 mg Q8W arm was 8.3%, which exceeds the 5% threshold). However, this was not considered an ADR because a higher incidence was observed at the lower dose level. Overall, this logic was agreed, and that the probability of influenza being a true ADR for donidalorsen is not high.

Other safety topics of interest

Prekallikrein/kallikrein reduction broader toxicity discussion

Upon request, the applicant provided a discussion of the potential for reduced expression of PKK to affect physiological processes, specifically coagulation and complement systems, haemodynamics/vascular tone. It should be noted that it is expected that donidalorsen should bring a reduction of 75% in PK, not a complete elimination.

Coagulation: The applicant discussed congenital PK deficiency, a rare condition, thought to be asymptomatic, but has been linked to increased risk of thrombotic events. It is probable that a majority of individuals with PK deficiency are presumed to go unrecognised given its largely asymptomatic nature. The validity of the claim that PK deficiency increases thrombotic risks is hampered by the lack of adequate control groups and the risks of both selection and publication biases. If an increased thrombotic risk of PK deficiency were to exist, this can be caused by either enhanced clot formation via the intrinsic coagulation cascade or via decreased fibrinolytic activity. Increased thrombin generation could be caused by enhanced intrinsic factor XI (FXI) activation due to increased binding to high molecular weight kininogen (HK), as the latter protein is less bound to plasma PK (PKa) in this scenario. Elevated FXI levels are associated with an increased risk of arterial and venous thromboses. However, KLKB1-/- mice have similar FXI plasma levels compared with wild-type mice.

In CS2 study, extended coagulation and fibrinolytic activity assays were performed in samples obtained from 22 HAE patients before and after 4 months (hereinafter referred to as follow-up) of treatment with either

80 mg donidalorsen or placebo. All generic and specific assays measured at follow-up in the donidalorsen group were comparable with baseline. There was also no signal of increased thrombotic risk in the clinical donidalorsen development to date. The Applicant's results are consistent with in vivo experiments in mice, as well as findings in HAE patients treated with lanadelumab. There also has been no signal of increased bleeding risk in the clinical development for donidalorsen to date.

Arising from some discussion on prekallikrein (PKK) deficiency in the context of identifying potential toxicities of donidalorsen), the applicant stated that aPTT may sometimes be prolonged in patients with prekallikrein deficiency, but that this prolongation is not indicative of an increased tendency to bleed, rather, is indicative of vitro artifact due to delayed initiation of contact activation. The applicant states that while there were a few isolated and transient abnormal aPTT values in the HAE patients treated with donidalorsen, there was no apparent clinically meaningful impact on aPTT or prolongation of aPTT as a laboratory artefact. Additionally, HAE itself can influence coagulation pathways, including the intrinsic pathway, potentially resulting in prolonged aPTT. As such, it was not considered necessary to add a warning to the product information about potential interference with aPTT as an in vitro artefact for donidalorsen in the treatment of HAE.

Complement: Donidalorsen may theoretically influence the complement system through 2 distinct mechanisms: (1) nonspecific, class-related activation of the alternative complement pathway, and (2) specific inhibition of PK expression.

Class-related complement activation is of interest as it has been observed, but largely only in nonhuman primates following administration of ASO therapeutics. NHPs seems to exhibit an exaggerated sensitivity to alternative complement pathway activation following SC ASO administration, which is not representative of the human response to date across various ASO clinical programmes. C5a, which was used to assess any impact on complement systems in the donidalorsen programme, a well-established biomarker indicative of terminal complement activation as it reflects activity across both the classical and alternative pathways. In donidalorsen HAE clinical programme, no increases in C5a were observed in the CS5 and CS7 studies.

With respect to the on-target action of donidalorsen, namely the suppression of PKK expression, the applicant cites some in vitro studies which suggest kallikrein can potentially activate the alternative complement pathway. However, there is currently no in vivo evidence, nonclinical or clinical, that supports a functional role for plasma kallikrein in modulating complement activity through this mechanism. No clinically significant changes or patterns in C5a were detected in the donidalorsen clinical studies.

Haemodynamics/vascular tone: The KKS, including prekallikrein and bradykinin seems to play a role in maintaining a balance with the RAS by counteracting its vasoconstricting effects and promoting vasodilation. Based on the above, PK plays a role in blood pressure regulation. Whether PK might play a role in hypertension is not clear- some studies suggest elevated PK levels in individuals with hypertension, while others suggest PK deficiency could be associated with hypertension. Importantly no clinically relevant changes/trends in vital signs including blood pressure were identified in CS5 and CS7.

Injection site reactions

In CS5 a greater proportion of patients in the donidalorsen-4 group (24.4%) experienced injection site reaction compared with the donidalorsen-8 (4.3%) group and the placebo group (4.5%). All injection site reactions were nonserious, mild in severity, and did not result in any action taken with study drug. Most injection site reactions resolved spontaneously without sequelae or any treatment.

In Pool 1, donidalorsen-4 group (21.0%) vs donidalorsen-8 (4.3%) group vs placebo group (7.1%) had ISRs. In Pool 2, with additional long-term exposure to donidalorsen 32.5% of the donidalorsen 80mg 4 weekly

treated subjects, and 4.7% of the 80mg 8 weekly treated subjects had ISRs. All injection site reactions were nonserious, the majority were mild in severity.

ISRs are included in Section 4.8 at a frequency of very common, and details of the most common ISR seen are also described in Section 4.8 (erythema, discolouration, pain, pruritus, induration, haematoma, bruising, exfoliation, hypersensitivity and swelling).

The rate of ISRs is considerably lower for the 8-week versus the 4-week dosing regimen in CS5, Pool 1 and Pool 2. In this context, the flexibility in the posology to reduce the dosing frequency to every 2 months if a patient is well controlled may be in the interests of patients.

The product information includes advice in SmPC Section 4.2 on rotation of injection sites, and avoidance of injecting into areas where skin is tender, bruised, red, hard, infected or discoloured, and into the site of the previous injection.

Local Cutaneous Reactions at Injection Site

Local cutaneous reactions at the injection site were defined by the applicant to try and identify localised skin inflammatory reactions that were longer lasting than a typical injection site reaction (> 2 days), and that have been reported following subcutaneous injection of other oligonucleotides.

There was no LCRIS event reported in CS5 or Pool 1. Overall, there were 19 LCRIS events reported in subjects treated with donidalorsen (4 subjects, 2.3%), in Pool 2, none for placebo. 3 of the 4 subjects concerned were dosed with the 80mg 4 weekly regime, and the fourth was dosed with 100mg 4 weekly. The events of LCRIS reported included injection site pruritus (n = 7), injection site discolouration (n = 6), and injection site erythema (n = 6), with a TTO from the most recent dose of 1 day. Average duration of LCRIS events was 26 days (range: 1 – 89 days). One patient experienced one event of moderate injection site reactions/discolouration which led to that patient's discontinuation.

Given that LCRIS has been shown to be associated with other ASOs, it is considered important that LCRIS specifically is listed in Section 4.8 of the SmPC. The applicant agreed to add information in Section 4.8 in a paragraph about ISRs that persisted for more than a few days, and also to mention the one subject who had repeated injection site discoloration who eventually had to discontinue treatment due to these events,

Description of selected adverse events.

Platelets

The laboratory data across all studies in patients with HAE do not suggest that donidalorsen has a clinically meaningful impact on platelet count. However, the dataset size is that of a rare disease, and it is possible that rarer incidences of thrombocytopenia may not have been picked up. It is also noted that there were 2 cases of severe thrombocytopenia in monkeys treated with donidalorsen, albeit at much higher margins/exposure, and that thrombocytopenia has been associated with other ASOs. Bleeding/thrombocytopenia has been included in the RMP as an important potential risk and will be followed up in the ongoing OLE CS7 study.

Bleeding events

Bleeding events were looked at in particular detail given that the mechanism of donidalorsen involves interference with the plasma kallikrein-kinin system (KKS) as kallikrein plays an integral role in many biological processes including coagulation. The main function of kallikrein in coagulation is the amplification of

activated factor XIIa (FXIIa) generation which ultimately leads to thrombin generation and fibrin clot formation.

Bleeding events are also of interest in the context of the potential for thrombocytopenia as a class effect of ONs.

In CS5, 8.9% of the donidalorsen-4 treated subjects, and 4.3% of the donidalorsen-8 treated subjects, and 4.5% of the placebo subjects experienced any bleeding TEAE. None of the events were SAEs, and a majority were mild, none were severe. In Pool 2, 14.7% of the donidalorsen-4 treated subjects, 6.5% of the donidalorsen-8 treated subjects, and 10.7% of the placebo subjects experienced any bleeding TEAE. Of the 23 donidalorsen-4 treated subjects that had a bleeding TEAE- at least 11 events are clearly related to injection site, and this might explain why there are less bleeding TEAEs for the 8-weekly regime, where there are less frequent injections.

In exposure-adjusted analysis of bleeding TEAEs the incidence rate (per 100 patient years of exposure) of bleeding TEAEs was 15.5% in the donidalorsen-4 group, 5.7% in the donidalorsen-8 group, and 26.2% in the placebo group. Overall, it is agreed that there does not seem to have been an increase in bleeding TEAEs in the donidalorsen treated subjects.

Flu like reactions

No patient in any treatment group in any study or pool experienced a flu-like reaction post injection as defined in the protocols.

Hypersensitivity AEs

In Pool 1, the highest rate of hypersensitivity reaction was in the placebo group: placebo group (21.4%) experienced at least 1 hypersensitivity TEAE compared with the donidalorsen-4 (14.5%) and donidalorsen-8 (4.3%) groups. In Pool 2, 27.6% of the donidalorsen 80 mg 4 weekly subjects had any hypersensitivity reaction, versus 7% in the 8-weekly group, 66.7% (2 of the 3 subjects) in the 100 mg group, and 21.4% in the placebo group. Overall, 6 TEAEs (1 serious and 5 nonserious) in 3 (1.6%) patients who received donidalorsen have been assessed by the applicant as reactions, including 1 event in one subject discussed below that met Sampson's criteria for anaphylaxis reaction.

The Applicant stated that based on review of the safety data, a causal relationship (considering the temporal associations and a lack of alternate aetiologies) between the events of hypersensitivity and use of donidalorsen is assessed possibly related, which is agreed. Therefore, 4.4 section of the SmPC has been updated by the Applicant with a warning on hypersensitivity including anaphylaxis. In addition, hypersensitivity has been added in section 4.8 of the SmPC, as a specific adverse reaction describing serious hypersensitivity under this heading.

Renal impairment or other renal related AEs

In CS5 there was no donidalorsen treated patient that experienced a renal impairment TEAE, and there was no occurrence of raised creatinine, or blood urea nitrogen. There was an isolated subject in the donidalorsen-4 group that had had an elevated baseline UPCR and then an isolated further elevation in UPCR > 1000 mg/g on D138 which returned to within reference range by the next visit. There was a second patient also in the donidalorsen-4 group with ≥ 25% reduction from Baseline for eGFR for a sustained period, but that gradually returned to near Baseline.

In Pool 2, there were 6 renal TEAEs, all mild, 2 raised creatinine, one raised urea, and 3 proteinuria cases- all in donidalorsen treated arms; no renal TEAEs were reported for placebo. There was a small number of

patients with isolated (once off) reductions in eGFR, or mildly elevated creatinine, or raised blood urea nitrogen where the patients did not experience any renal impairment TEAEs during the study and no action was taken with study drug.

Overall, it can be agreed that there does not appear to be any signal to date to indicate that donidalorsen could be causing renal impairment in HAE treated patients. That said, however, it is noted that no patients with moderate or severe renal impairment have been studied on donidalorsen. It is also the case that the available safety data is limited both in terms of numbers and extent of follow up. Renal toxicity has been included in the RMP and will be further explored in a post-marketing study.

Liver AEs and Liver function

Liver TEAEs and liver parameter changes are of particular interest based on the targeted delivery of donidalorsen to hepatocytes. It is understood that it would be expected that this type of conjugated ASO should have less impact on the liver due to lower doses, and more targeted delivery to hepatocytes.

Liver AEs:

In pivotal study CS5, the rate of Abnormal liver function TEAE was low overall, and similar for both the donidalorsen-4, donidalorsen-8 and placebo groups, at 1.6%, 4.3% and 3.6% respectively. In Pool 2, the rate of abnormal liver function TEAE for the donidalorsen-4 and donidalorsen-8 groups was 12.9% and 11.6% respectively.

Across the 4 studies, most hepatic TEAEs related to liver enzyme changes. Only 6 hepatic TEAEs did not relate to liver enzyme changes, and all 6 concerned hepatic steatosis. Overall, the applicant has reasonably discussed these 6 hepatic steatosis cases, and based on the data available, it would seem unlikely that these cases of hepatic steatosis are related to the use of donidalorsen, and therefore no action is required with respect to the SmPC/PI at this point. There were no additional cases of hepatic steatosis reported since the time of MAA submission.

The applicant states that across the 4 clinical studies, 25 cases with 49 TEAEs related to hepatic enzymes were observed. All events were nonserious. The majority of the events were mild in severity (41 of 49 events [84%]) and a few were moderate (8 of 49 events [16%]). No cases were severe, no case met Hy's law criteria, and no pattern suggestive of drug-induced liver injury was identified.

Regarding causality, it is agreed that there are many potentially confounding concomitant medications, previous medications and other medical factors in the patient population, making it difficult to determine with certainty causality to donidalorsen. Nonetheless, it is notable that of 48 liver enzyme related events reported, a majority 29/48 were considered related by the investigator.

Liver enzyme parameters:

Over time there is a gradual increase in mean ALT from values in late teens to mid/late thirties U/L, and (possibly to a lesser extent) also for AST in donidalorsen treated subjects. This does not seem to have occurred in the placebo patients. While this rise does not place mean ALT or AST outside of the normal range- there does seem to be some low-grade effect on liver enzymes.

It is agreed that the majority of liver enzyme elevations are mild or moderate, and that none were serious. However, the liver enzyme TEAE numbers presented by the applicant do not necessarily match the liver enzyme increase rates; it seems that some LFT rises, even severe rises may not have to have been reported as TEAEs.

In terms of comparison to placebo, Pool 1 is probably the most useful analysis as in Pool 2 the placebo arm is diluted in terms of exposure. There appears to have been more mild ALT, AST and GGT rises in the donidalorsen subjects than in placebo. In Pool 1, 3.6% of placebo patients had ALT or AST rises (any grade)- versus 21.3% of donidalorsen patients (both doses combined), 7.2% of placebo patients had bilirubin rises (any grade) versus 5.9% of donidalorsen patients (both doses combined), and 7.1% of placebo patients had GGT rises (any grade) versus 17.7% of donidalorsen patients(both doses combined). No subject in Pool 1 had raised Alkaline phosphatase.

Looking at Pool 2 (updated), 71 subjects (27.6%) of subjects had a mild ALT increase in the 80mg 4 weekly arm, and 8 subjects (14.9%) of subjects had a mild increase in ALT in the 80mg 8 weekly subjects, which is considered a high enough rate. 18.7% of subjects had a mild ALT increase in the 80mg 4 weekly arm, and 11.2% of subjects had a mild increase in ALT in the 80mg 8 weekly subjects. There were similarly high rates of mild GGT rises in donidalorsen treated subjects.

In the exposure-adjusted analysis of liver function abnormalities, the incidence rate (per 100 patient years of exposure) of liver function TEAEs and moderate to severe liver function abnormalities was similar between the donidalorsen and placebo groups, but a numerical difference in mild ALT and AST increased was noted between the donidalorsen treatment groups and the placebo group. For mild ALT rises the exposure adjusted incidence rate was 27.6% for the 80mg 4 weekly subjects, and 14.9% for the 80mg 8 weekly subjects, versus 8.7% for placebo. For mild AST the exposure adjusted incidence rate was 18.7% for the 80mg 4 weekly subjects, and 11.2% for the 80mg 8 weekly subjects, versus 0% for placebo.

Additionally, there were 2 subjects who discontinued from the study on account of raised liver enzymes.

The data, as provided do not particularly indicate the presence of a dose effect. Looking at Pool 2, 'any grade' rise rate for ALT, AST, Bilirubin and GGT are quite similar between the donidalorsen-4 and donidalorsen-8 subjects, however the particularly low numbers treated at the 8 weekly dose limits such comparisons.

Taken together, it is agreed the available data do not suggest that donidalorsen has any serious effect on liver function or liver injury. However, the dataset is small in keeping with the rarity of HAE. There does appear to be a generally mild (up to 3 x ULN) effect on liver function, mainly on AST, ALT and GGT, which was seen commonly in the donidalorsen treated subjects. However, there have been 2 cases of LFT rises where treatment was discontinued, and in both cases the investigator considered the changes to be related to IMP.

Looking at liver enzyme TEAEs and liver enzyme rises, while causality assessment is challenging in many of the cases due to confounding factors, for a majority of the liver enzyme TEAEs reported, the investigator considered the event to be related to donidalorsen. It is also the case that the clinical trial population excluded patients with any significant baseline elevations in liver enzymes (ALT/AST> 3 ×ULN, Bilirubin > 1.5×ULN) and any significant hepatic disease. Patients with moderate or severe hepatic impairment have not received donidalorsen in studies.

To summarise liver enzyme elevations, which were in the main mild or moderate, have occurred with donidalorsen treatment, and therefore ‘hepatic enzyme increased’ has been included in Section 4.8 of the SmPC in the ADR table; frequency ‘very common’.

SAEs, deaths

There was no SAE reported in any patient treated with donidalorsen in CS5, or in Pool 1. In Pool 2, 12 SAEs were reported, and all bar one (spontaneous abortion) occurred while on donidalorsen-4. The SAEs are varied. In all but one case there is a clear indicator from the background history to suggest non causal

relationship with donidalorsen. There has been one in study death since the data cut-off date- the death by suicide, considered not related by the investigator. The SAE that does appears to be related to donidalorsen concerns a second hypersensitivity severe allergic anaphylactic type reaction in a patient which fulfilled Sampon's criteria.

AEs resulting in discontinuation

Overall, there was a low rate of treatment discontinuation due to AEs. Looking at Pool 2 there were 8 subjects that had AEs that resulted in discontinuation, and most were considered related.

Antidrug antibodies

In CS5, the incidence rate of treatment-emergent ADA was 20.0% in the donidalorsen-4 group and 21.7% in the donidalorsen-8 group following treatment for 25 weeks. The incidence rate of ADA positive does seem to increase with continued use. In the CS7 study (safety set), 56.8% (84/148) of patients were ADA positive with the majority of them with treatment-emergent ADA (91.7% [77/84]).

Based on further data from the CS3 and CS7 studies received during this procedure, it is agreed that generally, there were no clinically important differences of effect on safety profile, including ISRs, between ADA negative, treatment-unaffected and treatment-emergent ADA. One potential exception is with respect to hypersensitivity- a higher incidence of patients reported in the treatment-emergent subgroup from the CS7 study who experienced any treatment-emergent hypersensitivity reactions (29.9%) compared to 18.8% of the patients in the ADA-negative subgroup and 14.3% of patients in treatment-unaffected subgroup, suggesting a possible impact of treatment-emergent ADA in hypersensitivity reactions.

Taken together, the rate of ADA positivity in donidalorsen treated subjects across the 4 studies is not insignificant. Based on the data provided there does not seem to be a correlation with an increase in toxicity, except possibly with respect to hypersensitivity reactions, however, small sample sizes limit interpretation of the data.

Special populations

Pregnancy

There are very limited data from the use of donidalorsen in pregnant women.

Across all clinical studies, 6 patients on donidalorsen experienced a pregnancy or partner pregnancy, and no adverse effects are reported.

Section 4.6 of the SmPC states to avoid the use in pregnancy which is appropriate, see also non-clinical discussion.

Breastfeeding

There are no data from donidalorsen breastfeeding women, hence the presence of donidalorsen and/or metabolites in human milk or on their effects on the breastfed child or on milk production has not evaluated.

Elderly

There are very limited data available from the use of donidalorsen in patients over the age of 65 years. Only 3 subjects over the age of 65 years were treated in the controlled trials, all 3 received active, and of 2 of these 3 subjects continued into the OLE studies. A further 2 elderly subjects were recruited as Switch patients into open label study CS7.

Renal impairment

Small numbers of patients with mild renal impairment were recruited into the donidalorsen studies. However, no patients with moderate or severe renal disease, or end stage renal disease have received donidalorsen. Mild renal impairment does not appear to influence PK or PD- see Clinical Pharmacology section.

Hepatic impairment

Small numbers of patients with mild hepatic impairment were recruited into the donidalorsen studies. However, no patients with moderate or severe hepatic disease have received donidalorsen. Mild hepatic impairment does not appear to influence PK or PD- see Clinical Pharmacology section.

Race/ethnicity/gender/study region

The data do not suggest any meaningful differences in the safety profile according to gender and study region. There is not a sufficiently diverse representation across races/ethnicities to make any interpretation about potential differences in the safety profile, the vast majority of subjects were White, non-Hispanic/Latino.

Body weight

It is noted that there was a very low number of patients with low body weight recruited (e.g. under 50kg), and it is understood that body weight can influence exposure (See Clinical pharmacology section) The applicant provided details of all TEAEs in the 3 patients who were < 50 kg in BW at baseline. While there are no concerns from those 3 cases, considering such small numbers are in question, no firm conclusions can be drawn on the safety profile in lighter patients. This will be monitored via PSURs.

Cardiac Safety

A formal QTc study was not conducted, see assessment of QT prolongation potential in the Clinical Pharmacology Section. There were no notable trends or safety concerns observed for ECG findings in any of the 4 studies or integrated pools. No patient experienced a QTcF interval > 500 msec in any treatment group.

Laboratory measurements, and vitals

See Liver enzyme discussion, renal toxicity discussion, and platelets discussion. Otherwise, no meaningful trends were seen in the laboratory monitoring (haematology, chemistry, coagulation, complement 5a), vitals or physical exam.

Exposure response relationship for safety

As discussed in the Clinical Pharmacology section- an analysis of AEs in CS5 versus exposure metrics did not reveal any substantial differences in safety endpoints across exposure tertiles; moderate and severe TEAEs, and renal/hepatic function and platelets were analysed.

Assessment of paediatric data on clinical safety

There are very limited data available from the use of donidalorsen in adolescent subjects, (aged 12 years to 17 years). Only 7 adolescents were treated in the controlled trials; all received active, and 6/7 entered OLE CS7 as adolescents. For patients aged 12 to <18 years subgroup, the AE analysis does not suggestive any meaningful trends or differences in safety profile versus older subjects; however, given the low number of adolescents the interpretability of such analysis is limited. Further data will be collected in the ongoing OLE CS7 study.

2.6.10. Conclusions on the clinical safety

Overall, it is agreed that the safety data provided demonstrate an acceptable safety and tolerability profile in HAE patients from age 12 years and into adulthood.

2.7. Risk Management Plan

2.7.1. Safety concerns

Important Identified Risks	• None
Important Potential Risks	• Hepatotoxicity • Renal toxicity • Bleeding/Thrombocytopenia
Missing Information	• Use in pregnancy • Use during breastfeeding • Long-term use

2.7.2. Pharmacovigilance plan

Study Number **Title** **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 3- Required additional pharmacovigilance activities				
ISIS 721744-CS7 An Open-Label, Long Term Safety and Efficacy Study of Donidalorsen in the Prophylactic Treatment of Hereditary Angioedema (HAE). **Ongoing**	- Evaluate the safety of long-term dosing with donidalorsen in patients with HAE. - Evaluate the long-term efficacy and the effects of donidalorsen on the number of HAE attacks and their impact on the quality of life (QoL) of patients with HAE. - Further characterize the effects of donidalorsen on HAE attacks, additional Patient Reported Outcomes (PROs) and biomarkers.	Long-term use Hepatotoxicity Renal toxicity Bleeding/Thrombocytopenia	Final Report	LPLV: December 2027 Final Study Report: Dec 2028

2.7.3. Risk minimisation measures

Safety Concern	**Risk Minimisation Measures**	**Pharmacovigilance Activities**
Hepatotoxicity	Routine Risk Minimization measures: Prescription only medicine Additional risk minimization measure: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: ISIS 721744-CS7

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Renal Toxicity	Routine Risk Minimization measures: Prescription only medicine Additional risk minimization measure: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: ISIS 721744-CS7
Bleeding/ Thrombocytopenia	Routine Risk Minimization measures: Prescription only medicine Additional risk minimization measure: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: ISIS 721744-CS7
Use in pregnancy	Routine Risk Minimization measures: SmPC Section 4.6 PL Section 2 Prescription only medicine Additional risk minimization measure: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None
Use during breastfeeding	Routine Risk Minimization measures: SmPC Section 4.6 PL Section 2 Prescription only medicine Additional risk minimization measure: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: None
Long-term use	Routine Risk Minimization measures: Prescription only medicine Additional risk minimization measure: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: Study ISIS 721744-CS7

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.1 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

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

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 21.08.2025. The new EURD list entry will therefore use the IBD to

determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. 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.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Dawnzera (Donidalorsen) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

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

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Hereditary angioedema (HAE) is a rare and potentially life-threatening autosomal dominant inherited disease of vascular permeability. Patients with HAE present with unpredictable, recurrent, painful, and often debilitating swelling episodes referred to as attacks, which affect subcutaneous tissue (face, upper or lower extremities, genitals), abdominal organs (stomach, intestines, bladder), and the upper airway (larynx, tongue) (Petersen et al. 2024; Wilkerson and Moellman 2023; Bernstein 2018). Symptoms of this lifelong disease usually appear within the first 2 decades of life, most often by puberty, and may increase in severity after puberty (Bernstein 2018). This rare disease has an estimated prevalence of approximately 1 in 50,000 individuals worldwide (Sinnathamby et al. 2023) with no known differences in prevalence among ethnic groups (Neako et al. 2001).

3.1.2. Available therapies and unmet medical need

Both the US HAE Association Medical Advisory Board (Busse et al. 2021), and the World Allergy Organization (WAO), in collaboration with the European Academy of Allergy and Clinical Immunology (EAACI; Maurer et al. 2022), recommend a 3-method approach for the management of HAE, including on-demand treatment for acute swelling attacks, short-term or situational prophylactic treatment with the intent of minimizing the risk of an attack during exposure situations (i.e. surgical trauma, dental surgery, etc), in addition to long-term

prophylactic treatment with the goal of reducing the number of HAE attacks, and, in turn, increasing the QoL for the patient. The indication to initiate long-term prophylaxis in adolescents follows the same guidelines as in adults (Maurer et al. 2022), to achieve complete control of the disease, and to "normalize patients' lives" (Maurer et al. 2022).

Long-term prophylaxis treatment is essential to achieving this goal of reducing HAE attacks with limited burden of treatment (Betschel et al. 2023). Current long-term prophylactic regimens for HAE include plasma-derived C1-INH concentrate (administered either intravenously or subcutaneously), monoclonal antibodies directed against plasma kallikrein (pKa), a small molecule inhibitor of pKa, and attenuated androgens. These options reduce the number and severity of attacks due to HAE, although breakthrough attacks still occur. Therefore, there is still need for new more effective treatments of this condition.

3.1.3. Main clinical studies

The applicant submitted 4 efficacy studies in support of this application including one phase 3 pivotal study (ISIS 721744-CS5), one phase 2 study (ISIS 721744-CS2) and two open-label, long term safety and efficacy studies (ISIS 721744-CS7 and ISIS 721744-CS3).

Studies ISIS 721744-CS5 and ISIS 721744-CS7 enrolled only patients with type I HAE (which presents with a deficiency of C1-INH) and type II HAE (which presents with a dysfunctional C1-INH) whereas studies ISIS 721744-CS2 and ISIS 721744-CS3 also enrolled as a small number of patients with type III HAE (hereditary angioedema with normal C1-INH activity).

3.2. Favourable effects

Donidalorsen 80 mg every 4 weeks dosing regimen

For the primary endpoint in the ISIS 721744-CS5 study, the LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 0.44 (95% CI: 0.265, 0.727) for the donidalorsen-4 group and 2.26 (95% CI: 1.657, 3.085) for the placebo group, representing an 81% (95% CI: (-89.3%, -64.9%) reduction relative to placebo for the donidalorsen-4 group (statistically significant, $p < 0.001$). A significant effect was found also for the reduction of moderate or severe HAE attack rate (-89%, 95% CI: -96.5%, -66.1%) and attacks requiring acute therapy (-92%, 95% CI -97.0%, -76.6%).

The primary endpoint result was supported by the secondary endpoints results. The relative reduction of investigator-confirmed HAE attacks (from Week 5 to Week 25) in the donidalorsen-4 group as compared to placebo was higher (87%) than that reported for the whole treatment period (from Week 1 to Week 25).

The percentage of patients with a $\geq$ 70% reduction from Baseline in Investigator-confirmed HAE attacks from Week 5 and Week 25 was 82.2%, compared with 18.2% in the placebo group. These differences were statistically significant ($p < 0.001$). Of the 45 patients in the donidalorsen-4 group, 24 (53.3%) were HAE attack-free from Week 5 to Week 25, compared with 2 of 22 (9.1%) patients in the placebo group.

Further, the time-normalized number of attacks requiring Acute Therapy from Week 5 to Week 25 was much lower in the donidalorsen-4 arm than in the placebo arm (mean (SD): 0.46 (1.290) versus 1.88 (1.834), respectively).

The LSM reduction (improvement) from Baseline in the AE-QoL total score at Week 25 was 24.8 points for the donidalorsen-4 group, compared with 6.2 points for the placebo group ($p < 0.001$).

Additionally, the results from the longer-term open label trials support the maintenance of efficacy over time.

The mean (SD, SEM) time-normalized Investigator confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 53 in the extension study (ISIS 721744-CS7) was 0.22 (0.41, 0.05); Index Run-in Baseline rate was 3.42 (2.12, 0.23).

For patients transferred from the pivotal study, the mean percent change in time-normalized, Investigator-confirmed HAE attack rate (per 4 weeks) for Week 1 to Week 53 of the ISIS 721744-CS7 study was a reduction of 93.11% (95% CI: 90.34%, 95.87%) from the Index Run-in Baseline.

Donidalorsen 80 mg every 8 weeks dosing regimen

As shown in the ISIS 721744-CS5 study, the efficacy of donidalorsen every 8 weeks was numerically inferior as compared to donidalorsen every 4 weeks. For the donidalorsen-8 group, the LSM time-normalized Investigator-confirmed HAE attack rate (per 4 weeks) from Week 1 to Week 25 was 1.02 (95% CI: 0.651, 1.594) and 2.26 (95% CI: 1.657, 3.085) for the placebo group, representing a 55% reduction (95% CI: -73.9%, -22.3%) relative to placebo for the donidalorsen-8 group (statistically significant, p = 0.004).

For 14 patients who maintained less frequent dosing in the OLE study (-CS7), the mean percent change in time-normalized, Investigator-confirmed HAE attack rate (per 4 weeks) for the OLE donidalorsen-8 group was a reduction of 92.01% (-100.81%, -83.20%).

During the Flexible Dosing Period of the ISIS 721744 CS3 study for 8 out of 20 patients the frequency of dosing could be reduced. Of these 8 patients, 5 patients continued to receive treatment every 8 weeks for a mean duration of 703 days. Patients who are attack free for ≥ 12 weeks on treatment with donidalorsen 80 mg once every 4 weeks can be switched to a less frequent every 8 weeks dosing regimen

Patients with nC1-INH-HAE

In 3 patients with nC1-INH-HAE treated with donidalorsen 80 mg every 8 weeks in the ISIS 72144 CS2 study, the number of attacks deceased from the monthly mean attack rate 4.23 (during the Run-in Period) to 1.52 from Week 1 to Week 17 (76% reduction). One patient was attack free from Week 1 to end of treatment. A responder analysis showed that 2 of 3 (66.7%) patients had a reduction of 50% or more in attack rate, 2 of 3 (66.7%) patients had a 70% or more in attack rate, and 1 of 3 (33.3%) patients had a 90% or more reduction in attack rate.

Adolescents ≥ 12 years of age

For adolescent patients in the pivotal study, a 97.1% decrease (95% CI: -106.26%, -88.01%) from baseline in the HAE attack rate from Week 1 to Week 25 was observed, which was similar to the 88.5% (95% CI: -94.75%, -82.30%) decrease for patients aged 18 to 39 years; patients aged 40 to 64 years had a numerically lower percent change from baseline (72.6% decrease [95% CI: -86.56%, -58.66%]).

Regarding the OLE study CS7, 7 adolescents from the pivotal study and 4 newly enrolled adolescents who switched from other prophylactic treatment (1 lanadelumab, 3 C1-esterase inhibitor) were included. Nine adolescents were treated with donidalorsen Q4W while two adolescents from the pivotal study continued with Q8W dosage.

3.3. Uncertainties and limitations about favourable effects

Efficacy in patients with nC1-INH-HAE (type III HAE)

Only 3 patients with nC1-INH-HAE were exposed to donidalorsen in studies ISIS 721744-CS2 and ISIS 721744-CS3.

In the ISIS 721744-CS2 study, a clinical diagnosis was confirmed by either threshold-stimulated kallikrein activity results and Investigator-confirmed response to acute use of a BK targeted treatment (icatibant or ecallantide) or the presence of one of the established mutations associated with HAE (i.e. mutation in factor XII gene, plasminogen gene or angiopoietin-1 gene. The SmPC (section 4.4) includes recommendation to perform genetic testing to confirm diagnosis.

None of the three patients enrolled in the CS2 study had an established mutation associated with HAE. The lack of identified mutation does not preclude the use of the product in patients with HAE-nC1-INH provided that bradykinin overproduction is confirmed.

Further, the response to treatment should be monitored in these patients and the treatment should be discontinued if clinical response is not observed. This was highlighted in the SmPC (section 4.2).

The mutation status of nC1-INH HAE patients who are not responding to treatment will be collected post-marketing and discussed in the lack of efficacy section of the PSUR.

Action of the product is delayed

Most secondary endpoints for the donidalorsen-4 group were analysed for the period from Week 5 to Week 25. This can be justified considering the expected delay in the onset action of the product: because donidalorsen targets PKK mRNA in hepatocytes, existing plasma PKK must degrade before the onset of the clinical effect. As the estimated plasma half-life of PKK is approximately 5 days, the efficacy of the product would be expected towards the end of the first 4-week dosing period.

The number of attacks during first the two weeks and separately during the first month of treatment were provided and showed no meaningful clinical response during the first week of treatment in particular in the less frequent dosing group. The mean percent change from run-in period (SD, SEM) was -12.72 (105.761, 22.548) for the placebo group, -33.33 (100.281, 14.949) for the Donidalorsen 80 mg Every 4 Weeks group and 9.37 (148.031, 30.867) for the Donidalorsen 80 mg Every 8 Weeks group.

In the following week of treatment (week 2 to 3) both treatment groups achieved more than 50 % reduction in the rate of HAE Attacks. The mean percent change from run-in period (SD, SEM) was -28.63 (108.383, 23.107) for the placebo group, -55.29 (82.218, 12.256) for the Donidalorsen 80 mg Every 4 Weeks group and -67.13 (65.235, 13.603) for the Donidalorsen 80 mg Every 8 Weeks group.

Consequently, the following statement was added to the SmPC (section 4.2): "Based on clinical data, a gradual reduction in attack rate is seen as early as Week 1 after the initial dose of donidalorsen with an expected maximum effect after 1 month."

Limited number of adolescents

Only a limited number of adolescent subjects were included in the provided donidalorsen studies: 7 adolescents in the pivotal study (4 in the donidalorsen-4 group, and 3 in the donidalorsen-8 group) and 4 newly enrolled adolescents in the OLE study CS7, switched from other prophylactic treatments. Although

based on limited data available, it is agreed that the efficacy results for adolescent OLE and Switch patients in study ISIS 721744-CS7 is in line with those observed in the overall study population.

3.4. Unfavourable effects

The most frequently occurring TEAEs, in pivotal study CS5, for the donidalorsen-4 group were headaches (13.3%), injection site erythema (13.3%), followed by nasopharyngitis (11.1%), UTI (8.9%), URTI (8.9%), and a number of other injection site AEs (injection site discolouration, injection site pain and injection site pruritis), each at 6.7%. In the donidalorsen-8 group the most commonly reported AEs were influenza (17.4%), nasopharyngitis (13%), headache (8.7%), URTI (8.7%), UTI (8.7%), oral herpes (8.7%), vomiting (8.7%) and injection site discoloration (4.3%).

There was no SAE reported in any patient treated with donidalorsen in CS5, or in Safety Pool 1 (CS2 and CS5). In Pool 2, 10 SAEs were reported in 7 donidalorsen treated subjects, and all bar one (spontaneous abortion) occurred while on donidalorsen 80 mg 4 weekly. The SAEs are varied. In all but one case there is a clear indicator from the background history or details to suggest non causal relationship with donidalorsen, the exception being one case of serious hypersensitivity/anaphylactic reaction.

There has been a low dropout rate in the two controlled studies and also in the two OLE studies. In CS5, of the 91 patients randomised the majority completed treatment, with a higher proportion in the donidalorsen groups completing versus placebo: donidalorsen-4 group (95.7% completion rate) and donidalorsen-8 group (91.3% completion rate). 83 subjects then rolled into the OLE CS7, of which only 2 had left the trial as of the data lock date at time of submission. There was a low rate of treatment discontinuation due to AEs.

ISRs/LCRIS

In CS5 a considerably higher proportion of patients in the donidalorsen-4 group (24.4%) experienced injection site reactions (ISRs) compared with the donidalorsen-8 (4.3%) group and the placebo group (4.5%), and similar consistent trends are seen in Safety Pools 1 and 2. All injection site reactions were nonserious, and the majority were mild in severity. However, there is a subset of the ISR adverse events that met the definitions for LCRIS (local cutaneous reactions at the injection site). There were 19 LCRIS events in 4 subjects (2.3%) in Pool 2. One of these subjects (donidalorsen 100 mg every 4 weeks) had recurrent injection site reaction/Local cutaneous reaction at the injection site, leading to permanent study drug discontinuation. ISRs and LCRIS events are consistent with the safety profiles of other ASOs. ISR/LCRIS are adequately represented in the product information.

Liver toxicity

Donidalorsen also appears to have an effect on liver function, mainly on AST, ALT and GGT, generally mild in severity, but with some moderate cases also. In Pool 1, 3.6% of placebo patients had ALT or AST rises (any grade) versus 21.3% of donidalorsen patients (both doses combined); 7.2% of placebo patients had bilirubin rises (any grade) versus 5.9% of donidalorsen patients (both doses combined); and 7.1% of placebo patients had GGT rises (any grade) versus 17.7% of donidalorsen patients (both doses combined). Two subjects discontinued donidalorsen on account of liver enzyme rises, which in both cases were considered related by the investigator. One of these had met the liver stopping criteria. Hepatic enzyme increases is included as an ADR in Section 4.8, under the SOC Investigations, with a frequency of very common.

ADA

In CS5, the incidence rate of treatment-emergent ADA was 20.0% in the donidalorsen-4 group and 21.7% in the donidalorsen-8 group following treatment for 25 weeks. The incidence rate of ADA positive does seem to increase with continued use. In the CS7 study (safety set), 56.8% (84/148) of patients were ADA positive with the majority of them with treatment-emergent ADA (91.7% [77/84]). Generally, based on the data provided there does not seem to be a correlation between positive ADA status and toxicity.

Platelets

Two AESIs related to thrombocytopenia +/- bleeding were predefined for examination, in recognition of the potential for ASOs to cause reductions in platelets. No subject treated with donidalorsen had an AESI related to low platelets with or without bleeding. The laboratory data across all studies in patients with HAE also do not suggest that donidalorsen has a clinically meaningful impact on platelet count.
Bleeding/thrombocytopenia has been added as an important potential risk and will be further characterised in a post-authorisation study.

3.5. Uncertainties and limitations about unfavourable effects

Dataset size and duration of exposure

In terms of total number of subjects dosed with donidalorsen, the number is low: 275 subjects have been dosed in total, including phase 1. 173 patients have been dosed in the 4 key clinical studies in HAE patients (CS5, CS2 and their respective OLE studies, CS7 and CS3), of which CS7 is ongoing.

At any dose, 166 patients have been dosed for more than 24 weeks and 154 have been dosed for more than 52 weeks. Specifically, for the 80mg 4 weekly dose, 151 have been dosed for more than 24 weeks, and 130 have been dosed for more than 52 weeks. The exposure in terms of duration of therapy meets minimum requirements in terms of duration on therapy - as outlined in ICH E1, which allays much of this uncertainty. There is still uncertainty about safety of longer-term treatment, which will be addressed from further safety data to come from the OLE studies. Longer term use has been added to the safety specification under Missing Information in the RMP.

Liver toxicity potential

While the data from the pivotal study CS5 and integrated safety pools do not, to date, suggest that donidalorsen has any serious effect on liver function or a risk of drug-induced liver injury in patients with HAE, there does appear to be an effect on liver function, mainly on AST, ALT and GGT. `Hepatic enzyme increased is therefore included as an ADR (frequency – very common) in SmPC section 4.8. Hepatotoxicity is a plausible side effect based on the mechanism of action of donidalorsen and has been seen with other ASOs. It is also the case that the clinical trial population excluded patients with any significant baseline elevations in liver enzymes (ALT/AST> 3 x ULN, Bilirubin > 1.5 x ULN) and any significant hepatic disease. Further, the available safety data are limited both in terms of numbers and extent of follow up and as such the risk of hepatoxicity cannot be considered to be fully investigated. Hepatotoxicity has been added to the safety specification under Important Potential Risks and will be further characterised in a post-authorisation study.

Unstudied group, or groups with minimal representation in the studies

Very limited number of adolescents, and elderly patients, have been treated across the clinical development programme, and this is adequately reflected in the product information.

No patients with moderate or severe (or end stage) renal impairment have been treated with donidalorsen. While it is not necessarily expected that renal impairment might affect exposure, this is an unknown, and the safety in this cohort is also not known. This is adequately reflected in the product information.

No subjects with moderate or severe hepatic failure have been treated with donidalorsen. This is adequately reflected in the product information. While it is not necessarily expected that hepatic impairment might affect exposure, this is an unknown, and the safety in this cohort is also not known. It is unknown but possible that patients with underlying liver dysfunction may be more susceptible to liver toxicity. Use in moderate and severe hepatic impairment will be monitored via routine pharmacovigilance activities.

3.6. Effects Table

Table 33. Effects Table for Dawnzera for routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older.

Effect	Short Description	Unit	Treatment	Control (placebo)	Uncertainties/ Strength of evidence	References
Favourable Effects						
Time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25	For Donidalorsen 80 mg Every **4** Weeks	Mean (SD, SEM) Least Square Mean Rate (95% CI)	0.89 (1.851, 0.276) 0.44 (0.265, 0.727)	2.29 (1.807, 0.385) 2.26 (1.657, 3.085)	Model Adjusted Mean Rate Ratio (95% CI): 0.19 (0.107, 0.351) Percentage Difference Relative to Placebo (95%CI) -81% (-89.3%, -64.9%) **The observed difference is also considered as clinically relevant**	Primary endpoint study ISIS 721744-CS5
Time-Normalized Investigator-Confirmed HAE Attack Rate (Per 4 Weeks) from Week 5 to Week 25	For Donidalorsen 80 mg Every **4** Weeks	Mean (SD, SEM) Least Square Mean Rate (95% CI)	0.75 (1.898, 0.283) 0.30 (0.151, 0.581)	2.32 (1.926, 0.411) 2.25 (1.594, 3.183)	Model Adjusted Mean Rate Ratio (95% CI): 0.13 (0.062, 0.281) Percentage Difference Relative to Placebo (95%CI) -87% (-93.8%, -71.9%) **The action of the product is expected towards the end of the first 4-week dosing period.**	Secondary endpoint study ISIS 721744-CS5
Patients with HAE Attack Rate Reductions ≥ 70%	For Donidalorsen 80 mg Every **4** Weeks	n (%)	37 (82.2)	4 (18.2)	Odds Ratio (95% CI) 17.04 (3.36, 86.42) P value <0.001 **The observed difference is also considered as clinically relevant**	Secondary endpoint study ISIS 721744-CS5
Time-normalized number of Investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25	For Donidalorsen 80 mg Every **8** Weeks	Mean (SD, SEM) Least Square Mean Rate (95% CI	1.19 (1.629, 0.340) 1.02 (0.651, 1.594)	2.29 (1.807, 0.385) 2.26 (1.657, 3.085)	Model Adjusted Mean Rate Ratio (95% CI): 0.45 (0.261, 0.777) Percentage Difference Relative to Placebo (95%CI) -55% (-73.9%, -22.3%)	Primary endpoint study ISIS 721744-CS5
Unfavourable Effects						

Effect	Short Description	Unit	Treatment	Control (placebo)	Uncertainties/ Strength of evidence	References
ISR *(note LCRIS not specifically discussed in this table as LCRIS did not arise in either controlled study- see detailed discussion in report on LCRIS)*	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	24.5	4.5		Pivotal study CS5
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	4.3	4.5		Pivotal study CS5
Bleeding TEAE	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	8.9	4.5		Pivotal study CS5
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	4.3	4.5		Pivotal study CS5
Liver function-raised AST or ALT (any grade)	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	20	0	**Uncertainty:** Patients with moderate or severe hepatic impairment were excluded from the study.	Pivotal study CS5
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	30.4	0	**Uncertainty:** Patients with moderate or severe hepatic impairment were excluded from the study.	Pivotal study CS5
Liver function-raised GGT (any grade)	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	15.6	4.5	**Uncertainty:** Patients with moderate or severe hepatic impairment were excluded from the study.	Pivotal study CS5
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	26.1	4.5	**Uncertainty:** Patients with moderate or severe hepatic impairment were excluded from the study.	Pivotal study CS5
Thrombocytopenia, Drop of ≥ 30% from baseline	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	2.2	9.1		Pivotal study CS5
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	8.7	9.1		Pivotal study CS5
Thrombocytopenia, Drop of ≥ 50% from baseline	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	0	0		Pivotal study CS5
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	4.3	0		Pivotal study CS5
ADA (treatment emergent)	For Donidalorsen 80 mg Every 4 Weeks	Incidence %	20	4.5		Pivotal study CS5

Effect	Short Description	Unit	Treatment	Control (placebo)	Uncertainties/ Strength of evidence	References
	For Donidalorsen 80 mg Every 8 Weeks	Incidence %	21.7	4.5		Pivotal study CS5

Abbreviations: ISR - Injection Site Reactions. LCRIS - Local cutaneous reaction at the injection site.
Notes: Open label data is not presented in the Effects table. LCRIS was only reported in the OLE studies, see relevant sections of report for specific discussion on this aspect.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Efficacy:

Donidalorsen 80 mg given every 4 weeks significantly reduced the number of HAE attacks as compared to placebo (primary endpoint). The secondary endpoints results showed similar findings, and similar effects were demonstrated in pre-specified sub-populations. For the primary endpoint and secondary endpoints, the observed differences are considered as not only statistically significant but also clinically relevant. The percentage of patients with a ≥ 70% reduction from baseline in HAE attacks was 82.2%, compared with 18.2% in the placebo group ($p < 0.001$). More subjects were attack-free in the Q4W arm (24 (53.3%)) compared to the placebo arm (2 (9.1%)). The change from Baseline in AE-QoL questionnaire total score at Week 25 showed a significant improvement in QoL for patients treated with donidalorsen Q4W compared to placebo group.

The efficacy is also supported by results from a second double-blind study (ISIS 721744-CS2) including 3 patients with nC1-INH-HAE. Moreover, the results from the longer-term OLE studies support the maintenance of efficacy over time.

The donidalorsen 80 mg every 8 weeks regimen also showed efficacy although lower than Q4W.

Overall, efficacy of donidalorsen in the routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older is demonstrated.

Safety:

While the safety dataset is small, this needs to be considered in the context of HAE being a rare disease, estimated prevalence of approximately 1 in 50,000 individuals worldwide (Sinnathamby et al. 2023), and the limited numbers treated can be acceptable based on safety results to date. Overall, the safety profile of donidalorsen is acceptable. The OLE study CS7 is still ongoing and will provide further long-term information once completed by December 2027.

3.7.2. Balance of benefits and risks

Long-term prophylactic treatment is essential to achieving the goal of reducing HAE attacks with limited burden of treatment. Although the currently approved long-term prophylactic regimens for HAE reduce the number and severity of attacks due to HAE, breakthrough attacks still occur and therefore there is still need for new more effective treatments of this condition.

The pivotal study ISIS 721744-CS5 met its primary endpoint, and the effect of the treatment is considered clinically relevant for patients with C1-INH-HAE from 12 years of age. The efficacy is also supported by the secondary endpoint results in the pivotal study and the second double-blind study (ISIS 721744-CS2). Moreover, the results from the longer-term OLE studies support the maintenance of efficacy over time.

The proposed indication is not limited to type 1 and type 2 HAE. Although the data on the use in patients with nC1-INH-HAE is limited, a broad indication is agreed taking into account the unmet medical need in this specific sub-population and that relevant information for nC1-INH HAE patients has been included in the SmPC (sections 4.2, 4.4 and 5.1).

From a safety perspective, the tolerability profile of donidalorsen in the treatment of hereditary angioedema is acceptable and the ADRs and uncertainties are considered appropriately managed by routine risk minimisation measures and pharmacovigilance activities.

Therefore, it is considered that the benefits of donidalorsen outweighs its risks in the routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older.

3.7.3. Additional considerations on the benefit-risk balance

Not applicable.

3.8. Conclusions

The overall benefit/risk balance of Dawnzera is positive in the routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Dawnzera is not similar to Takhzyro and Ekterly within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity

Outcome

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

Dawnzera is indicated for routine prevention of recurrent attacks of hereditary angioedema (HAE) in adults and adolescents aged 12 years and older.

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

Conditions or restrictions regarding supply and use

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

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

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

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

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

- **Risk Management Plan (RMP)**

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

An updated RMP should be submitted:

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

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

Not applicable.

New Active Substance Status

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

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0246/2024 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.