

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

219407Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	NDA
Application number(s)	219407
Priority or standard	Standard
Submit date(s)	8/21/2024
Received date(s)	8/21/2024
PDUFA goal date	8/21/2025
Division/office	Division of Pulmonology, Allergy, and Critical Care (DPACC)
Review completion date	8/20/2025
Established/proper name	donidalorsen
(Proposed) proprietary name	Dawnzera
Pharmacologic class	Prekallikrein-directed antisense oligonucleotide
Other product name(s)	ISIS 721744
Applicant	IONIS PHARMACEUTICALS INC
Dosage form(s)/formulation(s)	INJECTION, SOLUTION
Dosing regimen	80 mg administered subcutaneously (b) (4). A dosing interval of once every (b) (4) may be considered (b) (4) (b) (4)
Applicant-proposed indication(s)/ population(s)	Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older.
SNOMED CT code for proposed indication disease term(s)¹	82966003 Hereditary angioedema (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	80 mg administered subcutaneously every 4 weeks. A dosage of 80 mg every 8 weeks may also be considered.
Approved indication(s)/ population(s) (if applicable)	Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older.
SNOMED CT code for approved indication disease term(s)¹	82966003 Hereditary angioedema (disorder)

¹ For internal tracking purposes only.

Abbreviations: PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

ACE	angiotensin-converting enzyme
ADA	antidrug antibody
ADME	absorption, distribution, metabolism, and excretion
AE	adverse event
AECT	Angioedema Control Test
AE-QoL	Angioedema Quality of Life
AI	autoinjector
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ASGPR	asialoglycoprotein receptor
AST	aspartate aminotransferase
ASO	antisense oligonucleotide
AUC	area under the concentration-time curve
BCRP	Breast Cancer Resistance Protein
BLQ	below limit of quantification
BSEP	bile salt export pump
C1-INH	C1-esterase inhibitor
CDRH	Center for Devices and Radiological Health
CE	confirmatory evidence
cHK	cleaved high molecular weight kininogen
CHL	Chinese Hamster Lung
C _{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
CNS	central nervous system
CSR	clinical study report
C _{trough}	trough concentration
DCOA	Division of Clinical Outcomes Assessment
DILI	drug-induced liver injury
DRF	dose range finding
EBV	Epstein-Barr virus
ECAC	Executive Carcinogenicity Assessment Committee
ECG	electrocardiogram
ECL	electrochemiluminescence
EFD	embryo-fetal development
EPC	established pharmacologic class
E-R	exposure-response
FAS	full analysis set
FDA	Food and Drug Administration
FXIIa	activated Factor XII
GalNAc	<i>N</i> -acetylgalactosamine
GD	Gestation Day
GGT	gamma-glutamyl transferase
GLP	good laboratory practice

HAE	hereditary angioedema
HEK-293	human embryonic kidney-293
hERG	human ether-a-go-go related gene
HF	human factors
HPLC-MS/MS	high-performance liquid chromatography-tandem mass spectrometry
IC ₅₀	half-maximal inhibitory concentration
ICH	International Council for Harmonisation
IND	investigational new drug
INR	international normalized ratio
ISS	integrated summary of safety
ISR	injection site reaction
ITT	intent-to-treat
LD	Lactation Day
LFT	liver function test
LLN	lower limit of normal
LLOQ	lower limit of quantification
LS	least squares
MATE	multidrug and toxin extrusion protein
MDCK-II	Madin-Darby Canine Kidney
MTD	maximum tolerated dose
NDA	new drug application
NOAEL	no observed adverse effect level
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCMQ	Office of New Drugs custom medical query
OCT	organic cation transporter
OLE	open-label extension
OSIS	Office of Study Integrity and Surveillance
PD	pharmacodynamic
PFS	prefilled syringe
P-gp	P-glycoprotein
PI	Prescribing Information
PK	pharmacokinetic
PKK	prekallikrein
PMR	postmarketing requirement
PPND	pre- and postnatal development
PRO	patient-reported outcome
PT	preferred term
Q1M	every month
Q2M	every 2 months
Q2W	every 2 weeks
Q4W	every 4 weeks
Q8W	every 8 weeks
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous

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SEE	substantial evidence of effectiveness
SPA	special protocol assessment
TAM	test article mixture
TEAE	treatment-emergent adverse event
THA	trihexylamino
TK	toxicokinetic
T _{max}	time to maximum concentration
ULN	upper limit of normal

I. Executive Summary

1. Overview

1.1. Summary of Regulatory Action

The Applicant (Ionis Pharmaceuticals) submitted an NDA for donidalorsen (proposed tradename: Dawnzera), an antisense oligonucleotide (ASO) targeting prekallikrein mRNA, for prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and adolescent patients 12 years and older at a dose of 80 mg subcutaneous (SC) every 4 weeks; a dosing interval of once every (b) (4) may be considered (b) (4). The ASO is conjugated to a ligand containing three *N*-acetylgalactosamine (GalNAc) residues to enable delivery of the ASO to hepatocytes, where prekallikrein is synthesized, supporting a lower dose than for nonconjugated ASOs. The NDA was reviewed by a multidisciplinary review team; no issues were identified from any discipline that preclude approval.

Substantial evidence of effectiveness of donidalorsen in patients with HAE was established using data from a single adequate and well-controlled trial (CS5) studying two dosing regimens (80 mg every 4 weeks [Q4W] and 80 mg every 8 weeks [Q8W]) and placebo, and confirmatory evidence (CE) based on mechanistic data; see the description in Section 1.2. The available safety data demonstrated that donidalorsen is safe for its intended use. Anaphylaxis occurred in one subject. Common adverse reactions included injection site reactions, upper respiratory tract infection, urinary tract infection, and abdominal discomfort. Laboratory findings with donidalorsen treatment included decreased platelet counts and increased liver enzymes, consistent with the ASO class effect. The safety profile for donidalorsen is acceptable for an HAE population and can be adequately managed through labeling and further evaluated during routine pharmacovigilance. A clinical postmarketing requirement will be included with this approval to complete the ongoing multi-year open-label extension (OLE) trials (CS3 and CS7) to evaluate the potential for drug-induced liver injury with donidalorsen.

Based on the positive dose response relationship of the 80 mg Q4W dosing regimen, the recommended dosage of donidalorsen is 80 mg administered subcutaneously every 4 weeks. Because Q8W also independently met the primary efficacy endpoint and some secondary endpoints a dosage of 80 mg every 8 weeks may also be considered at the discretion of the prescriber.

The NDA includes appropriate preapproval nonclinical studies, but a nonclinical postmarketing requirement will be included with this approval to complete the ongoing 2-year carcinogenicity study in rats administered donidalorsen (see Section 24).

The submitted clinical program is adequate to support the efficacy and safety of donidalorsen for the proposed indication. The clinical review team concludes that the reduction in acute HAE attacks observed with donidalorsen treatment compared to placebo outweighs the risks, and that

donidalorsen will provide a novel option in the treatment armamentarium for HAE prophylaxis. The benefit-risk assessment is favorable and supports the approval of donidalorsen for “prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older.” The recommended dose is 80 mg administered subcutaneously every 4 weeks. A dosage of 80 mg every 8 weeks may also be considered.

The action for this application will be an **Approval**, with nonclinical and clinical postmarketing requirements.

1.2. Conclusions on Substantial Evidence of Effectiveness

Substantial evidence of effectiveness (SEE) was established with one adequate and well-controlled clinical investigation and confirmatory evidence.

The single adequate and well-controlled trial (CS5), was a 24-week multicenter, randomized, double-blind, placebo-controlled trial evaluating 90 adult and adolescent subjects with HAE Type I or II. Subjects were randomized to one of the following treatment arms:

- Donidalorsen 80 mg SC every 4 weeks (hereafter referred to as the donidalorsen-4 group).
- Donidalorsen 80 mg SC every 8 weeks (hereafter referred to as the donidalorsen-8 group).
- Placebo SC every 4 or 8 weeks (hereafter referred to as the pooled placebo group).

Trial CS5 met its primary endpoint, demonstrating a statistically significant and clinically meaningful reduction in the number of HAE attacks per 4 weeks in the donidalorsen-4 group (least squares [LS] mean =0.44, 95% CI =0.27, 0.73) and the donidalorsen-8 group (LS mean =1.02, 95% CI =0.65, 1.59), compared to the pooled placebo group (LS mean =2.26, 95% CI =1.66, 3.09); this represents a percentage reduction in the means relative to placebo for the donidalorsen-4 group of 81% (95% CI =65%, 89%) and for the donidalorsen-8 group of 55% (95% CI =22%, 74%). These findings were supported by the results of secondary endpoints, including the proportion of subjects with a $\geq 70\%$ reduction in attacks, attack-free subjects, moderate-to-severe HAE attacks, and HAE attacks requiring on-demand therapy. While both dosing regimens demonstrated efficacy compared to placebo, donidalorsen-4 demonstrated greater efficacy compared to donidalorsen-8 across primary and secondary endpoints.

The CE providing strong mechanistic support includes the well-established pathophysiology of the disease and the mechanism of action of the therapy. Donidalorsen is conjugated to a ligand containing three GalNAc residues that target the ASO to the liver. Donidalorsen inhibits production of prekallikrein, a key protein in the contact/kallikrein-kinin system pathway that is primarily synthesized in the liver, thereby reducing the downstream production of bradykinin, the main mediator of angioedema in HAE. The mean percentage reduction from baseline to Week 24 in trough plasma prekallikrein (PKK) concentrations were 73% and 47% following treatment in the donidalorsen-4 and donidalorsen-8 groups, respectively, compared with a slight increase (2%) observed in the pooled placebo group. The mean percentage reduction from baseline at Week 4 across both treatment groups was 48%. Based on the well-understood

pathophysiology of this genetic disease, demonstrated pharmacologic effect of donidalorsen on the kallikrein-kinin pathway, and established efficacy of numerous approved HAE therapies (for prophylaxis and acute attacks) that inhibit plasma kallikrein, the Applicant has provided adequate mechanistic data to serve as CE to support SEE for the proposed indication.

Extrapolation of efficacy from adults with HAE to adolescent patients 12 to 17 years of age with HAE is supported by the identical disease pathophysiology, mechanism of action, anticipated treatment response, and clinical manifestations across age groups.

Overall, SEE was demonstrated by the results from Trial CS5 and the mechanistic data provided by the Applicant. The use of a single adequate and well-controlled trial plus CE to demonstrate SEE is supported by: (1) hereditary angioedema being a rare, serious, and potentially life-threatening condition; (2) the efficacy results from the single trial are statistically significant and clinically meaningful across primary and secondary endpoints; and (3) conduct of a second trial would be infeasible. Use of a single adequate and well-controlled trial to demonstrate SEE is consistent with the approach used for other HAE drug development programs.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	• HAE is a rare condition caused by autosomal dominant mutations in the <i>SERPING1</i> gene, resulting in deficiency or dysfunction of C1-INH protein. • Approximately 85% of patients have Type I HAE, characterized by low production of normal C1-INH protein. The remaining 15% of patients have Type II HAE, characterized by production of dysfunctional C1-INH. Types I and II have the same clinical phenotypes and responses to available treatments. • Insufficient quantities of functional C1-INH lead to dysregulation of the kallikrein-kinin system, a plasma protease cascade initiated by Factor XII and culminating in the generation of bradykinin. Uncontrolled bradykinin production leads to increased vascular permeability and the subcutaneous/submucosal swelling seen in acute HAE attacks. • Acute HAE attacks are sporadic, recurrent, unpredictable, and typically self-resolve after 48 to 72 hours. Attacks often involve the gastrointestinal and genitourinary tract, extremities (hands/feet), and face/upper airway. Attacks are associated with significant morbidity due to pain, impact on daily functioning, and disfigurement, and can be life-threatening in cases of laryngeal edema resulting in airway compromise.¹	HAE is a rare, genetic, potentially life-threatening disease characterized by unpredictable, recurrent, and self-limited skin and submucosal swelling attacks. The pathophysiology of the disease is well-characterized.

¹ U.S. Food and Drug Administration’s Patient-focused drug development meeting summary report: September 25, 2017, externally led Patient-Focused Drug Development for Hereditary Angioedema

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current treatment options	- No cure exists; however, there are several approved therapies for prophylaxis, as well as for on-demand treatment of acute attacks. - Approved therapies for HAE prophylaxis include plasma-derived C1-INH replacement therapies (intravenous and subcutaneous), attenuated androgens (oral), a factor XIIa inhibitor (subcutaneous), and plasma kallikrein inhibitors (oral and subcutaneous).	Although there are numerous approved prophylactic therapies for patients with HAE, the availability of additional treatment options from a new pharmacologic class is desirable.
Benefit	- Efficacy for both donidalorsen-4 and donidalorsen-8 dosing regimens is based on the results from a single, adequate and well-controlled trial (CS5) in 90 adults and adolescents with Type I or II HAE. Treatment with both dosing regimens of donidalorsen resulted in statistically significant and clinically persuasive reductions in the primary endpoint, number of HAE attacks per month, throughout the 24-week Treatment Period compared to placebo. - Clinically meaningful secondary endpoints, such as number of attack-free subjects, number of moderate to severe HAE attacks, and number of attacks requiring acute treatment, further supported the beneficial treatment effect of donidalorsen compared to placebo. - Confirmatory evidence is provided by mechanistic data for the well-established role of prekallikrein in the pathophysiology of HAE attacks: this is supported by the effectiveness of approved products of the same pharmacological class (kallikrein inhibitors). - Efficacy in adolescents is based on extrapolation from adults.	Donidalorsen-4 and donidalorsen-8 dosing regimens demonstrated statistically significant and clinically relevant treatment benefits on the reduction of number of acute HAE attacks in adult and adolescent subjects with Types I and II HAE, with further support from secondary endpoints. Donidalorsen-4 demonstrated greater efficacy than donidalorsen-8 across primary and secondary endpoints, although both dosing intervals independently met the primary efficacy endpoint. Although both dosing regimens are being approved, based on the greater efficacy of the donidalorsen-4 dosing regimen, the recommended dosage of donidalorsen is 80 mg administered subcutaneously every 4 weeks. A dosage of 80 mg every 8 weeks may also be considered at the discretion of the prescriber. SEE is based on efficacy results from a single adequate and well-controlled clinical trial, with confirmatory evidence from mechanistic data.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and risk management	• Treatment-emergent adverse events that were serious, severe, or led to treatment discontinuation were rare. • Common TEAEs were injection site reactions, upper respiratory tract infection, urinary tract infection, and abdominal discomfort. The majority of TEAEs were mild or moderate in severity. • Anaphylaxis occurred in one subject. • Platelet count decreased; no donidalorsen-treated subjects had a platelet count of $<50,000/\text{mm}^3$. No major bleeding events were associated with a low platelet count. • Increases in liver enzymes were observed.	The safety profile of donidalorsen for the proposed HAE population and intended use is favorable. Due to the known ASO class effect of hepatotoxicity, the accumulation of ASOs in the liver over time, the small safety database, and the unknown consequences of lifetime treatment with ASOs, a clinical PMR is included with this approval to evaluate the potential for drug-induced liver injury with donidalorsen. A nonclinical PMR is included with this approval to assess carcinogenicity. No REMS is proposed. The other potential risks can be managed through labeling and routine pharmacovigilance.

Abbreviations: ASO, antisense oligonucleotide; C1-INH, C1-esterase inhibitor; GI, gastrointestinal; GU, genitourinary; HAE, hereditary angioedema; IV, intravenous; PMR, postmarketing requirement; REMS, risk evaluation and mitigation strategy; SC, subcutaneous; SEE, substantial evidence of effectiveness; TEAE, treatment-emergent adverse event.

2.2. Conclusions Regarding Benefit-Risk

Hereditary angioedema is a rare, autosomal dominant condition, most often caused by pathogenic variants in the *SERPING1* gene that encodes C1-esterase inhibitor (C1-INH), a serine protease-inhibitor involved in the kallikrein-kinin pathway, as well as the classical and lectin complement, contact system/intrinsic coagulation, and fibrinolytic pathways. Under homeostatic circumstances, activated Factor XII (FXIIa) triggers the kallikrein-kinin system, resulting in the downstream production of bradykinin, a potent vasodilator and enhancer of vascular permeability. C1-INH normally serves as an inhibitory check on the system by inhibiting both kallikrein and FXIIa; in the absence of sufficient quantities of functional C1-INH, bradykinin production is relatively unchecked and leads to recurrent subcutaneous and submucosal swelling (or angioedema). Clinically, HAE is characterized by episodic, spontaneous, unpredictable attacks of nonhistaminergic angioedema involving the subcutaneous and submucosal tissues, and can include the face, gastrointestinal tract, genitourinary tract, extremities (hands/feet), and/or throat and upper airway, the last of which can be particularly serious and potentially life-threatening. Several products are approved for the prophylaxis of HAE attacks as well as for the treatment of acute HAE attacks; however, breakthrough attacks still occur, and additional prophylactic therapy options for HAE patients are beneficial.

Substantial evidence of effectiveness for donidalorsen in the prophylactic treatment of HAE is based on the efficacy results from the single adequate and well-controlled clinical trial (CS5), with confirmatory evidence from mechanistic data, as presented in Section 1.2. Although the donidalorsen-4 dosing regimen demonstrated greater efficacy than the donidalorsen-8 dosing regimen across primary and secondary endpoints, because both dosing intervals independently met the primary efficacy endpoint, both dosing options are being approved for use. However, based on the greater efficacy of the donidalorsen-4 dosing regimen, the recommended dosage of donidalorsen is 80 mg administered subcutaneously every 4 weeks. A dosage of donidalorsen 80 mg administered subcutaneously every 8 weeks may also be considered, as included in labeling, at the discretion of the prescriber.

Although the safety database is small, strengths include the placebo-controlled design, use of two doses to assess dose-response, the 6-month treatment duration with additional long-term exposure in the open-label extension period, the low study drop-out rate, and the small amount of missing data. The safety assessment of donidalorsen revealed no concerning safety signals that would preclude approval. No deaths occurred in the clinical development program. Serious adverse events, severe adverse events, and adverse events leading to treatment discontinuation were rare with no major imbalances between treatment groups. Anaphylaxis occurred in one subject. Overall, treatment-emergent adverse events were predominantly mild or moderate in severity, with the most common adverse reactions being injection site reactions, upper respiratory tract infection, urinary tract infection, and abdominal discomfort.

As with other GalNac-ASOs, effects on clinical laboratory results including decreased platelet counts, increased liver enzymes, and an increase in international normalized ratio (INR) were observed, but these did not result in a safety signal in terms of adverse events. Generally, the safety profile of donidalorsen is consistent with expected class effects of ASOs and kallikrein-directed therapeutics. Potential risks can be addressed through labeling and routine

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pharmacovigilance, as well as postmarketing requirements to assess for drug-induced liver injury in the multi-year OLE trials (CS3 and CS7) and to complete the ongoing 2-year carcinogenicity study in rats.

Although Trial CS5 enrolled a limited number of adolescent subjects (seven in the donidalorsen group and zero in the placebo group), the efficacy and safety of donidalorsen in adolescent patients 12 to <18 years of age is based on extrapolation from adults and matching pharmacokinetic (PK) exposure and pharmacodynamic (PD) response. Extrapolation of efficacy is supported by the same underlying pathophysiology and clinical manifestations of disease, mechanism of action of donidalorsen, and similar expected response to treatment across age groups.

Overall, the Agency concludes that the benefit-risk assessment is favorable and supports the approval of donidalorsen 80 mg SC every 4 weeks or every 8 weeks for the proposed indication of prophylaxis to prevent HAE attacks in adults and adolescents 12 years of age and above.

II. Interdisciplinary Assessment

3. Introduction

This review serves as the interdisciplinary assessment for the NDA for donidalorsen. Donidalorsen is a new molecular entity, first-in-class chimeric GalNAc-conjugated 2'-O-methoxyethyl-modified antisense oligonucleotide targeting prekallikrein mRNA. The proposed indication is for prophylaxis to prevent attacks of HAE in adult and adolescent patients aged 12 years and older. The proposed dosage is 80 mg administered SC (b) (4). A dosing interval of 80 mg (b) (4) may also be considered (b) (4). (b) (4).

Hereditary angioedema is a rare genetic condition characterized by recurrent episodes of swelling in various body parts, including the hands/feet, genitals, gastrointestinal tract, face, and/or throat ([USHAA 2024](#)). It is most often caused by pathogenic variants in the *SERPING1* gene that encodes C1-INH, resulting in either low levels of C1-INH (Type I) or normal levels of dysfunctional C1-INH (Type II) ([Busse et al. 2021](#)). The inheritance of HAE is autosomal dominant, with approximately 25% of patients having de novo mutations. Patients with HAE experience recurrent episodes of swelling that typically last for 2 to 3 days, but occasionally up to 5 days. Symptom onset usually occurs in childhood, with 50% of patients experiencing angioedema episodes by 10 years of age, and symptoms often increase after puberty. There is a high degree of inter- and inpatient variability in both the frequency and severity of attacks. Characteristically, HAE symptoms do not respond to mast-cell targeted treatments, such as antihistamines, corticosteroids, or epinephrine. Pharmacologic treatment of HAE consists of access to on-demand therapies to treat acute episodes of angioedema and consideration for long-term prophylaxis on an individual basis. Approved therapies for the on-demand treatment of acute attacks consist of products that target the kallikrein-kinin pathway (i.e., C1-INH replacements, plasma kallikrein inhibitors (ecallantide and sebetralstat), and a bradykinin B2-receptor antagonist (icatibant)). Similarly, approved therapies for maintenance prophylaxis to prevent acute attacks consist of products that target the kallikrein-kinin pathway (i.e., C1-INH replacement, a Factor XIIa inhibitor (garadacimab), and plasma kallikrein inhibitors (lanadelumab and berotralstat)). Danazol, an attenuated androgen, is also approved for prophylaxis, although its mechanism of action in HAE is unclear.

The Applicant submitted an IND application (IND 142564; July 15, 2019) to investigate donidalorsen for the prophylaxis of HAE attacks. To support the NDA, the Applicant submitted the results of a single, adequate, and well-controlled phase 3 trial (CS5). This 24-week, randomized, double-blind, placebo-controlled trial evaluated 90 adult and adolescent subjects with HAE Type I or II. The primary efficacy endpoint was the time-normalized number of investigator-confirmed HAE attacks per 4 weeks from Week 1 to Week 25.

During the course of the application review, the Agency identified several key efficacy and safety review issues, as listed below. In-depth analyses and assessments of these issues can be found in Sections [6.3](#) and [7.7](#).

3.1. Review Issue List

3.1.1. Key Efficacy Review Issues

3.1.1.1. Dosing Interval of Once Every 2 Months

3.1.1.2. Confirmatory Evidence

3.1.2. Key Safety Review Issues

3.1.2.1. Injection Site Reactions

3.1.2.2. Bleeding Events, Thrombocytopenia, and Coagulation

3.1.2.3. Hepatic Function Effects

3.1.2.4. Hypersensitivity Reactions

3.2. Approach to the Clinical Review

[Table 3](#) provides an overview of the clinical trials used to support the benefit-risk assessment of donidalorsen for the prophylaxis of HAE attacks in adults and adolescents 12 years of age and above. Trial CS5 served as the primary basis for the efficacy and safety of donidalorsen. Trial CS2 provided data to support dose selection as well as supportive efficacy and safety results. Trial CS2 also included Part B which enrolled 3 subjects with Type III HAE. Subjects with Type III HAE were excluded from the analysis as these patients are not included in the indicated population. Additionally, the ongoing OLE trials (CS3 and CS7) provided data on the long-term safety of donidalorsen.

3.3. Approach To Establishing Substantial Evidence of Effectiveness

1. Verbatim indication:

Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older.

2. SEE was established with:

a. Adequate and well-controlled clinical investigation(s):

- i. Two or more adequate and well-controlled clinical investigations, **OR**
- ii. One adequate and well-controlled clinical investigation with highly persuasive results that is considered to be the scientific equivalent of two clinical investigations

OR

b. One adequate and well-controlled clinical investigation and confirmatory evidence^{2,3,4}

OR

c. Evidence that supported SEE from a prior approval (e.g., 505(b)(2) application relying only on a previous determination of effectiveness; extrapolation; over-the-counter switch)⁵

3. Complete response, if applicable

- a. SEE was established
- b. SEE was not established (*if checked, omit item 2*)

² FDA draft guidance for industry *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products* ([December 2019](#))

³ FDA guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products* ([May 1998](#))

⁴ FDA guidance for industry *Demonstrating Substantial Evidence of Effectiveness Based on One Adequate and Well-Controlled Clinical Investigation and Confirmatory Evidence* ([September 2023](#))

⁵ See Footnote [3](#)

Table 3. Clinical Trials Submitted in Support of Efficacy and/or Safety Determinations^a for Donidalorsen

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
ISIS 721744-CS2 Part A	Patients with HAE 1 or HAE 2	<u>Control Type:</u> Placebo concurrent <u>Randomization:</u> Standard randomization <u>Randomization Ratio:</u> 2:1 <u>Blinding:</u> Double-blind	<u>Drug (established name):</u> Donidalorsen <u>Dose:</u> 80 mg SC Q4W <u>Number treated:</u> Donidalorsen: 14 Placebo: 6 <u>Duration (quantity and units):</u> 16 weeks	<u>Primary:</u> - The time-normalized number of HAE attacks (per month) from Week 1 to Week 17. <u>Secondary:</u> - 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 subjects with a clinical response (defined as a $\geq 50\%$, $\geq 70\%$, or $\geq 90\%$ 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 levels at Weeks 9 and 17 - Prekallikrein activity at Weeks 9 and 17 - Consumption of on-demand medication at Weeks 9 and 17	<u>Planned:</u> 18 <u>Actual:</u> 20	<u>Centers:</u> 8 <u>Countries:</u> 2

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
ISIS 721744-CS3	Rollover patients from CS2 with HAE 1, HAE 2, or HAE-nC1-INH	<u>Randomization:</u> No randomization <u>Blinding:</u> Open-label	<u>Drug:</u> Donidalorsen <u>Dose:</u> 80 mg SC Q4W or 80 mg SC Q8W or 100 mg SC Q4W <u>Number treated:</u> 20 Rollover subjects from CS2 <u>Duration:</u> 208 weeks	<u>Primary:</u> - The incidence and severity of TEAEs <u>Secondary:</u> - The time-normalized HAE attacks (per 4 weeks) by treatment - Consumption of on-demand medications	<u>Planned:</u> 20 <u>Actual:</u> 20	<u>Centers:</u> 7 <u>Countries:</u> 2
ISIS 721744-CS5	Patients with HAE	<u>Control Type:</u> Placebo concurrent <u>Randomization:</u> Standard randomization <u>Randomization Ratio:</u> 2:1 to either Cohort A (Q4W) or Cohort B (Q8W), within each cohort 3:1 randomization to donidalorsen or placebo <u>Blinding:</u> Double-blind	<u>Drug:</u> Donidalorsen <u>Dose:</u> 80 mg SC Q4W or 80 mg SC Q8W <u>Number treated:</u> 90 <u>Duration:</u> 24 weeks	<u>Primary:</u> - The time-normalized number of investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 25 compared to placebo for donidalorsen 80 mg Q4W. <u>Secondary:</u> - The time-normalized number of investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25 compared to placebo for donidalorsen 80 mg Q4W. - The number of subjects with a $\geq 70\%$ reduction from baseline in investigator-confirmed HAE attack rate (per 4 weeks) between Week 5 to Week 25 compared to placebo for donidalorsen 80 mg Q4W. - The percentage of investigator-confirmed HAE attack-free subjects from Week 5 to Week 25 compared to placebo for donidalorsen 80 mg Q4W.	<u>Planned:</u> 84 <u>Actual:</u> 91	<u>Centers:</u> 39 <u>Countries:</u> 13

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
				• The time-normalized number of moderate or severe investigator-confirmed HAE attacks (per 4 weeks) from Week 5 to Week 25 compared to placebo for donidalorsen 80 mg Q4W. • The number of investigator-confirmed HAE attacks requiring acute therapy (per 4 weeks) from Week 5 to Week 25 compared to placebo for donidalorsen 80 mg Q4W. • Change in AE-QoL total score at Week 25 compared to placebo for donidalorsen 80 mg Q4W. • All listed secondary endpoints were also included, in order, for the donidalorsen 80 mg Q8W.		

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
ISIS 721744-CS7	Rollover patients from CS5 with HAE	<u>Randomization:</u> No randomization <u>Blinding:</u> Open-label	<u>Drug:</u> Donidalorsen <u>Dose:</u> 80 mg SC Q4W or 80 mg Q8W <u>Number treated:</u> 147 (83 Rollover subjects from CS5+64 switch subjects) <u>Duration:</u> 146 weeks	<u>Primary:</u> - Incidence and severity of TEAEs. <u>Secondary:</u> - 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 subjects 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 - AE-QoL questionnaire total score over 52 weeks	<u>Planned:</u> N/A (dependent on CS5 enrollment and eligibility) <u>Actual:</u> 147	<u>Centers:</u> 50 <u>Countries:</u> 13

Source: Clinical study report and adsl.xpt

^a Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and pharmacokinetic trials.

Abbreviations: AE-QoL, Angioedema Quality of Life; HAE, hereditary angioedema; nC1-INH-HAE (Type III), hereditary angioedema formerly type III; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous; TEAE, treatment-emergent adverse event

4. Patient Experience Data

Patient experience information about donidalorsen is summarized in [Table 4](#).

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	Type of Data	Section Where Discussed, if Applicable
Clinical Outcome Assessment Data Submitted in the Application		
☒	Patient-reported outcome	Section 6.2.2.4.6.2
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	Type of Data	Section Where Discussed, if Applicable
☐	Perspectives shared at patient stakeholder meeting	Section 2.1 Analysis of Condition
☒	Patient-focused drug development meeting summary report: September 25, 2017, externally led Patient-Focused Drug Development for Hereditary Angioedema	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

The unconjugated human PKK ASO parent compound of donidalorsen, ISIS 546254, which has the same nucleobase sequence as donidalorsen, is perfectly matched to only PKK mRNA in the human transcriptome (targets site free of meaningful sequence variation).

NDA 219407

Dawnzera (donidalorsen)

Donidalorsen does not align perfectly to the mouse PKK gene sequence and is not pharmacologically active in the mouse. The human sequence targeted by donidalorsen has a single-base sequence mismatch within the binding site of monkey PKK mRNA (position 19). Despite this mismatch, donidalorsen demonstrated dose-dependent inhibition of liver target mRNA expression and reductions in PKK protein and activity levels in peripheral circulation of Cynomolgus monkeys.

Incubation of donidalorsen with HepatoPac® (co-culture system of primary human hepatocytes and stromal cells [mouse fibroblasts]) reduced PKK mRNA in a dose-dependent manner and resulted in a half-maximal inhibitory concentration (IC₅₀) value of 0.19µM.

In an in vivo study in human KLKB1/PKK transgenic mice, single SC administration of donidalorsen on Day 0 resulted in a dose- and time-dependent reduction of plasma PKK protein that approached 91% at the highest dose tested (10 mg/kg) on Day 14 after treatment. PKK levels were still 20% below baseline levels by Day 56 (last time point evaluated) at 10 mg/kg.

The established pharmacologic class (EPC) for donidalorsen has been designated as “prekallikrein-directed antisense oligonucleotide” based on in vitro and in vivo pharmacology data.

5.2. Clinical Pharmacology/Pharmacokinetics

Donidalorsen (ISIS 721744) is a chimeric GalNAc-conjugated 2'-O-methoxyethyl (2'-MOE)-modified ASO. The ASO is covalently bound to GalNAc via a trishexylamino (THA) linker. GalNAc is a ligand with affinity for the hepatocyte-specific asialoglycoprotein receptor (ASGPR). This GalNAc conjugate approach results in ASO delivery to hepatocytes. The modifications in ASO chains have been seen in other approved ASO products, such as eplontersen (NDA 217388), tofersen (NDA 215887), inotersen (NDA 211172), and olezarsen (NDA 218614). Eplontersen also contains an identical THA linker to that used in donidalorsen.

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information														
	Pharmacologic Activity														
EPC	Prekallikrein-directed antisense oligonucleotide (FDA 2025)														
Mechanism of action	Donidalorsen is an ASO-GalNAc conjugate that causes RNase H1-mediated degradation of PKK mRNA through binding to PKK mRNA, which results in reduced production of PKK protein. PKK is mainly synthesized by the liver and it is a pro-enzyme for plasma kallikrein, which results in the release of bradykinin, a vasodilator causing swelling and pain in HAE.														
Active moieties	Unconjugated ASO of donidalorsen (ISIS 546254)														
QT prolongation	At the maximum recommended dose of donidalorsen 80 mg Q4W, clinically significant QTc interval prolongation was not observed.														
	General Information														
Bioanalysis	The plasma concentrations of donidalorsen, including fully conjugated, partially conjugated (i.e., with 1, 2, or 3 GalNAc sugar deletions), and unconjugated donidalorsen, was assessed with a validated hybridization ECL method ((b) (4)) with an analytical range of 0.1735 to 86.73 ng/mL. In addition, dilutions up to 10,000-fold were evaluated and deemed acceptable. The plasma PKK concentrations in Trials CS3, CS5, and CS7 were assessed by a validated ELISA method with a detection range of 3.76 µg/mL to 480 µg/mL after a MRD of 1:8000. Plasma PKK in CS1 and CS2 were analyzed by a chromogenic ELISA method with a detection range between 7.52 and 2560 µg/mL ((b) (4)). See Section 14.3 for more details.														
Healthy subjects versus patients	Following the same dosing regimen of 80 mg Q4W, the pharmacokinetics of donidalorsen was similar between subjects with HAE and healthy subjects (mean C _{trough,ss} for both populations was around 1 ng/mL), while the maximum plasma PKK reduction from baseline was greater in healthy subjects (94%) compared to subjects with HAE (73%). The baseline plasma PKK concentrations were similar between subjects with HAE and healthy subjects.														
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	The steady state PK parameters in subjects with HAE based on PopPK simulation are shown below: Table 6. Steady State PK Parameters in Subjects With HAE Based on PopPK Simulation <table border="1"> <thead> <tr> <th rowspan="2">Parameter</th> <th colspan="2">Geometric Mean (Range)</th> </tr> <tr> <th>80 mg Q4W</th> <th>80 mg Q8W</th> </tr> </thead> <tbody> <tr> <td>C_{max,ss} (ng/mL)</td> <td>417 (43, 3560)</td> <td>416 (41.4, 4310)</td> </tr> <tr> <td>AUC_{0-tau,ss} (ng*h/mL)</td> <td>5240 (1180, 23600)</td> <td>5210 (1070, 27500)</td> </tr> <tr> <td>C_{trough,ss} (ng/mL)</td> <td>0.755 (0.128, 5.72)</td> <td>0.255 (0.03, 2.03)</td> </tr> </tbody> </table> Source: Table 13, Report 721744-PKK01 Abbreviations: AUC_{0-tau,ss}, area under the plasma concentration-time curve to the end of the dosing period at steady state; C_{max,ss}, maximum plasma concentration at steady-state; C_{trough,ss}, trough plasma concentration at steady-state; HAE, hereditary angioedema; PopPK, population pharmacokinetics; Q4W, every 4 weeks; Q8W, every 8 weeks	Parameter	Geometric Mean (Range)		80 mg Q4W	80 mg Q8W	C _{max,ss} (ng/mL)	417 (43, 3560)	416 (41.4, 4310)	AUC _{0-tau,ss} (ng*h/mL)	5240 (1180, 23600)	5210 (1070, 27500)	C _{trough,ss} (ng/mL)	0.755 (0.128, 5.72)	0.255 (0.03, 2.03)
Parameter	Geometric Mean (Range)														
	80 mg Q4W	80 mg Q8W													
C _{max,ss} (ng/mL)	417 (43, 3560)	416 (41.4, 4310)													
AUC _{0-tau,ss} (ng*h/mL)	5240 (1180, 23600)	5210 (1070, 27500)													
C _{trough,ss} (ng/mL)	0.755 (0.128, 5.72)	0.255 (0.03, 2.03)													

Characteristic	Drug Information
Range of effective dose(s) or exposure	Two dosing regimens, 80 mg Q4W and 80 mg Q8W, were evaluated and both demonstrated efficacy and safety for the prevention of HAE attacks in the pivotal phase 3 trial (CS5). Refer to Section 6 for more details.
Maximally tolerated dose or exposure	80 mg SC Q4W was the highest dose tested in the phase 3 trial (CS5). One subject received 100 mg Q4W in Trial CS3, an open-label extension to the phase 2 trial (CS2).
Dose proportionality	Donidalorsen AUC _{tau,ss} increased in a greater than dose-proportional manner between dosages of 20 mg Q4W and 80 mg Q4W. Donidalorsen AUC _{tau,ss} increased by about 6-fold for a 4-fold increase in dose.
Accumulation	No accumulation in C _{max} and AUC was observed following administration of 4 doses of 80 mg Q4W donidalorsen in healthy subjects as well as in subjects with HAE. However, a 2-fold increase in mean C _{trough} was observed in both healthy subjects and subjects with HAE at steady state. Of note, the observed C _{trough} is about 1% of the value of C _{max} .
Time to achieve steady-state	The steady-state C _{trough} following administration of 80 mg Q4W was reached by Day 85 for healthy subjects and by Day 113 for subjects with HAE. The steady state for change from baseline in plasma PKK was reached by around Day 85 in subjects with HAE following administration of 80 mg Q4W or Q8W donidalorsen.
Bridge between to-be-marketed and clinical trial/study formulations	The drug product used in clinical trials was a vial product while the to-be-marketed formulation is proposed as a prefilled syringe with autoinjector. Bioequivalence based on C _{max} and AUC between the drug product presentations was demonstrated in Trial CS9 conducted in healthy subjects.
Absorption	
Bioavailability	Absolute bioavailability after SC administration of donidalorsen has not been determined.
T _{max}	Following a single SC dose of 80 mg in healthy subjects, the median (range) T _{max} for donidalorsen in plasma is 2 (0.25, 8) hours.
Food effect (fed/fasted) Geometric least square mean and 90% CI	Not applicable.
Distribution	
Volume of distribution	Central compartment volume (V _{c/F}) is 69.8 L Peripheral compartment volume (V _{p/F}) is 1840 L
Plasma protein binding	Donidalorsen is highly plasma protein bound (>98%) with minimal change in protein binding over a concentration range of 5 to 150 µg/mL.
Drug as substrate of transporters	Donidalorsen is not a substrate of P-gp, BCRP, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2K, and BSEP. THA-linker major metabolite M8 is not a substrate of OAT1, OAT3, OCT1, OCT2, OATP1B1, MATE1, MATE2-K, BCRP, and P-gp. However, M8 is a substrate of BSEP and OATP1B3.

Characteristic	Drug Information
	<i>Elimination</i>
Mass balance results	Human mass balance study was not conducted.
Clearance	Apparent clearance (CL/F) is 12.8 L/h
Half-life	Terminal elimination half-life is 31.4 days, which likely reflects the half-life in tissue (i.e., liver). The apparent half-life in plasma, reflecting tissue distribution, is estimated to be 4 to 5 hours.
Metabolic pathway(s)	The unconjugated ASO portion is expected to be metabolized by endo- and exo-nucleases to form fragmented oligonucleotide metabolites. The GalNAc-THA linker moiety is hydrolyzed intracellularly following uptake into tissue. After hydrolysis, GalNAc sugars undergo endogenous carbohydrate catabolism, while the THA linker undergoes oxidative metabolism. No specific CYP enzyme was identified to majorly contribute to the metabolism of donidalorsen. Minimal linker-related metabolites (e.g., M8 and M12) were detected in human plasma, with mean concentrations <2% of those determined for parent drug at 2-hours postdose.
Primary excretion pathways (% dose)	The mean fraction of donidalorsen (including fully conjugated, partially conjugated, and unconjugated donidalorsen) excreted in the urine within 24 hours is <1% of the administered dose in healthy subjects. The fragmented oligonucleotide metabolites formed by metabolism of unconjugated ASO are excreted in urine or feces. Metabolites of the THA linker are also excreted in the urine, representing ≤6.4% of the dose detected in urine samples collected over 24 hours following the four doses of 80 mg Q4W on Day 85.
	<i>Intrinsic Factors and Specific Populations</i>
Body weight	No clinically meaningful differences in the pharmacokinetics or pharmacodynamics of donidalorsen were observed based on body weight (37 to 152 kg). Based on population PK analysis, the estimated steady-state plasma donidalorsen systemic exposure ($C_{max,ss}$, $AUC_{tau,ss}$ and $C_{trough,ss}$) in a subject weighing 100 kg is about 38% to 54% lower as compared to that in a subject weighing 70 kg.
Age	No clinically meaningful differences in the pharmacokinetics or pharmacodynamics of donidalorsen were observed based on age (12 to 68 years).
Renal impairment	No clinically meaningful differences in the pharmacokinetics or pharmacodynamics of donidalorsen were observed in subjects with mild renal impairment (i.e., eGFR ≥60 to <90 mL/min/1.73 m ²) compared to subjects with normal renal function. Donidalorsen was not studied in subjects with moderate renal impairment, severe renal impairment, or ESRD.
Hepatic impairment	No clinically meaningful differences in the pharmacokinetics or pharmacodynamics of donidalorsen were observed in subjects with mild hepatic impairment (defined using NCI-ODWG criteria: total bilirubin ≤1× ULN and AST >1× ULN, or total bilirubin >1 to 1.5× ULN and any AST) compared to subjects with normal hepatic function. Donidalorsen was not evaluated in patients with moderate or severe hepatic impairment. Given that the site of action of donidalorsen is in the liver, the long-term safety and efficacy in patients with moderate or severe hepatic impairment is not known.

Characteristic	Drug Information
	<i>Drug Interaction Liability (Drug as Perpetrator)</i>
Inhibition/induction of metabolism	In vitro, donidalorsen did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. Donidalorsen did not induce CYP1A2, CYP2B6, or CYP3A4. In vitro, THA-linker major metabolite M8 did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4. M8 did not induce CYP1A2, CYP2B6, or CYP3A4.
Inhibition/induction of transporter systems	In vitro, donidalorsen did not inhibit OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, BCRP, P-gp, or BSEP. In vitro, THA-linker metabolite M8 did not inhibit OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE2-K, BCRP, P-gp, or BSEP. M8 inhibited MATE1 with an IC ₅₀ of 62.7 μM.
	<i>Immunogenicity (if Applicable)</i>
Bioanalysis	Antidrug antibodies against donidalorsen in human plasma were assessed using a validated multitiered ELISA.
Incidence	In the pivotal trial (CS5) with a treatment duration of 24 weeks, the incidence rates of treatment-emergent ADAs were 20% (9 out of 45 subjects) and 22% (5 out of 23 subjects) among subjects who received donidalorsen 80 mg Q4W and 80 mg Q8W, respectively. In the ongoing OLE trial (CS7), subjects who were rolled over from Trial CS5 continued treatment with donidalorsen in 80 mg Q4W or Q8W groups for up to 3 years (median exposure duration of 227 days). The incidence rate of treatment-emergent ADAs was 35% (22 of 63 subjects) in the Q4W group, including subjects initially randomized to Q4W (36%, 16 out of 44 subjects) in Trial CS5 and subjects initially randomized to placebo in Trial CS5 (32%, 6 out of 19 subjects). The incidence rate of treatment-emergent ADAs was 21% (3 of 14 subjects) among subjects who received Q8W in both Trial CS5 and Trial CS7.
Clinical impact	The presence of ADAs was not found to impact the pharmacodynamics (i.e., change from baseline in plasma PKK), safety, or efficacy of donidalorsen. An increase in donidalorsen plasma C _{trough} was observed in ADA-positive subjects with high titers. Because of the small sample size, the effect of ADAs on the PK, PD, safety, and effectiveness of donidalorsen is inconclusive.

Source: Reviewer-generated table

Abbreviations: ADA, antidrug antibody; ASO, antisense oligonucleotide; AST, aspartate aminotransferase; AUC, area under the concentration-time curve; BCRP, Breast Cancer Resistance Protein; BSEP, bile salt export pump; C_{max}, maximum plasma concentration; C_{trough}, trough concentration; CYP, cytochrome P450 enzyme; ECL, electrochemiluminescence; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; EPC, established pharmacologic class; ESRD, end stage renal dysfunction; GalNAc, N-acetylgalactosamine; HAE, hereditary angioedema; IC₅₀, half-maximal inhibitory concentration; MATE, multidrug and toxin extrusion protein; MRD, minimum required dilution; NCI-ODWG, National Cancer Institute-Organ Dysfunction Working Group; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; OLE, open-label extension; PD, pharmacodynamic; P-gp, P-glycoprotein; PK, pharmacokinetic; PKK, prekallikrein; PopPK, population pharmacokinetics; Q4W, every 4 weeks; Q8W, every 8 weeks; Rnase-H1, ribonuclease H1; THA, tris(2-hydroxyethyl)amino; T_{max}, time to maximum concentration; ULN, upper limit of normal

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

The Applicant's proposed dosing regimen for donidalorsen is 80 mg given subcutaneously (b) (4)
(b) (4)
(b) (4)

The donidalorsen-4 (every 4 weeks) and donidalorsen-8 (every 8 weeks) dosing regimens were investigated in one phase 3 trial (CS5), while switching from donidalorsen-8 to donidalorsen-4 was evaluated in the open-label extension trial for CS5 (Trial CS7). The donidalorsen-4 regimen was also assessed in a phase 2 trial (CS2) and switching from donidalorsen-4 to donidalorsen-8 dosing regimens were assessed in its open-label extension trial (CS3). Dose selection for CS5 was based on CS1 and CS2 (See Section 14).

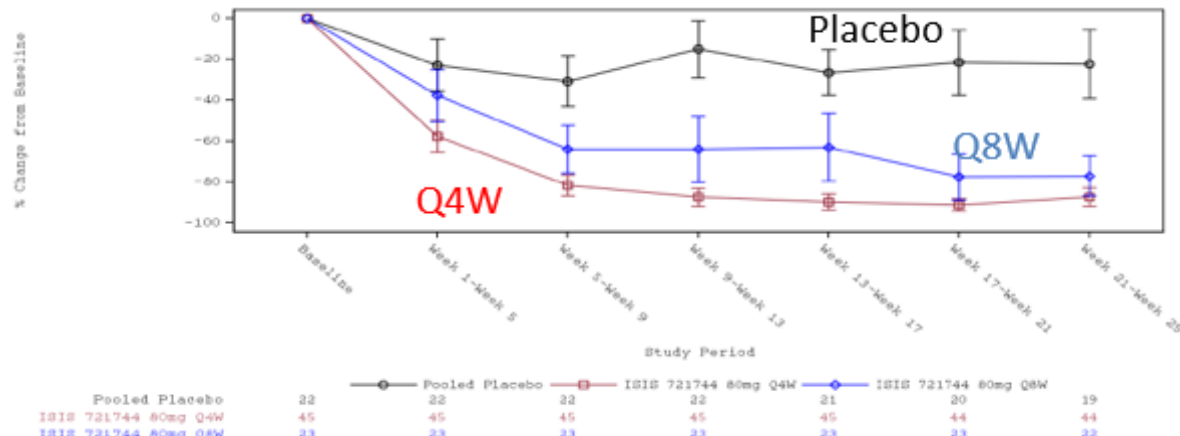
Based on an assessment of the submitted data, the recommended dosage regimen is 80 mg every 4 weeks. A dosage of 80 mg every 8 weeks may also be considered. (b) (4)
(b) (4) switching from a more frequent dosing regimen (donidalorsen-4) to a less frequent dosing regimen (donidalorsen-8) for all patients based on the disease control for at least 3 months, (b) (4) However, as a positive dose response relationship was demonstrated between donidalorsen-4 and donidalorsen-8 in CS5, the donidalorsen-4 is the preferred dosing regimen. The donidalorsen-8 may also be considered for some subjects based on the efficacy demonstrated in Trial CS5 and the sustained efficacy demonstrated in CS7.

(b) (4)
However, since clinical trials only evaluated donidalorsen-4 and donidalorsen-8 regimens, the recommended dosing regimen should reflect what has been studied in clinical trials, namely every 4 weeks or every 8 weeks.

Exposure/Pharmacodynamics-Response Relationship

In Trial CS5, a positive dose-response relationship was observed for HAE attack rate reduction between the donidalorsen-4 and donidalorsen-8 dosing regimens, with a greater HAE attack rate reduction observed with donidalorsen-4 compared to donidalorsen-8. Subjects randomized to both the donidalorsen-4 and donidalorsen-8 dosing regimens demonstrated a reduction in HAE attack rate as compared to subjects who received placebo (Figure 1).

Figure 1. Mean (SEM) Percent Change From Baseline in HAE Attack Rate (Per 4 Weeks) Over Successive 4-Week Periods From Weeks 1 to 25, Trial CS5



APPEARS THIS WAY ON ORIGINAL

Source: Adapted from Figure 14.2.1.4.1. CSR CS5

For investigator-confirmed HAE attacks, the baseline rate, (i.e., Run-In Period investigator-confirmed HAE attack rate) was calculated for each subject 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.

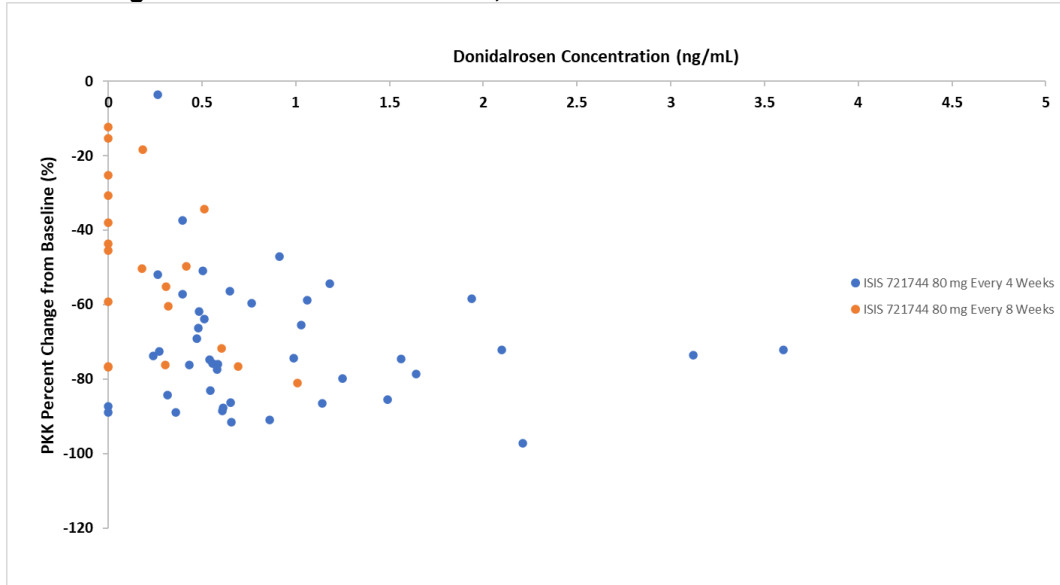
The HAE attack rate (per 4 weeks) was calculated for each subject as number of HAE attacks occurring during each 4-week period divided by the number of days the subject contributed to the period multiplied by 28. Week 1 to Week 5 starts from Day 1 to Day 28; Week 5 to Week 9 starts from Day 29 to Day 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.

Abbreviations: CSR, clinical study report; HAE, hereditary angioedema; ISIS 721744, donidalorsen; Q4W, every 4 weeks; Q8W, every 8 weeks; SEM, standard error of mean

The PK/PD relationship between plasma donidalorsen trough concentrations and percent change in plasma PKK trough concentrations at steady state (Week 17) based on observed data from Trial CS5 demonstrated a weak positive association (Figure 2). A numerically smaller percent reduction in plasma PKK trough concentration was more often observed in subjects in the donidalorsen-8 group, who had lower donidalorsen exposure. This is consistent with the observed dose-response in Trial CS5 for the mean percent reduction in plasma PKK concentrations. Among subjects randomized to the donidalorsen-4 and donidalorsen-8 dosing regimens, the mean (SD) percent change from baseline in PKK concentrations was -73% (17) and -47% (21) at Week 25, respectively.

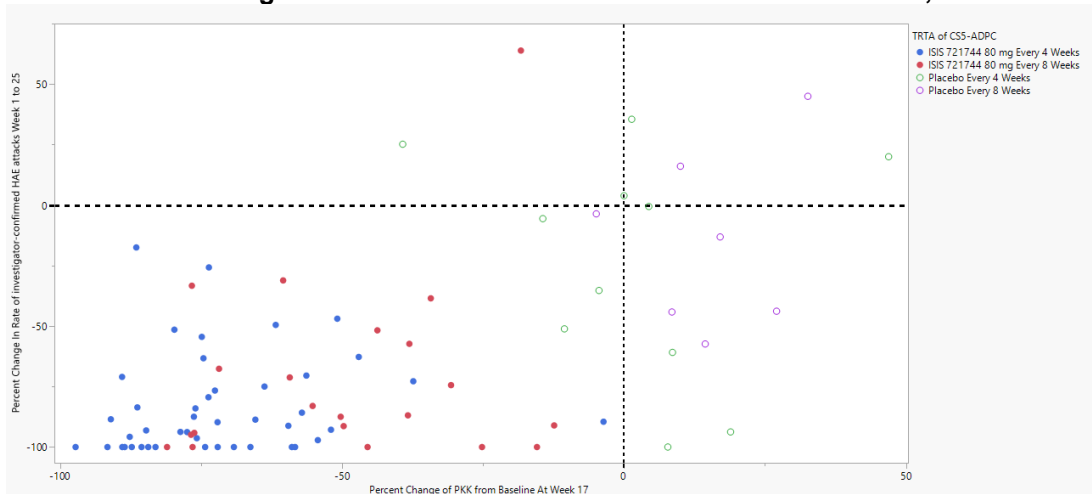
Figure 2. Individual Plasma Donidalorsen Trough Concentration vs. Percent Change in Plasma PKK Trough Concentration at Week 17, Trial CS5



Source: Reviewer-generated graph based on adpc.xpt and adpd.xpt for CS5
 Abbreviations: ISIS 721744, donidalorsen; PKK, prekallikrein

The observed positive PK/PD relationship is consistent with the dose-response relationship for mean HAE attack rate reduction between the donidalorsen-4 and donidalorsen-8 dosing regimens in Trial CS5. At the individual level, a reduction from baseline in plasma PKK trough concentrations was generally associated with the reduction in HAE attack rate. However, there was no clear quantitative correlation between the magnitude of plasma PKK reduction and HAE attack rate reduction based on observed data in Trial CS5 (Figure 3).

Figure 3. Individual Percent Change in Plasma PKK Trough Concentration From Baseline at Week 17 vs. Percent Change in HAE Attack Rate Between Week 1 and Week 25, Trial CS5



Source: Clinical pharmacology reviewer-generated graph based on adpd.xpt and adefx.xpt for CS5
 X axis = percent change of plasma PKK trough concentration from baseline at Week 17.
 Y axis = percent change in rate of investigator-confirmed HAE attacks Week 1 to 25.
 Abbreviations: HAE, hereditary angioedema; ISIS 721744, donidalorsen; PKK, prekallikrein

Dosing Regimen Switching

The Applicant-proposed dosing regimen is 80 mg given subcutaneously (b) (4)
(b) (4)
(b) (4)

Switching from the donidalorsen-4 to donidalorsen-8 dosing regimen was not evaluated in the pivotal trial (CS5). However, some subjects switched between the donidalorsen-4 and donidalorsen-8 dosing regimen in the open-label extension trial (CS3) and from donidalorsen-8 to donidalorsen-4 in the open-label extension trial (CS7).

Trial CS7

In the open-label Trial CS7 (an extension of the pivotal trial [CS5]), no subject was switched from the donidalorsen-4 to donidalorsen-8 dosing regimen. Subjects who received the donidalorsen-8 dosing regimen during Trial CS5 remained in the same dosing regimen (donidalorsen-8) if well controlled (N=14), or were switched to a more frequent dosing regimen (donidalorsen-4) if they experienced any attack between Week 17 to Week 25 during Trial CS5 (N=6); subjects who received the donidalorsen-4 dosing regimen during Trial CS5 remained in the same dosing regimen (donidalorsen-4) (N=44). In addition, subjects who received placebo during Trial CS5 received the donidalorsen-4 dosing regimen during Trial CS7 (N=19). For additional details, refer to Section [14.2.6](#).

A total of 14 subjects in the donidalorsen-8 group who were adequately controlled during Trial CS5 (i.e., no HAE attack $\geq$ 8 weeks after last dose in Trial CS5 [Week 17 to Week 25]) continued on the donidalorsen-8 regimen in Trial CS7. In these 14 subjects, the mean percent change from baseline (i.e., Run-In Period for Trial CS5) in plasma PKK concentration was -45% at Week 9 in Trial CS7; this is similar to the mean percent reduction in plasma PKK concentrations observed among subjects in the donidalorsen-8 group in Trial CS5 (i.e., -47.2% at Week 25). Although subjects in the donidalorsen-8 group had a smaller percent reduction in plasma PKK concentrations as compared to subjects in the donidalorsen-4 group (mean -73.8% at Week 25 in Trial CS5), no subject who continued receiving the donidalorsen-8 dosing regimen in Trial CS7 was reported to have switched to a more frequent regimen by the NDA cutoff date.

The mean (SD) HAE attack rate (per 4 weeks) in Rollover subjects from Trial CS5 to Trial CS7 is shown in [Table 7](#). The HAE attack rate in Trial CS7 in subjects in the donidalorsen-8 group in both Trial CS5 and Trial CS7 remained consistent compared to their mean rate at the end of Trial CS5. Notably, the mean HAE attack rate determined in Trial CS7 was comparable between subjects in the donidalorsen-8 group in Trial CS5 and Trial CS7, and subjects in the donidalorsen-4 group in Trial CS5 and Trial CS7. See Sections [6.2.2.4.3](#) and [6.2.2.4.4](#) for more details on the assessment of efficacy and Section [6.2.2.4.6.5](#) for the treatment effect durability of the donidalorsen-8 regimen.

Table 7. Mean (SD) Investigator-Confirmed HAE Attack Rate Per 4 Weeks in Rollover Subjects, Trial CS7

Timepoints	80 mg Q4W in CS7 (N=69)			80 mg Q8W in CS7 (N=14)
	Q4W in CS5 (N=44)	Q8W in CS5 (N=6)	Placebo in CS5 (N=19)	Q8W in CS5 (N=14)
Index Run-In baseline (at CS5)	3.66 (2.24)	4.32 (2.44)	2.97 (1.70)	2.87 (2.06)
OLE baseline (CS5 Week 1 to Week 25)	0.89 (1.87)	3.08 (2.02)	2.35 (1.87)	0.32 (0.36)
OLE Week 1 to Week 53 ^a	0.15 (0.29)	1.11 (0.82)	0.18 (0.26)	0.12 (0.13)

Source: Adapted from Table 23 and 14.2.1.1.3. CSR CS7

^a Trial was ongoing at NDA data cutoff. Some subjects did not complete Week 53. The Week 1 to Week 53 period HAE attack rate was calculated as the number of HAE attacks occurring during this period divided by the number of days the subject contributed to the period multiplied by 28 days.

Abbreviations: CSR, clinical study report; HAE, hereditary angioedema; N, number of subjects; NDA, new drug application; OLE, open-label extension; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

Trial CS3

Switching from the donidalorsen-4 to donidalorsen-8 dosing regimen was investigated in the open-label Trial CS3 (an extension of the phase 2 trial [CS2]). In Trial CS3, Rollover subjects from Trial CS2 initially entered a 12-week Fixed Treatment Period where all subjects received the donidalorsen-4 dosing regimen. Following completion of the Fixed Treatment Period, the dosing regimen could be switched in the subsequent Flexible Dosing Period. For additional details, refer to Section 14.2.5. In Trial CS3, eight subjects who were attack free for at least 12 weeks on the donidalorsen-4 regimen during the Fixed Treatment Period were switched to the less frequent donidalorsen-8 regimen during the Flexible Treatment Period. However, three of these eight subjects switched back to the donidalorsen-4 regimen due to experiencing HAE attacks.

Thus, based on observations from Trial CS3, it is not clear that subjects who were responsive on the donidalorsen-4 regimen would also be responsive to the donidalorsen-8 regimen. Therefore, as three out of eight subjects experienced an HAE attack after extension of the dosing interval despite being controlled for >12 weeks on the donidalorsen-4 regimen, the available data are not sufficient to support a recommendation to switch from the donidalorsen-4 to the donidalorsen-8 regimen based on HAE attack incidence. Given that HAE is a heterogeneous disease, the response from the more frequent donidalorsen-4 dosing regimen may not be predictive of who may benefit from the longer donidalorsen-8 dosing interval.

Although a positive dose-response relationship was observed in Trial CS5 between the donidalorsen-4 and donidalorsen-8 dosing regimens, both for the mean percent reduction from baseline in plasma PKK concentrations and HAE attack rate, variability in these responses was observed at the individual level within each dosing group (Figure 2 and Figure 3). The data suggest that there is heterogeneity in the disease and response to donidalorsen. While the reduction in plasma PKK trough concentrations following donidalorsen treatment was generally associated with the reduction in HAE attack rate, there are no dedicated data showing a clear association between the magnitude of change from baseline in plasma PKK trough concentration and HAE attack rate at the individual level.

Taken together, the effectiveness of both the donidalorsen-4 and donidalorsen-8 dosing regimens compared to placebo are supported by efficacy data from the pivotal trial (CS5). However, the available data do not support the switch from the donidalorsen-4 to the donidalorsen-8 dosing

regimens for all subjects who are responsive with the donidalorsen-4 regimen. The positive dose-response relationship between donidalorsen-4 and donidalorsen-8 observed in CS5 supported that donidalorsen-4 is the preferred regimen. In addition, as there were subjects who remained controlled and continued treatment with donidalorsen-8 when rolling over from CS5 to CS7, donidalorsen-8 regimen may also be considered.

6.2. Clinical Studies/Trials Intended To Demonstrate Efficacy

6.2.1. Results of Pooled Analyses

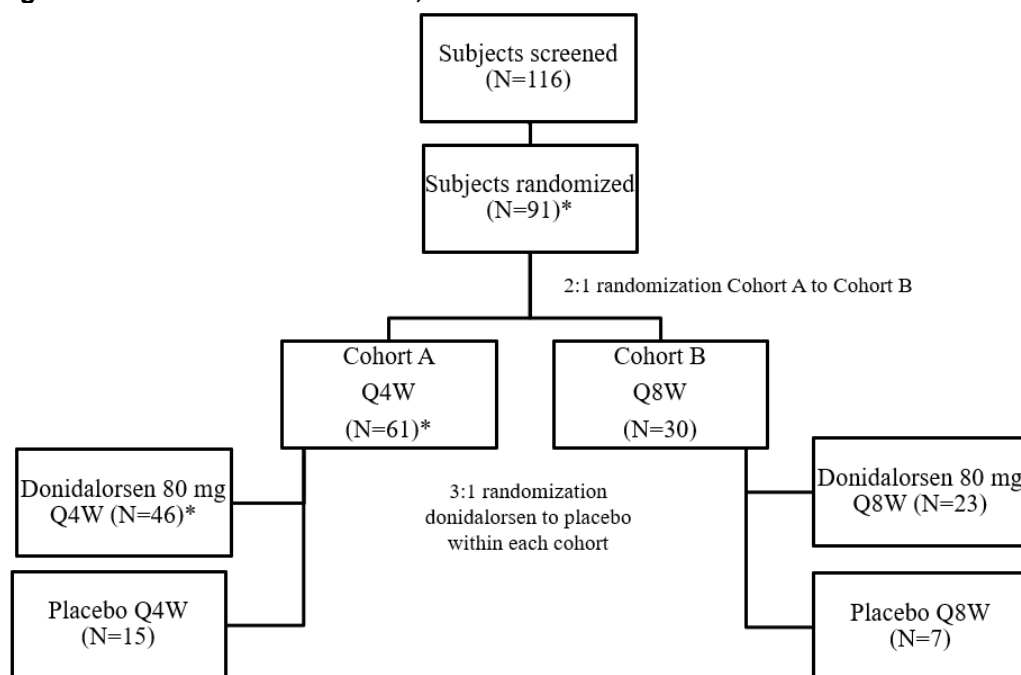
The evaluation of efficacy was based on the analyses of the individual trials. Pooled efficacy analyses will not be discussed in this review.

6.2.2. Trial CS5

6.2.2.1. Design and Conduct, Trial CS5

Results of the pivotal trial (CS5) served as the basis for efficacy evaluation. This was a 24-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter, phase 3 trial that treated 90 subjects aged 12 years or older with Type I or II HAE who had ≥ 2 HAE attacks during the Screening Period. Subjects were randomized 2:1 to either Cohort A (donidalorsen-4 or matched placebo) or Cohort B (donidalorsen-8 or matched placebo), respectively (see [Figure 4](#)). Within each cohort, subjects were randomized 3:1 to receive donidalorsen or a matching volume of placebo. Treatment was provided over a 24-week Treatment Period. Data from subjects receiving placebo in Cohorts A and B were pooled for analyses, and this is referred to as the pooled placebo group for primary analyses.

Figure 4. Randomization Schema, Trial CS5



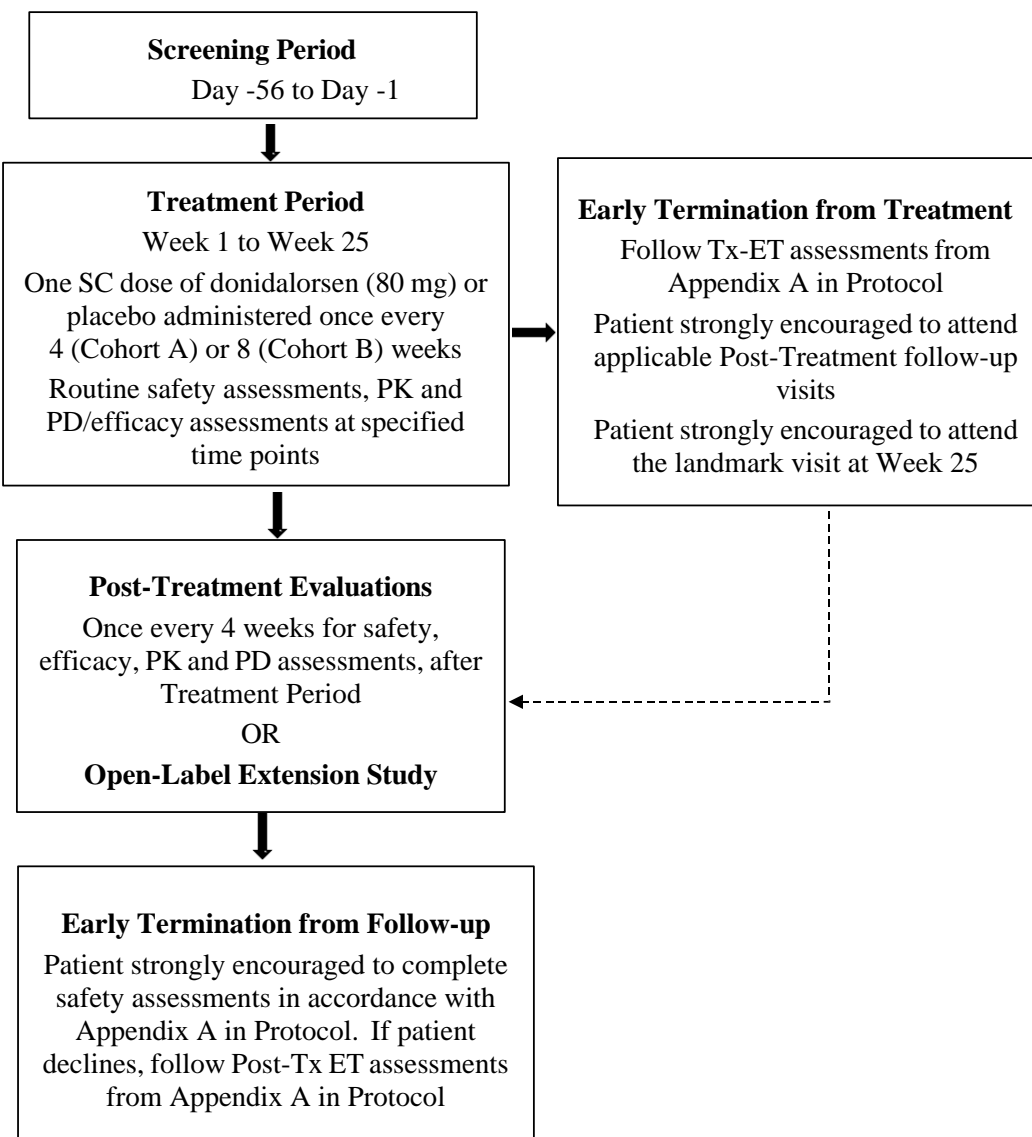
Source: FDA Clinical Data Scientist and Clinical Reviewer

* One subject in 80 mg Q4W withdrew consent after randomization but prior to receiving trial drug.

Abbreviations: Q4W, every 4 weeks; Q8W, every 8 weeks

As shown in [Figure 5](#), the trial consisted of three periods: the Screening Period (up to 8 weeks in duration; this period provided the baseline HAE attack rate for each subject), the Treatment Period (24 weeks in duration), and the Post-Treatment Period (up to 13 weeks in duration). Subjects were allowed to choose to enroll in an OLE trial (CS7 [see Section [15.2](#)]), rather than complete the Post-Treatment Period. The length of trial participation for each subject was up to 11 months. Written and signed informed consent (and assent for minors) was obtained prior to the performance of any trial-related procedures, including Screening. Each enrolled subject was randomized to begin trial treatment as soon as the subject experienced ≥ 2 HAE attacks during the Screening Period.

Figure 5. Trial Design, Trial CS5



Source: CS5 clinical study report

Abbreviations: ET, early termination; PD, pharmacodynamics; PK, pharmacokinetic; SC, subcutaneous; Tx, treatment

Subjects who experienced ≥ 5 attacks per month for 2 consecutive months after Week 5 (deemed lack of efficacy) had the opportunity to discontinue from treatment in the current trial and either progress to the Post-Treatment Period or enroll in the OLE trial (CS7). If a subject chose to enroll in Trial CS7, the subject was discontinued from Trial CS5.

Subjects not participating in Trial CS7 entered a 13-week Post-Treatment Period. Subjects who discontinued treatment prior to the end of the scheduled Treatment Period attended the Early Termination Visit and were encouraged to complete the Post-Treatment Period unless consent or assent (subjects not of legal age) was withdrawn.

The End of Study was defined as the date of the last visit of the last subject in the trial. For individual subjects, the End of Study was defined as the date of their last trial visit.

NDA 219407
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For details regarding the collection and investigator confirmation of HAE attacks, see Section [15.1.1](#).

The overall design of Trial CS5 is consistent with previous programs for HAE prophylaxis. The duration of the controlled Treatment Period (24 weeks) is acceptable for this indication.

Concomitant Medications

Consistent with the eligibility criteria, continued use of a stable dose of androgens or tranexamic acid for HAE prophylaxis was allowed while on trial; this differs from some HAE products' development programs, which disallow the use of these agents. Once enrolled, including during the Screening Period, subjects were not allowed to use other HAE prophylaxis agents, such as lanadelumab or berotralstat. Other disallowed concomitant therapies included angiotensin-converting enzyme (ACE) inhibitors and estrogen-containing medications with systemic absorption.

Subjects were required to have access to, and the ability to use, on-demand medication to treat acute HAE attacks, per the recommendation of their treating physician.

Primary Objective and Endpoint

The primary objective of Trial CS5 was to evaluate the efficacy of donidalorsen as prophylaxis in patients with HAE. The primary efficacy endpoint was the time-normalized number of investigator-confirmed HAE attacks per 4 weeks during the Treatment Period (from Week 1 to Week 25) for the donidalorsen-4 group compared to pooled placebo. The primary endpoint was calculated per subject as:

$$[\text{number of HAE attacks} \div \text{length subject contributed to analysis period in days}] \times 28$$

Length subject contributed to analysis period (in days) was calculated as:

$$[\text{End date of Treatment Period (if completed) or Date of 25 weeks after first dose (if did not complete) or Date of follow-up discontinuation or Date of end of participation (whichever is first)}] - \text{Date of first dose}$$

Treatment Period in the above formula is defined as the period from the first dose date/time to the last dose date/time +28 days for Cohort A and +56 days for Cohort B.

The selected primary endpoint is acceptable and consistent with previous programs for HAE prophylaxis.

Secondary and Exploratory Objective and Endpoints

The secondary objectives included characterizing the efficacy of donidalorsen (donidalorsen-4 or donidalorsen-8) on the quality and pattern of HAE attacks and their impact on quality of life. Only investigator-confirmed HAE attacks, as defined in Section [15.1.1](#), were included in the efficacy evaluation. The secondary endpoints, in the order of the multiplicity control hierarchy, were:

- The time-normalized number of investigator-confirmed HAE attacks per 4 weeks from Week 5 to Week 25 for the donidalorsen-4 group compared to pooled placebo
- Subjects with $\geq 70\%$ reduction from baseline in investigator-confirmed HAE attack rate between Week 5 to Week 25 in the donidalorsen-4 group compared to pooled placebo
- The percentage of investigator-confirmed HAE attack-free subjects from Week 5 to Week 25 in the donidalorsen-4 group compared to pooled placebo
- The time-normalized number of moderate or severe investigator-confirmed HAE attacks per 4 weeks from Week 5 to Week 25 in the donidalorsen-4 group compared to pooled placebo
- The number of investigator-confirmed HAE attacks requiring acute HAE therapy per 4 weeks from Week 5 to Week 25 in the donidalorsen-4 group compared to pooled placebo
- Change in Angioedema Quality of Life (AE-QoL) total score at Week 25 in the donidalorsen-4 group compared to pooled placebo
- The time-normalized number of investigator-confirmed HAE attacks per 4 weeks during the Treatment Period (from Week 1 to Week 25) for the donidalorsen-8 group compared to pooled placebo
- The time-normalized number of investigator-confirmed HAE attacks per 4 weeks during the Treatment Period (from Week 5 to Week 25) for the donidalorsen-8 group compared to pooled placebo
- Subjects with $\geq 70\%$ reduction from baseline in investigator-confirmed HAE attack rate between Week 5 to Week 25 in the donidalorsen-8 group compared to pooled placebo
- The percentage of investigator-confirmed HAE attack-free subjects from Week 5 to Week 25 in the donidalorsen-8 group compared to pooled placebo
- The time-normalized number of moderate or severe investigator-confirmed HAE attacks per 4 weeks from Week 5 to Week 25 in the donidalorsen-8 group compared to pooled placebo
- The number of investigator-confirmed HAE attacks requiring acute HAE therapy per 4 weeks from Week 5 to Week 25 in the donidalorsen-8 group compared to pooled placebo
- Change in AE-QoL total score at Week 25 in the donidalorsen-8 group compared to pooled placebo
- Change in AE-QoL domain score of Functioning at Week 25 in the donidalorsen-4 group compared to pooled placebo

- Change in AE-QoL domain score of Fears/Shame at Week 25 in the donidalorsen-4 group compared to pooled placebo
- Change in AE-QoL domain score of Fatigue/Mood at Week 25 in the donidalorsen-4 group compared to pooled placebo
- Change in AE-QoL domain score of Nutrition at Week 25 in the donidalorsen-4 group compared to pooled placebo
- Change in AE-QoL domain score of Functioning at Week 25 in the donidalorsen-8 group compared to pooled placebo
- Change in AE-QoL domain score of Fears/Shame at Week 25 in the donidalorsen-8 group compared to pooled placebo
- Change in AE-QoL domain score of Fatigue/Mood at Week 25 in the donidalorsen-8 group compared to pooled placebo
- Change in AE-QoL domain score of Nutrition at Week 25 in the donidalorsen-8 group compared to pooled placebo
- Percent of subjects who are well controlled on the Angioedema Control Test (AECT) at Week 25 in the donidalorsen-4 group compared to pooled placebo
- Percent of subjects who are well controlled on the AECT at Week 25 in the donidalorsen-8 group compared to pooled placebo

The secondary endpoints encompass the most clinically relevant endpoints that are typically evaluated in HAE prophylaxis programs. Some objectives/endpoints aimed to evaluate quality of life via the clinical outcome tools AE-QoL and AECT. These tools have not been found to be fit-for-purpose for the regulatory assessment of efficacy in HAE (see Section [6.2.2.4.6.2](#)), and as such, these endpoints are not appropriate to support efficacy or appear in product labeling.

6.2.2.2. Eligibility Criteria, Trial CS5

Key eligibility criteria are summarized below. Trial CS5 enrolled subjects ≥ 12 years of age with clinically confirmed HAE and an attack frequency that would likely warrant prophylactic HAE therapy. The inclusion and exclusion criteria are acceptable and consistent with those used in previous trials of approved products for HAE prophylaxis.

Key Inclusion Criteria

- Male or female ≥ 12 years of age at the time of informed consent.
- Diagnosed with clinically confirmed C1-INH (Type I or Type II) HAE based on all of the following:
 - Documented clinical history consistent with HAE (SC or mucosal, nonpruritic swelling episodes without accompanying urticaria).
 - 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.
 - At least one of the following: age at HAE onset ≤ 30 years, family history consistent with Type I or II HAE, or complement component 1q within the normal range.
- Experienced ≥ 2 HAE attacks during the Screening Period.
- Female subjects: nonpregnant, nonlactating, and either surgically sterile, postmenopausal, abstinent, or using acceptable contraceptive methods.
- Male subjects: either surgically sterile or using an acceptable contraceptive method.

Key Exclusion Criteria

- Concomitant diagnosis of another form of angioedema, such as idiopathic or acquired angioedema, recurrent angioedema associated with urticaria, or Type III HAE.
- Exposure to any of the following medications:
 - ACE inhibitors or any estrogen containing medications with systemic absorption (such as oral contraceptive or hormonal replacement therapy) within 4 weeks prior to Screening.
 - 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/Beriner 2000/3000® subcutaneously/Cinryze/Ruconest within 2 weeks prior to Screening, or Orladeyo within 3 weeks prior to Screening).
 - Oligonucleotides within 4 months of Screening (if a single administration) or 12 months of Screening (if multiple doses received); does not apply to vaccines.

- Any clinically significant abnormalities in Screening laboratory values that would render a patient unsuitable for inclusion; the following values are exclusionary:
 - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $>3\times$ upper limit of normal (ULN).
 - Total bilirubin $>1.5\times$ ULN or, if due to Gilbert's syndrome, >5 mg/dL.
 - Platelet count <130 K/mm³.
 - For patients ≥ 18 years old: eGFR <45 mL/min (per Chronic Kidney Disease Epidemiology Collaboration formula).
 - For patients 12 to <18 years old: eGFR <60 mL/min (per Bedside Schwartz formula).
- History of acquired coagulopathies or bleeding diathesis, inherited bleeding disorders, hypercoagulability.
- Any clinically significant renal or hepatic diseases.

6.2.2.3. Statistical Analysis Plan, Trial CS5

The statistical analysis plan (SAP) for Trial CS5 was Version 2 dated December 13, 2023. The primary efficacy endpoint was the time-normalized HAE attack rate per month (28 days) comparing the donidalorsen-4 group to pooled placebo for the full analysis set (FAS). The FAS was defined as all randomized subjects who received at least one dose of trial drug; subjects were analyzed according to the treatment group to which they were randomized. The definition of the Safety set was the same as the FAS. Only investigator-confirmed HAE attacks were included in the evaluation of efficacy for all primary and secondary endpoints. Treatment policy strategy was used for the intercurrent events of use of not allowed concomitant therapy or procedures and early termination of treatment, which included subjects escaping early to the OLE trial (CS7) due to experiencing ≥ 5 recurrent HAE attacks per month for 2 consecutive months during the Treatment Period, subjects discontinuing the trial treatment early due to recurrent HAE attacks and not enrolling in Trial CS7, and subjects discontinuing trial treatment early due to COVID-19 or war-related events.

Primary Analysis

The primary analysis used a Poisson regression model and Pearson chi-square scaling of standard errors to account for potential overdispersion on the FAS, including subjects from the donidalorsen-4, donidalorsen-8, and pooled placebo groups. The model included fixed effects for treatment group (three categories), baseline (the time-normalized Run-In Period attack rate, continuous), and the treatment-by-baseline interaction as covariates, and the logarithm of time each subject was observed from Week 1 to Week 25 as an offset variable.

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 donidalorsen-4 relative to the pooled placebo group and the corresponding 95% confidence interval were estimated. The comparison between donidalorsen-8 and pooled placebo was calculated from this model as a key secondary endpoint. These estimates were reported as mean

event rates per 4 weeks 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 HAE attack rate between donidalorsen-4 and the pooled placebo was calculated as $100\% \times (\text{model adjusted mean rate ratio} - 1)$. Similarly, the estimated upper and lower confidence limits for the model adjusted mean rate ratio were transformed by subtracting 1 and multiplying by 100% to calculate 95% confidence intervals for the percentage change.

Sensitivity and Supplementary Analyses

A sensitivity analysis was prespecified that investigated whether the results for the primary endpoint were robust to departures from the assumption that the mean and variance are equal, as in Poisson regression. The analysis was the same as the primary analysis, except that negative binomial regression was performed instead of Poisson regression.

The primary analysis assumed missing data were missing at random for subjects who discontinued the Treatment Period early. A sensitivity analysis was prespecified to investigate the robustness of the conclusions to the missing at random assumption. The analysis was performed on imputed data by using a multiple imputation method assuming jump to reference, used the Poisson regression model of the primary analysis, and combined the results using Rubin's rules.

A tipping point sensitivity analysis was prespecified to investigate the effect of missing data on the reliability of the primary efficacy analysis. A range of progressively more conservative assumptions about the number of events occurring in the post withdrawal period was explored to search for the assumption which would reverse the conclusion.

A supplementary analysis repeated the primary analysis and the negative binomial sensitivity analysis on the per-protocol set, which included all subjects in the FAS who were treated according to the protocol without any major deviations that could compromise the interpretation of efficacy.

Subgroup Analyses

The Applicant prespecified subgroup analyses based on the following:

- Age category in years (12 to 17, 18 to 39, 40 to 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)

The Agency conducted subgroup analyses by race (American Indian or Alaska Native, Asian, Black or African American, Multiple, White, Other), by ethnicity (Hispanic or Latino, Not

Hispanic or Latino), and by HAE type (Type I, Type II). The final SAP specified that HAE attacks (per 4 weeks) from Week 1 to Week 25 would be summarized by each subgroup. The primary endpoint was also analyzed by adding region and the interaction of region and treatment group as covariates. The Agency analyzed all other subgroups similarly to the prespecified analysis of region.

A subgroup analyses that excluded subjects considered to be not “typical patients” was prespecified. These subjects were identified prior to trial unblinding based on clinical history and/or Screening Period characteristics considered to be atypical for Type I/II HAE. The analyses restricted the analysis set to “typical patients” and reevaluated the primary endpoint of time-normalized number of HAE attacks (per 4 weeks) from Week 1 to 25, as well as the secondary endpoints from Week 5 to 25 of time-normalized number of HAE attacks (per 4 weeks), time-normalized number of moderate or severe HAE attacks (per 4 weeks), and time-normalized number of HAE attacks requiring acute therapy (per 4 weeks).

Additional analyses by HAE attack location and severity were completed by the Agency to inform regulatory decision-making, presented in Section [6.2.2.4.6.4](#).

Secondary Endpoints

The Applicant prespecified key secondary endpoints for both the comparison between donidalorsen-4 and pooled placebo, and the comparison between donidalorsen-8 and pooled placebo. All methods used data from all subjects in the FAS and for each endpoint the same model was used to calculate comparisons between both treatment groups and pooled placebo. Section [6.2.2.1](#) describes the order of multiplicity-controlled hierarchical testing of the secondary endpoints. At each level of the hierarchy, a test was performed at two-sided alpha of 5% if the test of the previous null hypothesis was rejected.

The time-normalized number of HAE attacks (per 4 weeks) from Week 5 to Week 25 was analyzed using the same methods as described for the primary efficacy endpoint, based on the Poisson regression model on FAS with the treatment policy strategy. The time-normalized number of moderate or severe HAE attacks (per 4 weeks) from Week 5 to Week 25 and the time-normalized number of HAE attacks (per 4 weeks) requiring acute therapy from Week 5 to Week 25 were analyzed similarly.

A responder analysis was conducted for subjects who achieved a clinical response, defined as a $\geq 70\%$ reduction from baseline in HAE attack rate. The percentage reduction is defined as:

$$(\text{Postbaseline rate} - \text{Baseline rate}) \div \text{Baseline rate} \times 100\%$$

The postbaseline is the HAE attack rate from Week 5 to Week 25 and baseline is Run-In Period HAE attack rate.

The odds ratio, corresponding 95% confidence intervals, and p-value were calculated using a logistic regression model with the baseline, treatment, and the treatment by-baseline interaction as covariates. The clinical response of HAE attack-free from Week 5 to Week 25 was analyzed using a similar logistic regression model. Exploratory analyses which defined clinical response as $\geq 50\%$ and $\geq 90\%$ reduction from baseline in HAE attack rate were conducted similarly.

6.2.2.4. Results of Analyses, Trial CS5

This section summarizes subject disposition, baseline demographics and clinical characteristics, and the primary, secondary, and exploratory efficacy results for the endpoints listed in Section [6.2.2.1](#). The results of additional analyses of interest to the Agency are also reported.

6.2.2.4.1. Subject Disposition, Trial CS5

Disposition information is presented in [Table 8](#) and [Table 9](#). A total of 91 subjects were randomized. One subject withdrew consent prior to receiving trial drug and 90 subjects received at least one dose of trial drug. Lack of efficacy was defined per protocol as subjects who experienced at least five HAE attacks per month for 2 consecutive months after Week 5. One subject in each of the donidalorsen groups and three subjects in the pooled placebo group discontinued the trial drug due to lack of efficacy, and these subjects were rolled over into the open-label extension trial (CS7). One subject in the donidalorsen-8 group discontinued trial drug due to the adverse event of ALT increased. One subject in the pooled placebo group discontinued trial drug due to pregnancy.

Most subjects completed the Trial CS5 treatment and then rolled over to Trial CS7 for open-label extended treatment.

Table 8. Subject Screening and Enrollment, Trial CS5

Disposition	Trial CS5
Screened	116
Screening failures	25
Inclusion/exclusion criteria	21
Withdrawal of consent	4
Randomized	91

Source: Clinical study report Section 14 Table 14.1.2 and Listing 16.2.1.1-2; findings reproduced by the statistical reviewer using ds.xpt

Table 9. Subject Disposition, Trial CS5

Disposition Outcome	Donidalorsen	Donidalorsen	Pooled Placebo
	80 mg Every 8 Weeks	80 mg Every 4 Weeks	
Randomized	23 (100)	46 (100)	22 (100)
Analysis set			
FAS	23 (100)	45 (97.8)	22 (100)
PP	21 (91.3)	43 (93.5)	20 (90.9)
Safety population	23 (100)	45 (97.8)	22 (100)
Completed CS5 trial treatment	21 (91.3)	44 (95.7)	18 (81.8)
Discontinued trial	20 (87)	45 (97.8)	22 (100)
Pregnancy	0	0	1 (4.5)
Roll over into CS7	20 (87.0)	44 (95.7)	19 (86.4)
Voluntary withdrawal	0	1 (2.2)	2 (9.1)

Disposition Outcome	Donidalorsen 80 mg Every 8 Weeks	Donidalorsen 80 mg Every 4 Weeks	Pooled Placebo
Discontinued trial drug	2 (8.7)	2 (4.3)	4 (18.2)
Adverse event	1 (4.3)	0	0
Lack of efficacy	1 (4.3)	1 (2.2)	3 (13.6)
Pregnancy	0	0	1 (4.5)
Voluntary withdrawal	0	1 ^a (2.2)	0

Source: Clinical study report Table 7 and Table 9 (Pages 70-71, 73); findings reproduced by statistical reviewer using ds.xpt and adsl.xpt

Duration is up to 24 weeks.

All values expressed as n (%) unless stated otherwise. Percentages out of number of subjects randomized.

^a One subject withdrew consent after randomization but prior to receiving trial drug.

Abbreviations: FAS, full analysis set; PP, per-protocol

6.2.2.4.2. Baseline Demographics and Clinical Characteristics, Trial CS5

The demographics and clinical characteristics of the Full Analysis Set at baseline are summarized in [Table 10](#) and were reasonably similar between the treatment groups given the small sample size. Seven adolescents were enrolled, with none randomized to the placebo group; acknowledging that this is small, note that the pathophysiology of HAE Type I or II is similar across age groups. Racial and ethnic group representation in Trial CS5 is congruent with the overall subject population. Most subjects had Type I HAE (93%) and a history of laryngeal HAE attacks (52%; data not shown). Some subjects (17%) were previously on prophylaxis treatment. A total of seven (8%) subjects continued their allowed androgen or tranexamic acid prophylaxis (six (7%) on danazol and one (1%) on tranexamic acid; data not shown). Enrollment occurred at sites in the United States (20%) and outside the United States.

Table 10. Baseline Demographics and Clinical Characteristics, Full Analysis Set Population, Trial CS5

Demographic Characteristic	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22	Total N=90
Sex, n (%)				
Female	12 (52.2)	28 (62.2)	8 (36.4)	48 (53.3)
Male	11 (47.8)	17 (37.8)	14 (63.6)	42 (46.7)
Age, years				
Mean (SD)	34.1 (13.2)	39.6 (15.2)	35.4 (11.0)	37.2 (13.9)
Median	33.0	39.0	33.0	35.5
IQR	24, 45	29, 52	27, 44	27, 48
Min, max	12, 66	12, 68	21, 59	12, 68
Age categories, n (%)				
12-17	3 (13.0)	4 (8.9)	0	7 (7.8)
18-39	12 (52.2)	19 (42.2)	15 (68.2)	46 (51.1)
40-64	7 (30.4)	21 (46.7)	7 (31.8)	35 (38.9)
≥65	1 (4.3)	1 (2.2)	0	2 (2.2)

Demographic Characteristic	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22	Total N=90
Race, n (%)				
American Indian or Alaska Native	1 (4.3)	0	2 (9.1)	3 (3.3)
Asian	0	1 (2.2)	0	1 (1.1)
Black or African American	0	1 (2.2)	1 (4.5)	2 (2.2)
Multiple	0	0	1 (4.5)	1 (1.1)
White	22 (95.7)	42 (93.3)	18 (81.8)	82 (91.1)
Other	0	1 (2.2)	0	1 (1.1)
Ethnicity, n (%)				
Hispanic or Latino	3 (13.0)	2 (4.4)	1 (4.5)	6 (6.7)
Not Hispanic or Latino	20 (87.0)	43 (95.6)	21 (95.5)	84 (93.3)
Baseline BMI (kg/m²)				
Mean (SD)	27.0 (5.4)	28.0 (6.7)	28.9 (9.2)	28.0 (7.1)
Median	26.2	26.8	26.0	26.3
IQR	22.8, 32.2	23.8, 31.1	23.7, 32.2	23.1, 31.9
Min, max	19.7, 36.8	16.0, 43.6	18.9, 57.4	16.0, 57.4
Missing	0	0	1	1
Analysis country, n (%)				
Belgium	0	1 (2.2)	0	1 (1.1)
Bulgaria	0	3 (6.7)	0	3 (3.3)
Canada	0	2 (4.4)	1 (4.5)	3 (3.3)
France	1 (4.3)	2 (4.4)	0	3 (3.3)
Germany	0	4 (8.9)	0	4 (4.4)
Israel	2 (8.7)	0	3 (13.6)	5 (5.6)
Italy	3 (13.0)	5 (11.1)	1 (4.5)	9 (10.0)
Netherlands	2 (8.7)	5 (11.1)	1 (4.5)	8 (8.9)
Poland	1 (4.3)	2 (4.4)	2 (9.1)	5 (5.6)
Spain	1 (4.3)	3 (6.7)	2 (9.1)	6 (6.7)
Turkey	6 (26.1)	10 (22.2)	4 (18.2)	20 (22.2)
United Kingdom	3 (13.0)	1 (2.2)	1 (4.5)	5 (5.6)
United States	4 (17.4)	7 (15.6)	7 (31.8)	18 (20.0)
HAE subtype, n (%)				
Type I	22 (95.7)	42 (93.3)	20 (90.9)	84 (93.3)
Type II	1 (4.3)	3 (6.7)	2 (9.1)	6 (6.7)
Number of attacks in the 12 months prior to Screening				
Mean (SD)	33.3 (21.95)	45.7 (43.04)	29.1 (21.13)	38.5 (34.57)
Median	36.0	36.0	24.0	35.0
IQR	14.0, 40.0	15.0, 57.0	12.0, 47.0	14.0, 47.8
Min, max	1.0, 80.0	0.0, 208.0	1.0, 75.0	0.0, 208.0
Family history of HAE, n (%)	20 (87.0)	41 (91.1)	17 (77.3)	78 (86.7)
Previous prophylactic use^a, n (%)	5 (21.7)	6 (13.3)	5 (22.7)	16 (17.8)
Screening Period HAE attack rate per 4-weeks, n (%)				
<2	10 (43.5)	12 (26.7)	6 (27.3)	28 (31.1)
≥2	13 (56.5)	33 (73.3)	16 (72.7)	62 (68.9)

Source: Clinical study report Table 10 and Table 11 (Pages 74-75, 76-77); findings reproduced by statistical reviewer using adsl.xpt and adefx.xpt

^a Previous prophylactic use defined as first-line prophylactic agents and excluded attenuated androgens and tranexamic acid. Abbreviations: BMI, body mass index; HAE, hereditary angioedema; IQR, interquartile range; max, maximum; min, minimum; SD, standard deviation

6.2.2.4.3. Primary Efficacy Results, Trial CS5

The primary efficacy endpoint was the time-normalized number of HAE attacks per 4 weeks from Week 1 to Week 25. This endpoint is clinically meaningful and consistent with previous programs for HAE prophylaxis. Details regarding the reporting, collection, and investigator confirmation of HAE attacks are provided in Section [15.1.1](#).

[Table 11](#) summarizes the efficacy results for the time-normalized number of HAE attacks per 4 weeks from Week 1 to Week 25 for the primary endpoint comparison between donidalorsen-4 and pooled placebo, as well as the key secondary results for the comparison between donidalorsen-8 and pooled placebo. Note that the latter comparison was tested eighth in the Applicant’s prespecified testing hierarchy, and all preceding endpoints achieved statistical significance. After adjusting for baseline attack rate by treatment group, the estimated least square mean time-normalized number of HAE attacks per 4 weeks was lower in both the donidalorsen-4 (LS mean =0.44, 95% CI =0.27, 0.73) and donidalorsen-8 (LS mean =1.02, 95% CI =0.65, 1.59) groups compared to pooled placebo (LS mean =2.26, 95% CI =1.66, 3.09). The estimated attack rate ratio relative to placebo was 0.19 (95% CI =0.11, 0.35) for the donidalorsen-4 group and 0.45 (95% CI =0.26, 0.78) for the donidalorsen-8 group. Additionally, the percentage reduction in the means for the donidalorsen-4 group was 81% (95% CI =65%, 89%) and for the donidalorsen-8 group was 55% (95% CI =22%, 74%) relative to placebo.

Table 11. Primary Analysis: Time-Normalized HAE Attack Rate Per 4 Weeks From Week 1 to Week 25 in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22
	Screening Period HAE attack rate, mean ^a (SD)	3.18 (2.15)	3.61 (2.24)
Time-normalized HAE attack rate from Week 1 to Week 25, mean ^b (SD)	1.19 (1.63)	0.89 (1.85)	2.29 (1.81)
Least square mean rate ^c (95% CI)	1.02 (0.65, 1.59)	0.44 (0.27, 0.73)	2.26 (1.66, 3.09)
Model adjusted mean rate ratio ^c (95% CI)	0.45 (0.26, 0.78)	0.19 (0.11, 0.35)	-
Percentage difference relative to pooled placebo ^d (95% CI)	-55 (-74, -22)	-81 (-89, -65)	-
p-value	0.004	<0.0001	-

Source: Clinical study report Table 14 (Page 86) and Table 15 (Page 91); findings reproduced by the statistical reviewer using adefx.xpt

^a The Screening Period HAE attack rate (per 4 weeks) for each subject was calculated as the number of HAE attacks that occurred during the Screening Period divided by the number of days the subject contributed to the Screening Period, and then multiplied by 28.

^b The time-normalized HAE attack rate (per 4 weeks) was calculated as number of HAE attacks that occurred from Week 1 to Week 25, divided by the number of days the subject contributed to that period, and then multiplied by 28.

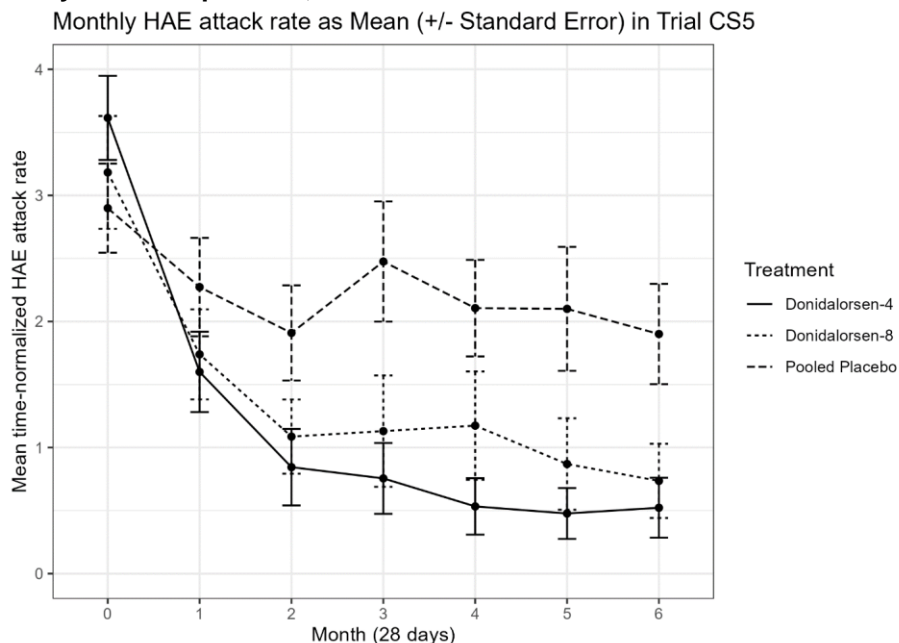
^c The Poisson regression model included treatment groups, baseline (the Screening Period HAE attack rate), the treatment-by-baseline interaction as a covariate, and the logarithm of time in every 4 weeks that each subject was observed from Week 1 to Week 25 used as an offset variable (e.g., log (6) if the subject stayed in the trial for 24 weeks). Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

^d The percentage difference in mean 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 were 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; SE, standard error

The mean HAE attack rate per 4 weeks from Week 1 through Week 25 with standard error bars is shown in [Figure 6](#).

Figure 6. Plot of Time-Normalized HAE Attack Rate Per 4 Weeks From Week 1 to Week 25 in Full Analysis Set Population, Trial CS5



Source: Statistical Reviewer Analysis; adefx.xpt
Abbreviations: HAE, hereditary angioedema

The Agency was able to replicate the Applicant's analyses of the primary endpoint, and the results demonstrate superiority compared to placebo for donidalorsen-4 and donidalorsen-8 in Trial CS5.

6.2.2.4.4. Key Secondary Efficacy Results, Trial CS5

[Table 12](#) presents the results for the time-normalized number of HAE attacks per 4 weeks from Week 5 to Week 25 for the comparison of both donidalorsen-4 and donidalorsen-8 to pooled placebo. After adjusting for baseline attack rate by treatment group, the estimated least square mean time-normalized number of HAE attacks per 4 weeks was lower in both the donidalorsen-4 (LS mean =0.30, 95% CI =0.15, 0.58) and donidalorsen-8 (LS mean =0.90 95% CI =0.53, 1.52) groups compared to pooled placebo (LS mean =2.25, 95% CI =1.59, 3.18). The estimated attack rate ratio relative to placebo was 0.13 (95% CI =0.06, 0.28) for the donidalorsen-4 group and 0.40 (95% CI =0.21, 0.75) for the donidalorsen-8 group. Additionally, the percentage reduction in the means for the donidalorsen-4 group was 87% (95% CI =72%, 94%) and for the donidalorsen-8 group was 60% (95% CI =25%, 79%) relative to placebo. The results of analyses of this secondary endpoint agree with the primary efficacy results. This provides limited additional information for evidence of effectiveness of the trial drug as it is the same as the primary endpoint with a shortened time window starting at Week 5.

Table 12. Secondary Analysis: Time-Normalized HAE Attack Rate From Week 5 to Week 25 in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22
Screening Period HAE attack rate, mean ^a (SD)	3.18 (2.15)	3.61 (2.24)	2.90 (1.66)
Time-normalized HAE attack rate from Week 5 to Week 25, mean ^b (SD)	1.09 (1.71)	0.75 (1.90)	2.32 (1.93)
Least square mean rate ^c (95% CI)	0.90 (0.53, 1.52)	0.30 (0.15, 0.58)	2.25 (1.59, 3.18)
Model adjusted mean rate ratio ^c (95% CI)	0.40 (0.21, 0.75)	0.13 (0.06, 0.28)	-
Percentage difference relative to pooled placebo ^d (95% CI)	-60 (-79, -25)	-87 (-94, -72)	-
p-value	0.004	<0.0001	-

Source: Clinical study report Table 16 (Page 92-93); findings reproduced by the statistical reviewer using adefx.xpt

^a The Screening Period attack rate (per 4 weeks) for each subject was calculated as the number of HAE attacks that occurred during the Screening Period divided by the number of days the subject contributed to the Screening Period, and then multiplied by 28.

^b The time-normalized HAE attack rate (per 4 weeks) was calculated as number of HAE attacks that occurred from Week 5 to Week 25, divided by the number of days the subject contributed to that period, and then multiplied by 28.

^c The Poisson regression model included treatment groups, baseline (the Screening Period HAE attack rate), the treatment-by-baseline interaction as a covariate, and the logarithm of time in every 4 weeks that each subject was observed from Week 5 to Week 25 used as an offset variable. Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

^d The percentage difference in mean 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 were 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; SE, standard error

[Table 13](#) presents the results for the clinical response of $\geq 70\%$ reduction from baseline in HAE attack rate from Week 5 to Week 25 for the comparison of both donidalorsen-4 and donidalorsen-8 to pooled placebo. A total of 56/90 subjects (62%) experienced clinical response by this definition, of which 37 were in the donidalorsen-4 group (82% of the donidalorsen-4 group), 15 were in the donidalorsen-8 group (65% of the donidalorsen-8 group), and 4 were in the pooled placebo group (18% of the pooled placebo group). After adjusting for baseline HAE attack rate by treatment group, the odds ratio of clinical response of $\geq 70\%$ reduction from baseline in HAE attack rate relative to pooled placebo was 34.74 (95% CI = 7.32, 164.87) for donidalorsen-4 and 9.17 (95% CI = 2.05, 41.09) for donidalorsen-8. Exploratory analyses considered the clinical response of $\geq 50\%$ and $\geq 90\%$ reduction from baseline in HAE attack rate between Week 5 to Week 25 and the proportion of responders, respectively, were 42/45 (93%) and 28/45 (62%) in the donidalorsen-4 group, 19/22 (83%) and 11/22 (48%) in the donidalorsen-8 group, and 6/23 (27%) and 2/23 (9%) in the pooled placebo group. These results provide further evidence supporting the efficacy of donidalorsen.

Table 13. Secondary Analysis: Clinical Response of $\geq 70\%$ Reduction From Baseline in HAE Attack Rate From Week 5 to Week 25 in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22
	Number (%) of subjects with $\geq 70\%$ reduction from baseline in HAE attack rate	15 (65.2)	37 (82.2)
Odds ratio (95% CI)	9.17 (2.05, 41.09)	34.74 (7.32, 164.87)	-
p-value	0.004	<0.0001	-

Source: Clinical study report Table 19 (Page 101); findings reproduced by the statistical reviewer using adefx.xpt
The logistic regression model included treatment groups, Baseline (the Screening Period HAE attack rate), the Treatment-by-Baseline interaction as a covariate.

Abbreviations: CI, confidence interval; HAE, hereditary angioedema; SD, standard deviation; SE, standard error

[Table 14](#) presents the results for the clinical response of being HAE attack-free (i.e., experiencing zero HAE attacks) from Week 5 to Week 25 for the comparison of both donidalorsen-4 and donidalorsen-8 to pooled placebo. A total of 34/90 subjects (38%) did not experience any HAE attacks from Week 5 to Week 25, of which 24 were in the donidalorsen-4 group (53% of the donidalorsen-4 group), 8 were in the donidalorsen-8 group (35% of the donidalorsen-8 group), and 2 were in the pooled placebo group (9% of the pooled placebo group). After adjusting for baseline HAE attack rate by treatment group, the odds ratio of being HAE attack-free relative to pooled placebo was 11.79 (95% CI =2.34, 59.36) for donidalorsen-4 and 3.23 (95% CI =0.46, 22.85) for donidalorsen-8. The comparison of donidalorsen-8 to pooled placebo for the clinical response of being HAE attack-free from Week 5 to Week 25 was the first test in the prespecified testing hierarchy that failed to achieve statistical significance.

Table 14. Secondary Analysis: Clinical Response of HAE Attack-Free From Week 5 to Week 25 in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22
	Number (%) of subjects HAE attack-free from Week 5 to Week 25	8 (34.8)	24 (53.3)
Odds ratio (95% CI)	3.23 (0.46, 22.85)	11.79 (2.34, 59.36)	-
p-value	0.24	0.003	-

Source: Clinical study report Table 20 (Page 102); findings reproduced by the statistical reviewer using adefx.xpt
The logistic regression model included treatment groups, baseline (the Run-In Period HAE attack rate), the treatment-by-baseline interaction as a covariate.

Abbreviations: CI, confidence interval; HAE, hereditary angioedema; SD, standard deviation; SE, standard error

[Table 15](#) presents the results for the time-normalized number of moderate or severe HAE attacks per 4 weeks from Week 5 to Week 25 for the comparison of both donidalorsen-4 and donidalorsen-8 to pooled placebo; refer to Section [15.1.1](#) for the severity grading guidelines for HAE attacks. After adjusting for baseline moderate or severe HAE attack rate by treatment group, the estimated least square mean time-normalized number of moderate or severe HAE attacks per 4 weeks was numerically lower in both the donidalorsen-4 (LS mean =0.12, 95% CI =0.04, 0.35) and donidalorsen-8 (LS mean =0.68, 95% CI =0.37, 1.23) groups compared to pooled placebo (LS mean =1.15, 95% CI =0.72, 1.83). The estimated attack rate ratio relative to placebo was 0.11 (95% CI =0.04, 0.34) for the donidalorsen-4 group and 0.59 (95% CI =0.28, 1.26) for the donidalorsen-8 group. Additionally, the percentage reduction in the LS means for

the donidalorsen-4 group was 89% (95% CI =66%, 97%) and for the donidalorsen-8 group was 41% (95% CI =-26%, 72%) relative to placebo.

Table 15. Secondary Analysis: Time-Normalized Moderate or Severe HAE Attack Rate From Week 5 to Week 25 in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen	Donidalorsen	Pooled Placebo N=22
	80 mg Every 8 Weeks N=23	80 mg Every 4 Weeks N=45	
Screening Period moderate or severe HAE attack rate, mean ^a (SD)	2.04 (2.10)	2.38 (2.02)	2.03 (1.85)
Time-normalized moderate or severe HAE attack rate from Week 5 to Week 25, mean ^b (SD)	0.90 (1.62)	0.37 (1.03)	1.35 (1.71)
Least square mean rate ^c (95% CI)	0.68 (0.37, 1.23)	0.12 (0.04, 0.35)	1.15 (0.72, 1.83)
Model adjusted mean rate ratio ^c (95% CI)	0.59 (0.28, 1.26)	0.11 (0.04, 0.34)	-
Percentage difference relative to pooled placebo ^d (95% CI)	-41 (-72, 26)	-89 (-97, -66)	-
p-value	NS ^e	0.0001	-

Source: Clinical study report Table 17 (Page 94); findings reproduced by the statistical reviewer using adefx.xpt

^a The Screening Period moderate or severe HAE attack rate (per 4 weeks) for each subject was calculated as the number of moderate or severe HAE attacks that occurred during the Screening Period divided by the number of days the subject contributed to the Screening Period, and then multiplied by 28.

^b The time-normalized HAE attack rate (per 4 weeks) was calculated as number of moderate or severe HAE attacks that occurred from Week 5 to Week 25, divided by the number of days the subject contributed to that period, and then multiplied by 28.

^c The Poisson regression model included treatment groups, baseline (the Screening Period moderate or severe HAE attack rate), the Treatment-by-baseline interaction as a covariate, and the logarithm of time in every 4 weeks that each subject was observed from Week 5 to Week 25 used as an offset variable. Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

^d The percentage difference in mean HAE attack rate between donidalorsen and placebo groups were 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% confidence intervals for the percentage change.

^e Endpoint was not tested due to nonsignificant result higher in testing hierarchy.

Abbreviations: CI, confidence interval; HAE, hereditary angioedema; NS, not significant; SD, standard deviation; SE, standard error

Table 16 presents the results for the time-normalized number of HAE attacks requiring acute therapy per 4 weeks from Week 5 to Week 25 for the comparison of both donidalorsen-4 and donidalorsen-8 to pooled placebo. After adjusting for rate of baseline HAE attack rates requiring acute therapy by treatment group, the estimated least square mean time-normalized number of HAE attacks requiring acute therapy per 4 weeks was numerically lower in both the donidalorsen-4 (LS mean =0.15, 95% CI =0.06, 0.39) and donidalorsen-8 (LS mean =0.59, 95% CI =0.31, 1.15) groups compared to pooled placebo (LS mean =1.80, 95% CI =1.23, 2.62). Only the comparison between donidalorsen-4 and pooled placebo achieved statistical significance in the prespecified testing hierarchy. The estimated attack rate ratio relative to placebo was 0.08 (95% CI =0.03, 0.23) for the donidalorsen-4 group and 0.33 (95% CI =0.16, 0.71) for the donidalorsen-8 group. Additionally, the percentage reduction in the means for the donidalorsen-4 group was 92% (95% CI =77%, 97%) and for the donidalorsen-8 group was 67% (95% CI =29%, 85%) relative to placebo.

Table 16. Secondary Analysis: Time-Normalized HAE Attacks Requiring Acute Therapy (Per 4 Weeks) From Week 5 to Week 25 in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22
	Run-In Period HAE attack requiring acute therapy rate, mean ^a (SD)	2.79 (2.30)	2.53 (2.18)
Time-normalized HAE attack requiring acute therapy rate from Week 5 to Week 25, mean ^b (SD)	0.94 (1.69)	0.46 (1.29)	1.88 (1.83)
Least square mean rate ^c (95% CI)	0.59 (0.31, 1.15)	0.15 (0.06, 0.39)	1.80 (1.23, 2.62)
Model adjusted mean rate ratio ^c (95% CI)	0.33 (0.16, 0.71)	0.08 (0.03, 0.23)	-
Percentage difference relative to pooled placebo ^d (95% CI)	-67 (-85, -29)	-92 (-97, -77)	-
p-value	NS ^e	<0.0001	-

Source: Clinical study report Table 18 (Page 98); findings reproduced by the statistical reviewer using adefx.xpt

^a The Run-In Period (screening HAE attack rate (per 4 weeks) requiring acute therapy for each subject was calculated as the number of HAE attacks requiring acute therapy that occurred during the Run-In Period divided by the number of days the subject contributed to the Run-In Period, and then multiplied by 28.

^b The time-normalized HAE attack rate (per 4 weeks) was calculated as number of HAE attacks requiring acute therapy that occurred from Week 5 to Week 25, divided by the number of days the subject contributed to that period, and then multiplied by 28.

^c The Poisson regression model included treatment groups, baseline (the Run-In Period HAE attack rate requiring acute therapy), the treatment-by-baseline interaction as a covariate, and the logarithm of time in every 4 weeks that each subject was observed from Week 5 to Week 25 used as an offset variable (e.g., log (6) if the subject stayed in the trial for 24 weeks). Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

^d The percentage difference in mean 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.

^e Endpoint was not tested due to nonsignificant result higher in testing hierarchy.

Abbreviations: CI, confidence interval; HAE, hereditary angioedema; NS, not significant; SD, standard deviation; SE, standard error

The Applicant proposed to include the endpoint of change in AE-QoL total score at Week 25 in the testing hierarchy. However, AE-QoL has been determined to be not fit-for-purpose for HAE (see Section [6.2.2.4.6.2](#)), and so results related to AE-QoL are not presented here.

Multiplicity-Controlled Testing

Hierarchical testing at two-sided alpha of 5% was performed. The primary endpoint of the time-normalized number of HAE attacks per 4 weeks from Week 1 to Week 25 comparing donidalorsen-4 to pooled placebo was statistically significant. Secondary endpoint comparisons between donidalorsen-4 and pooled placebo considering data from Week 5 to Week 25 were statistically significant for time-normalized number of HAE attacks per 4 weeks, clinical response of $\geq 70\%$ reduction from baseline in HAE attack rate, clinical response of being HAE attack-free, time-normalized number of moderate or severe HAE attacks per 4 weeks, and time-normalized number of HAE attacks requiring acute therapy per 4 weeks. Although change in AE-QoL total score at Week 25 was specified next in the testing hierarchy and statistically significant per the Applicant's analysis, AE-QoL has been determined to be not fit-for-purpose for HAE (see Section [6.2.2.4.6.2](#)). Secondary endpoints comparing donidalorsen-8 to pooled placebo were statistically significant for the time-normalized number of HAE attacks per 4 weeks from Week 1 to Week 25, the time-normalized number of HAE attacks per 4 weeks from Week 5 to Week 25, and clinical response of $\geq 70\%$ reduction from baseline in HAE attack rate. Clinical response of being HAE attack-free from Week 5 to Week 25 comparing donidalorsen-8 to pooled placebo was not statistically significant, and so no further hierarchical testing was conducted.

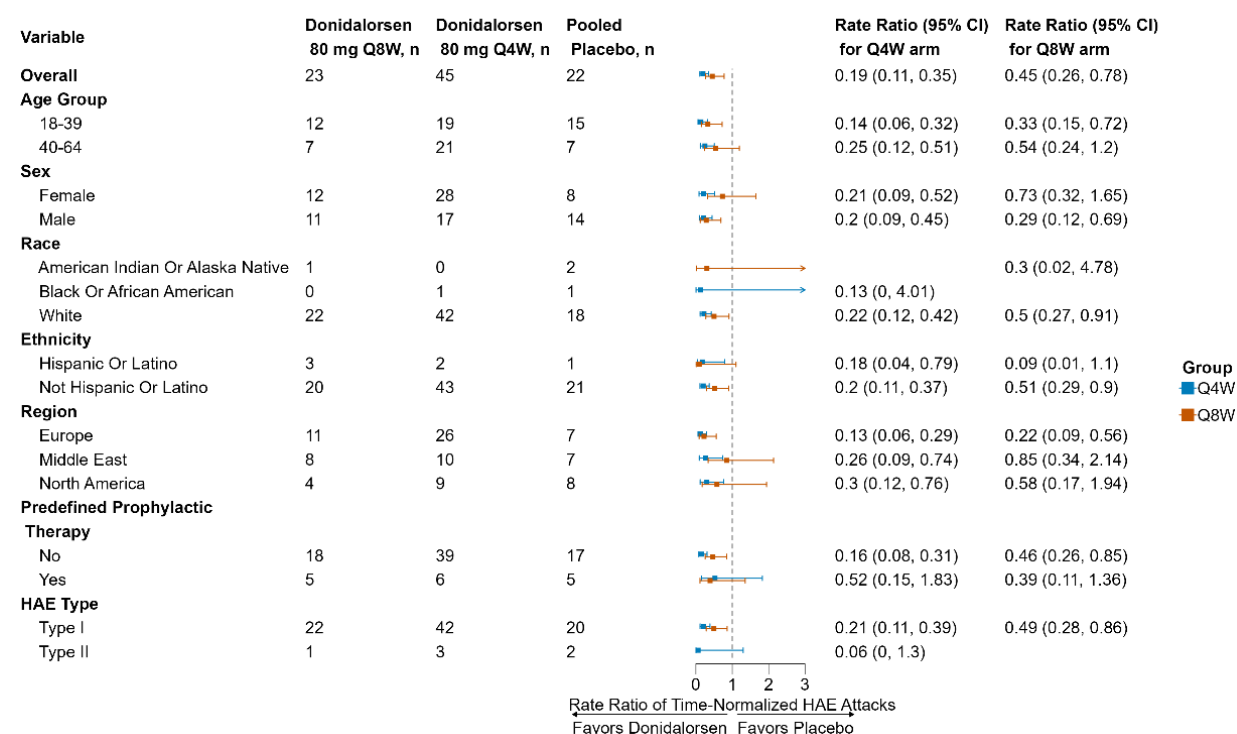
The efficacy endpoints in the multiplicity control hierarchy are generally supportive and are clinically and statistically convincing of the efficacy of donidalorsen at either donidalorsen-4 or donidalorsen-4 dosing regimens compared to placebo.

6.2.2.4.5. Subgroup Analyses, Trial CS5

The rate ratio of the time-normalized number of HAE attacks for the prespecified subgroups are presented in [Figure 7](#). No subjects aged 12 to 17 or ≥ 65 years of age were randomized to pooled placebo, and so no estimation of comparisons between donidalorsen and pooled placebo could be performed; these subgroups are not depicted in [Figure 7](#). One subject was enrolled in each of the race subgroups of Asian, Multiple, and Other, and so these subgroups have no comparisons between treatment groups; these subgroups are not depicted in [Figure 7](#). The rate ratios of the time-normalized number of HAE attacks per 4 weeks from Week 1 to Week 25 comparing both donidalorsen groups to pooled placebo were generally consistent across subgroups defined by age group, sex, race, ethnicity, region, predefined prophylactic therapy use, and HAE type.

Figure 7. Subgroup Analysis: Forest Plot of the Rate Ratio of the Time-Normalized Number of HAE Attacks Per 4 Weeks From Week 1 to Week 25 in Full Analysis Set Population, Trial CS5

Forest Plot of the Rate Ratio of the Time-Normalized Number of HAE Attacks per 4 Weeks from Week 1 to Week 25 Between Donidalorsen and Placebo



Source: Response to Agency Information Request dated February 14, 2025, Ad hoc Figure 14.2.1.5 (Pages 2-3); findings reproduced by the statistical reviewer using adeff.xpt
Abbreviations: CI, confidence interval; HAE, hereditary angioedema; n, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks

The rate ratio of time-normalized HAE attacks from Week 1 to Week 25 favored donidalorsen-4 compared to pooled placebo for all subgroups and for most subgroups. The 95% confidence

intervals overlapped with the null value of 1 for the female, Black or African American, Hispanic or Latino, and Yes predefined prophylactic therapy subgroups. The sample sizes in these four subgroups were small.

The rate ratio of time-normalized HAE attacks from Week 1 to Week 25 favored donidalorsen-8 compared to pooled placebo for all subgroups, but the 95% confidence intervals excluded the null value of 1 for most subgroups. Subgroups with 95% confidence intervals that excluded the null value of 1 were age group 18 to 39 years, male, White, Not Hispanic or Latino, region Europe, No predefined prophylactic therapy, and HAE Type I. The overall sample size was smaller for the donidalorsen-8 group than the donidalorsen-4 group by design, and so the subgroup sample sizes are small.

Given that the pathophysiology of the disease and response to therapy are expected to be the same across age groups, the efficacy of donidalorsen in adolescents is extrapolated from the adult efficacy results and supported by matching PK and PD exposure between the age groups (refer to Section [8.1.1](#)).

The efficacy of donidalorsen in geriatric patients was not adequately explored in Trial CS5 due to the limited enrollment of subjects aged 65 years and older (n=2; one enrolled in each donidalorsen treatment arm, 0 randomized to placebo). Although efficacy in this age group has not been established by direct evidence, it may be inferred from the overall efficacy results.

Conclusion

The interpretation of the subgroup analyses above is limited by small sample sizes. However, the treatment benefit of donidalorsen-4 and donidalorsen-8 based on the primary endpoint appears to be similar across subgroups based on age, sex, race, ethnicity, region, predefined prophylactic use, and HAE type.

6.2.2.4.6. Additional Efficacy Analyses, Trial CS5

6.2.2.4.6.1. Sensitivity Analysis, Trial CS5

Results of the sensitivity analyses based on negative binomial regression and multiple imputation using jump to reference were consistent with the primary analysis of the primary endpoint of time-normalized number of HAE attacks (per 4 weeks) from Week 1 to 25.

The number of subjects who discontinued early from the FAS was four in the pooled placebo group, one in the donidalorsen-4 group, and two in the donidalorsen-8 group. Of these subjects, three had at least 5 months of follow-up, three had between 3 and 5 months of follow-up, and one had almost 3 months of follow-up. The tipping point analysis investigated the effect of this missing data on the results of the primary analysis. The range of plausible assumptions explored did not identify a tipping point that would reverse the conclusion of the primary analysis.

Six subjects who were from the FAS were excluded from the per-protocol set. Results of the supplementary analysis based on the per-protocol set using both Poisson and negative binomial regression were consistent with the primary analysis.

6.2.2.4.6.2. Angioedema Quality of Life Total Score, Trial CS5

Issue

Determine whether the AE-QoL total score is fit-for-purpose for the context of use, that is, to support regulatory evaluation of effectiveness (b) (4)

(b) (4)

Background

HAE is characterized by unpredictable, recurrent episodes of angioedema, which impact quality of life and daily functioning. At the HAE Patient-Focused Drug Development meeting held at the Agency on September 25, 2017 ([CBER 2018](#)), patients with HAE shared the aspects of the disease that had the greatest impact on their lives, including the unpredictability of attack occurrence, the painful abdominal attacks, the dramatic change swelling had on their physical appearance, and the emotional toll the disease had on their lives.

The AE-QoL was used to derive secondary and exploratory endpoints, (b) (4)

(b) (4) There is currently no fit-for-purpose patient-reported outcomes (PROs) for use in HAE prophylaxis clinical trials.

During the course of the donidalorsen clinical development program, other developers of HAE therapies have also positioned AE-QoL as supportive of efficacy in their respective programs. During the FDA review of another prophylaxis HAE product, AE-QoL total score was reviewed by the Division of Clinical Outcomes Assessment (DCOA). Important deficiencies with the COA tool were identified, rendering AE-QoL unfit for purpose for the regulatory evaluation of efficacy. These deficiencies (outlined below) cannot be overcome with the supportive evidence dossier provided in the donidalorsen NDA.

Assessment

During development, the Applicant requested Agency feedback on the AE-QoL and endpoints. The NDA submission includes exact copies of the tool, associated materials, and a supportive evidence dossier.

The AE-QoL questionnaire was developed to assess quality of life impairment in recurrent angioedema patients, including patients with HAE ([Weller et al. 2012](#)). This 17-item instrument uses a 5-point Likert scale with response options of never, rarely, sometimes, often, and very often, rated over a recall period of the last 4 weeks. The 17 items within the AE-QoL are organized into 4 distinct domains labeled as follows: functioning, fatigue/mood, fears/shame, and food/nutrition.

Each domain has its own score, and a total score can also be calculated. The items are scored from 0 to 4, and they are summed to provide raw domain scores, which are then transformed into linear scores ranging from 0 to 100. The total score is the sum of all 17 items. Higher scores in the AE-QoL domains and total scores indicate greater levels of impairment of quality of life. In

Trial CS5, the AE-QoL was administered using electronic forms, displayed via a tablet device, on Days 1, 85, and 169 in the Treatment Period, and on Day 91 of the Post-Treatment Period.

There is inadequate qualitative evidence in the published literature, including (Weller et al. 2012), to support the content validity of the AE-QoL for efficacy evaluation in the Applicant's drug development program. In addition, the domains of the AE-QoL are not well-defined and reliable to inform regulatory decision-making. Specifically, for the AE-QoL functioning domain, asking patients about restrictions in their daily lives due to swelling episodes, regardless of whether or not they have actually experienced swelling episodes during the 4-week recall period, will not provide useful information for assessing treatment benefit in the context of a clinical trial. While the core concepts of disease-related symptoms and physical function are reasonable PRO measurement strategies for demonstrating treatment benefit, emotional impacts (such as anxiety and embarrassment) as assessed by the AE-QoL fears/shame domain are distal concepts, and as such, they may be affected by factors external to HAE disease or effect of the investigational product. In addition, the 4-week recall period of the AE-QoL is not appropriate as patients may not reliably recall the occurrence of their HAE attacks during this timeframe. As such, the AE-QoL is not content valid for this context of use.

Conclusion

Based on the construction of the instrument, the AE-QoL is not well-defined and reliable to inform regulatory decision-making [REDACTED] (b) (4)

6.2.2.4.6.3. Adequacy of Trial Blinding, Trial CS5

Issue

Trial CS5 studied two different dosing regimens: donidalorsen-4 and donidalorsen-8. Donidalorsen 80 mg was matched to a placebo group with the same dosing regimen. Subjects and investigators were aware of the assigned dosing regimen. An alternative design to maintain blinding to the dosing regimen is double dummy dosing, in which all subjects take either placebo or the donidalorsen-4 regimen. In the absence of double dummy dosing, it is of interest to examine the sensitivity of the results to the assumption that the two placebo groups were equivalent and trial blinding was adequate.

Background

Trial blinding is a fundamental feature of removing bias from experimental results. Because subjects in Trial CS5 were blinded to receipt of drug versus placebo but were aware if their dosing interval was donidalorsen-4 or donidalorsen-8, we sought to evaluate any potential bias introduced to the efficacy results by awareness of dosing interval.

Assessment

As an exploratory analysis, the Agency analyzed the time-normalized HAE attack rate (per 4-weeks) as in the primary analysis except that the placebo every 4 weeks and placebo every 8 weeks arms were considered as separate treatment groups. The comparison of each donidalorsen

80-mg regimen was to its matched placebo. The results of this analysis are presented in [Table 17](#). Although the confidence intervals are wider for this exploratory analysis than in the primary analysis ([Table 11](#)), the conclusions remain unchanged. This supports the robustness of the results despite the lack of blinding to the treatment interval. Additionally, an investigator-confirmed HAE attack is considered an objective clinical outcome, and as such, less susceptible to bias from knowledge of assigned treatment interval.

Table 17. Exploratory Analysis: Time-Normalized HAE Attack Rate From Week 1 to Week 25 Compared to Matched Placebo^a in Full Analysis Set Population, Trial CS5

Parameter	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Placebo Every 8 Weeks N=7	Placebo Every 4 Weeks N=15
	Run-In Period HAE attack rate, mean ^b (SD)	3.18 (2.15)	3.61 (2.24)	2.78 (1.18)
Time-normalized HAE attack rate from Week 1 to Week 25, mean ^c (SD)	1.19 (1.63)	0.89 (1.85)	2.46 (1.56)	2.21 (1.96)
Least square mean rate ^d (95% CI)	1.02 (0.65, 1.60)	0.44 (0.26, 0.73)	2.78 (1.65, 4.65)	2.09 (1.40, 3.10)
Model adjusted mean rate ratio relative to matched placebo ^d (95% CI)	0.37 (0.18, 0.73)	0.21 (0.11, 0.40)	-	-
Percentage difference relative to matched placebo ^e (95% CI)	-63 (-82, -27)	-79 (-89, -60)	-	-
p-value	0.004	<0.0001	-	-

Source: Statistical Reviewer Analysis; adefx.xpt

^a Compared to matched placebo means that the donidalorsen 80 mg every 8 weeks group is compared to placebo every 8 weeks group and donidalorsen 80 mg every 4 weeks group is compared to placebo every 4 weeks group

^b The Run-In Period (Screening) HAE attack rate (per 4 weeks) for each subject was calculated as the number of HAE attacks that occurred during the Run-In Period divided by the number of days the subject contributed to the Run-In Period, and then multiplied by 28.

^c The time-normalized HAE attack rate (per 4 weeks) was calculated as number of HAE attacks that occurred from Week 1 to Week 25, divided by the number of days the subject contributed to that period, and then multiplied by 28.

^d The Poisson regression model included 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 weeks that each subject was observed from Week 1 to Week 25 used as an offset variable (e.g., log (6) if the subject stayed in the trial for 24 weeks). Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

^e The percentage difference in mean 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.

Abbreviations: CI, confidence interval; HAE, hereditary angioedema; SD, standard deviation

Conclusion

The Trial CS5 efficacy results do not appear to have been impacted by the lack of blinding to dosing interval.

6.2.2.4.6.4. Efficacy in Reducing HAE Attacks by Location, Trial CS5

Issue

An exploratory analysis of efficacy of donidalorsen in reducing HAE attack incidence, analyzed by anatomic location of the attack, was conducted.

Background

HAE attacks can result in serious and/or life-threatening sequelae if they affect certain anatomic locations, particularly the larynx. Laryngeal attacks can result in asphyxiation and death. Given this potentially serious outcome, we sought to analyze the efficacy by anatomic location of HAE attacks that occurred during Trial CS5.

Assessment

A summary of the history of attacks by location (abdominal, laryngeal, peripheral) is provided in [Table 18](#). Almost all subjects had a history of abdominal (94%) and peripheral (94%) attacks, and the majority of subjects had a history of laryngeal attacks (52%).

A summary of the HAE attacks experienced during the Treatment Period by location and severity is provided in [Table 19](#). The percentage of subjects experiencing abdominal attacks (regardless of severity) were approximately equal in all three treatment groups, while a lower percentage of subjects experienced laryngeal or peripheral attacks in the donidalorsen-4 and donidalorsen-8 groups compared to placebo. Few subjects experienced laryngeal attacks, so limited conclusions can be drawn from this data. The severity of attacks in each location followed a trend consistent with the results regardless of severity.

Table 18. Subjects With a History of HAE Attack by Location in Full Analysis Set Population, n (%),^a Trial CS5

Location	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22	Total N=90
Abdominal	22 (95.7)	43 (95.6)	20 (90.9)	85 (94.4)
Laryngeal	13 (56.5)	22 (48.9)	12 (54.5)	47 (52.2)
Peripheral	22 (95.7)	43 (95.6)	20 (90.9)	85 (94.4)

Source: Clinical study report Table 11 (Pages 76-77); findings reproduced by statistical reviewer using adsl.xpt

^a Percentages are based on the number of subjects in the full analysis set population.

Abbreviations: HAE, hereditary angioedema; N, number of subjects

Table 19. HAE Attacks During Treatment Period by Location and Severity in Full Analysis Set Population, n (%)^a [Number of Attacks], Trial CS5

Location Severity	Donidalorsen 80 mg Every 8 Weeks N=23	Donidalorsen 80 mg Every 4 Weeks N=45	Pooled Placebo N=22	Total N=90
Abdominal	13 (56.5) [121]	23 (51.1) [134]	11 (50.0) [56]	47 (52.2) [341]
Mild	9 (39.1) [26]	13 (28.9) [40]	7 (31.8) [28]	29 (32.2) [94]
Moderate	11 (47.8) [46]	16 (35.6) [84]	10 (45.5) [43]	37 (41.1) [173]
Severe	6 (26.1) [49]	6 (13.3) [10]	4 (18.2) [15]	16 (17.8) [74]
Laryngeal	2 (8.7) [3]	2 (4.4) [5]	5 (22.7) [7]	9 (10.0) [15]
Mild	0 (0.0) [0]	1 (2.2) [1]	1 (4.5) [2]	2 (2.2) [3]
Moderate	2 (8.7) [3]	1 (2.2) [2]	2 (9.1) [2]	5 (5.6) [7]
Severe	0 (0.0) [0]	1 (2.2) [2]	2 (9.1) [3]	3 (3.3) [5]
Peripheral	14 (60.9) [30]	21 (46.7) [73]	20 (90.9) [176]	55 (61.1) [279]
Mild	9 (39.1) [12]	14 (31.1) [48]	15 (68.2) [79]	38 (42.2) [139]
Moderate	8 (34.8) [11]	11 (24.4) [22]	15 (68.2) [77]	34 (37.8) [110]
Severe	5 (21.7) [7]	1 (2.2) [3]	6 (27.3) [20]	12 (13.3) [30]

Source: Statistical Reviewer Analysis; adsl.xpt

^a Percentages are based on the number of subjects in the FAS population.

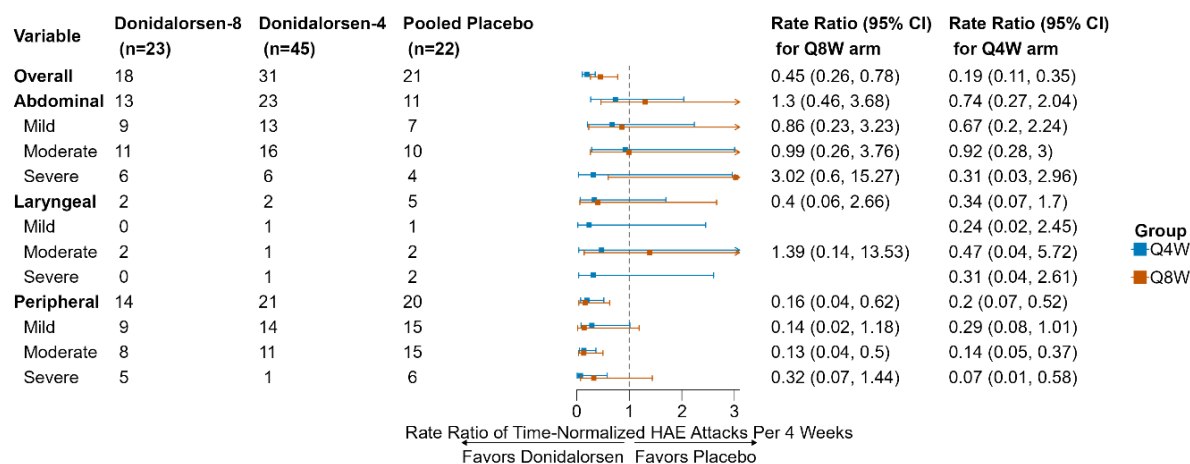
Abbreviations: FAS, full analysis set; HAE, hereditary angioedema; N, number of subjects

Subgroup analyses were performed by the Agency by location alone, and location and severity of HAE attacks. The rate ratio of the time-normalized number of HAE attacks from Week 1 to Week 25 was estimated using a Poisson regression model which included treatment groups and the logarithm of time in every 4 weeks that each subject was observed from Week 1 to Week 25 used as an offset variable. Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

Subgroup analyses of the primary efficacy endpoint for the comparison between donidalorsen and pooled placebo by HAE attack location and severity are shown in [Figure 8](#), including the number of subjects who experienced a particular type of HAE attack and the rate ratio. The rate ratio of the time-normalized number of HAE attacks from Week 1 to Week 25 favored donidalorsen-4 for all locations and severities, and favored donidalorsen-8 for most locations and severities, although 95% confidence intervals overlapped with the null value of 1 for most categories, possibly due to small sample sizes. Point estimates for abdominal attacks, severe abdominal attacks, and moderate laryngeal attacks favored pooled placebo compared to donidalorsen-8, although the confidence intervals were wide and sample sizes were small, so these results should be cautiously interpreted.

Figure 8. Exploratory Subgroup Analysis: Forest Plot of the Time-Normalized Number of HAE Attacks Per 4 Weeks From Week 1 to Week 25 in Full Analysis Set Population, Trial CS5

Forest Plot of the Rate Ratio the Time-Normalized Number of HAE Attacks per 4 weeks from Week 1 to Week 25 Between Donidalorsen and Placebo



Source: Statistical Reviewer Analysis; adce.xpt and adsl.xpt
Abbreviations: CI, confidence interval; HAE, hereditary angioedema; n, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks

Conclusion

Overall, the results for specific anatomic location and severity align with the primary efficacy analysis results, supporting the effectiveness of donidalorsen in reducing HAE attacks regardless of attack location and severity.

6.2.2.4.6.5. Durability of Treatment Effect, Trial CS5

Issue

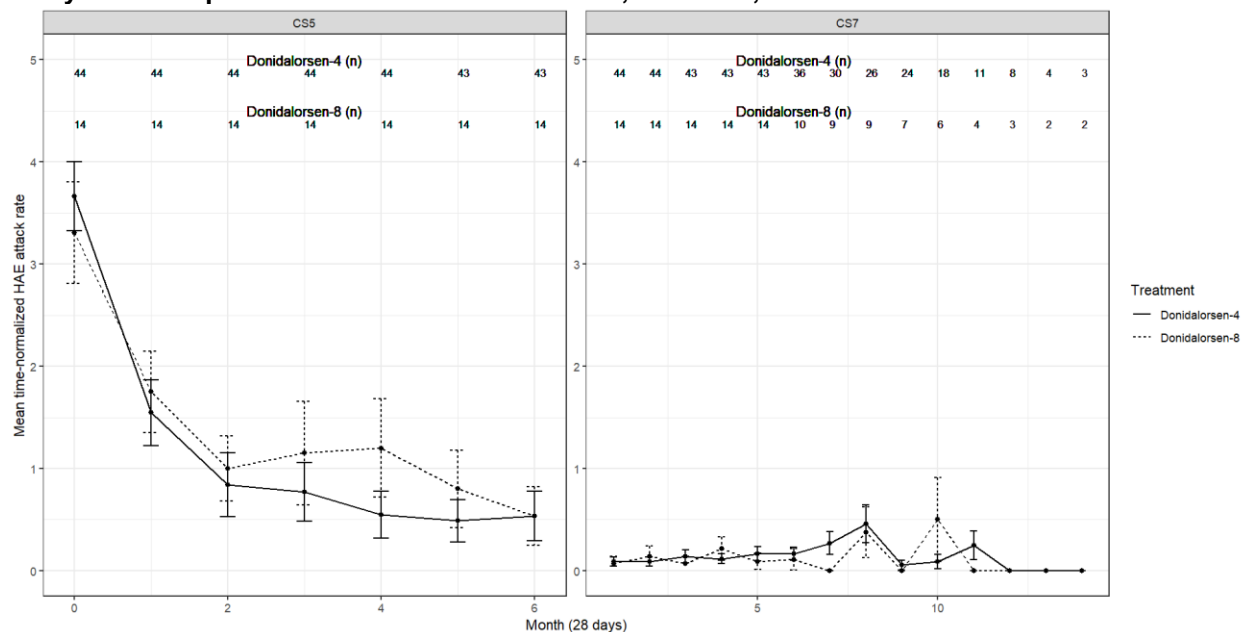
Because HAE is a chronic disease, the durability of the efficacy of donidalorsen is of particular interest and was explored during the development program.

Background

The Applicant conducted two OLE trials: Trial CS7 (extension of the pivotal trial [CS5]) and Trial CS3 (extension of the phase 2 trial [CS2]). See [Table 3](#) for more information. Long-term data from these trials allowed for exploration of durability of efficacy.

Assessment

A total of 44 subjects on the donidalorsen-4 regimen for Trial CS5 were enrolled in the open-label extension trial (CS7) and continued the donidalorsen-4 regimen. An additional 14 subjects on the donidalorsen-8 regimen for Trial CS5 who were attack-free for Weeks 17 to 25 were enrolled in the open-label extension Trial (CS7) and continued on the donidalorsen-8 regimen. [Figure 9](#) presents the mean time-normalized HAE attack rate (per 4-weeks) during Trial CS5 and Trial CS7 for these subject groups.

Figure 9. Exploratory Analysis: Plot of Time-Normalized HAE Attack Rate Per 4 Weeks in Full Analysis Set Population Rolled Over to Trial CS7, Trial CS5, and Trial CS7

Source: Statistical Reviewer Analysis; adefx.xpt

Abbreviations: HAE, hereditary angioedema; n, number of subjects

The time-normalized HAE attack rate was generally consistent across Trial CS7 for the subjects who maintained the treatment as assigned in Trial CS5. However, Trial CS7 lacks a control group and blinding, and therefore these results should be interpreted with caution. Additionally, caution is needed in interpreting the results for the donidalorsen-8 group because the subjects who continued on the donidalorsen-8 regimen in Trial CS7 were a subset of the subjects randomized to the donidalorsen-8 regimen in Trial CS5. Subjects who experienced an HAE attack during Weeks 17 to 25 of Trial CS5 were switched to the donidalorsen-4 regimen for Trial CS7 and their data are not examined here in the evaluation of the durability of the treatment effect.

Although small in sample size, long-term data from Trial CS3 (not presented) were consistent with the results of Trial CS7 and supportive of the durability of efficacy.

Conclusion

While the OLE trial data are limited by the lack of randomization or a control comparator, due to the well-established natural history of HAE, these data remain useful for characterizing the durability of effectiveness over the longer treatment duration. The HAE attack rate in Trial CS7 supports the longer-term durability of efficacy of donidalorsen, at both donidalorsen-4 and donidalorsen-8 dosing intervals, for HAE.

6.3. Key Efficacy Review Issues

6.3.1. Dosing Interval (b) (4)

Issue

The Applicant proposed a dosing interval extension from donidalorsen-4 to donidalorsen-8 in subjects who are attack free with donidalorsen-4 for at least 3 months. The proposed labeling language for dosing states: “The recommended dosage of donidalorsen is 80 mg administered subcutaneously (b) (4)

(b) (4)

(b) (4)”

Background

The pivotal phase 3, placebo-controlled trial (CS5) evaluated both safety and efficacy of two dosing regimens (donidalorsen-4 and donidalorsen-8) in comparison to placebo. The phase 2, open-label extension trial (CS3) enrolled Rollover subjects from the phase 2 trial (CS2) who previously received either placebo or the donidalorsen-4 regimen and placed them all in the donidalorsen-4 regimen for 12 weeks; subjects then either continued the donidalorsen-4 regimen or received the donidalorsen-8 regimen based on clinical response. The phase 3, open-label extension trial (CS7) included enrollment of Rollover subjects from the phase 3 trial (CS5) who previously received the donidalorsen-8 regimen and placed them on the donidalorsen-8 regimen if they are attack free for ≥ 8 weeks (Weeks 17 to 25 in Trial CS5) and otherwise placed them on the donidalorsen-4 regimen.

Assessment

Both the donidalorsen-4 and donidalorsen-8 dosing regimens demonstrated significantly better efficacy compared to placebo based on the results of Trial CS5. On the primary endpoint, the donidalorsen-4 and donidalorsen-8 regimens showed greater reduction in HAE attack rate compared to placebo (-81% and -55% , respectively [[Table 11](#)]). Thus, the donidalorsen-4 demonstrated greater efficacy compared to donidalorsen-8, but both dosing intervals can be considered effective.

PK/PD results from Trial CS5 showed that the mean donidalorsen steady-state trough concentrations following administration of donidalorsen-4 (0.81 ng/mL) was about 2-fold higher than that following administration of donidalorsen-8 (0.39 ng/mL). In addition, the mean percent reduction from baseline in PKK following the donidalorsen-4 regimen was numerically greater than that following the donidalorsen-8 regimen during the Treatment Period (at Week 25: -73.8% and -47.2% for donidalorsen-4 and donidalorsen-8, respectively). The smaller PKK reduction following the donidalorsen-8 compared to the donidalorsen-4 dosing regimen is consistent with the dose response relationship for efficacy between these two regimens. Despite the positive dose-response relationship observed for mean change from baseline in plasma PKK trough concentrations and HAE attack rate, there was variability in these responses at the individual level, suggesting heterogeneity in the disease and response to donidalorsen. While the reduction in plasma PKK trough concentrations following donidalorsen treatment was generally

associated with the reduction in HAE attack rate, there are no dedicated data showing clear association between the magnitude of change from baseline in plasma PKK trough concentration and HAE attack rate at the individual level. See Section [6.1](#) for more details.

For subjects in the donidalorsen-8 group, fluctuations were observed in the change from baseline in plasma PKK trough concentrations, with an increase in PKK trough concentrations observed just prior to administration of the next dose (i.e., at Week 9 and Week 17). The data suggest that this fluctuation may be associated with the longer dosing interval. Nevertheless, the fluctuation in PKK reduction did not appear to affect clinical response, as no increase in HAE attacks was observed at predose, around the time when PKK was increased (in the donidalorsen-8 group). See Section [14](#) for more details.

Subjects who were attack-free with donidalorsen-4 for ≥ 12 weeks may not respond well with donidalorsen-8 based on Trial CS3, as three out of eight subjects who switched from the donidalorsen-4 to the donidalorsen-8 regimen switched back to the donidalorsen-4 regimen due to experiencing an HAE attack. Despite the small sample size leading to limited data, the Applicant's proposal to switch regimens if "attack free for at least 3 months" does not appear to be well supported.

Subjects who were attack free with donidalorsen-8 for Weeks 17 to 25 in Trial CS5 and rolled over to Trial CS7 on donidalorsen-8 (n=14) had a mean baseline HAE attack rate of 2.87 (SD 2.06) per 4 weeks and mean of 32.6 (SD 19.3) HAE attacks in the 12 months prior to Screening in Trial CS5. This group was comprised of five females (36%) and had a mean age of 30.2 (SD 9.0). Subjects who were not attack free with donidalorsen-8 for Weeks 17 to 25 in Trial CS5 and rolled over to Trial CS7 on donidalorsen-4 (n=6) had a mean baseline HAE attack rate of 4.32 (SD 2.44) per 4 weeks and mean of 34.8 (SD 27.3) HAE attacks in the 12 months prior to Screening in Trial CS5. This group was comprised of five females (83%) and had a mean age of 37.7 (SD 16.1). The Agency did not identify characteristics defining a difference between these two groups.

Safety

The risks of both dose intervals are similar and can be mitigated through labeling. See Sections [7.6.1](#) and [7.7](#) for more information.

Conclusion

Availability of both regimens will permit flexibility in dosing at the physician's discretion and permit engagement in shared decision-making with each patient.

The Agency recommends the following labeling for the dosing regimen: "Donidalorsen can be administered as either 80 mg Q4W or 80 mg Q8W."

6.3.2. Confirmatory Evidence

Issue

The substantial evidence of effectiveness of donidalorsen for HAE is established by the single adequate and well-controlled clinical trial (CS5) and CE based on mechanistic data. The purpose of this review issue is to evaluate the source(s) of CE.

Background

The CE providing strong mechanistic support includes the well-established pathophysiology of HAE: defective C1-INH leading to dysregulated activity of bradykinin-mediated angioedema. Donidalorsen is an ASO-GalNAc conjugate that causes RNase H1-mediated degradation of PKK mRNA through selective binding to PKK mRNA, which results in decreased production of PKK protein. PKK is a pro-enzyme for plasma kallikrein, which results in the release of bradykinin, a vasodilator causing swelling and pain in HAE. Less substrate for the bradykinin-mediated angioedema pathway leads to less HAE attacks. PKK is a target of several approved HAE therapies.

Additionally, CE is derived from the in vitro and in vivo pharmacology data, the PD data, and clinical dose-ranging data that were reviewed with the NDA.

Assessment

In Vitro Pharmacology

Treatment of HepatoPac® (co-culture system of primary human hepatocytes and stromal cells [mouse fibroblasts]) with ISIS 721744 and ISIS 546254 (unconjugated parent compound of ISIS 721744) resulted in a dose-dependent reduction of PKK mRNA. The IC₅₀ for ISIS 721744 and ISIS 546254 were 0.19µM and 8.73µM, respectively, indicating that ISIS 721744 is about 46-fold more potent than ISIS 546254 (likely due to cellular uptake through hepatocyte-specific ASGPR).

In Vivo Pharmacology

ISIS 721744 does not have perfect alignment to the mouse PKK gene sequence. As such, an unconjugated mouse-specific ASO (i.e., ISIS 482584) was used for in vivo pharmacology studies in mice.

Male C57Bl/6 albino mice were treated by SC administration with vehicle or PKK ASO ISIS 482584 (5, 10, 20, 40, 80, or 160 mg/kg/week) for 3 weeks. ISIS 482584 inhibited PKK liver mRNA and PKK plasma protein expression in a dose-dependent manner.

Permeability assays were performed in male C57Bl/6 albino mice treated with PKK ASO ISIS 482584 (80 mg/kg/week) for 3 weeks. Evans Blue tracer was injected into the tail vein of mice and analyzed for its accumulation in the colon tissue. Some mice were also treated intraperitoneally with the ACE inhibitor captopril (1 mg/kg) at the time of Evans Blue tracer injection or with a C1-inhibitor ASO (40 mg/kg/week for 3 weeks) to enhance vascular

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permeability. ISIS 482584 inhibited basal vascular permeability. Further ISIS 482584 inhibited vascular permeability induced by either captopril or C1-inhibitor ASO.

Male C57Bl/6 albino mice were treated by SC administration with vehicle or PKK ASO ISIS 482584 (5, 10, 20, 40, 80, or 160 mg/kg/week) for 3 weeks. ISIS 482584 (10 to 160 mg/kg for 3 weeks) reduced basal activated high molecular weight kininogen cleavage in blood of male mice.

Human KLKB1/PKK transgenic mice were treated by SC administration with ISIS 721744 (0.1, 0.3, 1, 3, or 10 mg/kg) on Day 0 and plasma was collected on Day -1, 3, 7, 14, 28, 42, and 56. A single dose of ISIS 721744 resulted in a dose and time-dependent reduction of plasma PKK protein in human KLKB1/PKK transgenic mice that approached 91% at 10 mg/kg on Day 14 after treatment. After reaching the lowest point at Days 7 to 14, plasma PKK levels were fully restored by Day 42 for the 1.0 mg/kg dose or Day 56 for the 3 mg/kg dose. PKK levels were still 20% below baseline levels by Day 56 for the 10 mg/kg dose.

Pharmacodynamic Data

In healthy subjects, a dose-dependent reduction in plasma PKK concentrations from baseline was observed between dosages of 20 and 80 mg SC Q4W. After 4 doses of donidalorsen-4 (last dose administered on Day 85), the nadir in plasma PKK reduction was achieved between Day 71 and Day 99. The mean percent change from baseline for plasma PKK on Day 99 was -53%, -65%, -87%, and -94%, respectively, for 20 mg, 40 mg, 60 mg, and 80 mg Q4W.

In subjects with HAE Type I or II who were treated in either the donidalorsen-4 or donidalorsen-8 groups for 25 weeks, the steady-state PKK change from baseline was achieved by Week 13 (Day 85) and maintained thereafter. The mean percent reduction in plasma PKK from baseline at Week 25 was greater with the donidalorsen-4 regimen (73%) relative to the donidalorsen-8 regimen (47%). With the donidalorsen-8 regimen, a greater fluctuation in plasma PKK change from baseline was observed with smaller reduction from baseline was observed prior to administration of the next dose. This greater fluctuation in plasma PKK is likely associated with the longer donidalorsen-8 dosing interval.

Clinical Dose Ranging

Refer to Section [6.3.1](#) for discussion of increased efficacy with more frequent dosing, in support of clinical dose ranging.

Conclusion

The mechanistic data are sufficiently strong and consistent to serve as CE contributing to SEE for this program.

7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The nonclinical safety of donidalorsen was evaluated in good laboratory practice (GLP)-compliant safety pharmacology (cardiovascular, respiratory, and central nervous system [CNS] in monkeys and human ether-a-go-go related gene [hERG] in human embryonic kidney-293 [HEK-293] cells), repeat-dose toxicity (13-week mouse, 26-week mouse, and 13-week interim/39-week monkey), genotoxicity (Ames, chromosome aberration, and mouse bone marrow micronucleus), carcinogenicity (6-month Tg.rasH2 mouse), and reproductive and developmental toxicity (combined fertility and development mouse and pre- and postnatal development [PPND] mouse) studies. Donidalorsen does not align perfectly to the mouse PKK gene sequence, so the mouse toxicity studies also evaluated mouse surrogate ISIS 722059 to assess on-target effects.

Absorption, Distribution, Metabolism, Excretion

Unconjugated donidalorsen was measurable in the liver and kidney after SC administration in mice and monkeys. Donidalorsen was highly bound ($\geq 96.81\%$) to whole blood plasma proteins in human, monkey, and mouse.

As determined by the Applicant, the primary route of elimination of donidalorsen is initial rapid hydrolysis of the GalNAc conjugate in tissues, followed by slow nuclease-mediated metabolism of the unconjugated oligonucleotide, and subsequent excretion of chain-shortened metabolites, as well as full-length oligonucleotides, albeit minor in urine and feces. There were no unique metabolites found in humans compared to animal species. For donidalorsen linker-related metabolites, the GalNAc moiety is hydrolyzed intracellularly following uptake into tissues and the THA linker undergoes extensive oxidative metabolism, and is nearly completely eliminated via biliary excretion, and to a lesser extent, renal excretion, within 24 hours after administration.

Safety Pharmacology

Donidalorsen (single doses of 12 and 30 mg/kg SC) had no adverse effects on cardiovascular, respiratory, or CNS parameters in monkeys. Donidalorsen did not statistically inhibit hERG current in HEK-293 cells stably transfected with hERG cDNA with an estimated IC_{50} greater than $300\mu M$. In general, oligonucleotides are not thought to interact with the hERG channel.

General Toxicology

Donidalorsen-related toxicity findings in the 26-week mouse study and/or the 13-week interim/39-week monkey study included effects on thrombocytopenia and hematology (monkeys), coagulation (monkeys), liver (mice and monkeys), kidney (monkeys), and injection site (mice and monkeys).

Thrombocytopenia, Hematology, and Coagulation

In monkeys, severe thrombocytopenia was noted in one female and one male at 12 and 30 mg/kg/dose donidalorsen, respectively. Thrombocytopenia was not seen in either animal during the recovery period. Platelet decreases were accompanied by mild to marked decreases in red cell mass with mild to moderate increases in reticulocyte counts and/or increased polychromasia. The female had mild SC tissue necrosis with fibrin and hemorrhage at one of the injection sites, and both the female and male had red nasal discharge, red skin discoloration, bruising, and/or petechial hemorrhage at various body sites, which may have accounted for the decrease in red cell mass and the increase in reticulocyte counts. Complement Bb levels (marker of alternative pathway activation) were slightly elevated in males at 12 mg/kg/dose donidalorsen and in females at 12 and 30 mg/kg/dose donidalorsen. Minimal prolongations in mean activated partial thromboplastin time (aPTT) time were seen in males and females at 30 mg/kg/dose donidalorsen but were not evident by 24 hours postdose.

Liver

In mice, liver histopathology findings included minimal or slight centrilobular hepatocellular hypertrophy in males (1, 4, and 12 mg/kg/dose donidalorsen) and one female (12 mg/kg/dose donidalorsen), which correlated with increased mean liver weights (4 and 12 mg/kg/dose donidalorsen) and liver enzymes (AST, ALT, and/or ALP). There was also an increase in the severity (slight to marked) of hepatocyte vacuolation in males (4 and 12 mg/kg/dose donidalorsen), which correlated with gross pathology findings of liver discoloration at all dose levels. These findings were consistent with uptake of donidalorsen.

In monkeys, liver histopathology findings included hepatocyte hypertrophy (≥ 6 mg/kg/dose donidalorsen terminal), accumulation of basophilic granules in hepatocytes (≥ 2 mg/kg/dose donidalorsen terminal and ≥ 6 mg/kg/dose donidalorsen interim) and Kupffer cells (≥ 6 mg/kg/dose donidalorsen interim and terminal), and sporadic perivascular/vascular inflammation in one male (at 12 mg/kg/dose donidalorsen interim).

Kidney

In monkeys, kidney histopathology findings included accumulation of basophilic granules in tubular epithelial cells (≥ 2 mg/kg/dose donidalorsen interim and terminal).

Injection Sites

In mice, injection site histopathology findings included increased incidences and/or severity of infiltrates of vacuolated macrophages, mononuclear cells, and mixed cells, as well as fibrocyte vacuolation in males and/or females at 1, 4, and/or 12 mg/kg/dose donidalorsen and/or 4 mg/kg/dose ISIS 722059. These findings at the SC injection sites correlated with increased leukocyte counts.

In monkeys, injection site histopathology findings at interim and/or terminal sacrifices (generally at all doses with dose-related incidence and/or severity), included: basophilic granules, macrophages, fibrosis, mixed leukocyte infiltration, mononuclear cell infiltration, mixed cell

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infiltration/inflammation, perivascular/vascular inflammation, myofiber degeneration/regeneration, hemorrhage, and necrosis.

Reproductive and Developmental Toxicology

The potential reproductive and developmental toxicity of donidalorsen and mouse surrogate ISIS 722059 were evaluated in combined fertility and developmental toxicity study in mice and a PPND study in mice. No adverse effects on reproductive and developmental parameters were seen.

Genotoxicity

Donidalorsen was negative for genotoxicity (mutagenicity or clastogenicity) in the in vitro bacterial reverse mutation test (Ames assay), in vitro chromosome aberration assay in cultured Chinese Hamster Lung (CHL) cells, and bone marrow micronucleus assay in mice.

Carcinogenicity

In a 6-month carcinogenicity study in transgenic (Tg.rasH2) mice, SC administration of donidalorsen (5, 10, or 20 mg/kg for males and 10, 20, or 60 mg/kg for females) or mouse-specific surrogate ISIS 722059 (10 mg/kg) once every 2 weeks (Q2W) did not result in a statistically significant increase in tumors compared to the vehicle control in male mice. The carcinogenicity study was inadequate for female mice because a maximum tolerated dose (MTD) was not achieved and no other valid basis to support the high dose was discernable. Of note, at the time of the special protocol assessment (SPA) for the 6-month carcinogenicity protocol, the Executive Carcinogenicity Assessment Committee could not recommend doses for female mice for the proposed study, as no acceptable basis for dose selection could be identified in the non-GLP-compliant 14-week dose range finding (DRF) study in Tg.rasH2 mice.

A 2-year carcinogenicity study in rats is ongoing. At the time of the pre-NDA meeting, the Division agreed that it would be acceptable to submit a summary of the in-life data for the 2-year carcinogenicity study in rats at the time of the NDA submission and to submit the final report as a postmarketing requirement (PMR).

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Donidalorsen is a prekallikrein-directed GalNAc-conjugated ASO. The potential safety concerns for donidalorsen are based on both the molecule and the target.

ASOs

FDA-approved ASOs for intravenous or subcutaneous administration include mipomersen, inotersen, eteplirsen, golodirsen, vilpolarсен, casimersen, tofersen, defibrotide, and nusinersen. Safety risks associated with systemically administered ASOs have included injection site reactions, liver toxicity (Boxed Warning for mipomersen), thrombocytopenia (Boxed Warning

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for inotersen), coagulation abnormalities, and kidney toxicity (Boxed Warning for inotersen). Hypersensitivity reactions have also been reported.

Approved GalNAc conjugated ASOs include eplontersen and olezarsen. GalNAc conjugation is thought to enhance the safety of ASOs by targeting delivery to hepatocytes which improves the activity of ASO, allowing for lower dosages and potentially improved safety. It is not known if liver-targeted ASOs accumulate in the liver over time. The known class effects of ASOs (liver toxicity, thrombocytopenia, and glomerulonephritis) were not identified as risks for eplontersen. Olezarsen is labeled for hypersensitivity reactions, decreased platelet counts, and increased liver enzymes.

Kallikrein Inhibitors

Approved HAE products which target prekallikrein include lanadelumab and berotralstat for prophylaxis, and sebetrolstat for acute treatment of HAE attacks. Safety risks include coagulation abnormalities and hypersensitivity reactions.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

Not applicable. This drug is not yet commercially available in any country.

7.4. FDA Approach to the Safety Review

The sources of the clinical trial safety database are shown in [Table 3](#).

The clinical data scientists and clinical reviewer within the Agency performed independent safety analyses using R Studio 4.1.3, JMP, MAED, and Office of Computational Science Analysis Studio software programs. The safety assessment is primarily based on data from Trial CS5; only safety data from Trial CS5 is included in labeling. Supportive safety data is provided by Trial CS2 part A and the OLE trials (CS7, a continuation of CS5, and CS3, a continuation of CS2) and is discussed in Section [17](#). Given the differences in design and duration across trials safety from each trial was reviewed independently. Given the small sample size of the clinical program, pooled safety data was used to support the review of the Key Safety Review Issues in Section [7.7](#). The pooled safety analysis incorporates data from Trials CS2, CS3, CS5, and CS7.

All safety analyses were conducted in the safety population, which consisted of any subject who received at least one dose of donidalorsen. For both Trial CS5 and Trial CS2, the safety population and the intent-to-treat (ITT) population were the same.

7.5. Adequacy of the Clinical Safety Database

No data quality or integrity issues were identified in the submitted datasets or CRFs. Definitions of adverse events (AEs) and serious adverse events (SAEs) were consistent with Code of Federal Regulations 312.32 ([21 CFR 312.12 2004](#)). The Applicant coded AEs using the Medical

Dictionary for Regulatory Activities version 26.1, and determined AE severity using the FDA guidance for industry *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* ([September 2007](#)). Any AE not listed in that grading scale was graded as mild, moderate, or severe (see Section [15.1.6](#) for definitions). The Applicant's translation of verbatim terms to the Medical Dictionary for Regulatory Activities preferred terms (PTs) for the events reported in the trial was reviewed and found to be acceptable. Safety monitoring in the clinical program was adequate to inform the risks of donidalorsen. See Section [15.1.5](#) for the schedule and review of safety assessments.

Overall Exposure

[Table 20](#) represents exposure to the proposed doses of donidalorsen-4 and donidalorsen-8 in the HAE development program. Across the four trials (CS2, CS3, CS5, CS7), 156 subjects with HAE received at least one dose of donidalorsen-4 and 31 subjects received at least one dose of donidalorsen-8. The mean duration of exposure was 50 weeks for donidalorsen-4, and 59 weeks for donidalorsen-8. About half of the subjects were exposed for >1 year.

Table 20. Duration of Exposure, Safety Population, Pooled Analyses, Trials CS2, CS3, CS5, and CS7

Parameter	Donidalorsen 80 mg Every 4 Weeks N=156	Donidalorsen 80 mg Every 8 Weeks N=31
Duration of treatment, weeks		
Mean (SD)	49.6 (36.5)	58.8 (47.1)
Median (Q1, Q3)	42.9 (29.3, 57.5)	44.4 (24.2, 68.2)
Min, max	4, 215.9	4.9, 183.7
Total exposure (person years)	148	35
Subjects treated, by duration, n (%)		
≥0 to <12 weeks	3 (1.9)	2 (6.5)
≥12 to <24 weeks	24 (15.4)	5 (16.1)
≥24 to <36 weeks	33 (21.2)	5 (16.1)
≥36 to <48 weeks	33 (21.2)	5 (16.1)
≥48 to <60 weeks	27 (17.3)	3 (9.7)
≥60 to <72 weeks	19 (12.2)	4 (12.9)
≥72 to <84 weeks	7 (4.5)	1 (3.2)
≥84 to <108 weeks	2 (1.3)	1 (3.2)
≥108 to <168 weeks	2 (1.3)	4 (12.9)
≥168 to <216 weeks	6 (3.8)	1 (3.2)
≥216 weeks	0	0

Source: adsl.xpt; Software: R

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

The safety for the prescribing information will include Trials CS5 and the two OLEs (CS3 and CS7). Across these three trials 171 subjects were exposed with an average duration of 14 months, as confirmed by the safety analyses using duration of exposure (days)/28. Safety for Trial CS2 is not included as it provided limited safety data due to the small sample size and short trial duration.

Exposure for the pivotal trial, CS5, is provided in [Table 21](#). Subjects who completed the Week 25 Treatment Period visit and met eligibility requirements could enroll in Trial CS7 any time after Week 25. If a subject chose to enroll in Trial CS7, the subject discontinued participation in Trial CS5 prior to the first visit in Trial CS7. Therefore, their Trial CS5 participation duration (and Trial CS5 exposure data) is shorter than for those subjects who completed the Post-Treatment Period. This may be considered an artifact of the way Trial CS5 discontinuation and Trial CS7 enrollment were handled. For this reason, 20 subjects in the donidalorsen-8 group who rolled over into Trial CS7 appeared to discontinue much sooner, as their last dose was 16 weeks after the start of the trial, 4 weeks prior to the donidalorsen-4 cohort's last dose at 20 weeks after the start of the trial. For additional information, see [Figure 10](#).

Table 21. Duration of Exposure, Safety Population, Trial CS5

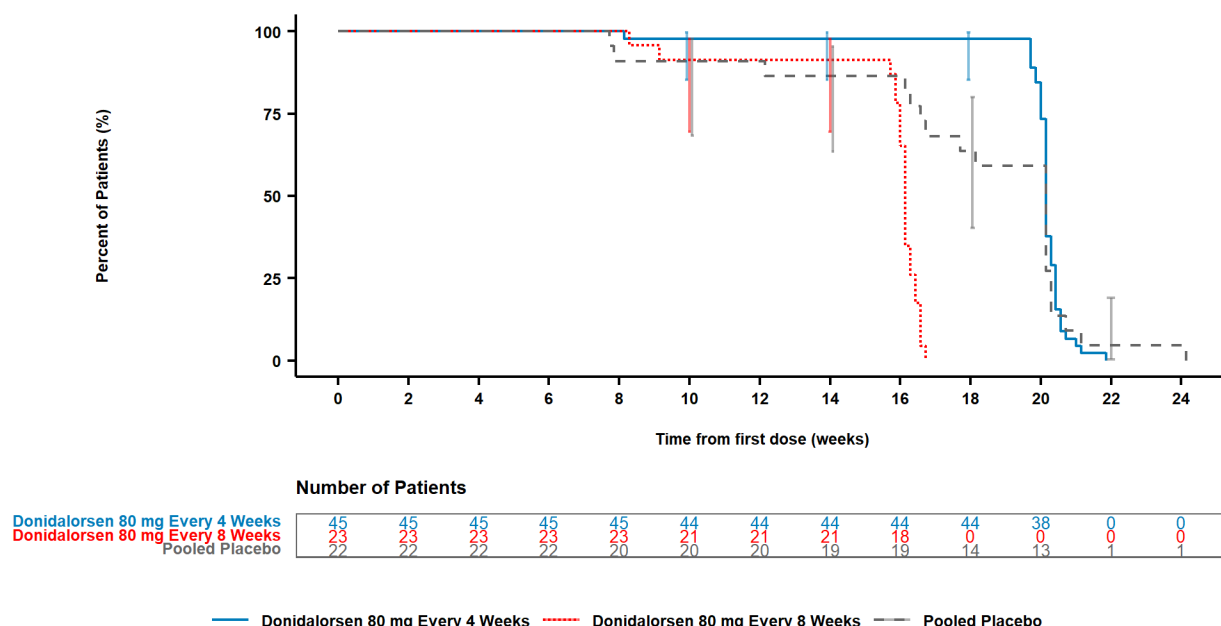
Parameter	Donidalorsen 80 mg Every 4 Weeks N=45	Donidalorsen 80 mg Every 8 Weeks N=23	Pooled Placebo N=22
Duration of treatment, weeks			
Mean (SD)	20 (1.8)	15.6 (2.2)	18.1 (4.1)
Median (Q1, Q3)	20.1 (20, 20.4)	16.1 (16, 16.4)	20.1 (16.6, 20.2)
Min, max	8.1, 21.9	8.3, 16.7	7.7, 24.1
Total exposure (person years)	17	7	8
Subjects treated, by duration, n (%)			
≥0 to <8 weeks	0	0	2 (9.1)
≥8 to <12 weeks	1 (2.2)	2 (8.7)	0
≥12 to <14 weeks	0	0	1 (4.5)
≥14 to <16 weeks	0	3 (13.0)	0
≥16 to <18 weeks	0	18 (78.3)	5 (22.7)
≥18 to <20 weeks	6 (13.3)	0	1 (4.5)
≥20 to <24 weeks	38 (84.4)	0	12 (54.5)
≥24 to <28 weeks	0	0	1 (4.5)
≥28 weeks	0	0	0

Source: adsl.xpt; Software: R

Duration is up to 24 weeks.

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation

Figure 10. Time to Permanent Discontinuation of Trial Drug, Safety Population, Trial CS5



Source: adsl.xpt; Software: R
Time to permanent discontinuation of trial drug defined as the duration of time from first dose to last dose of trial agent for each subject, regardless of the reason for trial agent discontinuation.

7.6. Safety Results

7.6.1. Safety Results, Trial CS5

7.6.1.1. Overview of Treatment-Emergent Adverse Events Summary, Trial CS5

An overview of treatment-emergent adverse event (TEAEs) is presented in [Table 22](#). Treatment-emergent adverse events occurred more commonly in subjects in the pooled placebo group (82%) than subjects in the donidalorsen-4 (73%) or donidalorsen-8 arms (61%). The greater % of TEAEs in the donidalorsen-4 group is primarily driven by injection-related reactions due to increased frequency of administered injections. Most TEAEs were mild to moderate. Serious adverse events and AEs leading to discontinuation were uncommon in all treatment groups.

Table 22. Overview of Treatment-Emergent Adverse Events,^a Safety Population, Trial CS5

Event Category	Donidalorsen 80 mg Every 4 Weeks N=45 n (%)	Donidalorsen 80 mg Every 8 Weeks N=23 n (%)	Pooled Placebo N=22 n (%)
Any AE	33 (73.3)	14 (60.9)	18 (81.8)
Severe	1 (2.2)	2 (8.7)	2 (9.1)
Moderate	15 (33.3)	7 (30.4)	9 (40.9)
Mild	17 (37.8)	5 (21.7)	7 (31.8)
AE leading to permanent discontinuation of trial drug	0	1 (4.3)	0
SAE	0	0	1 (4.5)

Source: adae.xpt; Software: R

^a Treatment-emergent adverse events defined as “an event that started or worsened on or after the first dose of trial drug.”

Duration is up to 24 weeks.

Severity as assessed by the investigator.

Abbreviations: AE, adverse event; N, number of subjects in treatment group; n, number of subjects with at least one event;

SAE, serious adverse event

7.6.1.2. Deaths, Trial CS5

No deaths were observed in Trial CS5.

7.6.1.3. Serious Treatment-Emergent Adverse Events, Trial CS5

There was one SAE in Trial CS5: a subject in the pooled placebo group experienced a limb injury. No SAEs occurred in the donidalorsen-4 or donidalorsen-8 treatment group.

7.6.1.4. Adverse Events and Office of New Drugs Custom Medical Queries Leading to Treatment Discontinuation, Trial CS5

There were no TEAEs leading to treatment discontinuation in the donidalorsen-4 and placebo groups. One TEAE, ALT increased (moderate, related), led to permanent treatment discontinuation in one (4%) subject in the donidalorsen-8 group. See Section [7.7.3](#) for more information on this subject’s experience.

7.6.1.5. Treatment-Emergent Adverse Events, Trial CS5

An analysis of common TEAEs (by PT with pooling of synonymous/similar terms) is shown in [Table 23](#). Due to the small sample size, a cutoff of 5% (or >2) subjects in the donidalorsen-4 group reporting the same/similar TEAE was used as the threshold for inclusion in labeling. Two PTs occurred ≥5% in the donidalorsen-8 group and more frequently than placebo (oral herpes: 2 (9%) and vomiting: 2 (9%)) with no events in the donidalorsen-4 group. These PTs were not included in [Table 23](#) due to the low overall incidence and the lack of AEs in the higher dose group suggests these PTs were not due to drug. The most common TEAEs in CS5 that occurred ≥5% in treatment group and greater than in placebo were injection site reactions, upper

respiratory tract infection, urinary tract infection, and abdominal discomfort. See Section [7.7.1](#) for a detailed review of injection-related reactions.

Table 23. Subjects With Common Treatment-Emergent Adverse Events^a Occurring at ≥5% Frequency and Occurring Most Frequently in the Donidalorsen Q4W Group, Safety Population, Trial CS5

Preferred Term	Donidalorsen 80 mg Every 4 Weeks	Donidalorsen 80 mg Every 8 Weeks	Pooled Placebo
	N=45 n (%)	N=23 n (%)	N=22 n (%)
Any AE	33 (73.3)	14 (60.9)	18 (81.8)
Injection site reaction ^b	11 (24.4)	1 (4.3)	1 (4.5)
Upper respiratory tract infection	4 (8.9)	2 (8.7)	1 (4.5)
Urinary tract infection	4 (8.9)	2 (8.7)	0
Abdominal discomfort	3 (6.7)	0	0

Source: adae.xpt; Software: R

^a Treatment-emergent adverse events defined as “an event that started or worsened on or after the first dose of trial drug.”

Duration is up to 24 weeks.

Coded as MedDRA preferred terms.

^b Injection site reaction terms: Injection site erythema, injection site discoloration, injection site pain, injection site pruritus, injection site induration, injection site bruising, injection site hematoma, injection site hypersensitivity, injection site swelling, injection site reaction, and injection site urticaria. All injection site reactions were mild in severity, and none resulted in trial discontinuation. Abbreviations: AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities; N, number of subjects in treatment group; n, number of subjects with adverse event; Q4W, every 4 weeks

The PTs for injection site reactions were identified based on the Office of New Drugs custom medical query ([OCMQ] narrow), as outlined in [Table 24](#).

Table 24. Subjects With Injection Site Reaction Adverse Events by OCMQ (Narrow) and Preferred Term, With OCMQ (Narrow) Safety Population, Trial CS5

OCMQ (Narrow) Preferred Term	Donidalorsen 80 mg Every 4 Weeks	Donidalorsen 80 mg Every 8 Weeks	Pooled Placebo
	N=45 n (%)	N=23 n (%)	N=22 n (%)
Local administration reaction (OCMQ)	11 (24.4)	1 (4.3)	1 (4.5)
Injection site erythema	6 (13.3)	1 (4.3)	0
Injection site discoloration	3 (6.7)	1 (4.3)	0
Injection site pain	3 (6.7)	0	0
Injection site pruritus	3 (6.7)	0	0
Injection site induration	2 (4.4)	0	0
Injection site bruising	1 (2.2)	0	0
Injection site hematoma	1 (2.2)	0	0
Injection site hypersensitivity	1 (2.2)	0	0
Injection site swelling	1 (2.2)	0	0
Injection site reaction	0	1 (4.3)	0
Injection site urticaria	0	0	1 (4.5)

Source: adae.xpt; Software: R. Edited by clinical reviewer

Treatment-emergent adverse events defined as “an event that started or worsened on or after the first dose of trial drug.”

Duration is up to 24 weeks.

The OCMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some OCMQs may contain PTs from more than one SOC.

Some preferred terms are not included in any OCMQ. Those preferred terms are not shown or counted in this table.

Abbreviations: N, number of subjects in treatment group; n, number of subjects with adverse event; OCMQ, Office of New Drugs custom medical query; PT, preferred term; Q4W, every 4 weeks; SOC, system organ class

There are no concerning safety signals based upon review of TEAEs that substantially affect the benefit-risk assessment of donidalorsen for HAE. Adverse events of special interest are discussed in Section [7.7](#).

7.6.1.6. Laboratory Findings, Trial CS5

Routine clinical testing (hematology, serum chemistry with liver function tests, and coagulation) were collected at baseline, Month 3, and Month 6 during the Treatment Period. The results of the platelet counts and liver function tests are discussed in Sections [7.7.2](#) and [7.7.3](#) respectively. Otherwise, there was no clinically significant difference between the donidalorsen and placebo groups in laboratory parameters during the trial.

7.6.1.7. Assessment of Drug-Induced Liver Injury, Trial CS5

Refer to the discussion in Section [7.7.3](#).

7.6.1.8. Vital Sign Analyses, Trial CS5

No clinically meaningful changes in vital signs (systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, temperature) were observed between treatment arms across trial visits.

7.6.1.9. Subgroup Analyses, Trial CS5

Subgroup analyses by age, sex, race, ethnicity, and geographic region revealed no major differences in safety; however, the number of subjects within certain subgroups was small, facilitating only limited analysis. For example, the sample sizes of adolescents and of non-White subjects were very small. For detailed analyses of TEAEs by subgroup, see Section [17](#).

7.7. Key Safety Review Issues

7.7.1. Injection Site Reactions

Issue

Injection site reactions are a common adverse reaction from SC injectable drug products and for antisense oligonucleotide products. Injection site reactions were observed during the donidalorsen development program. The Applicant proposes to include injection site reactions (ISRs) in the common adverse drug reaction table in Section 6 of the PI.

Background

Injection site reactions are a known class effect of ASOs, with reported rates as high as 84% for some ASOs (mipomersen).

Assessment

Findings at the SC injection site were noted in the 26-week mouse study with donidalorsen and mouse surrogate ISIS 722059, and the 39-week monkey study with donidalorsen.

Our primary safety review focuses on Trial CS5, with supportive safety analysis from the other clinical trials in the development program (see [Table 3](#) under Section [3](#)) due to the small size of the safety database.

In terms of ISR AEs in Trial CS5, there was an increased incidence of events in the donidalorsen-4 group, with 11 (24%) subjects experiencing an ISR based on the Local Administration Reaction narrow OCMQ compared to 4% for donidalorsen-8 and 5% for placebo (see [Table 24](#)). When accounting for the number of injections, the incidence remained higher for donidalorsen-4 (11/270 (6 injections x 45 subjects) = 4%) compared to donidalorsen-8 (1/69 (3 injections x 23 subjects) = 1%). Injection site erythema was the most frequently reported of the OCMQ AEs (n=6, 13%), followed by injection site discoloration, injection site pain, and injection site pruritus (n=3, 7% for each). All ISRs were nonserious, mild in severity, and did not result in discontinuation of IP. In contrast, only one subject per group in the donidalorsen-8 and placebo groups experienced an ISR.

In Trial CS7 (the OLE trial following Trial CS5), which lacked a placebo control group, a greater proportion of subjects in the Rollover donidalorsen-4 group (16%) experienced ISRs compared with the Rollover donidalorsen-8 group (6%) per the Local Administration Reaction narrow OCMQ. All ISRs were nonserious and did not result in any action taken with the trial drug. Except for one event of moderate severity, all ISRs were mild. Of Naïve subjects, 37% experienced ISRs per the Local Administration Reaction narrow OCMQ. Although this is the highest incidence noted in any cohort across the donidalorsen HAE program, all ISRs in Naïve subjects were non-serious and did not result in any action taken with the trial drug. The majority of ISRs were mild (25%) or moderate (6%) in severity.

In Trial CS3 (the OLE trial following Trial CS2), which lacked a placebo control group, 35% of subjects in the HAE-1/HAE-2 group (6/17 subjects, 40 events) experienced at least one ISR per the Local Administration Reaction narrow OCMQ. One subject in the HAE-1/HAE-2 group (donidalorsen 100 mg Q4W [Flexible Dosing Period]) permanently discontinued IP due to six moderate TEAEs of injection site discoloration.

The Applicant provided a pooled safety analysis of 173 subjects who received donidalorsen across Trials CS2, CS3, CS5 and CS7. The three subjects enrolled in Trial CS2 part B are excluded from the Agency's analysis due to their diagnosis with HAE Type III, bringing the sample size for the pooled safety analysis to 170 subjects.

Out of these 170 subjects, 44 (26%) subjects experienced at least one ISR, with the number of individual ISR events totaling 237. Thus, those subjects who do experience ISR may experience multiple and/or repeated events over time. Incidence of ISRs compared across dosing arms shows the highest incidence in the donidalorsen-4 dosing interval (28%), while donidalorsen-8 is similar to placebo (7% for both groups). The incidence of ISR appears generally stable with subsequent injections, i.e., a higher incidence of ISR was not observed with increased number of previous injections. The most common ISR reported in the any donidalorsen treatment group

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were Injection site erythema [20 (11.8%)], Injection site discoloration [12 (7.1%)], Injection site pruritus [11 (6.5%)], and Injection site pain [10 (5.9%)].

Regarding the timing of onset of ISRs, ISRs occurring after the first dose of donidalorsen occurred in 11 (25%) of the 44 subjects who developed ISRs: nine (21%) on the same day as the first dose and two on Day 2 after the first dose. Out of 44 subjects, 36 (82%) subjects experienced 135 ISRs within 2 days after the most recent dose of donidalorsen.

Regarding outcome and duration of ISRs, out of 237 ISRs, 209 (88%) were resolved at the time of database lock, with the remaining 28 (12%) ongoing. Out of 209 resolved ISRs, 45 (22%) ISRs resolved within 2 days and 84 (40%) ISRs resolved within 7 days. The most common ongoing ISRs were injection site discoloration (21 events in four subjects). As noted previously, one (2%) subject out of 44 experienced an ISR leading to permanent trial drug discontinuation; this subject received donidalorsen 100 mg Q4W and experienced six moderate events of injection site discoloration.

Regarding severity, out of 237 ISRs, 203 (86%) ISRs in 43 subjects were reported as mild, and 34 (14%) ISR in 7 subjects were reported as moderate. No serious or severe ISRs were reported. The severity of ISRs appears generally stable with subsequent injections, i.e., an increased severity of ISRs was not observed with increased number of previous injections.

Overall, the cumulative, pooled data regarding ISRs indicates that the pivotal trial safety data may provide an accurate overview of this observed risk of donidalorsen use, with additional supportive data about the characteristics of observed ISRs.

Conclusion

ISRs were observed in 24% of subjects in the donidalorsen-4 group of the pivotal trial (CS5), while 4 to 5% of subjects in the donidalorsen-8 and placebo groups experienced ISRs. The features of ISRs were similar between Trial CS5 and the cumulative, pooled safety data from the four clinical trials. ISRs were nonserious, with the vast majority mild in severity. The incidence and severity of ISRs did not vary with number of past injections. The most frequent AE PTs were injection site erythema, discoloration, pain, and pruritus. ISRs developed upon exposure to the first dose in 25% of subjects who eventually developed ISRs, and whether on the first or subsequent injection, they most commonly occurred within 1 to 2 days of the injection. The majority of ISRs resolved within 7 days. Treatment discontinuation due to ISRs was infrequent; across the program, one subject permanently discontinued donidalorsen due to six moderate ISR events. Availability of donidalorsen-8 provides options for prescribers to mitigate the risk of injection site reactions with donidalorsen-4.

7.7.2. Bleeding Events, Thrombocytopenia, and Coagulation

Issue

Bleeding risks, including thrombocytopenia and effects on the clotting cascade (prolonged aPTT) ([Crooke et al. 2019](#)) are known ASO class effects (regardless of the target protein). The donidalorsen clinical program was designed to monitor and mitigate this potential risk.

Background

ASO class effects, including thrombocytopenia, are generally thought to be dose-dependent, transient, and self-limited. Though the precise mechanism of thrombocytopenia is not known, it is thought to be due to ASOs binding to platelets and causing increased platelet clearance. Importantly, with chronic ASO administration, nonhuman primate and human clinical trials have revealed a risk of sporadic, unpredictable, severe thrombocytopenia. To our knowledge, such events have not been observed in humans receiving GalNAC-conjugated ASOs, which allow for reduced systemic dosage via increased potency, with the goal of reducing overall toxicities.

Additionally, some kallikrein inhibiting products are known to cause asymptomatic prolongation of aPTT in clinical use; however, bleeding events are not associated with this laboratory abnormality. The aPTT assay primarily evaluates the intrinsic coagulation pathway, which includes kallikrein and other clotting factors. Meanwhile, PT and INR evaluate the extrinsic pathway, and in the donidalorsen program they were utilized as a means to assess hepatic synthetic function.

Assessment

In the 13-week interim/39-week monkey study with a 26-week recovery period, severe thrombocytopenia was noted in one female and one male at 12 and 30 mg/kg/dose donidalorsen, respectively. Thrombocytopenia was not seen in either animal during the 26-week recovery period.

In the 13-week interim/39-week monkey study with a 26-week recovery period, minimal prolongations in mean aPTT time were seen in males and females at 30 mg/kg/dose donidalorsen but were not evident by 24-hours postdose.

Our primary safety review focuses on Trial CS5, which permits placebo-controlled analysis over 24 weeks, with supportive safety analysis from the other clinical trials in the development program (see [Table 3](#)). These other clinical trials, though they have limitations such as a shorter duration (for Trial CS2) and lack of placebo control (for the OLE trials), are useful due to the small size of the safety database and the longer-term data of the OLE trials. For each individual trial, and for pooled results from Trials CS5, CS2, CS7, and CS3, TEAEs and laboratory results were reviewed for signals of bleeding risk, thrombocytopenia, and aPTT prolongation.

Bleeding Adverse Events

Bleeding TEAEs were identified by the Hemorrhage OCMQ (Narrow).

In Trial CS5, there was a slight trend towards an increased incidence of bleeding TEAEs in the donidalorsen-4 group (see [Table 25](#)). However, the three TEAEs occurring in the donidalorsen-4 group were all mild and self-limited, without concomitant clinically significant abnormalities in coagulation laboratory results (including platelet counts, PT, and INR), and none led to treatment discontinuation. The events in donidalorsen-8 and placebo both started on Day 1, making them very unlikely to be related to the trial drug.

Table 25. Subjects With Hemorrhage Adverse Events by OCMQ (Narrow) and Preferred Term, With OCMQ (Narrow) Safety Population, Trial CS5

OCMQ (Narrow) Preferred Term	Donidalorsen 80 mg Every 4 Weeks N=45	Donidalorsen 80 mg Every 8 Weeks N=23	Pooled Placebo N=22
	n (%)	n (%)	n (%)
Hemorrhage (OCMQ)	3 (6.7)	1 (4.3)	1 (4.5)
Eye hematoma	1 (2.2)	0	0
Injection site bruising	1 (2.2)	0	0
Injection site hematoma	1 (2.2)	0	0
Intermenstrual bleeding	0	1 (4.3)	0
Vessel puncture site hematoma	0	0	1 (4.5)

Source: adae.xpt; Software: R. Edited by clinical reviewer

Treatment-emergent adverse events defined as “an event that started or worsened on or after the first dose of trial drug.”

Duration is up to 24 weeks.

The OCMQ is aligned to a single SOC based on clinical judgment. However, please be aware that some OCMQs may contain PTs from more than one SOC.

Some preferred terms are not included in any OCMQ. Those preferred terms are not shown or counted in this table.

Abbreviations: N, number of subjects in treatment group; n, number of subjects with adverse event; OCMQ, Office of New Drugs custom medical query; PT, preferred term; Q4W, every 4 weeks; SOC, system organ class

In Trial CS7 (the open-label trial following Trial CS5), which lacked a placebo control group, a higher incidence of bleeding TEAEs was observed in the Rollover donidalorsen-4 group compared to the Rollover donidalorsen-8 group (12% versus 0 subjects, respectively). The donidalorsen-naïve subjects (who received Q4W administration) had a similar incidence of bleeding TEAEs (14%). The PTs that occurred in >1 subject were injection site bruising (5; 3%), contusion (3; 2%), vaginal hemorrhage (3; 2%), and injection site hematoma (2; 1%). Regarding severity, most events were mild, with two subjects in the Rollover donidalorsen-4 group experiencing a moderate event (vaginal hemorrhage and PT prolonged; no bleeding occurred with this lab abnormality) and one in Rollover donidalorsen-8 (intermenstrual bleeding). None of the bleeding TEAEs led to treatment discontinuation, nor were there concomitant clinically significant abnormalities in coagulation laboratory results.

In the phase 2 trial (CS2 part A), bleeding TEAEs occurred at a higher incidence in the placebo group than the donidalorsen group.

In Trial CS3 (the OLE trial following Trial CS2), which lacked a placebo control group, two (10%) subjects experienced bleeding TEAEs, which were mild. These events required no action to be taken with the trial drug.

The Agency also examined concomitant use of anticoagulants, antiplatelet or blood-thinning medications in subjects with bleeding TEAEs; no subjects with concomitant use of such medications were identified.

Review of the 120-day safety update report did not reveal substantive, new information about bleeding TEAEs. Given the difference in exposure between the donidalorsen treatment groups and placebo in Trials CS5, CS2, CS7, and CS3, the Applicant conducted an exposure-adjusted analysis of bleeding TEAEs with the 120-day safety update report. The incidence rate (per 100 patient years of exposure) of bleeding TEAEs (per the Hemorrhages broad search SMQ) was 14% in the donidalorsen-4 group, 5% in the donidalorsen-8 group, and 26% in the placebo group. Thus, the incidence of bleeding TEAEs was higher in the placebo group than in the donidalorsen treatment groups.

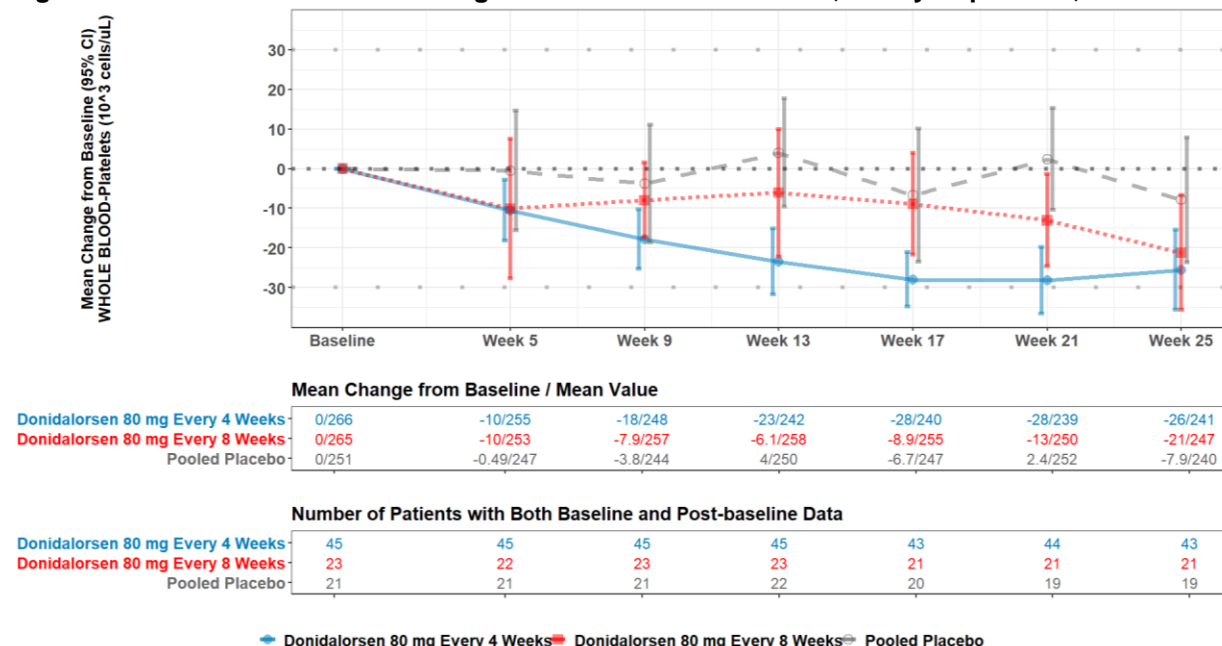
These results do not suggest an increased risk of bleeding TEAEs with donidalorsen exposure.

Platelet Counts

The effect of donidalorsen on platelet counts was also evaluated. There was no apparent exposure response between donidalorsen systemic exposure and change in platelet counts according to the clinical pharmacology analysis; see Section 8.1.2 for details.

Aggregate data on platelet counts over time were reviewed. Figure 11 shows the mean absolute change from baseline for platelet count; the mean values at each timepoint are displayed in the text box below the figure. Treatment with donidalorsen at both doses tended to decrease platelet counts, with a sharper downward slope in the donidalorsen-4 group, which converged with the donidalorsen-8 slope by Week 25. The platelet counts in the donidalorsen-4 group reached a plateau by Week 17, with the mean absolute value having decreased by 15,000 cells/mm³.

Figure 11. Mean Platelet Count Change From Baseline Over Time, Safety Population, Trial CS5



Source: adlb.xpt; Software: R

Figures do not include time points with data from fewer than 10% of randomized/enrolled patients in all treatment groups.

Only central laboratory data are included in the analysis.

Abbreviations: CI, confidence interval

Despite the trend to small decreases in platelet counts, clinically significant reductions below specified thresholds were rare, with no imbalance between treatment arms observed (see [Table 26](#)). One severely low platelet count was attributed to sample handling error. Overall, no clear trend of clinically relevant thrombocytopenia was noted in Trial CS5.

Table 26. Subjects With One or More Platelet Values Beyond Specified Levels, Safety Population, Trial CS5

	Donidalorsen 80 mg Every 4 Weeks N=45	Donidalorsen 80 mg Every 8 Weeks N=23	Pooled Placebo N=22
Platelets, Low (10^3 Cells/ μ L)	n/N _w (%)	n/N _w (%)	n/N _w (%)
Level 1 (<140)	1/45 (2.2)	1/23 (4.3)	2/22 (9.1)
Level 2 (<125)	1/45 (2.2)	1/23 (4.3)	1/22 (4.5)
Level 3 (<100)	0/45 (0)	1/23 (4.3)	0/22 (0)

Source: adlb.xpt; Software: R

Threshold levels 1, 2, and 3 as defined by the [Standard Safety Tables & Figures Integrated Guide](#).

Duration is up to 24 weeks.

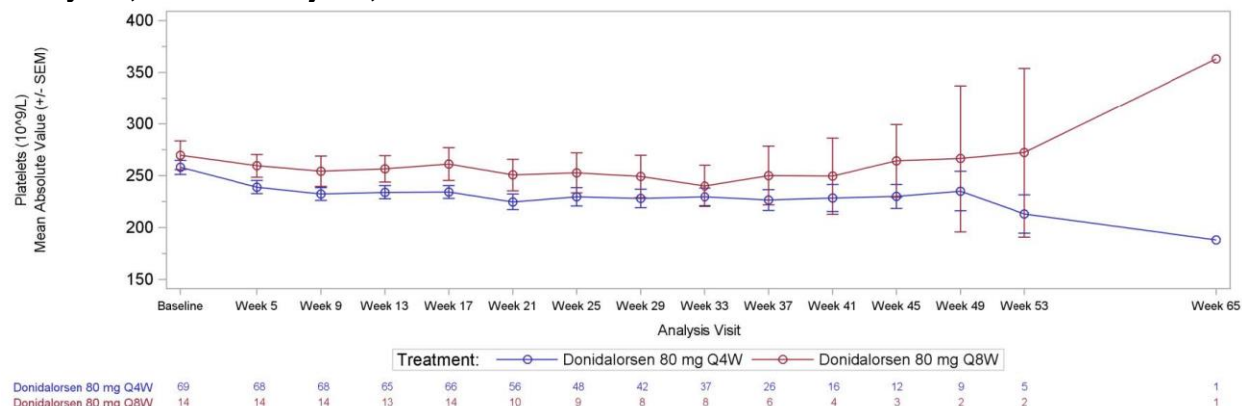
In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

Abbreviations: N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data

Overall, per the Applicant's analysis, the baseline mean platelet counts were similar across treatment groups (266,000/mm³ for donidalorsen-4, 265,000/mm³ for donidalorsen-8, and 245,000/mm³ for placebo). The donidalorsen treatment arms demonstrated a greater mean percent change in platelet count at Week 25 (9.6% for donidalorsen-4, 7.9% for donidalorsen-8) compared to placebo (1.4%).

In Trial CS7, 92 to 93% of subjects maintained platelet counts within the reference range at all timepoints. [Figure 12](#) shows the mean absolute platelet values over time for Rollover subjects; note that the sample size decreases over time due to the rolling enrollment from Trial CS5. As in Trial CS5, mean platelet counts decreased from baseline but remained within the reference range.

Figure 12. Mean (+/- SEM) Absolute Value Platelet Counts Over Time (On-Treatment Period), Safety Set, Rollover Subjects, Trial CS7



Source: Applicant CS7 CSR Appendix Figure 14.3.4.2.1

Baseline before first dose of donidalorsen is used. For subjects who received placebo in the Index Trial, baseline is defined as the average of all non-missing predose assessments prior to the first dose of the ISIS 721744-CS7 trial. For subjects who received donidalorsen in the Index Trial, baseline is defined as the average of all non-missing predose assessments prior to the first dose of the ISIS 721744-CS5 trial. If there is only one predose measurement available, then it is assigned as baseline.

Subjects who changed dosing could be plotted under both treatment groups (donidalorsen 80 mg Q4W and Q8W). Abbreviations: CSR, clinical study report; Q4W, every 4 weeks; Q8W, every 8 weeks; SEM, standard error of mean

Decreases in platelet counts were reviewed by specified thresholds and by percent reduction from baseline. Nadir platelet counts of $\geq 30\%$ decrease from baseline were observed in 17% of the Rollover donidalorsen-4 group and 21% of the donidalorsen-8 group (that is, a dose-response was not observed over longer-term exposure). The analyses revealed that abnormally low platelet counts, when they occurred, were usually mild, isolated, and returned to normal/baseline without any action required; some were suspected to be due to handling/laboratory error.

[Table 27](#) displays platelet counts at clinically relevant thresholds for the safety population in Trial CS7. One severely low platelet count was attributed to sample handling error, and the other was an isolated abnormality (nadir 99,000/mm³) that resolved without taking action with the trial drug.

Table 27. Subjects With One or More Platelet Values Beyond Specified Levels, Safety Population, Trial CS7

	Rollover Subjects Donidalorsen Q4W N=69	Rollover Subjects Donidalorsen Q8W N=14	Naïve Subjects N=64
Platelets, Low (10 ³ Cells/ μ L)	n/N _w (%)	n/N _w (%)	n/N _w (%)
Mild: 125 to <140	2/69 (2.9)	1/14 (7.1)	2/64 (3.2)
Moderate: 100 to <125	2/69 (2.9)	0/14 (0)	2/64 (3.2)
Severe: <100	1/69 (0)	0/14 (0)	1/64 (1.6)

Source: Adapted from CSR CS7 Table 71 and Table 72

Abbreviations: CSR, clinical study report; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data; Q4W, every 4 weeks; Q8W, every 8 weeks,

In the phase 2 trial (CS2 part A), no subjects had a confirmed platelet count below 140,000/mm³. The observed platelet count trend for the donidalorsen treatment group was consistent with observations in the other clinical trials.

In Trial CS3 (the OLE trial following Trial CS2), which lacked a placebo control group, one subject experienced a TEAE of platelet count decreased (mild), which was transient and resolved spontaneously, with no action taken with trial treatment. Regarding platelet count trends, the majority of subjects remained within the reference range for platelet counts, and the overall effects were consistent with those observed in Trial CS5 and Trial CS7. No subjects experienced a severe reduction ($<100,000/\text{mm}^3$). Three subjects in the HAE-1/HAE-2 Index donidalorsen-4 group experienced a decrease of $\geq 30\%$ from the Index Run-In Baseline. These decreases in platelet counts were not sustained, and all platelet counts remained between 100,000 to 140,000/ mm^3 . None of these platelet count decreases were reported as TEAEs.

Review of the 120-day safety update report did not reveal new, substantive information regarding platelet laboratory values.

Activated Partial Thromboplastin Time

In Trial CS5, mean aPTT remained within the reference range, and no imbalance in prolonged aPTT was observed compared to placebo. No clinically relevant changes in aPTT values were noted in Trial CS5. Review of safety data from Trials CS7, CS2, and CS3 showed, similarly, no apparent safety signal related to aPTT values.

Conclusion

Bleeding

In Trial CS5, there was a slight trend towards an increased incidence of bleeding TEAEs in the donidalorsen-4 group (see [Table 25](#)). However, the three TEAEs occurring in the donidalorsen-4 group were all mild and self-limited, without concomitant clinically significant abnormalities in coagulation laboratory results (including platelet counts, PT, and INR), and none led to treatment discontinuation.

In Trial CS7, 17 (12%) of all subjects experienced one or more bleeding TEAEs, with none occurring in the subjects who received the donidalorsen-8 dosing regimen. These events were typically mild or moderate in severity, did not lead to treatment discontinuation, and were not associated with concomitant clinically significant abnormalities in coagulation laboratory results. Results from Trials CS2 and CS3 were consistent with Trials CS5 and CS7.

Platelet Counts

The placebo-controlled safety data from Trial CS5 revealed a sustained decrease in platelet counts, with a magnitude of approximately 15,000 to 20,000 cells/ mm^3 , that stabilized in approximately 4 months and did not typically result in clinically significant thrombocytopenia or bleeding events. The donidalorsen treatment arms demonstrated a greater mean percent change in platelet count at Week 25 (9.6% for donidalorsen-4, 7.9% for donidalorsen-8) compared to placebo (1.4%). Results from CS2, CS3, and CS7 were consistent with Trial CS5.

Overall, although no serious clinical sequelae related to bleeding or thrombocytopenia were observed in the clinical program, the theoretical risk of uncommon, severe thrombocytopenia or

bleeding remains, due to the ASO class effects. Therefore, the decreased platelets observed in Trial CS5 will be included in labeling.

7.7.3. Hepatic Function Effects

Issue

Due to the known ASO class effect of hepatotoxicity and donidalorsen's pharmacologic mechanism, which relies on GalNAC binding hepatocytes, the occurrence of clinically significant hepatocellular injury risk with donidalorsen use was investigated.

Background

Antisense oligonucleotide class effects, including hepatotoxicity, are generally thought to be dose-dependent, transient, and self-limited. Risk of hepatotoxicity with elevations of ALT and AST, and hepatic steatosis were seen in the development programs for mipomersen and inotersen, which are ASOs targeting hepatic gene expression. Inotersen labeling recommends baseline and monthly monitoring of liver enzymes.

Olezarsen, a GalNAC-conjugated ASO for familial chylomicronemia, was approved in 2024 with a postmarketing requirement to evaluate the potential for drug-induced liver injury (DILI) with long-term use of this product. Olezarsen is labeled for increase in liver enzymes. Olezarsen was associated with average increases in ALT, AST, and gamma-glutamyl transferase (GGT) within the first 3 months which stabilized and remained within reference range. There were a higher proportion of olezarsen-treated subjects in whom serious hepatic events occurred and increases in liver enzymes led to discontinuation or interruption of the trial drug. Liver enzymes returned towards baseline with discontinuation of olezarsen. There were no cases of Hy's Law, an indicator of drug-induced liver injury.⁶

Assessment

Nonclinical

In the 26-week mouse study, liver histopathology findings included minimal or slight centrilobular hepatocellular hypertrophy (1, 4, and 12 mg/kg/dose donidalorsen), which correlated with increased mean liver weights (4 and 12 mg/kg/dose donidalorsen) and liver enzymes (AST, ALT, and/or ALP). There was also an increase in the severity (slight to marked) of hepatocyte vacuolation in males (4 and 12 mg/kg/dose donidalorsen), which correlated with gross pathology findings of liver discoloration at all dose levels. These findings were consistent with uptake of donidalorsen.

In the 13-week interim/39-week monkey study, liver histopathology findings included hepatocyte hypertrophy (≥ 6 mg/kg/dose donidalorsen terminal), accumulation of basophilic granules in hepatocytes (≥ 2 mg/kg/dose donidalorsen terminal and ≥ 6 mg/kg/dose donidalorsen

⁶ See FDA Summary Review for Olezarsen (NDA 218614), dated December 19, 2024, available at https://www.accessdata.fda.gov/drugsatfda_docs/nda/2025/218614Orig1s000IntegratedR.pdf.

interim) and Kupffer cells (≥ 6 mg/kg/dose donidalorsen interim and terminal), and sporadic perivascular/vascular inflammation (12 mg/kg/dose donidalorsen interim).

Our primary safety review focuses on Trial CS5, which permits placebo-controlled analysis over 24 weeks, with supportive safety analysis from the other clinical trials in the development program (see [Table 3](#), under Section [3](#)). These other clinical trials, though they have limitations such as a shorter duration (for Trial CS2) and lack of placebo control (for the OLE trials), are useful due to the small size of the safety database and the longer-term data of the OLE trials. For each individual trial, and for pooled data from Trials CS5, CS2, CS7, and CS3, TEAEs and laboratory results were reviewed for signals of hepatotoxicity.

Adverse Events

In Trial CS5, there were no clear imbalances between subjects receiving donidalorsen versus placebo in the incidence of TEAEs such as ALT and AST elevations, jaundice, PTs in the hepatic injury OCMQ (narrow or broad), or cases meeting Hy's Law criteria.

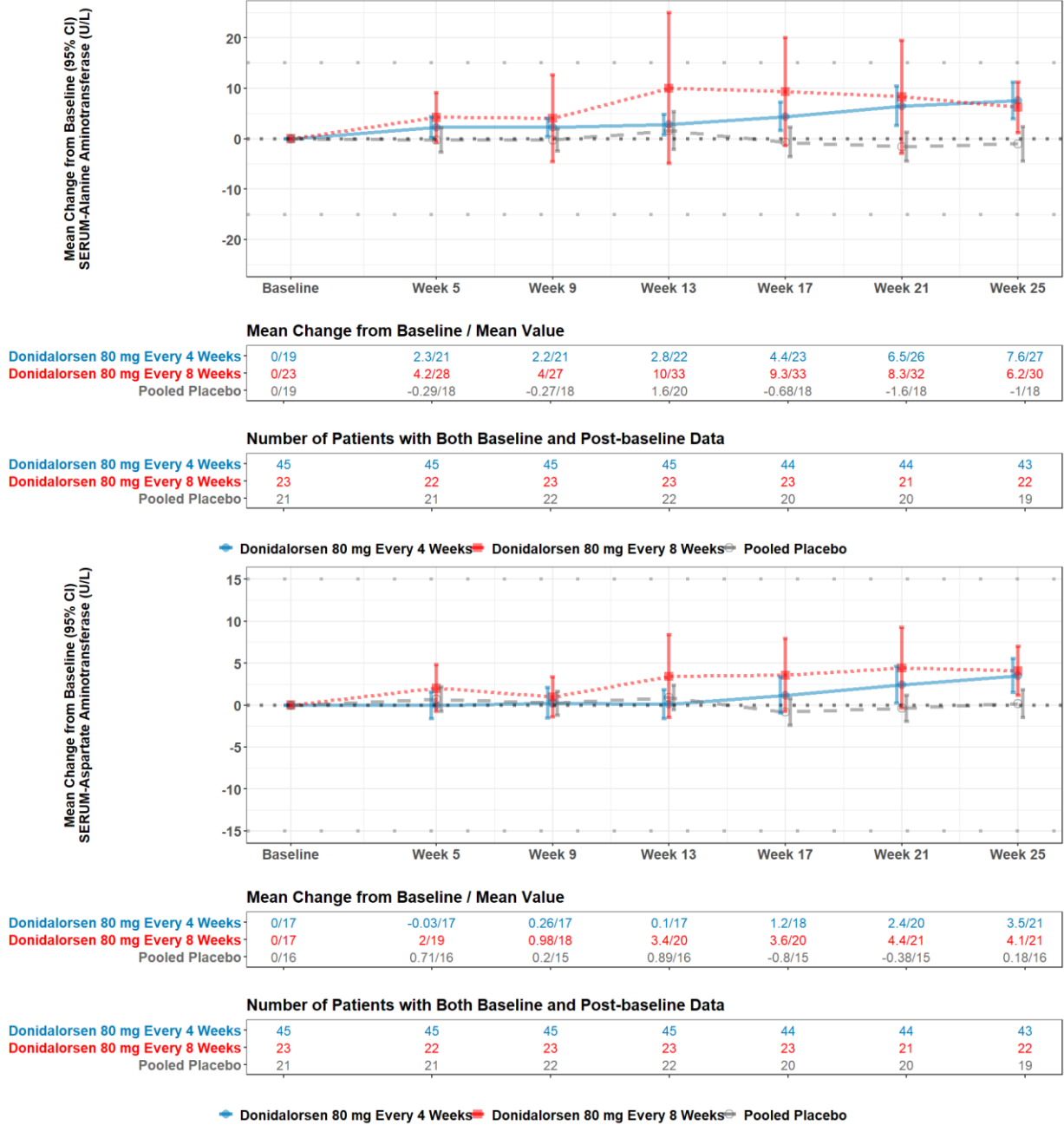
Overall, analysis of TEAEs did not reveal a signal of hepatic injury. However, given that this result could be consistent with products resulting in DILI, in which premarket clinical trials reveal only laboratory abnormalities not rising to the level of an imbalance in adverse events, further investigation was completed.

Liver Function Tests

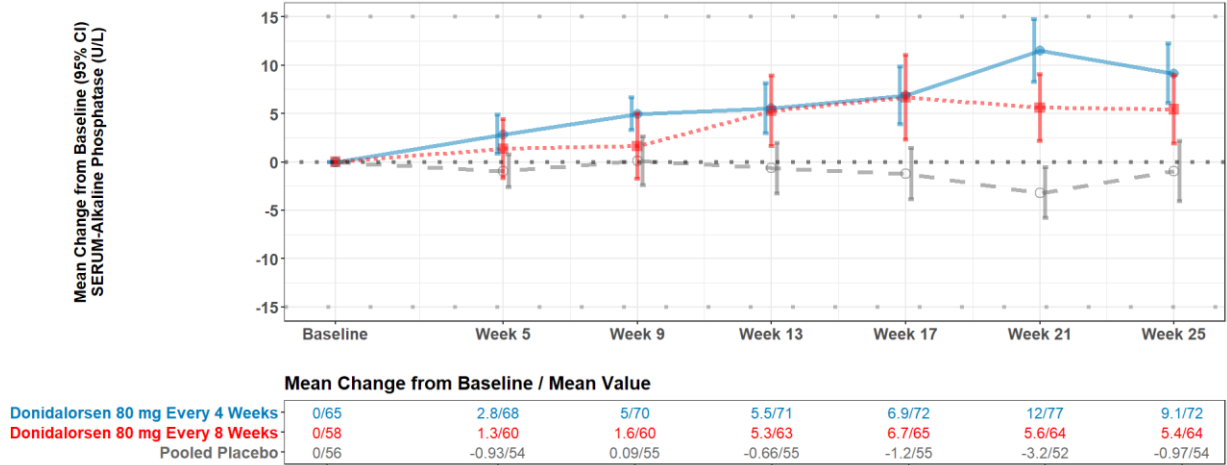
Because DILI is most commonly asymptomatic and diagnosed by laboratory testing, laboratory results for AST, ALT, ALP, GGT, bilirubin, and PT/INR were reviewed for each clinical trial.

[Figure 13](#) shows the mean absolute change from baseline for AST, ALT, ALP, and GGT during Trial CS5; total and direct bilirubin are not shown as these did not reveal a notable trend. The mean change from baseline and absolute values at each timepoint are displayed in the text boxes below each figure. Subclinical increases in AST and ALT were observed at a magnitude that was similar at both doses of donidalorsen, while the increases in ALP and GGT were of larger magnitude at higher doses. The increases in AST, ALT, and ALP appeared to reach a plateau during the trial, while there is not a clear plateau in GGT increase.

Figure 13. Mean Laboratory (Liver Biochemistry) Data Change From Baseline Over Time, Safety Population, Trial CS5

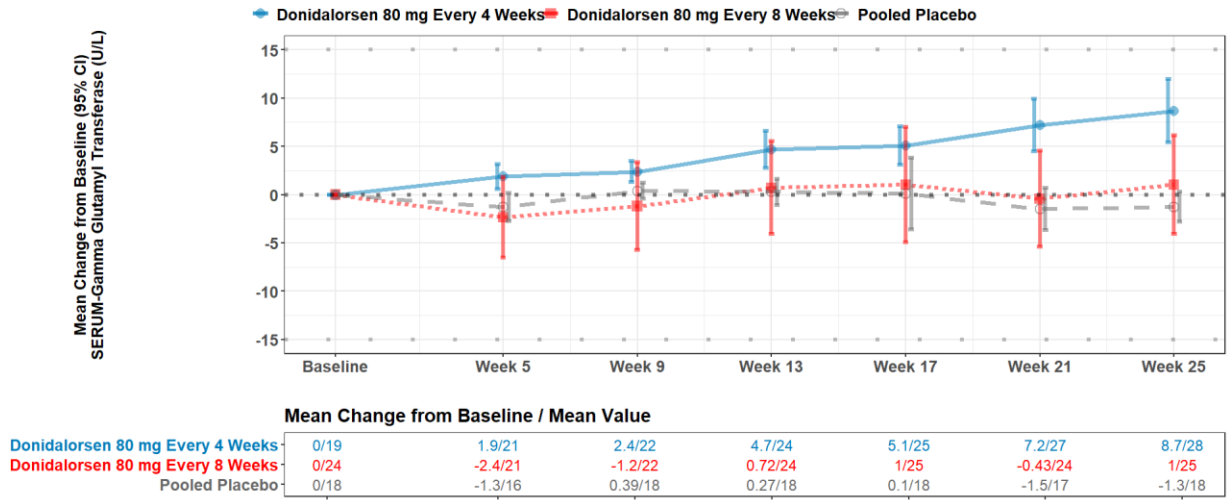


NDA 219407
Dawnzera (donidalorsen)



Number of Patients with Both Baseline and Post-baseline Data

	Baseline	Week 5	Week 9	Week 13	Week 17	Week 21	Week 25
Donidalorsen 80 mg Every 4 Weeks	45	45	45	45	44	44	43
Donidalorsen 80 mg Every 8 Weeks	23	22	23	23	23	21	22
Pooled Placebo	21	21	22	22	20	20	19

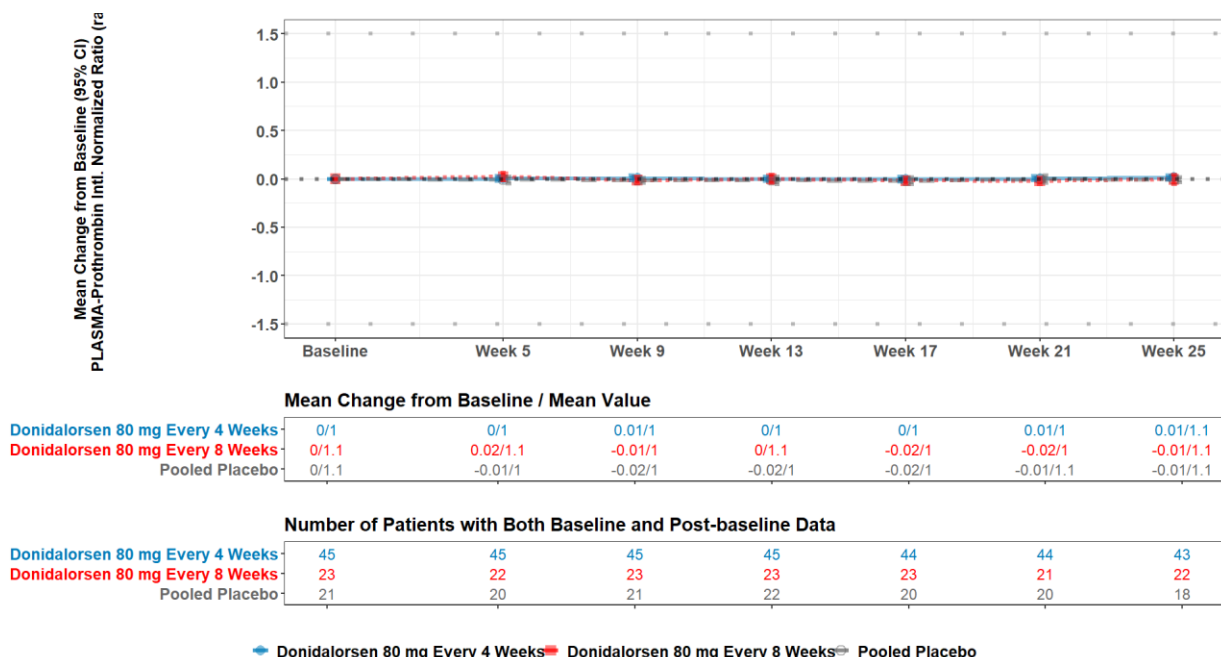


Number of Patients with Both Baseline and Post-baseline Data

	Baseline	Week 5	Week 9	Week 13	Week 17	Week 21	Week 25
Donidalorsen 80 mg Every 4 Weeks	45	45	45	45	44	44	43
Donidalorsen 80 mg Every 8 Weeks	23	22	23	23	23	21	22
Pooled Placebo	21	21	22	22	20	20	19

◆ Donidalorsen 80 mg Every 4 Weeks ■ Donidalorsen 80 mg Every 8 Weeks ○ Pooled Placebo

NDA 219407
Dawnzera (donidalorsen)



Source: adlb.xpt; Software: R. Made by CDS reviewer
 Figures do not include time points with data from fewer than 10% of randomized/enrolled patients in all treatment groups.
 Only central laboratory data are included in the analysis.
 Abbreviations: CDS, clinical data scientist; CI, confidence interval; Intl., international

Review of shift tables for liver function tests (LFTs) showed that 9% of Trial CS5 subjects in the donidalorsen-4 and donidalorsen-8 arms shifted from normal to mildly elevated values for ALT (mild: >1 and $<3 \times$ ULN), and 4% of subjects in donidalorsen-8 shifted from normal to moderate (moderate: 3 to $5 \times$ ULN). Similar trends were seen in AST. Clinically relevant levels of LFT elevation occurred infrequently (see [Table 28](#)). Two (9%) subjects in donidalorsen-8 and none in donidalorsen-4 had ALT $>3 \times$ ULN. One of the donidalorsen-8 subjects permanently discontinued treatment due to the ALT increase and was also diagnosed with hepatic steatosis (see more details below). One subject in the donidalorsen-4 group experienced a total bilirubin elevation of $>2 \times$ ULN. This subject had a medical history of Gilbert syndrome (see also [Figure 16](#)).

No subjects met protocol-defined stopping criteria for LFTs. Per the FDA guidance for industry *Drug-Induced Liver Injury: Premarketing Clinical Evaluation (July 2009)*,⁷ low-level elevations in AST and ALT ($<3 \times$ ULN) are common, nonspecific, and not concerning for DILI.

⁷ Guidances are updated periodically. For the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fdaguidance-documents>.

Table 28. Subjects With One or More Liver Biochemistry Analyte Values Exceeding Specified Levels, Safety Population, Trial CS5

Laboratory Parameter	Donidalorsen 80 mg Every 4 Weeks N=45	Donidalorsen 80 mg Every 8 Weeks N=23	Pooled Placebo N=22
	n/N _w (%)	n/N _w (%)	n/N _w (%)
Alkaline phosphatase, high (U/L)			
Level 1 (>1.5X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Level 2 (>2X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Level 3 (>3X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Alanine aminotransferase, high (U/L)			
Level 1 (>3X ULN)	0/45 (0)	2/23 (8.7)	0/22 (0)
Level 2 (>5X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Level 3 (>10X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Aspartate aminotransferase, high (U/L)			
Level 1 (>3X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Level 2 (>5X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Level 3 (>10X ULN)	0/45 (0)	0/23 (0)	0/22 (0)
Bilirubin, total, high (mg/dL)			
Level 1 (>1.5X ULN)	1/45 (2.2)	0/23 (0)	1/22 (4.5)
Level 2 (>2X ULN)	1/45 (2.2)	0/23 (0)	0/22 (0)
Level 3 (>3X ULN)	0/45 (0)	0/23 (0)	0/22 (0)

Source: adlb.xpt; Software: R

Threshold levels 1, 2, and 3 as defined by the [Standard Safety Tables & Figures Integrated Guide](#).

Duration is up to 24 weeks.

For specific evaluation of drug-induced liver injury, see [Figure 14](#).

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

Abbreviations: DILI, drug-induced liver injury; N, number of subjects in treatment group; n, number of subjects meeting criteria; N_w, number of subjects with data; ULN, upper limit of normal

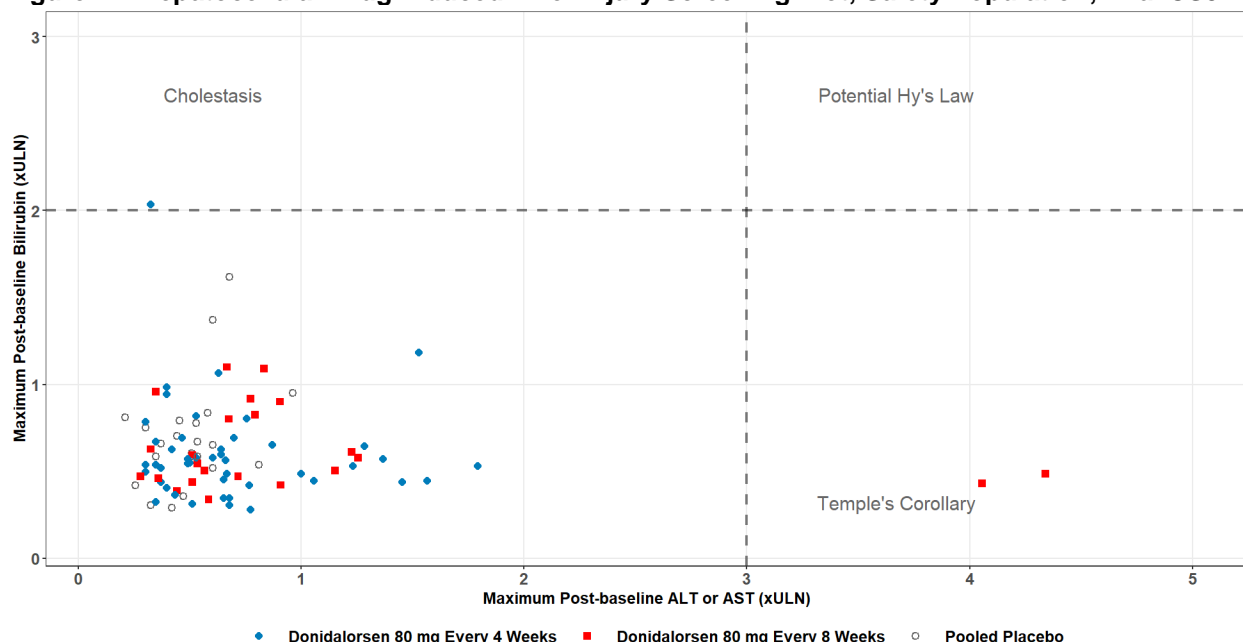
GGT

No subject in any treatment group experienced GGT elevations $>2.5 \times$ ULN. A greater proportion of subjects in the donidalorsen-4 group (16%) and donidalorsen-8 group (26%) experienced mild GGT elevations ($<2.5 \times$ ULN) compared with the placebo group (4.5%). Gamma-glutamyl transferase values shifted from normal to mildly elevated (>1 to $<2.5 \times$ ULN) in 11% of donidalorsen-4 and 17% of donidalorsen-8 subjects.

INR

No subject in any treatment group experienced INR elevations $>1.5 \times$ ULN. A greater proportion of subjects in the donidalorsen-4 group (17.8%) and donidalorsen-8 group (17.4%) experienced mild INR elevations ($<1.5 \times$ ULN) compared with the placebo group (9.1%). INR values shifted from normal to mildly elevated (>1 to $1.5 \times$ ULN) in 16% of donidalorsen-4 and 17% of donidalorsen-8 subjects.

As shown in [Figure 14](#), the drug-induced liver injury Screening plot of Trial CS5 revealed no cases in the potential Hy's Law quadrant. Two (9%) subjects in the donidalorsen-8 group fell in Temple's Corollary quadrant, and one (2%) subject in the donidalorsen-4 group fell in the cholestasis quadrant.

Figure 14. Hepatocellular Drug-Induced Liver Injury Screening Plot, Safety Population, Trial CS5

Source: adlb.xpt; Software: R

Each data point represents a subject plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period.

A potential Hy's Law case was defined as having any postbaseline total bilirubin equal to or exceeding 2X ULN after a postbaseline ALT or AST equal to or exceeding 3X ULN. Those subjects who meet total bilirubin equal to or exceeding 2X ULN criteria within 30 days of the ALT or AST equal to or exceeding 3X ULN criteria are circled in red.

The within 30 days analysis window rule does not apply to cholestasis and temple's corollary cases.

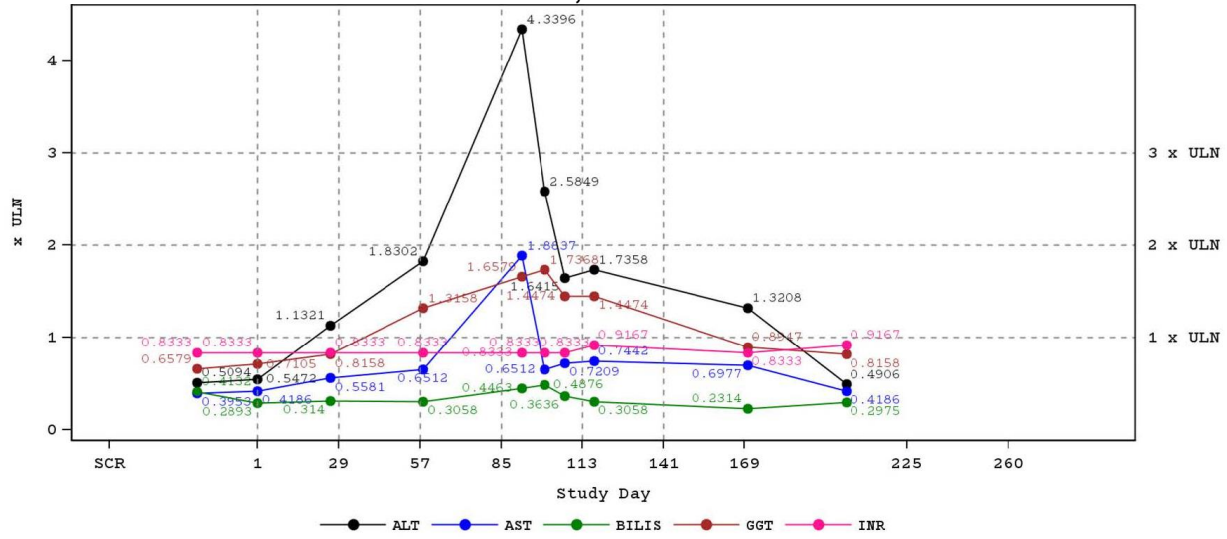
All subjects with at least one postbaseline ALT or AST, bilirubin and ULN are plotted.

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal

One TEAE, moderate related ALT increased, led to permanent treatment discontinuation in one subject in the donidalorsen-8 group (plotted in the Temple's Corollary quadrant in [Figure 14](#)). The subject was a 45-year-old White female with HAE type I and a history of cholecystectomy in [\(b\) \(6\)](#), enrolled in the donidalorsen-8 group. Relevant prior medications included danazol (taken for years and discontinued 3 weeks before starting trial treatment), desogestrel (started 3 weeks prior to first dose of trial treatment), and icatibant acetate. Relevant concomitant medications, taken while on trial, included C1 esterase inhibitor (Cinryze), icatibant acetate, and desogestrel. LFT results for the subject over the trial are shown in [Figure 15](#). The subject received her Week 9 dose of donidalorsen on Day 58, when ALT was elevated to 97 U/L (1.8× ULN), and the AE of ALT increased of mild intensity was reported. No treatment was recorded for the AE. On Day 92, ALT peaked at 230 U/L (4.3× ULN) and the AE severity worsened from mild to moderate. By Day 100, ALT decreased to 137 U/L (2.6× ULN), and the severity improved to mild. No treatment was reported for these AEs. AST, GGT, and total bilirubin were within normal range throughout the trial, except on Day 92, AST was elevated at 81 U/L (1.9× ULN). On Day 170, trial treatment was permanently discontinued due to the AE of ALT increased; the subject had received a total of two doses, on Days 1 and 58. On Day 204, ALT returned to normal at 26 U/L, and a liver ultrasound revealed that the liver was slightly enlarged with smooth contours and increased parenchymal echogenicity, consistent with grade 1 hepatic steatosis (judged by the investigator to be mild, not related to trial treatment). On Day 211, the subject's ALT was 31 U/L, and the subject completed her final trial visit.

Figure 15. Liver Function Test Results for One Subject in Donidalorsen Q8W Group Who Discontinued Treatment Due to ALT Increase, Trial CS5



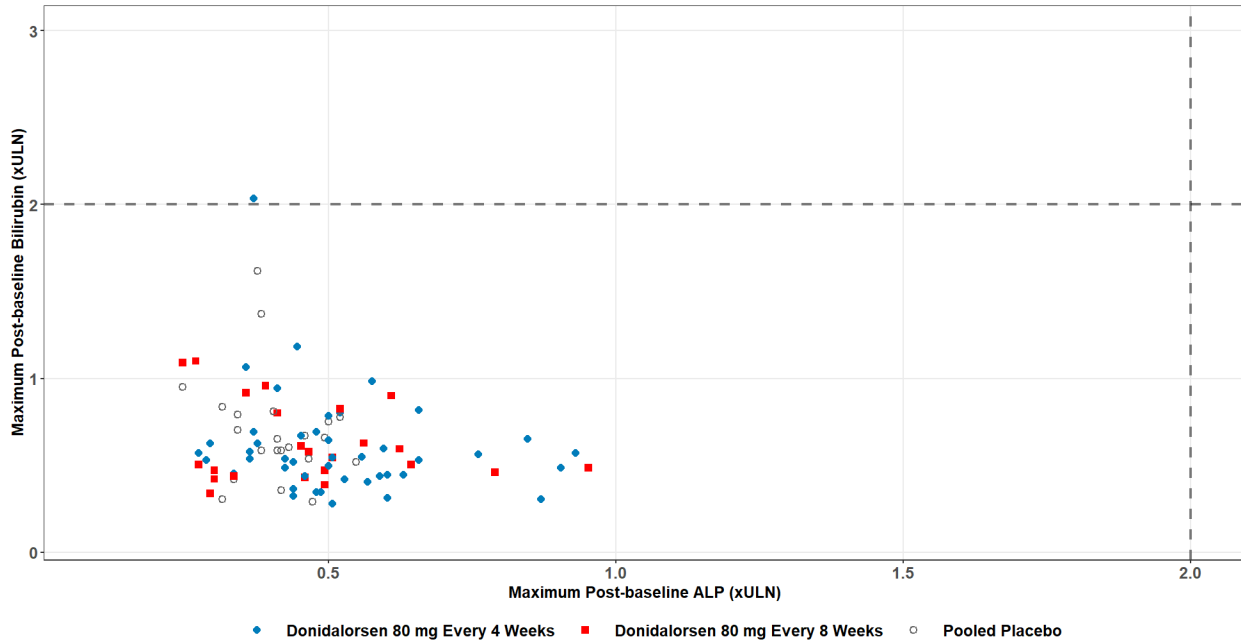
Source: Applicant CS5 CSR, Figure 14

The vertical dotted lines represent protocol-scheduled dosing days (this subject's last dose was given on Day 58) and horizontal dotted lines represent the highest upper limit and the lowest lower limit of the laboratory range. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILIS, total bilirubin; CSR, clinical study report; GGT, gamma-glutamyl transferase; INR, International normalized ratio; SCR, Screening; ULN, upper limit of normal

The second subject in the Temple's Corollary quadrant was a 25-year-old female with an ongoing medical history of hepatic steatosis and hepatosplenomegaly. At Screening, her LFTs were abnormal with ALT 113 U/L (reference range: 10 to 53 U/L), AST 53 U/L (reference range: 14 to 43 U/L), GGT 43 U/L (reference range: 7 to 38 U/L). LFTs were further elevated from Screening on Day 85 (ALT 168 U/L, AST 87 U/L, and GGT 58 U/L) and remained elevated on Day 111 (ALT 182 U/L, AST 88 U/L, and GGT 48 U/L); the subject received the last dose of the trial drug on the same day. On Day 161, the subject's ALT level was noted to be further elevated to 213 U/L (peak value >3.0 to <5.0x ULN); this lab abnormality was not recorded as a TEAE by the investigator. On Day 264, the subject's ALT level (109 U/L) decreased to baseline. Bilirubin levels remained within the reference range throughout trial; AST and GGT levels did not increase above 3.0x ULN.

The subject plotted in the Cholestasis quadrant (see [Figure 16](#)) was a 35-year-old female whose bilirubin levels remained elevated throughout the trial. She had a known medical history of Gilbert syndrome, to which the hyperbilirubinemia was attributed. Her elevated bilirubin was not recorded as a TEAE, and hepatic transaminases and GGT remained within the reference ranges throughout the trial.

Figure 16. Cholestatic Drug-Induced Liver Injury Screening Plot, Safety Population, Trial CS5



Source: adlb.xpt; Software: R

Each data point represents a subject plotted by their maximum ALP versus their maximum total bilirubin values in the postbaseline period.

A potential cholestatic DILI case (red circled) was defined as having a maximum postbaseline total bilirubin equal to or exceeding 2X ULN within 30 days after postbaseline ALP became equal to or exceeding 2X ULN.

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

For number of subjects in each quadrant, see the table "Subjects in Each Quadrant for Cholestatic DILI Screening Plot ..."

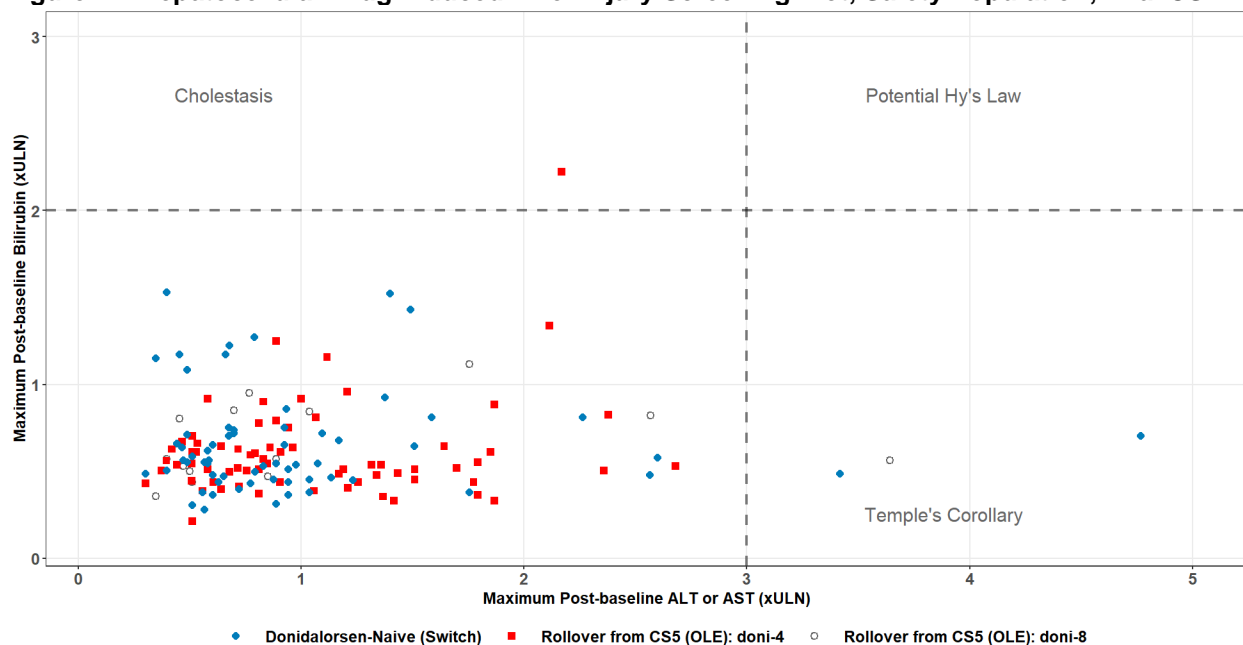
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

Trials CS3 and CS7

The open-label extension Trials CS3 and CS7, while uncontrolled, provide relevant liver safety results for longer term use of donidalorsen.

As shown in [Figure 17](#), the drug-induced liver injury Screening plot of Trial CS7 revealed no cases in the potential Hy's Law quadrant. One (7%) subject in the donidalorsen-8 group fell in Temple's Corollary quadrant, and two (3%) subjects in the naive group (receiving donidalorsen-4) fell in the cholestasis quadrant.

Figure 17. Hepatocellular Drug-Induced Liver Injury Screening Plot, Safety Population, Trial CS7



Source: adlb.xpt; Software: R

Each data point represents a subject plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period.

A potential Hy's Law case was defined as having any postbaseline total bilirubin equal to or exceeding 2X ULN after a postbaseline ALT or AST equal to or exceeding 3X ULN. Those subjects who meet total bilirubin equal to or exceeding 2X ULN criteria within 30 days of the ALT or AST equal to or exceeding 3X ULN criteria are circled in red.

The within 30 days analysis window rule does not apply to cholestasis and temple's corollary cases.

All subjects with at least one postbaseline ALT or AST, bilirubin and ULN are plotted.

In addition to central laboratory data, local laboratory data may be included in the analysis, if applicable.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; OLE, open-label extension; ULN, upper limit of normal

No subjects in Trial CS3 fell in Temple's Corollary or potential Hy's law quadrant (data not shown).

Pooled Hepatic Safety Results Through the 120-Day Safety Update Data Cutoff in CS5, CS2, and the Ongoing Open-Label Extension Trials (CS3 and CS7)

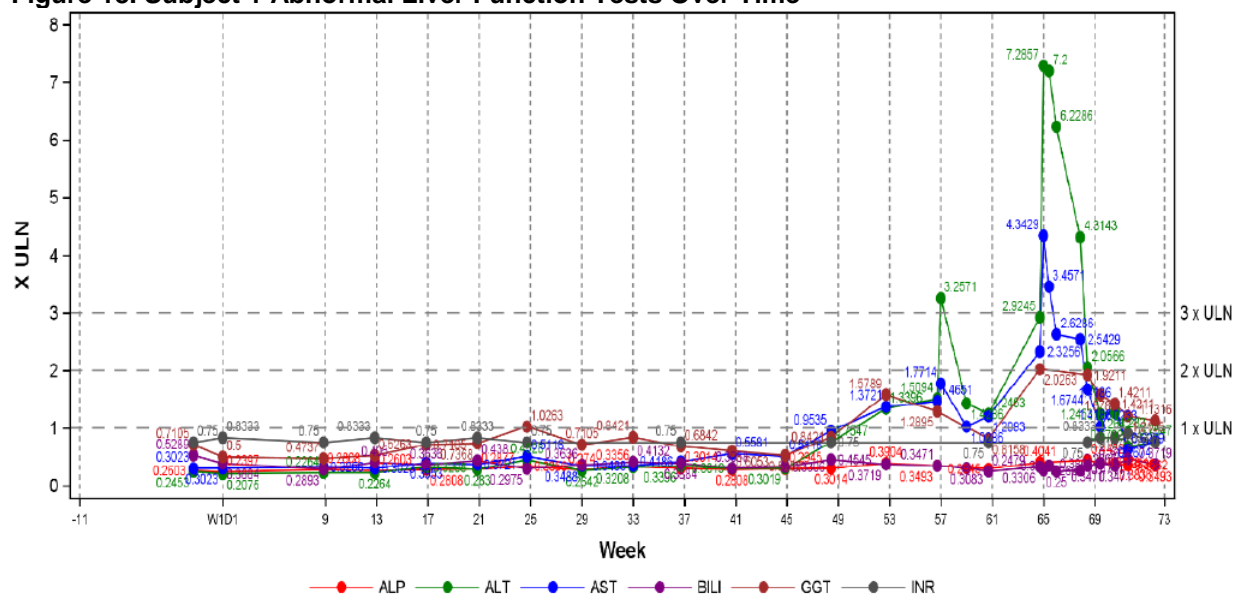
The Applicant provided a cumulative safety summary of Trials CS5, CS2, CS7, and CS3 with the 120-day safety update report. The most relevant new information is summarized below.

Since the NDA data cutoff, three (1.9%) subjects in the donidalorsen-4 group experienced new elevations in ALT $>3 \times$ ULN (all of whom also experienced ALT $\geq 5 \times$ ULN) and are described below. One of these subjects discontinued donidalorsen due to the ALT elevation meeting protocol-defined stopping rules.

A 45-year-old female subject who received donidalorsen-8 in Trial CS5 and received donidalorsen-4 in Trial CS7) experienced fluctuating ALT throughout Trial CS7. The subject experienced TEAEs of ALT increased (mild) and AST increased (mild) on integrated summary of safety (ISS) Trial Day 363 (CS7 Trial Day 197), which were upgraded to moderate severity on ISS Trial Day 447 (CS7 Trial Day 281) and then subsequently downgraded to mild severity on ISS Trial Day 480 (CS7 Trial Day 314). At Index Baseline, the subject's AST and ALT values

were within the reference range (13 and 12 U/L, respectively). No treatment was given for the elevated enzymes, which corrected gradually. Hepatitis viral serologies, autoimmune hepatitis, Epstein-Barr virus (EBV), and cytomegalovirus testing were negative. The subject's liver function tests over time are plotted in [Figure 18](#). The subject permanently discontinued the trial drug on ISS Trial Day 510 (CS7 Trial Day 344) since the ALT >5x ULN for over 2 weeks met the protocol stopping rule.

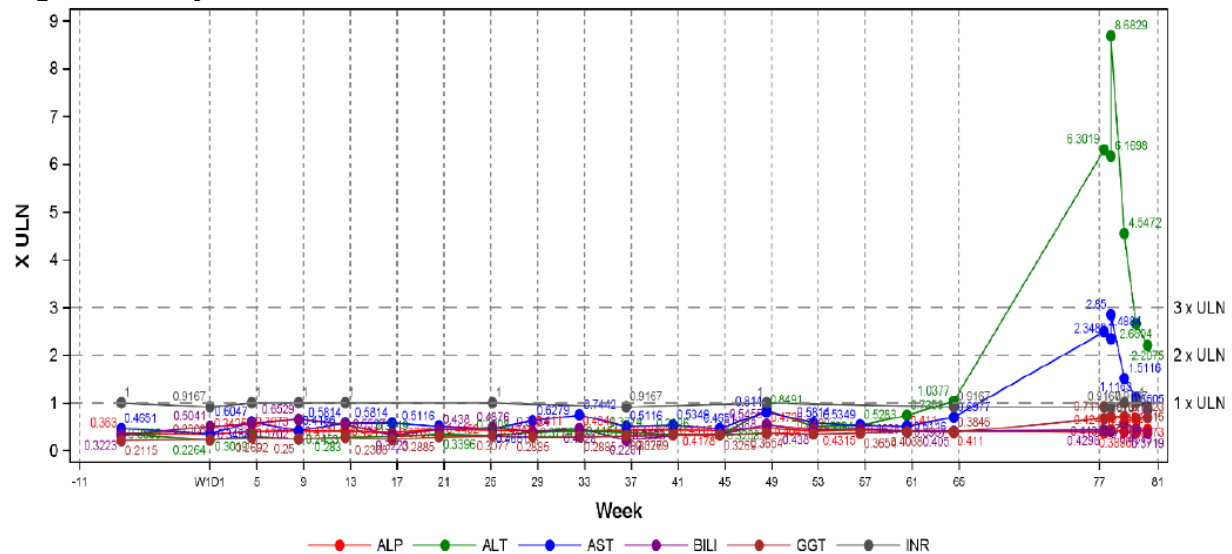
Figure 18. Subject 1 Abnormal Liver Function Tests Over Time



Source: Applicant's 120-day safety update report, Figure 1
 Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, bilirubin; GGT, gamma-glutamyl transferase; INR, international normalized ratio; ULN, upper limit of normal

A 27-year-old male subject from the Index placebo/OLE donidalorsen-4 group in Trial CS7 experienced severely elevated ALT levels while in Trial CS7. The subject's ALT level was within reference range at Index Baseline (15 U/L) and remained normal as of the NDA data cutoff. On ISS Trial Day 365 (CS7 Trial Day 365), the subject's ALT level was 55 U/L and on ISS Trial Day 455 (CS7 Trial Day 455), the subject's ALT level was noted to be elevated to 334 U/L (>5.0x ULN), which was also a ≥2 grade shift from normal (≤ ULN) to severe. On ISS Trial Day 459 (CS7 Trial Day 459), peak ALT value was 356 U/L; this elevation was not recorded as a TEAE. The subject did not receive any concomitant treatment for the event and no action was taken with the trial drug. ALT levels subsequently decreased to a moderate grade elevation at the following visit on ISS Trial Day 467 (CS7 Trial Day 467 [241 U/L]). ALT levels remained elevated at mild grade (> ULN to 3x ULN; 117 U/L) at the last trial visit at the time of the safety update data cutoff. The ALT elevations did not meet the protocol-defined stopping rules. On ISS Trial Day 500 (CS7 Trial Day 500), the subject was diagnosed with an EBV infection (immunoglobulin M titer of 82.9; reference range 0.0 to 39.9), which provides an alternative explanation for the increases in ALT and AST. The subject's liver function tests over time are plotted in [Figure 19](#).

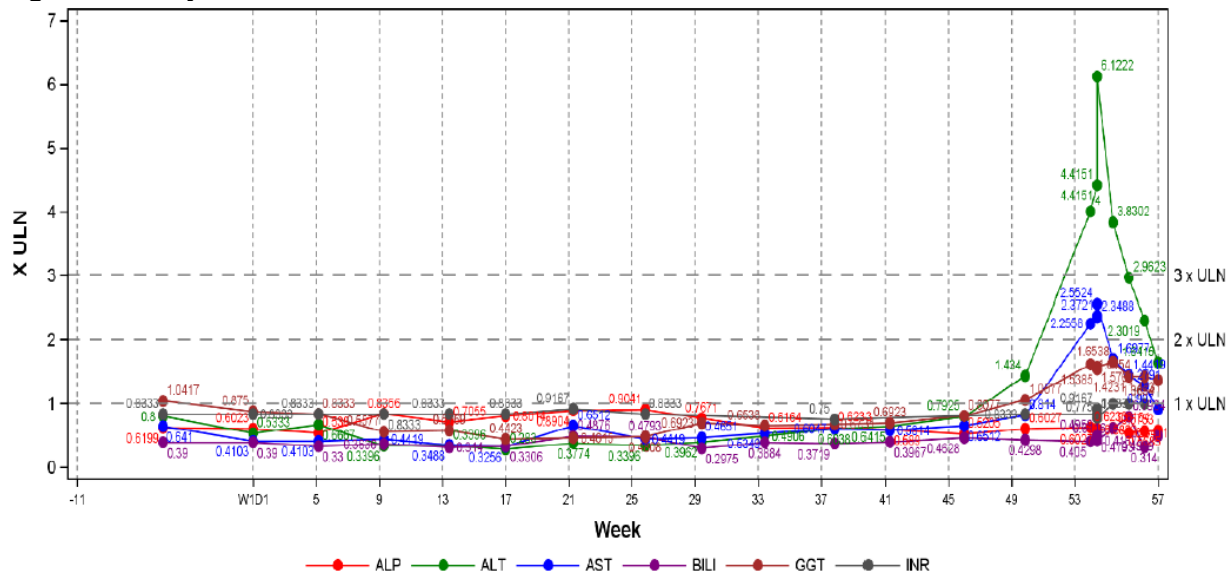
Figure 19. Subject 2 Abnormal Liver Function Tests Over Time



Source: Applicant's 120-day safety update report, Figure 2
 Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, bilirubin; GGT, gamma-glutamyl transferase; INR, international normalized ratio; ULN, upper limit of normal

A 17-year-old male subject from the Index/OLE donidalorsen-4 group in Trial CS7 experienced severe ALT levels while in Trial CS7. The subject's ALT level was within reference range at Index Baseline (20 U/L) and remained normal as of the NDA data cutoff. On ISS Trial Day 343 (CS7 Trial Day 169), the subject's ALT level was elevated to mild grade (76 U/L) and on ISS Trial Day 372 (CS7 Trial Day 198), the elevation reached moderate grade >3 to 5x ULN (212 U/L). On ISS Trial Day 375 (CS7 Trial Day 201), the subject's ALT level was noted to be elevated to 275.5 U/L (>5.0x ULN), which was also a ≥2 grade shift from normal (≤ ULN) to severe. The elevation was not recorded as a TEAE. ALT returned to moderate grade at the next trial visit (203 U/L). ALT levels remained elevated at mild grade (> ULN to 3x ULN; 87 U/L) at the last trial visit at the time of the safety update data cutoff. The ALT elevations did not meet the protocol-defined stopping rules. The subject did not receive any concomitant treatment for the event. No action was taken with the trial drug. The subject's liver function tests over time are plotted in [Figure 20](#).

Figure 20. Subject 3 Abnormal Liver Function Tests Over Time



Source: Applicant's 120-day safety update report, Figure 3
 Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, bilirubin; GGT, gamma-glutamyl transferase; INR, international normalized ratio; ULN, upper limit of normal

Bilirubin and GGT

One subject in Trial CS7 who was Naïve to donidalorsen, treated with donidalorsen-4, experienced severe bilirubin $>1.5 \times$ ULN, which was also a ≥ 2 -grade shift from normal, and GGT $>5.0 \times$ ULN with concurrent elevations in AST to $<3.0 \times$ ULN. The subject also had multiple comorbidities.

GGT elevation ($\geq 5 \times$ ULN) was reported in two subjects in Trial CS7. Both of these subjects experienced elevated GGT at baseline. No action was taken with donidalorsen as a result, and these subjects continued in the trial. Other confounding factors include long-term use of danazol, and congestive heart failure.

ALP

Across Trials CS2, CS3, CS5, and CS7, no subject in any treatment group experienced ALP elevations $\geq 2 \times$ ULN.

INR

In Trial CS7, one subject ((b) (6)) experienced concurrent TEAEs for increased PT (severity fluctuated between mild to moderate over 140 days with no dose change) and increased INR (mild, resolved after 92 days with no dose change); no bleeding events were recorded for this subject.

Integrated Summary

Due to the small sample size of the pivotal trial, safety data from the additional trials (the phase 2 Trial CS2 and the ongoing open-label Trials CS3 and CS7, including data from the 120-day

safety update) were included in evaluation of hepatic effects of donidalorsen. This summary incorporates findings across the program.

Regarding TEAEs, all abnormal liver function TEAEs were nonserious and mild to moderate in severity. Two subjects discontinued the trial drug due to these TEAEs: one for ALT increase related to donidalorsen, despite not meeting a stopping rule, and one for ALT increase that did meet a stopping rule.

No subject met criteria for Hy's law. Clinically relevant levels of LFT elevation occurred infrequently, while subclinical elevations in ALT, AST, GGT, and INR were observed more frequently with donidalorsen compared to placebo. Low-level elevations in AST and ALT ($<3\times$ ULN) are common, nonspecific, and not concerning for DILI.

A total of eight donidalorsen recipients (five in the donidalorsen-4 group and three in the donidalorsen-8 group) experienced ALT or AST elevations $\geq 3\times$ ULN. Of these, two subjects had ALT levels that peaked at a level $\geq 5\times$ ULN (one also experienced an AST $\geq 3\times$ ULN), and one subject peaked at ALT $>8\times$ ULN (possibly due to acute EBV infection). Two subjects discontinued donidalorsen due to LFT abnormalities.

Two subjects experienced severe ($\sim 1.5\times$ ULN) elevations of bilirubin and two subjects experienced severe ($\geq 5.0\times$ ULN) increases in GGT, but these did not require treatment discontinuation. Plausible etiologies were present for these subjects, including Gilbert syndrome, multiple comorbidities, elevated GGT level at baseline, prior long-term use of danazol, and congestive cardiac failure.

Conclusion

There was no clear imbalance for liver related TEAEs between donidalorsen and placebo. There were no cases of Hy's law. Clinically relevant levels of LFT elevation occurred infrequently, while subclinical elevations in ALT, AST, GGT, and INR were observed more frequently with donidalorsen compared to placebo. Low-level elevations in AST and ALT ($<3\times$ ULN) were common, nonspecific, and not concerning for DILI. A total of three subjects had ALT $\geq 5\times$ ULN.

Due to the known ASO class effect of hepatotoxicity, the accumulation of ASOs in the liver over time, the small safety database, and the unknown consequences of lifetime treatment with ASOs, a PMR will be issued to assess the potential for chronic drug-induced liver injury with long-term treatment.

7.7.4. Hypersensitivity Reactions

Issue

During the NDA review process, an adverse reaction of anaphylaxis in a subject enrolled in the ongoing trial (CS7) was reported as a Safety Report to IND 142564. The safety assessment relies on the response to an Information Request that was sent to the Applicant to provide relevant information to evaluate this safety signal.

Background

Hypersensitivity reactions are a known, labeled AE for several FDA-approved ASO products. Hypersensitivity reactions are listed in the Warning and Precautions section of labeling for at least four of these products: defibrotide, inotersen, eteplirsen, and golodirsen. The observed symptoms include rash, urticaria, skin exfoliation, chills, flushing, dysphagia, tachycardia, and bronchospasm. Serious hypersensitivity reactions occurred in the development program for volanesorsen, including suspected anaphylaxis in one subject.⁸

Assessment

For donidalorsen, the subject was a 20-year-old White female who received donidalorsen-4 in Trial CS5 and Trial CS7. The SAE with PT Hypersensitivity (Verbatim: Allergic reaction) occurred on Trial CS7 Day 816 and resulted in permanent treatment discontinuation. Per the case narrative, the event started 5 minutes after donidalorsen injection. The subject developed a red rash covering most of her body, dyspnea, perioral swelling, and chest pain. The investigator administered oral diphenhydramine and subcutaneous epinephrine. Her baseline vital signs were normal and she developed tachycardia to 145 bpm at the time of the event, but blood pressure was not measured. Thirty minutes later, when symptoms had subsided, heart rate was 113 and BP was 126/82 mm Hg. Tryptase was not drawn. The subject had also taken ibuprofen 200 mg to prevent postinjection site pain; she reported having tolerated ibuprofen in the past. The narrative states that a skin allergy test conducted with donidalorsen demonstrated a negative reaction, without providing details on the quality/results of this test, so this cannot be interpreted as meaningful. Given the fulfillment of Sampson's criteria ([Sampson et al. 2006](#)), the temporal relationship to donidalorsen, and the lack of an alternative diagnosis, this event is consistent with anaphylaxis due to donidalorsen.

Using a cumulative cutoff date of February 24, 2025, a search of the clinical and safety databases was conducted using the OCMQs of Hypersensitivity (broad) and Anaphylactic Reaction (broad) to identify potential cases of hypersensitivity and anaphylaxis, from two completed trials (CS2 and CS5) and two ongoing clinical trials (CS3 and CS7) in the donidalorsen clinical development program. The cumulative exposure across the four trials at the cutoff date was 177 subjects. Out of 53 events meeting the Hypersensitivity (broad) OCMQ criteria, the Applicant identified five events (PTs: four Hypersensitivity and one Rash) in three subjects (all in Trial CS7) that are consistent with non-serious hypersensitivity to donidalorsen (i.e., the temporal association and lack of alternate diagnosis make this the most likely diagnosis). One of these events led to treatment discontinuation.

There was no clear correlation between hypersensitivity and antidrug antibody (ADA) titers or systemic concentration.

⁸ See FDA Summary Review for Olezarsen (NDA 218614), dated December 19, 2024, available at https://www.accessdata.fda.gov/drugsatfda_docs/nda/2025/218614Orig1s000IntegratedR.pdf.

Conclusion

Overall, six AEs (one serious and five non-serious) in three (2%, 3/177) subjects who received donidalorsen were assessed as hypersensitivity reactions from Trials ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, and ISIS 721744-CS7. One subject (0.6%, 1/177) experienced a serious event of Hypersensitivity which fulfills NIAID criteria for the diagnosis of anaphylaxis was reported across all donidalorsen clinical trials. Two out of six AEs of hypersensitivity reactions led to permanent treatment discontinuation in two (1%) subjects.

This risk of donidalorsen use is important to communicate in labeling and will be described in Section 5 of the USPI.

8. Therapeutic Individualization

8.1. Intrinsic Factors

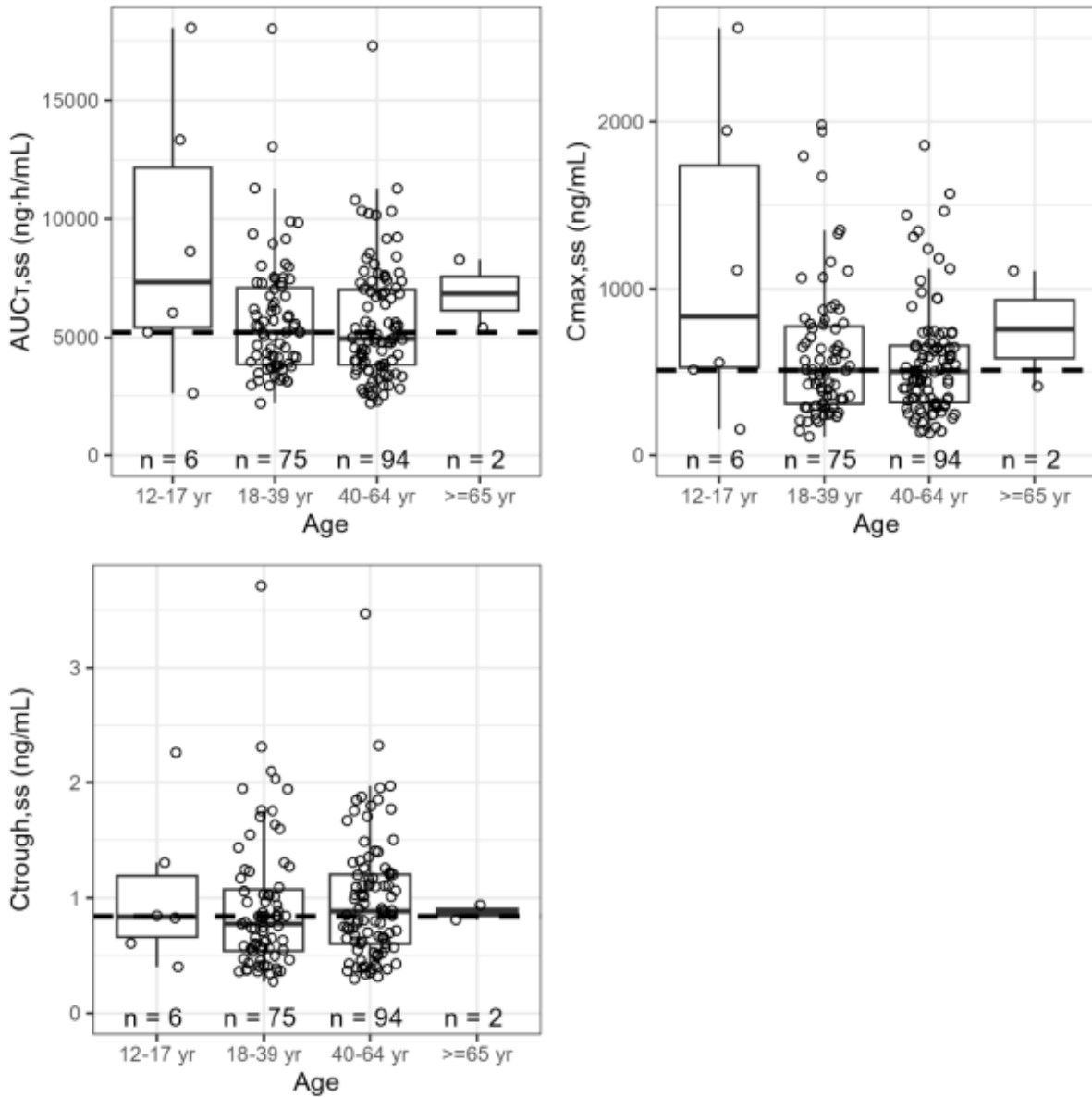
Population PK and PD analyses showed no clinically meaningful differences in the pharmacokinetics or pharmacodynamics of donidalorsen based on age, body weight, sex, race, ethnicity, disease status (i.e., healthy subjects or HAE patients), mild renal impairment (eGFR ≥ 60 to < 90 mL/min/1.73 m²), or mild hepatic impairment (total bilirubin $\leq 1 \times$ ULN and AST $> 1 \times$ ULN, or total bilirubin > 1 to $1.5 \times$ ULN and any AST). Donidalorsen has not been studied in patients with moderate or severe renal impairment, end-stage renal disease, or moderate or severe hepatic impairment.

8.1.1. Age

No dosage adjustments based on age are recommended.

The proposed indication for donidalorsen is for prophylaxis to prevent HAE attacks in adults and adolescents aged 12 years and older. No dose adjustment was proposed for adolescent subjects. Based on the Applicant's population PK analysis, the steady-state exposures following administration of the donidalorsen-4 regimen was similar across different age groups, as shown in [Figure 21](#). A higher mean exposure (area under the concentration-time curve [AUC]_{tau,ss} and maximum plasma concentration [C_{max}]_{ss}) was estimated for adolescents as compared to adults. The difference in body weight may contribute to this exposure difference (refer to Section [8.1.2](#)). The mean body weight for adolescents (12 to 17 years) and adults enrolled in Trial CS5 were 67.98 kg and 81.78 kg, respectively.

Figure 21. Population-Estimated Post Hoc PK Parameters at Steady State by Age



Source: Figure 86, Report 721744-PPK01

Horizontal lines (middle of the boxes), median; hinges (top and bottom of the boxes), 25th and 75th percentiles (i.e., IQR); whiskers, extend to largest and smallest values within $1.5 \times$ IQR of the hinges; circles, individual data; dashed lines, median across all categories.

Abbreviations: AUC, area under the plasma concentration-time curve; C_{max} , maximum plasma concentration; C_{trough} , trough plasma concentration; IQR, interquartile range; PK, pharmacokinetic; yr, year

The population PK analysis results were consistent with observed data from the pivotal phase 3 trial (CS5), in which seven adolescent subjects with Type I or II HAE were evaluated with the same dosing regimens as adults (three treated with the donidalorsen-8 regimen and four treated with the donidalorsen-4 regimen). The systemic exposure (trough concentration [C_{trough}]) in adolescent subjects was similar to that in adults who received the same dosing regimen. For donidalorsen-4 in Trial CS5, the observed median C_{trough} at Week 17 for both adults (N=39) and adolescents (N=4) was 0.61 ng/mL.

In terms of PD response, a greater percent reduction from baseline in mean plasma PKK was observed in adolescents relative to adults based on observed data in Trial CS5 ([Table 29](#)).

Table 29. Mean (SD) Observed Plasma PKK Concentration Over Time Between Adults and Adolescents, Trial CS5

Parameter	Q4W		Q8W	
	Adults	Adolescents	Adults	Adolescents
Run-In baseline value (µg/mL)	127.6 (43.3) (N=41)	133.8 (26.5) (N=4)	145.7 (61.0) (N=20)	132.1 (46.8) (N=3)
Week 21 value (µg/mL)	36.9 (28.5) (N=40)	10.5 (7.5) (N=4)	51.1 (26.7) (N=18)	33.4 (14.8) (N=3)
Percent change from baseline (%)	-70.8 (16.9) (N=40)	-92.5 (4.4) (N=4)	-59.6 (22.7) (N=18)	-70.0 (20.2) (N=3)

Source: Reviewer-generated table based on adpd.xpt for CS5

Abbreviations: PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

The mean (range) percent change in the HAE attack rate between adults and adolescents was similar among subjects in the same treatment group based on data from Trial CS5, as shown in [Table 30](#).

Table 30. Mean (Range) HAE Attack Rate and Percent Change From Baseline by Age Group, Trial CS5

Parameter	Adolescents (12 to 17 Years)		Adult (18 to 39 Years)		Adult (40 to 64 Years)	
	80 mg Q4W (N=4)	80 mg Q8W (N=3)	80 mg Q4W (N=19)	80 mg Q8W (N=20)	80 mg Q4W (N=21)	80 mg Q8W (N=7)
	Run-In baseline	1.74 (0.5, 3.9)	2.04 (1.0, 3.9)	3.49 (1.1, 8.0)	2.84 (0.7, 7.6)	4.18 (0.8, 10)
Week 1 to 25	0.04 (0.0, 0.2)	0.39 (0.2, 0.7)	0.36 (0.0, 1.8)	0.66 (0.0, 4.7)	1.55 (0.0, 9.6)	2.21 (0.3, 5.8)
Percent change from baseline	-97.13 (-100, -88.5)	-70.53 (-91.4, -33.3)	-88.52 (-100, -49.5)	-84.84 (-100, -38.5)	-72.61 (-100, 2.4)	-33.40 (-94.2, 155.2)

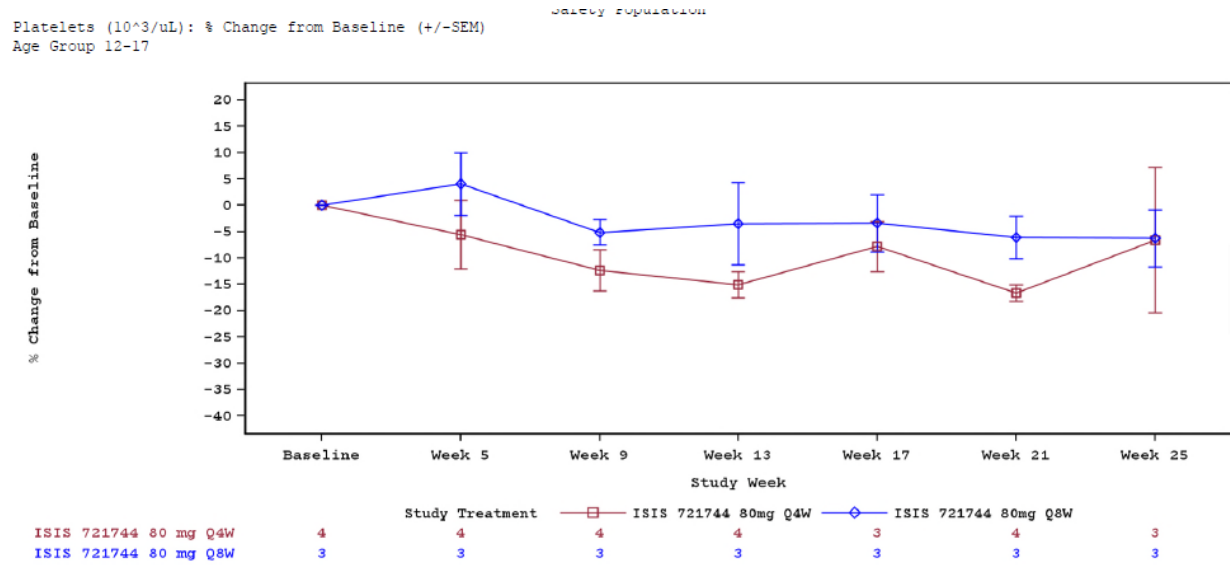
Source: Adapted from Table 14.2.1.5-1 CSR CS5

Abbreviations: CSR, clinical study report; HAE, hereditary angioedema; Q4W, every 4 weeks; Q8W, every 8 weeks

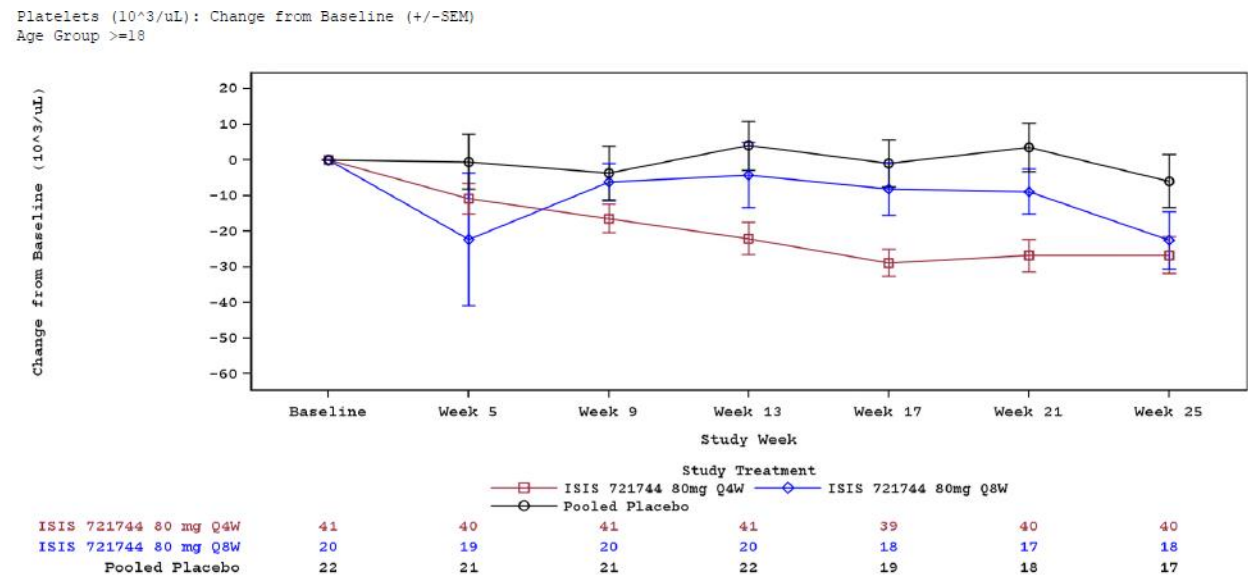
Antisense oligonucleotide therapeutics are associated with bleeding risks, including thrombocytopenia. Therefore, the impact of donidalorsen on platelet counts by age group was assessed. For additional details on the association between donidalorsen and bleeding events or thrombocytopenia, refer to Section [7.7.2](#). In Trial CS5, the mean (SE) percent change from baseline in platelet count was similar between adults and adolescents treated with the same dosing regimen, as shown in [Figure 22](#).

Figure 22. Mean (SE) Percent Change of Platelet From Baseline by Age Group, Trial CS5

A) Age 12 to 17 Years



B) Age 18 Years and Older



Source: Figure 14.3.4.1.2. CSR CS5

Abbreviations: CSR, clinical study report; ISIS 721744, donidalorsen; Q4W, every 4 weeks; Q8W, every 8 weeks; SE, standard error; SEM, standard error of mean

Overall, based on results from Trial CS5, adolescents and adults demonstrated similar donidalorsen systemic exposure with no clinically meaningful differences noted for PD (i.e., PKK) and efficacy response.

It was noted that one adolescent subject aged 17 years who rolled over from Trial CS5 to the OLE trial (CS7) and continued receiving the same donidalorsen-4 regimen across both trials experienced increases in ALT after the NDA data cutoff date. Given that similar safety events were also observed among adult subjects who received a long duration of treatment with

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donidalorsen, there is no notable difference in safety between adults and adolescents. See Section [7.7](#) for more details.

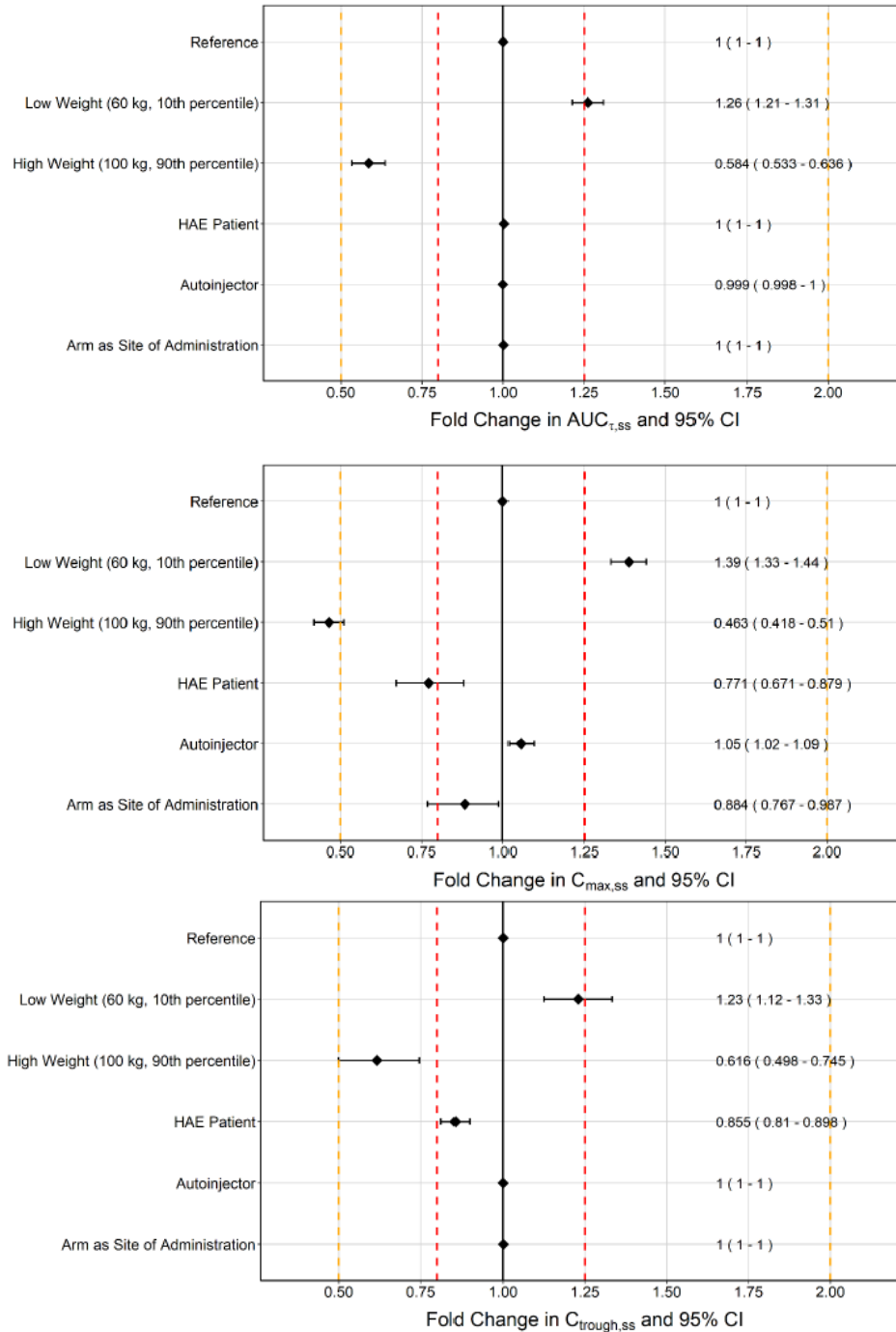
Overall, no dosage adjustments by age are recommended.

8.1.2. Body Weight

No dosage adjustments based on body weight are recommended.

No dosage adjustments were proposed based on body weight. Based on the Applicant's population analysis, body weight was identified as a significant covariate on donidalorsen pharmacokinetics. In general, donidalorsen exposure is higher in subjects with lower body weight. As compared with a reference subject with a body weight of 70 kg, the median donidalorsen exposure ($AUC_{\tau, ss}$, $C_{max, ss}$, $C_{trough, ss}$) for a subject at the 10th percentile of the body weight distribution (i.e., 60 kg) is expected to increase by 26%, 39%, and 23%, respectively. A subject at the 90th percentile of the body weight distribution (i.e., 100 kg) is expected to have a 42% lower $AUC_{\tau, ss}$, 54% lower $C_{max, ss}$, and 38% lower $C_{trough, ss}$ compared with a reference subject with a weight of 70 kg, as shown in [Figure 23](#).

Figure 23. Model-Predicted Covariate Effects on Donidalorsen PK Parameters



Source: Figure 37, 39, and 41. Report 721744-PPK01

The solid black line represents the median of the simulated reference subject, defined as a healthy volunteer with a body weight of 70 kg receiving 80 mg donidalorsen Q4W SC into abdomen or thigh using vial as the drug presentation.

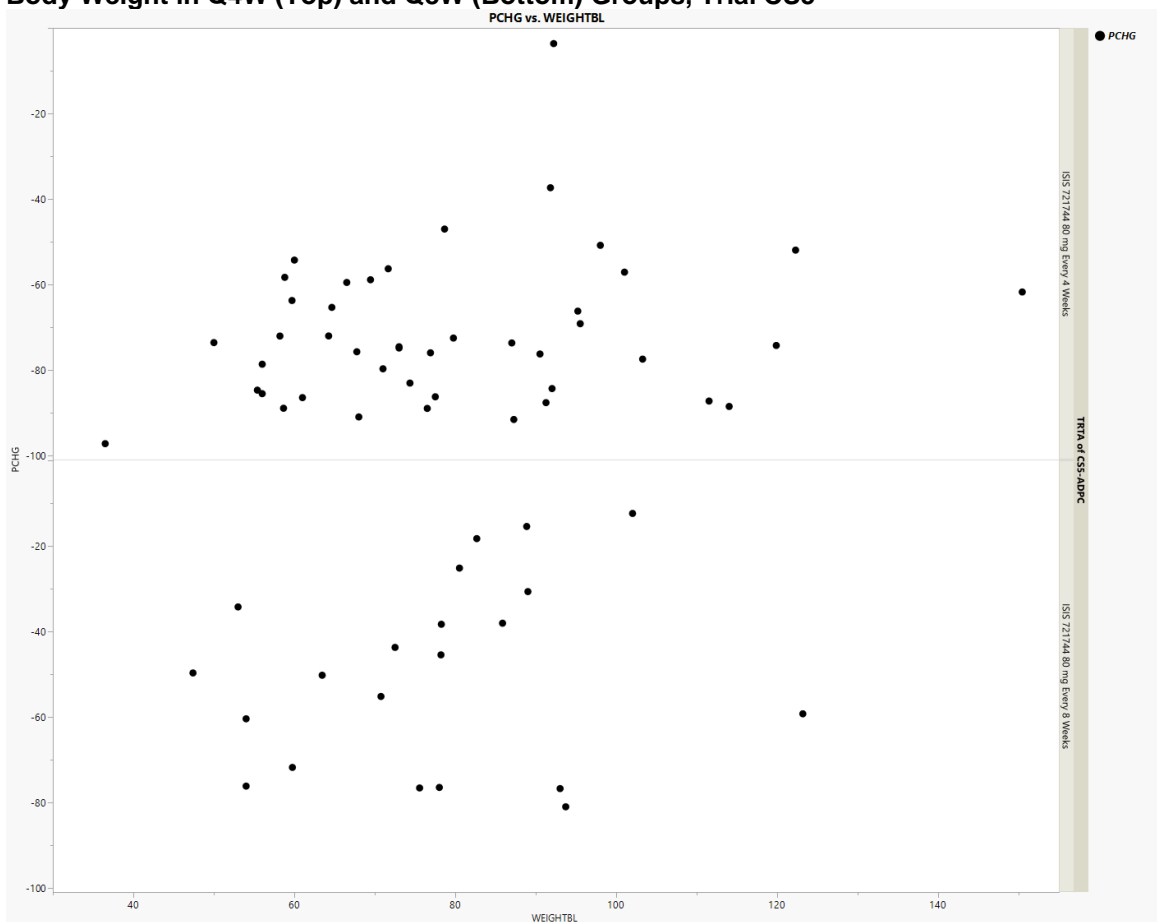
Dashed red lines represent the 80 to 125% range of the reference subject, and dashed orange lines represent the 50 to 200% range of the reference subject.

The symbols and error bars represent the median and 95% CI based on 1000 simulated subjects within each group including uncertainty on the fixed effect.

Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum plasma concentration; C_{trough}, trough plasma concentration; HAE, hereditary angioedema; PK, pharmacokinetic; Q4W, every 4 weeks; SC, subcutaneous

The Applicant's population PK simulation results on the impact of body weight were consistent with observations in Trial CS5. Despite differences in systemic exposure noted across different body weights, no notable difference in Week 17 pharmacodynamic response (i.e., percent change from baseline at steady state in plasma PKK) according to body weight was observed in Trial CS5, as shown in [Figure 24](#).

Figure 24. Percent Change From Baseline in Plasma Trough PKK Concentrations at Week 17 vs. Body Weight in Q4W (Top) and Q8W (Bottom) Groups, Trial CS5



Source: Reviewer's analysis based on adpd.xpt for CS5
X-axis represents body weight and Y-axis represents percent change from Baseline to Week 17 in plasma trough PKK concentrations.
Top and bottom panels show data from subjects who received the Q4W and Q8W regimens, respectively.
Abbreviations: PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks

The percent change in HAE attack rate was also similar across body weight quartiles for the donidalorsen-4 group in Trial CS5 ([Table 31](#)), which is consistent with lack of impact of body weight on PD response. Notably, among subjects who received donidalorsen-8, a lower reduction in HAE attack rate was observed among subjects in the third body weight quartile. This result appears to have been driven by data from an outlier subject ((b) (6)) who presented with an HAE attack rate between Weeks 1 and 25 of 5.9 (percent change from baseline of +155%). Excluding this subject, the mean (SD) HAE attack rate within the third body weight quartile was 0.9 (1.3) with mean (SD) percent change from baseline of -56.1% (69.3). Despite this result, no consistent trend of lower HAE attack rate in higher body weight quartiles was

observed. The data support that the change in plasma PKK concentration and HAE attack rate is more likely associated with clinical effect at the site of action (i.e., in the liver) rather than donidalorsen concentrations in plasma.

Table 31. Mean (SD) HAE Attack Rate Following Donidalorsen Q4W and Q8W by Body Weight Quartiles, Trial CS5

Parameter	Body Weight Quartiles			
	1 st Quartile	2 nd Quartile	3 rd Quartile	4 th Quartile
Q4W				
Run-In baseline	4.7 (2.2) (N=11)	3.1 (2.7) (N=11)	2.8 (1.2) (N=11)	3.8 (2.3) (N=12)
Week 1 to 25	1.3 (2.3) (N=11)	0.7 (1.1) (N=11)	0.5 (0.4) (N=11)	1.1 (2.7) (N=12)
Percent change from baseline to Week 1 to 25 (%)	-81.1 (30.3) (N=11)	-82.2 (18.8) (N=11)	-83.0 (12.1) (N=11)	-79.7 (32.0) (N=12)
Q8W				
Run-In baseline	6.2 (1.8) (N=5)	2.6 (1.7) (N=6)	2.2 (1.0) (N=6)	2.5 (1.5)
Week 1 to 25	2.2 (2.0) (N=5)	0.6 (0.5) (N=6)	1.7 (2.3) (N=6)	0.4 (0.5) (N=6)
Percent change from baseline to Week 1 to 25 (%)	-64.6 (29.2) (N=5)	-70.0 (24.8) (N=6)	-20.9 (106.2) (N=6)	-86.3 (12.8) (N=6)

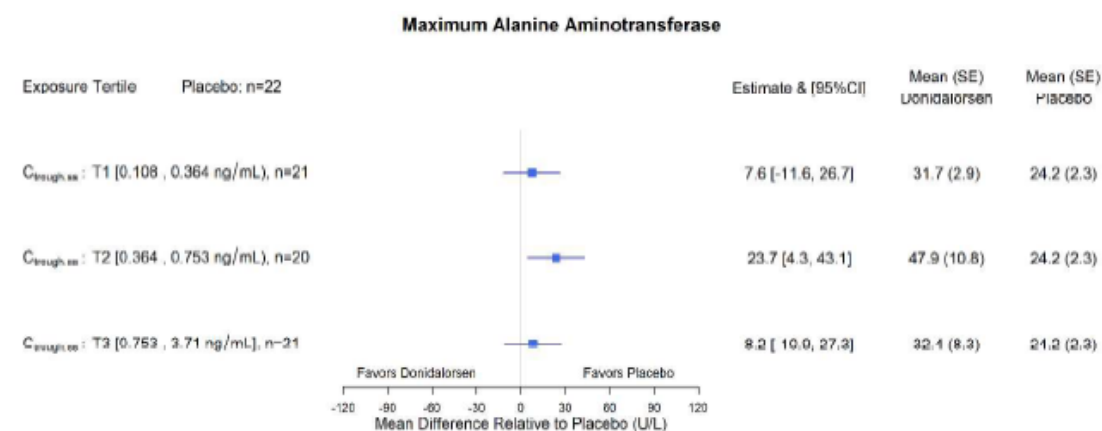
Source: Reviewer's analysis based on adefx.xpt for CS5

The body weight range for each quartile in Q4W was 1st [36.5, 64.3] kg, 2nd [64.3, 76.5] kg, 3rd [76.5, 92.2] and 4th [92.2, 150.4] kg. The body weight range for each quartile in Q8W was: 1st [47.4, 63.5] kg, 2nd [63.5, 78.2] kg, 3rd [78.2, 88.9] and 4th [88.9, 123.2] kg. Abbreviations: HAE, hereditary angioedema; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

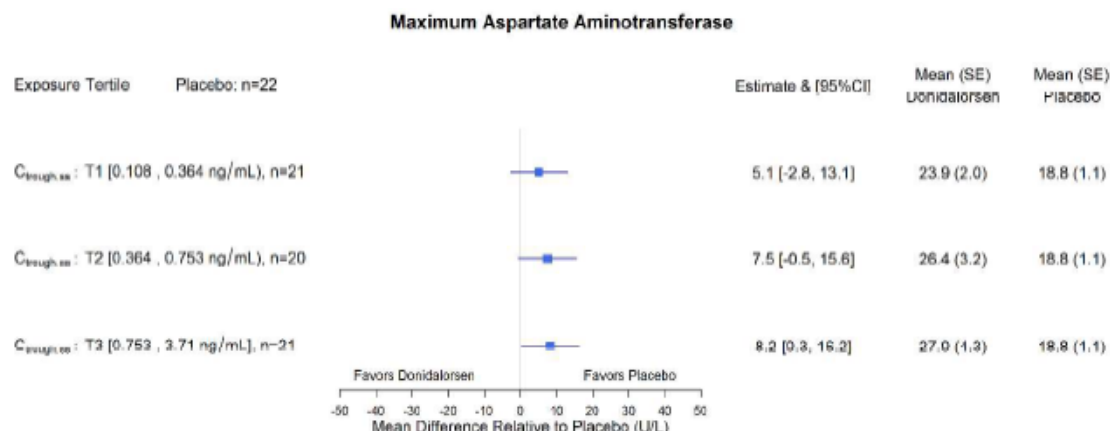
The potential impact of body weight-associated changes in donidalorsen systemic exposure on the safety of donidalorsen was also explored. Based on liver function tests in Trial CS5, an increase in donidalorsen systemic exposure was not associated with LFT elevations based on the Applicant's exposure-response analysis. However, for all donidalorsen tertiles, ALT and AST were greater as compared to placebo. The analysis for $C_{trough,ss}$ is shown in [Figure 25](#).

Figure 25. Exposure-Response Analysis for Safety Based on Trial CS5 (Liver Function Test)

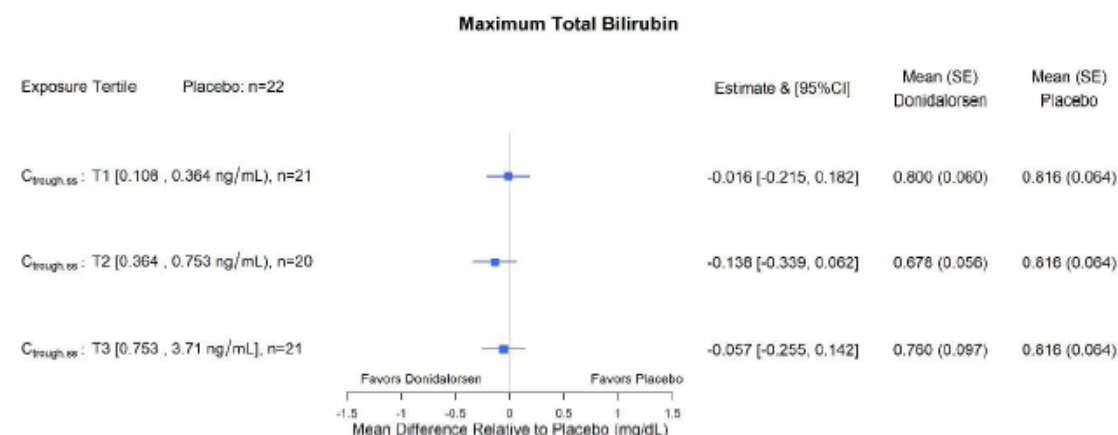
A) Maximum ALT vs. $C_{trough,ss}$ Tertiles



B) Maximum AST vs. $C_{trough,ss}$ Tertiles



C) Maximum Total Bilirubin vs. $C_{trough,ss}$ Tertiles



Source: Figure 11, 14, and 17. Report PPK02

Analysis was conducted on the safety population, consisting of subjects from Trial ISIS 721744-CS5, who received placebo or at least one dose of donidalorsen and were included in the population PK model, and had at least one safety endpoint measurement. Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; C_{trough} , trough concentration; PK, pharmacokinetic; SE, standard error

Other PK parameters including $C_{max,ss}$ and $AUC_{1344h,ss}$ were also used for the exposure-response (E-R) safety analysis. A similar trend was observed with these parameters as for $C_{trough,ss}$. There is no apparent E-R relationship for the magnitude of difference in ALT or AST relative to placebo. The Applicant's E-R analyses were consistent with the observed data in Trial CS5, where subclinical elevations in LFTs were observed with both donidalorsen-4 and donidalorsen-8 groups with no imbalance between treatment groups in clinically relevant LFT elevation: two subjects (9%) treated with donidalorsen-8 were reported to have ALT $>3\times$ ULN, while one subject (2.2%) treated with donidalorsen-4 was reported to have total bilirubin $>2\times$ ULN. The body weight of these subjects ranged from 60 kg to 93.7 kg.

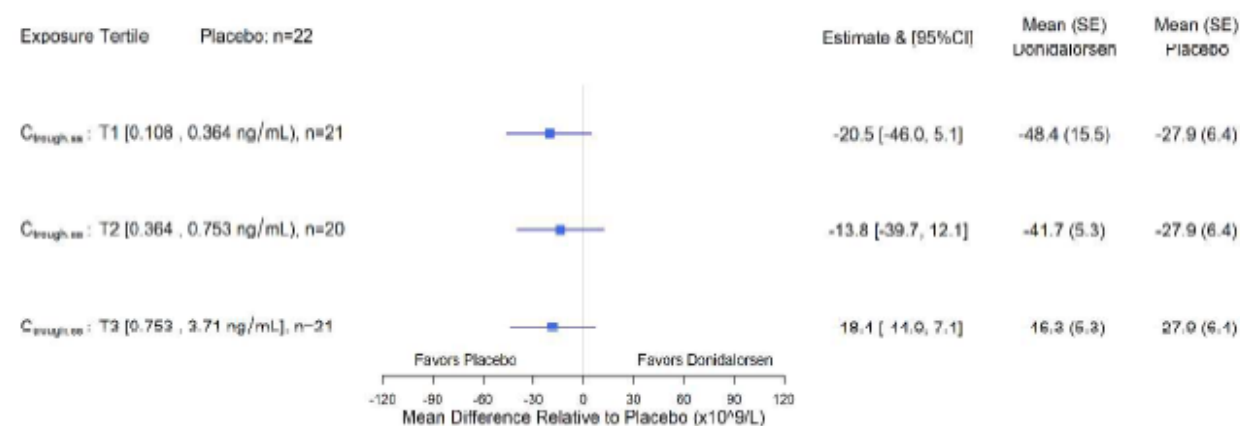
However, in the 120-day safety update, three Rollover subjects from Trial CS5 (Index) to the OLE trial (CS7) experienced an increase in ALT $>3\times$ ULN (one from Index Q8W/OLE Q4W, one from Index/OLE Q4W, and one from Index placebo/OLE Q4W). At the time of NDA data cutoff, these subjects had normal ALT levels at baseline and elevations were experienced after receiving donidalorsen for a longer duration (around over one year). See Section 7.7 for more details. Although two out of three subjects were treated with a greater dosing frequency

(donidalorsen-4) in Trial CS7 compared to their previously assigned regimen in Trial CS5 (placebo or donidalorsen-8), it is unclear whether the increase in dosing frequency, the duration of exposure, or both contributed to the ALT elevations.

As described above, platelet lowering is a class effect for oligonucleotide therapeutics. Therefore, the E-R relationship for platelet counts was also assessed based on data from Trial CS5, as shown in [Figure 26](#). Based on $C_{\text{trough,ss}}$, all exposure tertiles demonstrated a reduction in platelet counts as compared to placebo. However, no clear exposure-dependent trend was identified (i.e., greater reductions in platelets with higher $C_{\text{trough,ss}}$).

Figure 26. Exposure-Response Analysis for Safety Based on Trial CS5 (Platelet Count)

Maximum Decrease From Baseline Platelet Count vs. $C_{\text{trough,ss}}$ Tertiles



Source: Figure 35, Report PPK02

Analysis was conducted on the safety population, consisting of subjects from Trial ISIS 721744-CS5, who received placebo or at least one dose of donidalorsen and were included in the population PK model, and had at least one safety endpoint measurement. Abbreviations: CI, confidence interval; C_{trough} , through concentration; PK, pharmacokinetic; SE, standard error

Other PK parameters including $C_{\text{max,ss}}$ and $AUC_{1344h,ss}$ were also used for the E-R safety analysis. A similar trend was observed with these parameters as for $C_{\text{trough,ss}}$. The Applicant's E-R analyses were consistent with the observed data in Trial CS5, where no imbalance between treatment groups in clinically significant platelet count reduction was observed. See [Section 7.7](#) for more details.

AEs related to platelet reduction and liver enzyme elevations were reported across a wide range of body weights. In addition, there was no clear exposure-response relationship for these safety events. Therefore, the body weight-associated changes in donidalorsen plasma exposures are not likely to affect the safety of donidalorsen.

Thus, although body weight was identified as a significant covariate on donidalorsen pharmacokinetics, there was no clinically significant difference in PD response (PKK reduction from baseline), efficacy, or safety across different body weights. Therefore, no dosage adjustments based on body weight are recommended.

8.1.3. Hepatic Impairment

No dosage adjustments are recommended for patients with mild hepatic impairment.

Donidalorsen has not been studied in patients with moderate or severe hepatic impairment. Use of donidalorsen is not recommended in patients with moderate or severe hepatic impairment.

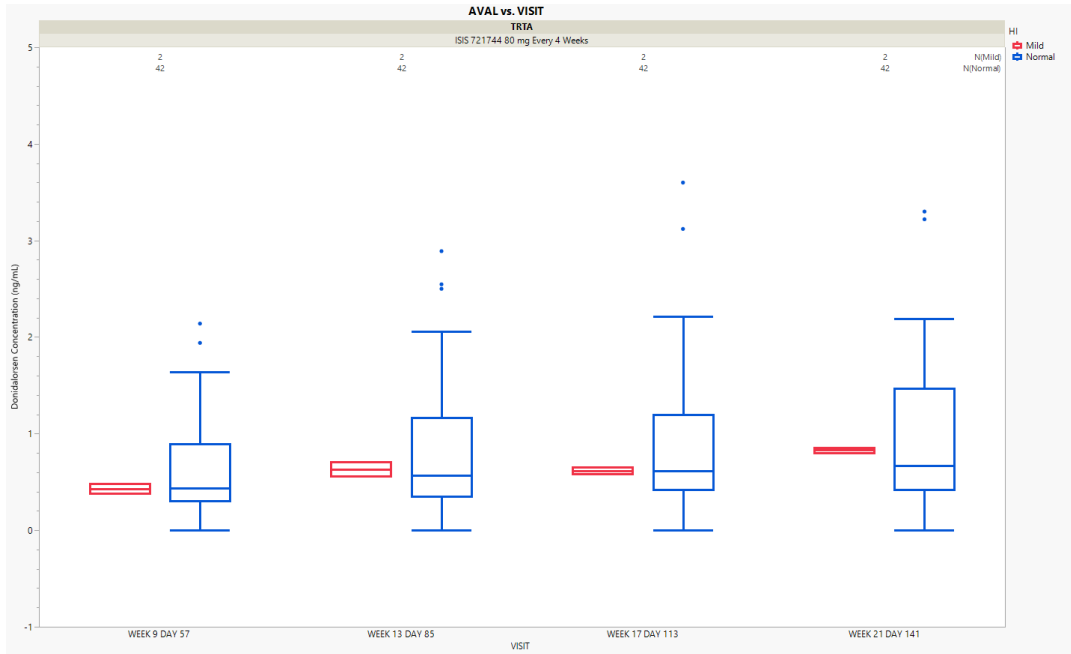
The effect of hepatic impairment on the pharmacokinetics and pharmacodynamics of donidalorsen was not assessed in a dedicated clinical pharmacology trial. No dose adjustment was proposed for subjects with mild hepatic impairment. Donidalorsen has not been studied in subjects with moderate or severe hepatic impairment. However, the Applicant has not proposed a restriction on the use of donidalorsen in patients with moderate or severe hepatic impairment.

In the pivotal trial (CS5), two subjects ((b) (6) and (b) (6)) with mild hepatic impairment (based on National Cancer Institute-Organ Dysfunction Working Group classification; total bilirubin $\leq 1 \times$ ULN and AST $> 1 \times$ ULN, or total bilirubin > 1 to $1.5 \times$ ULN and any AST level) were treated with donidalorsen-4. One subject (Subject (b) (6)) had normal total bilirubin but elevated AST $> 1 \times$ ULN at baseline (Week 1). The other subject ((b) (6)) had elevated bilirubin (> 1 to $1.5 \times$ ULN) but normal AST at baseline (Week 1). Both subjects also had mild elevation of ALT (1.1 to $1.2 \times$ ULN) at Week 1. No subject with moderate or severe hepatic impairment (i.e., total bilirubin $> 1.5 \times$ ULN irrespective of AST level) was included in the donidalorsen clinical program.

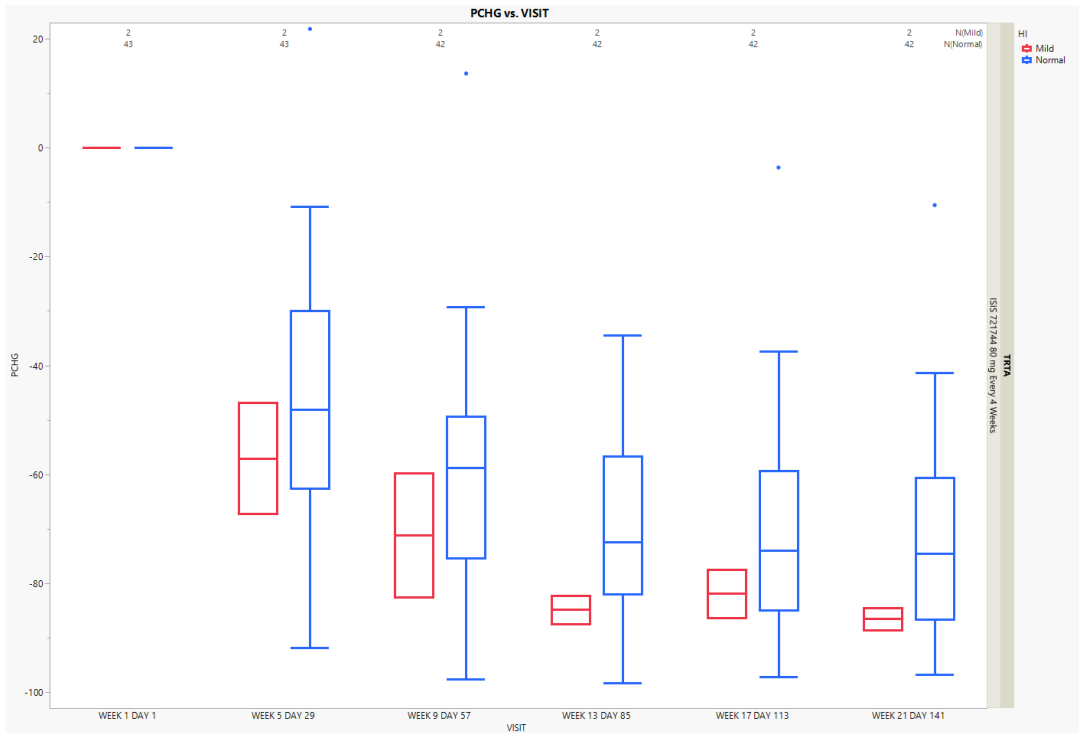
The systemic exposure of donidalorsen and PD response (i.e., PKC concentrations) in these two subjects were within the range of those observed in subjects with normal hepatic function in the same dosing group (i.e., donidalorsen-4), as shown in [Figure 27](#). Subjects with mild hepatic impairment achieved $> 80\%$ reduction from baseline in HAE attack rate between Week 5 and Week 25, which is similar to what was observed in subjects with normal hepatic function.

Figure 27. Box Plot of Observed Plasma Donidalorsen C_{trough} (Top) and Percent Change in Plasma Trough PKK Concentration (Bottom) Over Time in Subjects With Normal Hepatic Function and Two Subjects With Mild Hepatic Impairment Following Q4W Dosing, Trial CS5

A) Plasma Donidalorsen C_{trough} Over Time



B) Percent Change in Plasma Trough PKK Concentration Over Time



Source: Reviewer-generated graph based on adpc.xpt and adpd.xpt for CS5
 Red represents mild hepatic impairment (N=2). Blue represents normal subjects (N=43).
 Abbreviations: C_{trough} , trough plasma concentration; HI, hepatic impairment; PKK, prekallikrein; Q4W, every 4 weeks

Similar to other GalNAc-conjugated oligonucleotide products, donidalorsen is mainly distributed to the liver after subcutaneous administration with an initial rapid distribution phase followed by a much slower elimination phase. Therefore, the liver concentrations of donidalorsen and its metabolites are expected to be higher than those in plasma. Hepatic impairment may thus impact the accumulation of GalNAc-conjugated products in the liver. In addition, the production of PKK may be impaired in patients with hepatic impairment, which indicates that a lower dose of donidalorsen may also be effective in this patient population. As donidalorsen has not been evaluated in subjects with moderate to severe hepatic impairment, the long-term safety and efficacy of donidalorsen in subjects with moderate to severe hepatic impairment is not known.

Both subjects with mild HI enrolled in Trial CS5 and later rolled over into Trial CS7 and continued on the same donidalorsen-4 dosing regimen. Both subjects had ALT levels trending upwards in Trial CS7, although no ALT value exceeded $>3\times$ ULN as of the date of NDA data cutoff. Neither of these two subjects presented with any new ALT elevations after NDA data cutoff in the 120-day safety update. See Section 7.7 for more details regarding safety evaluation. The small sample size of mild HI subjects (N=2) limits the evaluation of safety in this population.

In subjects with normal hepatic function, although there was no clear exposure-response relationship observed between LFT elevation and plasma donidalorsen concentration in Trial CS5, subclinical elevations in LFTs were observed more frequently with donidalorsen compared to placebo. Some subjects with normal ALT at baseline who were treated with donidalorsen for a longer duration (>1 year) throughout Trial CS5 and Trial CS7 experienced clinically relevant ALT elevations ($\geq 5\times$ ULN). This observation increases the uncertainty regarding the long-term safety of donidalorsen in patients with moderate to severe hepatic impairment who may be more vulnerable to hepatic toxicity. Therefore, the use of donidalorsen is not recommended in patients with moderate to severe hepatic impairment from a safety perspective.

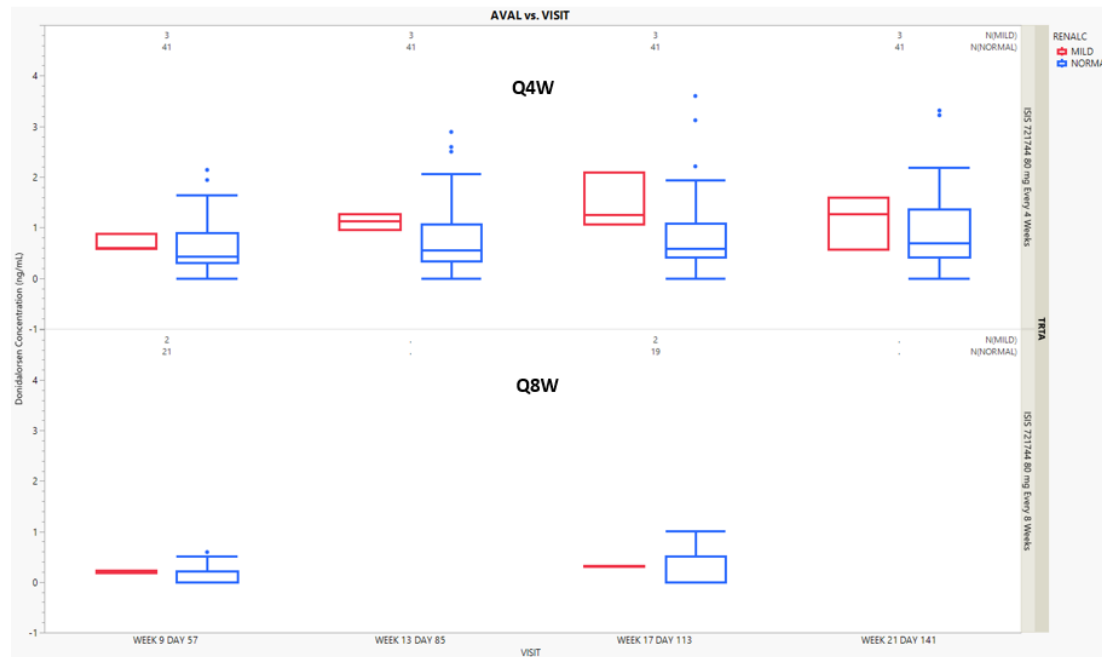
8.1.4. Renal Impairment

No dosage adjustments are recommended for patients with mild renal impairment. Donidalorsen has not been studied in patients with moderate or severe renal impairment or end-stage renal disease.

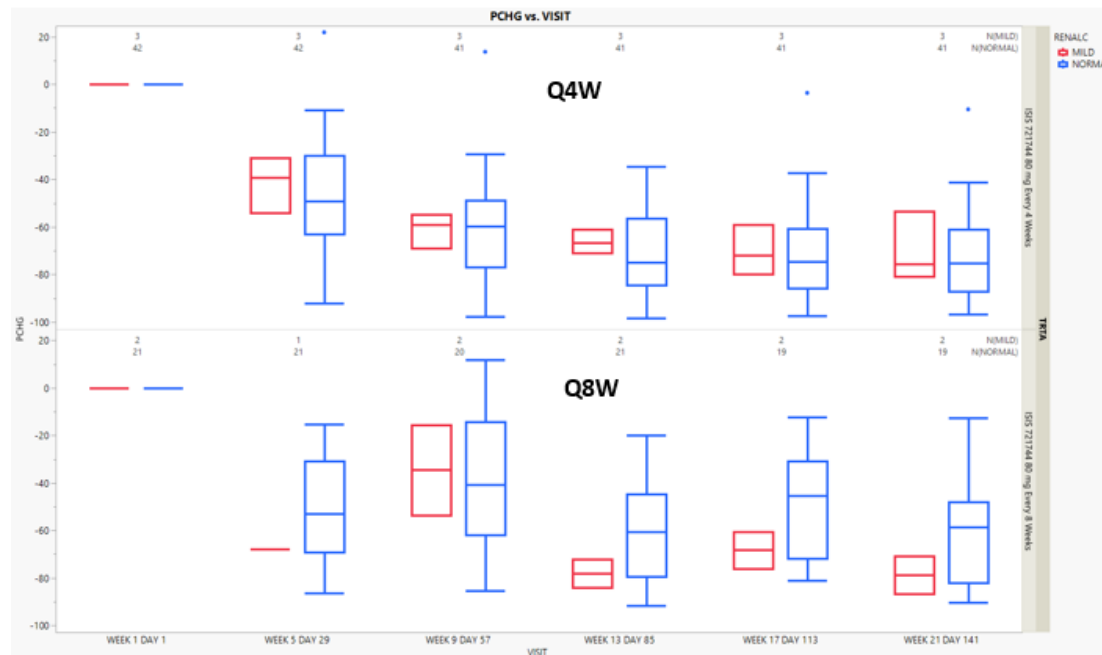
The effect of renal impairment on the pharmacokinetics and pharmacodynamics of donidalorsen was not assessed in a dedicated clinical pharmacology trial. Donidalorsen is highly bound to plasma proteins ($>98\%$) and less than 1% of the administered dose is excreted as total full-length oligonucleotide in urine within the first 24 hours after the fourth dose administered every 4 weeks. In addition, linker-related metabolites accounted for $\leq 6.4\%$ of the administered dose in urine. Renal impairment did not impact the pharmacokinetics and pharmacodynamics of donidalorsen. In the pivotal trial (CS5), five subjects with mild renal impairment (eGFR 60 to 89 mL/min/1.73 m²) were enrolled. Three of these subjects received donidalorsen-4. The remaining two received donidalorsen-8. Based on observed data from Trial CS5, the plasma C_{trough} of donidalorsen and change from baseline in plasma PKK were similar between subjects with normal renal function and subjects with mild renal impairment, as shown in [Figure 28](#).

Figure 28. Box Plot of Observed Plasma Donidalorsen C_{trough} (Top) and Percent Change in Plasma Trough PKK Concentration (Bottom) Over Time in Subjects With Normal Renal Function and Mild Renal Impairment, Trial CS5

A) Plasma Donidalorsen C_{trough} Over Time



B) Percent Change in Plasma Trough PKK Concentration Over Time



Source: Reviewer-generated graph based on adpc.xpt and adpd.xpt for CS5
 Red represents mild renal impairment (N=3 in Q4W and N=2 in Q8W). Blue represents normal subjects (N=42 in Q4W and N=21 in Q8W).
 Abbreviations: C_{trough} , trough plasma concentration; PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks

Mild renal impairment did not impact the pharmacokinetics and pharmacodynamics of donidalorsen based on observed data from Trial CS5. Therefore, no adjustment is recommended for subjects with mild renal impairment. Donidalorsen has not been studied in subjects with moderate severe renal impairment or end stage renal disease.

8.2. Extrinsic Factors

8.2.1. Drug Product Presentation

The proposed to-be-marketed presentation is solution for injection (80 mg/0.8 mL) in a single-dose autoinjector (AI). The clinical formulation used in the pivotal phase 3 trial (CS5) is donidalorsen solution (100 mg/mL) for injection in stopper glass vial.

To bridge the proposed AI and vial product, the Applicant evaluated plasma PK profiles of donidalorsen in healthy adult subjects following administration of a single dose of donidalorsen using vial and AI in a phase 1 bioequivalence trial (CS9).

The results of the bioequivalence trial demonstrated comparable systemic exposure between AI and vial presentations (geometric mean ratio [90% CI] for AI versus vial products were 113% [105, 121] for C_{max} and 107% [104, 111] for AUC_{0-336}). PD results based on PKK concentrations were also similar between both products. Refer to Section [14.2.1](#) for details. The AI and vial products are adequately bridged. The results therefore support use of the AI product as the to-be-marketed drug product.

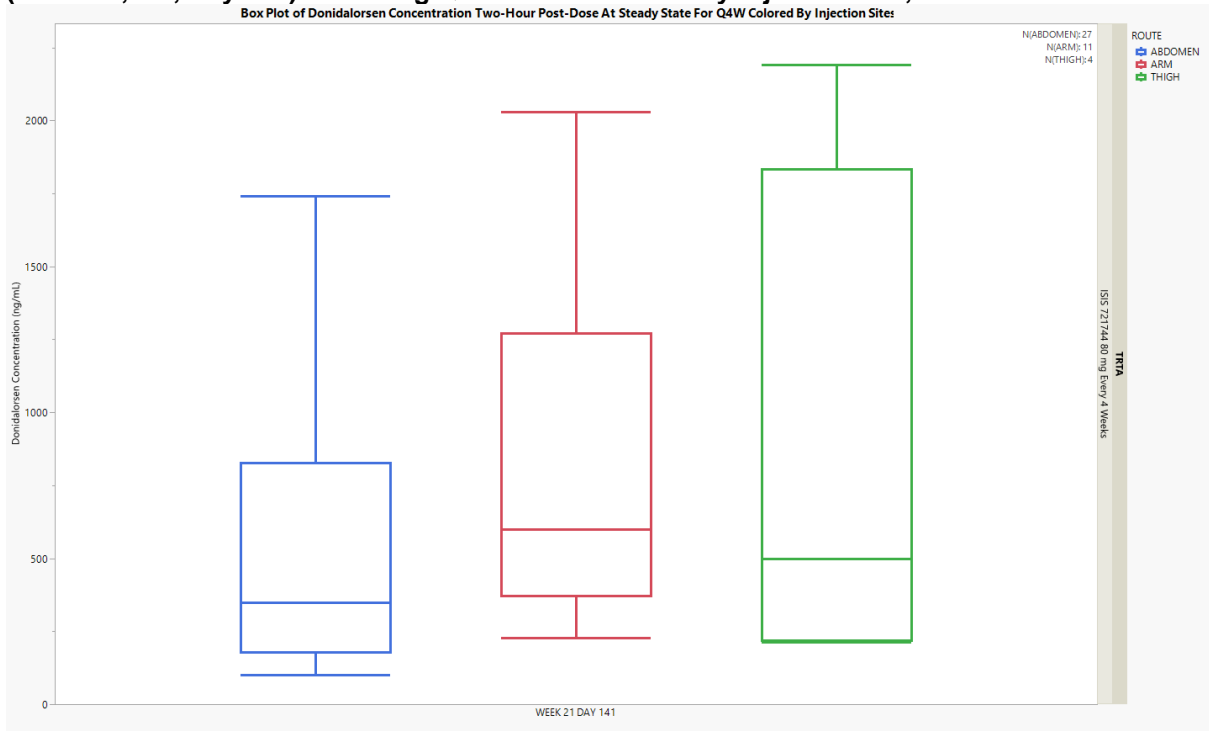
8.2.2. Injection Site

The Applicant proposes that donidalorsen can be administered SC into the abdomen, upper thigh, or the back of the upper arm.

Based on the Applicant's population PK analysis, injection into the arm is predicted to yield a 12% lower geometric mean $C_{max,ss}$ (440 ng/mL) as compared to injection into the abdomen (495 ng/mL) but a similar $C_{max,ss}$ following injection into the thigh (451 ng/mL). Injection into the arm is also predicted to yield a similar mean $AUC_{tau, ss}$ and $C_{trough, ss}$ compared to those following injection into the abdomen or thigh. The range of post hoc estimates for steady-state exposure of C_{max} , $AUC_{tau, ss}$, and $C_{trough, ss}$ were overlapping between subjects with injection into the arm, abdomen, and thigh in the Applicant's population PK analysis.

In the pivotal phase 3 trial (CS5), donidalorsen could be administered into any of three injection sites (abdomen, arm, or thigh) and alternating injection sites were used in some individuals. The range of donidalorsen concentrations collected 2 hours postdose at steady state (Week 21) following donidalorsen-4 administration was overlapping across injections. Although, a slightly higher median value was noted following injection into the arm as compared to the abdomen or thigh injection, in contrast to population PK predictions ([Figure 29](#)).

Figure 29. Box Plot of Donidalorsen Concentrations (ng/mL) Two-Hours Postdose at Steady State (Week 21, i.e., Day 141) Following Q4W Administration by Injection Site, Trial CS5



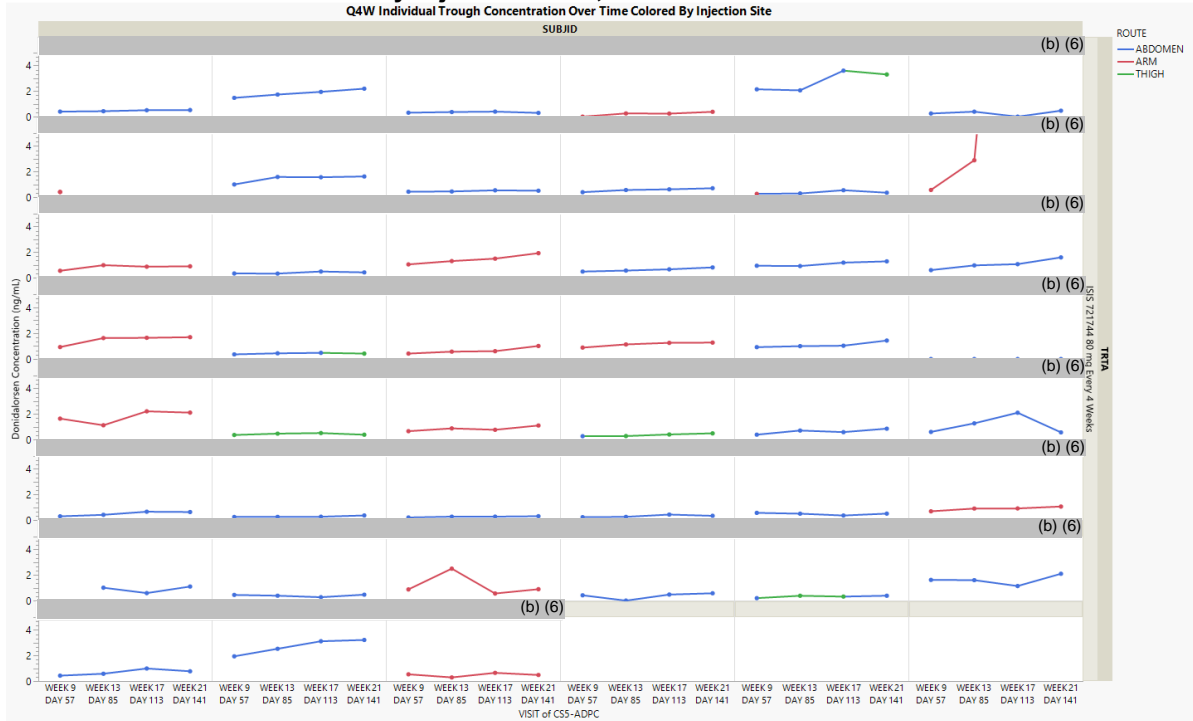
Source: Reviewer-generated graph based on adpc.xpt for CS5.

Blue represents injection into abdomen (N=27). Red represents injection into arm (N=11). Green represents injection into thigh (N=4).

Abbreviations: Q4W, every 4 weeks

Based on data from Trial CS5, there was no apparent change in $C_{\text{trough,ss}}$ after changing the site of injection. More subjects randomized to the donidalorsen-4 treatment group were noted to have switched injection sites. Therefore, individual $C_{\text{trough,ss}}$ values over time following donidalorsen-4 administration colored by injection sites are shown in [Figure 30](#).

Figure 30. Individual Plasma Donidalorsen C_{trough} (ng/mL) Over Time (Weeks 9 to 21) Following Q4W Administration Colored by Injection Sites, Trial CS5

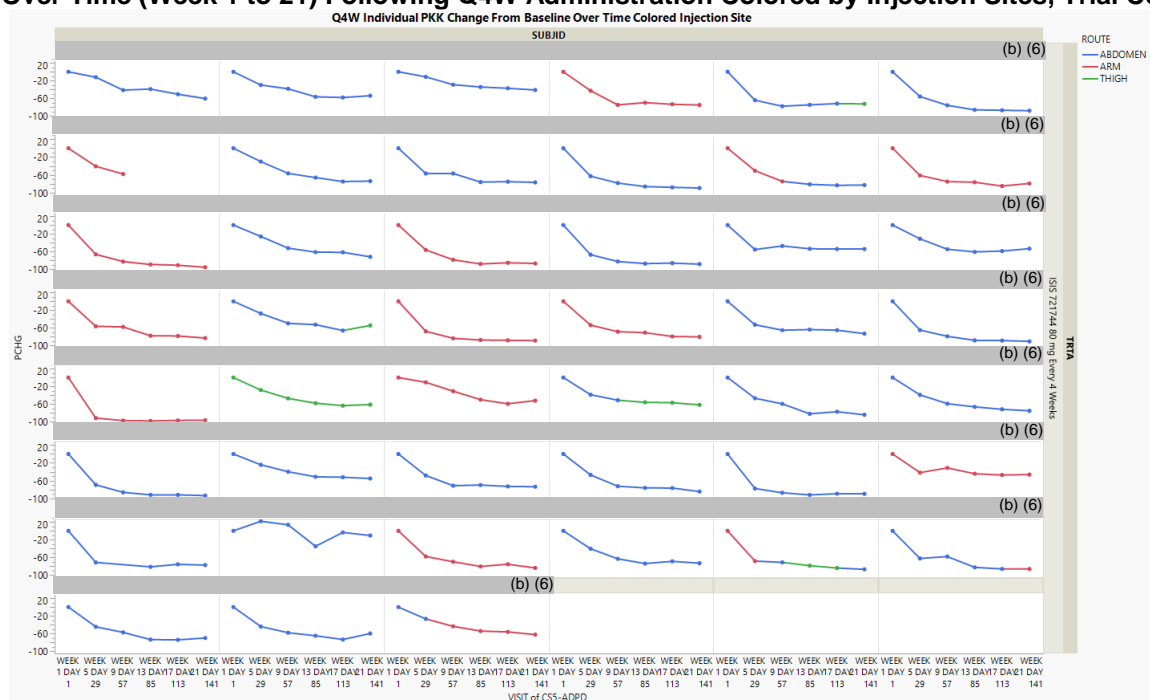


Source: Reviewer-generated graph based on adpc.xpt

Blue is C_{trough} after injection into abdomen. Red is C_{trough} after injection into arm. Green is C_{trough} after injection into thigh.

Abbreviations: C_{trough}, trough concentration; Q4W, every 4 weeks

As the donidalorsen site of action is in the liver, the clinical meaningfulness of changes in donidalorsen plasma exposure by injection site is unclear. Therefore, PD data (i.e., PKK concentrations) by injection site were also assessed. Similar to observations with the PK data, there was no apparent change in plasma PKK trough concentrations by injection site or following switching of injection sites in Trial CS5 (Figure 31).

Figure 31. Individual Percent (%) Change in Plasma PKK Trough Concentration From Baseline Over Time (Week 1 to 21) Following Q4W Administration Colored by Injection Sites, Trial CS5

Source: Reviewer-generated graph based on adpc.xpt

Blue is plasma PKK concentration after injection into abdomen. Red is plasma PKK concentration after injection into arm. Green is plasma PKK concentration after injection into thigh.

Abbreviations: PKK, prekallikrein; Q4W, every 4 weeks

Donidalorsen C_{trough} and plasma PKK trough concentrations were consistent by injection site and following switching of injection sites among subjects in Trial CS5. The data suggest that changes in injection site are not likely to yield clinically meaningful differences in efficacy or safety.

8.2.3. Drug-Drug Interactions

No dose adjustment strategies with respect to drug-drug interactions are warranted for donidalorsen.

In vitro studies indicate that donidalorsen and its main linker-related metabolite (i.e., M8) are not inducers or inhibitors of CYP-mediated oxidative metabolism. Donidalorsen was not identified to be a substrate of any CYP enzymes. Linker-related metabolite M8 was determined to be a substrate of CYP3A4. With respect to transporters, donidalorsen was not found to be a substrate or inhibitor of major drug transporters. Linker-related metabolite M8 was identified as a substrate of the transporters organic anion transporting polypeptide 1B3 and bile salt export pump (BSEP). Linker-related metabolite M8 was also determined to be an inhibitor of multidrug and toxin extrusion protein (MATE) 1. Notably, although M8 was identified as the main linker-related metabolite, its abundance in human plasma is low (1.3% relative to parent). Lastly, no in vitro DDIs were observed with regards to plasma protein binding displacement between donidalorsen and other highly plasma protein-bound drugs, such as ibuprofen and warfarin. For additional details, refer to Section [14.1](#).

8.3. Plans for Pediatric Drug Development

An initial pediatric study plan (iPSP) was submitted by the Applicant on June 3, 2022, and an agreement letter for the agreed iPSP was issued on July 6, 2022. The Applicant requested a waiver for pediatric patients under 2 years of age on the grounds that clinical manifestations of the disease state (HAE attacks) do not exist in this age group. Ages 12 to less than 18 years were included in Trial CS5 and Trials CS7. The Applicant requested a deferral for ages 2 to less than 12 years; this age group will be targeted in a future open-label clinical trial (CS8) that will target enrolling 20 patients for a treatment duration of 25 weeks.

On September 19, 2023, donidalorsen received orphan designation for the treatment of HAE exempting pediatric studies under PREA.

Nonclinical

A juvenile toxicity study was not needed to support usage in pediatric patients 12 years of age and older. A repeat-dose 13-week toxicity study in juvenile mice has been conducted (report pending) [REDACTED] (b) (4)

[REDACTED] (b) (4) The design of the juvenile toxicity study was discussed with the Applicant in the context of the iPSP.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

The potential reproductive and developmental toxicity of donidalorsen was evaluated in GLP-compliant combined fertility and embryofetal (embryo-fetal development [EFD]) developmental and PPND studies in mice. Donidalorsen does not have perfect alignment to the mouse PKK gene sequence and mice are not considered a pharmacologically relevant species. As such, the reproductive and developmental toxicity studies in mice also evaluated mouse surrogate antisense oligonucleotide ISIS 722059, which targets mouse PKK, to assess potential toxicities related to the intended pharmacology of PKK inhibition.

The following nonclinical information was used in support of the drug's labeling. Additional details are available in Section [13](#).

Table 32. Nonclinical Data Supporting Labeling on Fertility, Pregnancy, and Lactation

Labeling Section	Nonclinical Data
8.1 Pregnancy	In a combined fertility and EFD study, male and female F0 mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection), donidalorsen (1, 4, or 10 mg/kg/week), or mouse surrogate ISIS 722059 (4 mg/kg/week) weekly, prior to and during mating, and continuing every other day in females throughout the periods of implantation and organogenesis (GD 0 to 16). There were no adverse effects on embryofetal development. There was no evidence of maternal toxicity. The NOAEL for developmental toxicity was the high dose of 10 mg/kg/week donidalorsen. In a PPND study, F0 female mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection), donidalorsen (5, 10, or 20 mg/kg/week), or mouse surrogate ISIS 722059 (5 mg/kg/week) every other day from GD 6 to GD 18 and weekly from LD 1 to LD 20. There were no adverse effects on behavioral, fertility, and reproductive development parameters in the F1 offspring. There was no evidence of maternal toxicity. The NOAEL for the PPND study was the high dose of 20 mg/kg/week donidalorsen. The unconjugated form of donidalorsen was not detected (i.e., was below the limit of quantitation) in fetal tissues. It was unclear if donidalorsen or unconjugated donidalorsen crossed the placenta to reach fetal tissues.
8.2 Lactation	In the PPND study in mice, the concentrations of donidalorsen in breast milk from lactating mice on LD 15 increased in a dose-dependent manner at doses ≥ 10 mg/kg/week, but these concentrations of donidalorsen in breast milk were lower than the observed tissue concentrations in the liver where the drug is preferentially taken up. Even though donidalorsen was detected in the maternal mouse milk, systemic exposure in pups was not expected due to the lack of oral absorption of donidalorsen.
13. Carcinogenesis, Mutagenesis, Impairment of Fertility	In the combined fertility and EFD study in mice, fertility and reproductive performance were unaffected by SC administration of donidalorsen up to 10 mg/kg/week or a mouse surrogate ISIS 722059 (4 mg/kg/week) to male and female F0 mice weekly, prior to and during mating, and continuing every other day in females throughout the periods of implantation and organogenesis.

Source: Table generated by reviewer

Abbreviations: EFD, embryo-fetal development; GD, Gestation Day; LD, Lactation Day; NOAEL, no observed adverse effect level; PPND, pre- and postnatal development; SC, subcutaneous

Table 33. Reproductive and Developmental Toxicity Safety Margins^a

Animal Study	Animal NOAEL	HED	BSA (mg/m²) Margins^b	Body Weight (mg/kg) Margins^b
Mouse		0.81 mg/kg		
Combined Male and Female Fertility and EFD SC	10 mg/kg/week	49 mg adult	0.6x	7.7x
		24 mg 12-year-old	2.4x	30.8x
			0.3x	3.7x
			1.2x	14.8x
Mouse		1.63 mg/kg		
PPND		98 mg adult	1.2x	15.4x
Maternal; Offspring Development SC	20 mg/kg/week	49 mg 12-year-old	4.9x	61.5x
			0.6x	7.4x
			2.4x	29.6x

Source: Table generated by reviewer

^a Mouse studies did not have toxicokinetic data, therefore exposure margins for C_{max} and AUC were not calculated.

^b The BSA and body weight margins are calculated based on the maximum recommended dosage of donidalorsen of 80 mg (i.e., 1.3 mg/kg for a 60 kg adult and 2.7 mg/kg for a 30 kg 12-year-old) Q4W. The margins shown are unadjusted and adjusted margins, respectively (i.e., do not account for and do account for, respectively, the more frequent dosing frequency in the animal studies compared to the recommended clinical dose).

Abbreviations: AUC, area under the concentration-time curve; BSA, body surface area; C_{max}, maximum plasma concentration; EFD, embryo-fetal development; HED, human equivalent dose; NOAEL, no observed adverse effect level; PPND, pre- and postnatal development; Q4W, every 4 weeks; SC, subcutaneous

9. Product Quality

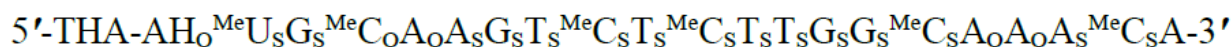
Approval

The Office of Pharmaceutical Quality (OPQ) has assessed NDA 219407 with respect to chemistry, manufacturing, and controls (CMC), and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such OPQ recommends approval of this NDA from a quality perspective.

9.1. Device or Combination Product Considerations

Drug Substance

Donidalorsen sodium drug substance is a chimeric 2'-O-methoxyethyl-modified ASO that is covalently bonded to a GalNAc moiety. GalNAc is a ligand for the hepatocyte-specific asialoglycoprotein receptor. The donidalorsen sequence is:



Where:

- Underlined residues are 2'-O-(2-methoxyethyl) nucleosides (MOE).
- All other residues are 2'-deoxynucleosides.
- The locations of phosphorothioate and phosphate diester linkages are designated by subscripted 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.

The drug substance is synthesized

(b) (4)
(b) (4)

The starting materials for the manufacture of donidalorsen are

(b) (4)

(b) (4)

these materials are adequately justified and are acceptable based on the principles in International Council for Harmonisation (ICH) guidance for industry *Q11 Development and Manufacture of Drug Substances--Questions and Answers (Chemical Entities and Biotechnological/Biological Entities)* ([February 2018](#)). Proven acceptable ranges have been established for the critical process parameters which ensure the quality of the drug substance.

The drug substance structure has been characterized by proton, carbon, and phosphorous nuclear magnetic resonance spectroscopy, mass spectrometry, and failure sequence analysis.

The drug substance is an (b) (4) solid and is freely soluble in water at physiological pH.

The application included sufficient information about the structures, origins, fates, and control strategies for the oligonucleotide-related impurities and these have been quantified and qualified in groups based on their structures and mechanisms of formation, which is acceptable considering the complexities of characterizing oligonucleotide impurities and with the expectation that impurities with similar structural changes will have similar toxicity profiles. The

NDA 219407

Dawnzera (donidalorsen)

specified impurities of the drug substance were qualified through toxicology studies. No ICH Q3C Class 1 solvents are used in the manufacture and there are no genotoxic or (b) (4) impurities in the drug substance.

The drug substance specification includes tests and acceptance criteria to ensure the identity, purity, strength, and quality of the drug substance. The non-compendial analytical methods are sufficiently described and validated and are appropriate for their intended uses for quality control. Analyses data provided were compliant with the specification.

The drug substance is stored in (b) (4). The retest period for the drug substance granted is (b) (4) months (b) (4) (b) (4) which is supported by long-term stability results from both primary stability batches (b) (4) month data available) and supporting stability batches (b) (4) month data available).

Product

Donidalorsen injection product is a sterile, single-dose, ready-to-use parenteral solution containing an ASO in prefilled syringe within an AI device. The drug/device combination product is intended for subcutaneous injection by the patient or caregiver at home and is indicated for the prophylaxis of HAE attacks in adult and pediatric patients aged 12 years and older. Donidalorsen, an ASO, reduces the production of PKK protein. Each product unit contains 80 mg/0.8 mL of donidalorsen drug. The labeling recommends a starting dose for patients aged 12 years and older of 80 mg donidalorsen (b) (4) (equivalent to 84 mg of donidalorsen sodium), (b) (4) (b) (4)

All excipients in the product are of USP/NF grade and in quantities that do not exceed the maximum daily amounts approved for other injection drug products or products, according to the Inactive Ingredient Report. None of the excipients are novel and none are derived from animal sources.

The product is manufactured through a process involving several key steps: (b) (4) (b) (4) The manufacturing process is based on prior knowledge for similar approved products from the same Applicant with similar oligonucleotide-based drugs.

The specification for the product is aligned with the relevant ICH guidances for industry, *Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances* (December 2000) and *Q3B(R2) Impurities in New Drug Products (Revision 3)* (August 2006), and the acceptance criteria are adequate with respect to the batch release and stability data in the application. The non-compendial analytical methods and associated validation reports have been evaluated and the methods are deemed suitable for regulatory purposes.

The primary container closure system for the product consists of a 1 mL staked needle syringe barrel and a plunger stopper. The filled primary container is assembled into an AI and packaged in an opaque carton (donidalorsen is photolabile). The AI is based on the (b) (4)

AI. This AI platform has been used as part of other approved combination products. Refer to the Center for Devices and Radiological Health (CDRH) consult review for a review of the (b) (4) AI/device from an engineering/device perspective (recommended approvable).

A vial was used for clinical trials CS2, CS3, and CS5. Trial CS7 used both the vial and AI. To bridge the proposed AI and vial product, the Applicant evaluated plasma PK profiles of donidalorsen in healthy adult subjects following administration of a single dose of donidalorsen using vial and AI in a phase 1 bioequivalence trial (CS9); the results are presented in Section [14.2.2](#).

An HF study validation study was conducted with the AI and the instructions for use were modified based on the identified use errors. Refer to the Office of Surveillance and Epidemiology review dated May 23, 2025.

The Applicant submitted product analysis data for three batches (CP721744-004-AI-A, CP721744-005-AI-B, and CP721744-006-AI-A) and these batches were used for clinical, registration stability, and bioequivalence trials. All batches were manufactured using the proposed commercial manufacturing process (Section 3.2.P.3.3) and they all met the specification acceptance criteria.

Stability studies were conducted to evaluate the product at long-term (2 to 8°C) and accelerated (25°C/60%RH) storage conditions. Registration stability studies are ongoing for product batches CP721744-004-AI-A, CP721744-005-AI-B, and CP721744-006-AI-A. Based on the long-term and accelerated stability data available, an expiration dating period of 30 months is granted when stored in the proposed container closure system and carton at 2 to 8°C (36°F to 46°F). Data have also shown that the product may also be stored at room temperature (up to 86°F or 30°C) in the original carton for up to 6 weeks.

Manufacturing/Facilities

The product manufacturing process consists of (b) (4) (b) (4)

(b) (4) The next unit operation includes the AI assembly process, and final QC testing and release of the AI packaged in a secondary packaging. The proposed critical process parameters, in-process controls, as well as overall control strategy for the commercial manufacturing of the product are supported by the registration batch data.

The production of the bulk PFS and assembly of the AI occur at a (b) (4) (b) (4). Bulk PFS batches for registration stability were manufactured using (b) (4) (b) (4) the commercial batch size ((b) (4) L for registration stability batches versus (b) (4) L for commercial batches).

Fifteen facilities are used to produce the product and all are found to be adequate, with the exception of the two (b) (4) facilities responsible for secondary packaging and labeling, as these did not require evaluation.

Microbiology

Donidalorsen injection is sterilized by (b) (4) and is subsequently filled into PFS, which are then assembled into nonsterile AI devices. (b) (4)
(b) (4). The syringes and the rubber stoppers are purchased ready to use.

Conclusion

Based on the recommendations of the CMC review team and CDRH, the application is recommended for approval.⁹

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

Human Subjects Protections

As stated by the Applicant, the clinical trials were conducted in compliance with Good Clinical Practice, as required by the ICH guidance for industry *E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1)* ([March 2018](#)). The clinical trials also met the requirements of the Declaration of Helsinki and, where applicable, national laws and regulations regarding ethical committee review, informed consent, and protection of human subjects participating in biomedical research. All trials submitted in this application were conducted in accordance with FDA requirements under IND 142564.

Clinical Site Inspections

FDA clinical site inspections were performed at one site in the United States for Trial CS5. Refer to Section [22](#) for details.

Financial Disclosure

The Applicant adequately disclosed financial interests/arrangements with clinical investigators as recommended in the FDA guidance for industry, *Financial Disclosure by Clinical Investigators* ([February 2013](#)), and by 21 CFR 54.4 ([21 CFR 54.4 2025](#)). None of the investigators in the clinical trials were employed by the Applicant. One clinical investigator disclosed financial interests and arrangements with the Applicant (see Section [25](#)). To minimize bias, an independent data monitoring committee was established to monitor the safety and efficacy data generated from the clinical trials. Additionally, the small number of subjects who participated at each site also minimized the potential for bias.

⁹ The CMC-related labeling review decision is pending for this IQA due to ongoing negotiations with the Applicant. There are only minor labeling deficiencies from the CMC perspective and we expect the Applicant will accept our recommendations. Refer to the CMC sections of the final approved labeling.

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In conclusion, the likelihood that trial results were biased by financial interests is minimal and should not affect the approvability of the application.

11. Advisory Committee Summary

An advisory committee was not held for this application. The application did not raise challenging efficacy or safety issues that required external input.

III. Additional Analyses and Information

12. Summary of Regulatory History

On July 15, 2019, Ionis Pharmaceuticals submitted IND 142564 with a proposed trial, ISIS 721744-CS2, entitled “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,” which was allowed to proceed on August 14, 2019. A phase 1 study, Protocol No. ISIS 721744-CS1, was previously conducted in Canada to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of ISIS 721744 in healthy volunteers.

On August 30, 2021, an end-of-phase 2 meeting was held to discuss the adequacy of the nonclinical and clinical development to support a marketing application. FDA recommended an increase in the safety database size, evaluating an alternative lower dosing regimen, shared concerns and requested additional information regarding regulatory acceptability of the proposed PRO tools (AE-QoL and AECT), and recommended evaluating mild-to-moderate hepatic impairment and/or pre-existing hepatic diseases for generalizability. FDA stated the acceptability of a single pivotal trial to provide substantial evidence of effectiveness would be a review issue. Regarding the nonclinical development, FDA recommended that prior to the start of phase 3 clinical trials, a characterization of the metabolism of ISIS 721744 and its THA linker should be provided, as recommended by the Pharmacology and Toxicology and Clinical Pharmacology Reviewers within the Agency. Further, a toxicologic assessment of the liver inflammation and necrosis in female mice in the 26-week toxicity study should be provided. It was also agreed that an EFD study of ISIS 721744 in rabbits is not needed. The meeting minutes were issued on October 18, 2021.

On July 6, 2022, FDA provided initial agreement on the pediatric trial plan for a partial waiver for <2 years old, assessment of 12 to less than 18 years old as part of the proposed phase 3 trial, and a deferred trial of patients 2 to <12 years old postmarketing.

SPA 1 for the 2-year study in rats was submitted on August 5, 2022, and was subsequently denied due to lack of the Standard for Exchange of Nonclinical Data datasets for the supporting 16-week dose range finding study in rats and resubmitted on September 30, 2022, with the Standard for Exchange of Nonclinical Datasets. FDA agreed with the proposed 2-year carcinogenicity study in rats on November 4, 2022. SPA 2, which proposed a 6-month carcinogenicity study in Tg.rasH2 mice, was submitted on October 12, 2022, and was agreed upon for male mice but not for female mice (as no acceptable basis for dose selection could be identified) on November 16, 2022.

Ionis requested a use-related risk analysis review on January 20, 2023, and included information on the target disease and human factors (HF) development. In the June 17, 2024, response, FDA did not identify any new or unique risks for the proposed product ISIS 721744 AI for intended adults as compared to currently approved Wainua AI and noted prematurity in determining the need for additional HF validation trials for adolescent users. FDA recommended submission of the full results report for the HF validation trial with the marketing application.

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On February 3, 2023, a Type C Written Response Only was issued, which discussed the acceptability of the proposed bioequivalence trial design and protocol (Trial ISIS 721744-CS9) to support bridging from the current syringe/vial drug product to an AI configuration. FDA agreed the proposed trial design is acceptable and provided AI guidance from CDRH.

On September 19, 2023, donidalorsen received orphan designation for the treatment of HAE.

A Type C Written Response Only was issued on December 19, 2023, where FDA reiterated previous concerns regarding the small size of the safety database and suggested inclusion of safety support from other GalNAc-conjugated products, especially those with the identical linker. FDA commented the AE-QoL Questionnaire is not fit-for-purpose in the absence of results of the Sponsor's planned qualitative and quantitative trials and reiterated comments regarding the AE-QoL conveyed in the August 26, 2021, Meeting Preliminary Comments and provided additional DCOA/Patient-Focused Statistical Scientists comments on AE-QoL for Ionis' consideration.

Proprietary name review request was submitted on November 30, 2023, and was deemed conditionally acceptable on May 7, 2024.

On January 10, 2024, the Sponsor submitted a summary of the nonclinical and clinical data and relevant antisense oligonucleotide class data to enable an assessment of a thorough QT study waiver. FDA granted the waiver on April 5, 2024, concluding that the QT/QTc risk assessment showed low risk to prolong QTc and that the data from this QTc assessment can be used as a substitute for a thorough QT study.

The Sponsor submitted a pre-NDA meeting request on February 6, 2024, was granted on February 20, 2024. The meeting was withdrawn by the Sponsor upon receipt of the April 10, 2024, preliminary comments, which conveyed acceptability of the clinical and nonclinical data package. FDA agreed that the final report for the 27-week Tg.rasH2 mouse carcinogenicity study and a summary of the in-life data for the 2-year carcinogenicity study in rats should be available at the time of the NDA submission, and the final report can be submitted as a PMR.

The NDA 219407 was submitted on August 21, 2024, as prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older.

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

The pivotal and supporting pharmacology (primary and safety), absorption and distribution, and toxicology (repeat-dose toxicity, genotoxicity, carcinogenicity, and reproductive and developmental toxicity) studies submitted with both IND 142564 and NDA 219407 have been reviewed under either the IND or NDA and are summarized here.

13.1.1. Pharmacology

13.1.1.1. Primary Pharmacodynamics

Donidalorsen is a triantennary GalNAc₃-conjugated ASO that causes ribonuclease H1 (RNase H1)-mediated degradation of PKK messenger ribonucleic acid (mRNA) through 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-INH deficiency or dysfunction leads to excessive plasma kallikrein activity, bradykinin generation, and angioedema attacks. Donidalorsen lowers PKK concentration, which is intended to prevent excessive bradykinin production in patients with HAE.

The primary pharmacodynamics of donidalorsen have been evaluated in both in vitro and in vivo primary pharmacology studies.

In Vitro Primary Pharmacology Studies

As described by the Applicant, over 1300 ASOs were designed to target various sites within the human PKK gene and were evaluated for their ability to inhibit the level of PKK mRNA in vitro in the human hepatocellular carcinoma cell line HepaRG. Of those ASOs, ISIS 546254, an unconjugated 2'-*O*-methoxyethyl (2'-MOE) gapmer ASO with a uniform phosphorothioate (PS) backbone was identified as a lead candidate. Donidalorsen was derived from ISIS 546254 as a 2'-MOE gapmer ASO with the same nucleobase sequence as ISIS 546254 but with a mixed PS and phosphodiester (PO) backbone and conjugated to a trivalent GalNAc₃.

In Silico and In Vitro Characterization of Human PKK ASO ISIS 721744 Specificity (Study No. ECID129370)

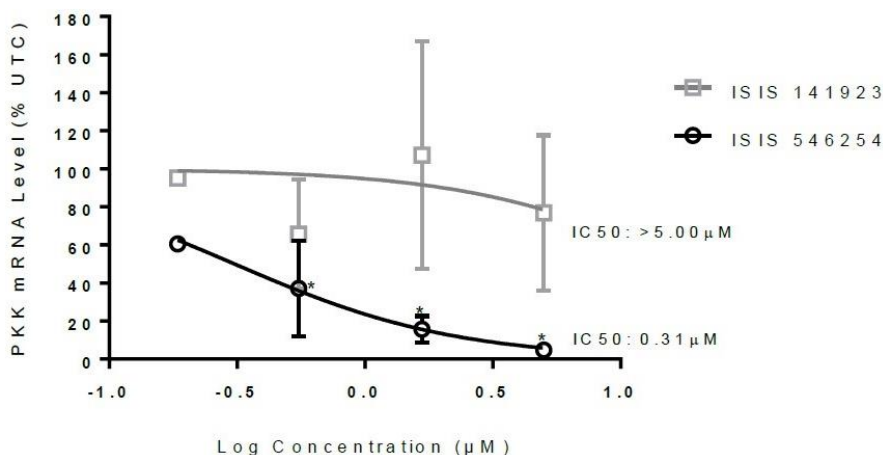
The specificity of donidalorsen to PKK mRNA was characterized in silico and in vitro with the unconjugated human PKK ASO parent compound, ISIS 546254, which has the same nucleobase sequence as donidalorsen. ISIS 546254 is perfectly matched to only PKK mRNA in the human transcriptome (targets site free of meaningful sequence variation). Based on the in silico Basic Local Alignment Search Tool analysis, there were no identified potential off-target genes with a 0-, 1-, or 2-base mismatch or a 3-base mismatch with 17 matched bases in a row. Alignment of the ISIS 546254 sequence against the human transcriptome and mRNA reference sequences predicted seven human genes (i.e., ACACA, CTAGE5, IFIH1, PRKN, ANKS1B, CDH4, and LOC442028) with mismatched counts of two bases. Of these, one gene (i.e., LOC442028) had no reported expression in human tissues and two genes (ANKS1B and CDH4) were reported to be expressed only in the human brain, which is not a preferential tissue for GalNAc₃ ASO accumulation. The effect of ISIS 546254 on the expression of PKK mRNA and the remaining four predicted off-target mRNAs (i.e., ACACA, CTAGE5, IFIH1, and PRKN), which are ubiquitously expressed in human tissue, was evaluated in the human hepatocellular cell line Hep3B. In this experiment, ISIS 546254 (doses up to 20µM) potentially reduced PKK mRNA (IC₅₀=0.35µM) and in comparison, only minimally reduced ACACA, CTAGE5, IFIH1, and PRKN mRNA (IC₅₀=>20, >20, 9.1, and 8.8µM, respectively). These data show that ISIS 546254 is selective for PKK mRNA compared to potential off-target mRNAs.

In Vitro Characterization of Human Prekallikrein Antisense Oligonucleotide ISIS 721744 Activity (Study No. ECID129371)

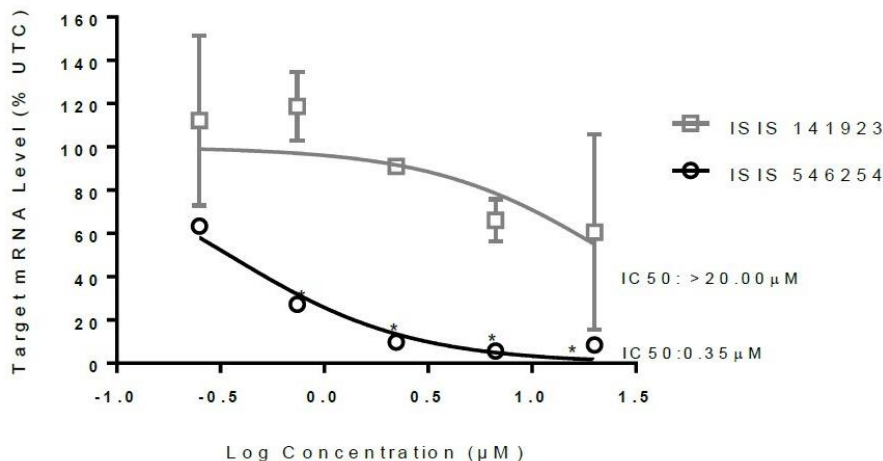
The in vitro characterization of donidalorsen to reduce human PKK mRNA was based on an experiment using two human hepatocellular carcinoma cell lines (HepaRG and Hep3b) treated with ISIS 546254 (unconjugated human PKK ASO parent compound of donidalorsen with the same nucleobase sequence) or ISIS 141923 (control oligonucleotide with no matches to any sites within the human transcriptome). ISIS 546254 reduced human PKK mRNA levels in human hepatocellular cell lines HepaRG and Hep3b by greater than 90% in a concentration-dependent manner with IC_{50} of $0.31\mu\text{M}$ and $0.35\mu\text{M}$, respectively (Figure 32). In comparison, the control oligonucleotide ISIS 141923 did not reduce PKK mRNA in either cell line.

Figure 32. Concentration-Dependent Reduction of Human PKK mRNA by ISIS 546254 in Human Hepatocellular Carcinoma Cells

A.



B.



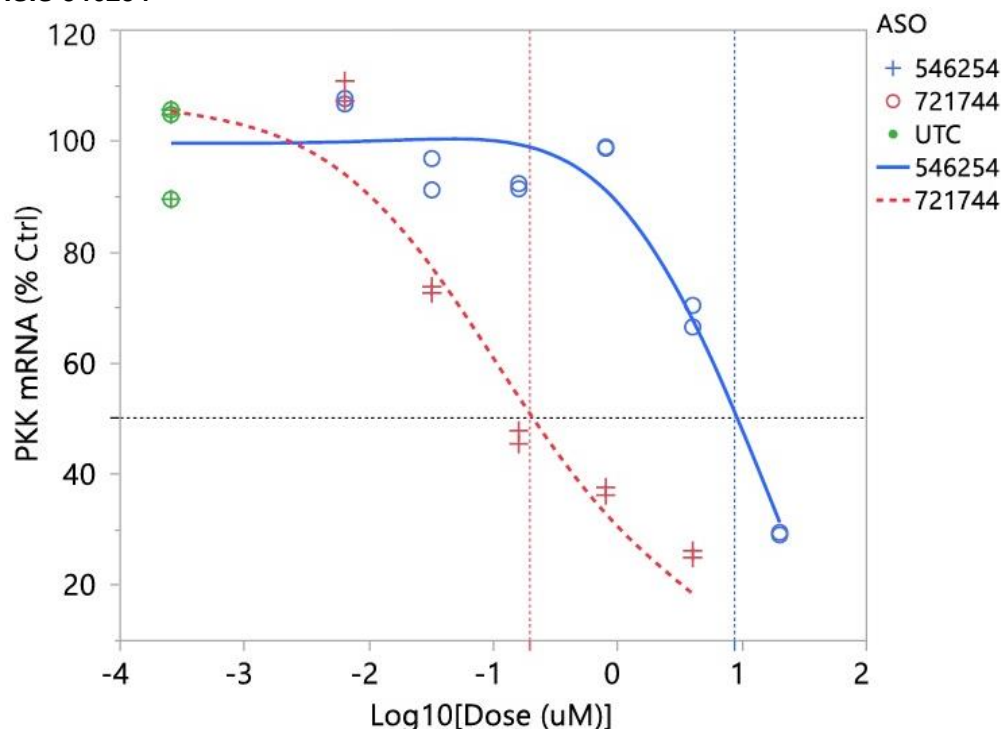
Source: Excerpted from the Applicant's submission

Abbreviations: IC_{50} , half-maximal inhibitory concentration; mRNA, messenger ribonucleic acid; PKK, prekallikrein; UTC, untransfected control

Characterization of ISIS 721744 Pharmacology in Primary Human Hepatocytes Co-Cultured With Mouse Fibroblasts (HepatoPac®) (Study No. GEN2.5-RS83 (PART F))

Treatment of HepatoPac® (co-culture system of primary human hepatocytes and supporting stromal cells [mouse fibroblasts]) with donidalorsen (6.4 to 20000nM) and ISIS 546254 (unconjugated human PKK ASO parent compound of donidalorsen with the same nucleobase sequence) reduced PKK mRNA in a dose-dependent manner (Figure 33). The IC₅₀ for donidalorsen and ISIS 546254 were 0.19μM and 8.73μM, respectively, indicating that donidalorsen is about 46-fold more potent than ISIS 546254 (likely due to cellular uptake through hepatocyte-specific ASGPR).

Figure 33. Dose-Dependent Reduction of PKK mRNA in Human Hepatocytes by Donidalorsen and ISIS 546254



Source: Excerpted from the Applicant's submission

Abbreviations: ASO, antisense oligonucleotide; mRNA, messenger ribonucleic acid; PKK, prekallikrein; UTC, untransfected control

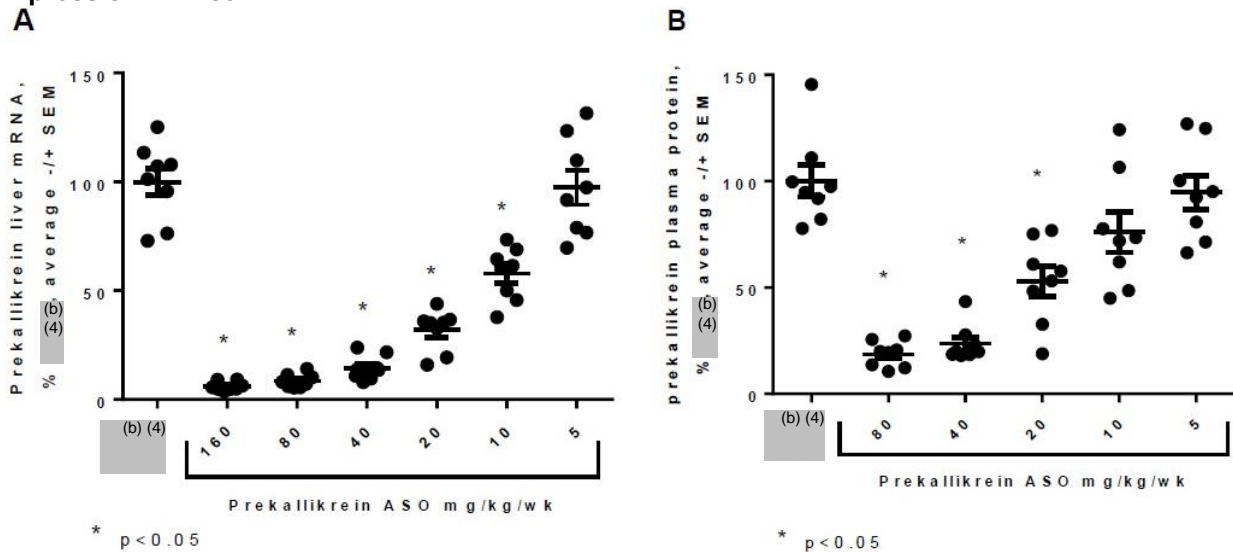
In Vivo Primary Pharmacology Studies

Donidalorsen does not align perfectly to the mouse PKK gene sequence. As such, the unconjugated PKK ASO mouse surrogate ISIS 482584 was used for in vivo pharmacology studies in mouse disease models.

Evaluation of the Effect of Prekallikrein ASO ISIS 482584 on the Basal Vascular Permeability (Study Nos. 721744-1887 and 721744-3223), Captopril Induced Vascular Permeability (721744-1888 and 721744-2064), and C1 Inhibitor ASO-Induced Vascular Permeability (EXP-19-AE2418) in Mice

Treatment of male C57Bl/6 albino mice with vehicle or unconjugated PKK ASO mouse surrogate ISIS 482584 (5, 10, 20, 40, 80, or 160 mg/kg/week for 3 weeks) inhibited PKK liver mRNA and PKK plasma protein expression in a dose-dependent manner ([Figure 34](#)).

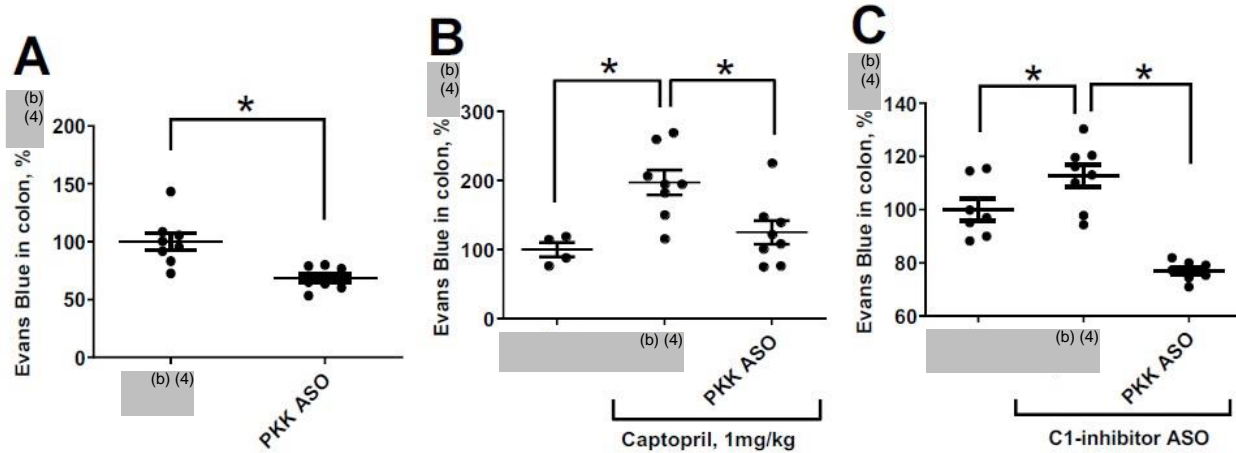
Figure 34. Mouse PKK ASO ISIS 482584 Inhibits Liver PKK mRNA and Plasma PKK Protein Expression in Mice



Source: Excerpted from the Applicant's submission
Abbreviations: ASO, antisense oligonucleotide; mRNA, messenger ribonucleic acid; (b) (4); PKK, prekallikrein; SEM, standard error of the mean

Permeability assays were performed in male C57Bl/6 albino mice treated with unconjugated PKK ASO mouse surrogate ISIS 482584 (80 mg/kg/week for 3 weeks). Evans Blue tracer was injected into the tail vein of mice and analyzed for its accumulation in the colon tissue. Some mice were also treated intraperitoneally with the ACE inhibitor captopril (1 mg/kg) at the time of Evans Blue tracer injection or with a C1-INH ASO (40 mg/kg/week for 3 weeks) to enhance vascular permeability. ISIS 482584 inhibited basal vascular permeability ([Figure 35](#); panel A) as well as vascular permeability induced by either captopril ([Figure 35](#); panel B) or C1-INH ASO ([Figure 35](#); panel C).

Figure 35. Mouse PKK ASO ISIS 482584 Reduces Basal and Induced Vascular Permeability in Mice (Analysis of Evans Blue Tracer Accumulation in the Colon Tissue)

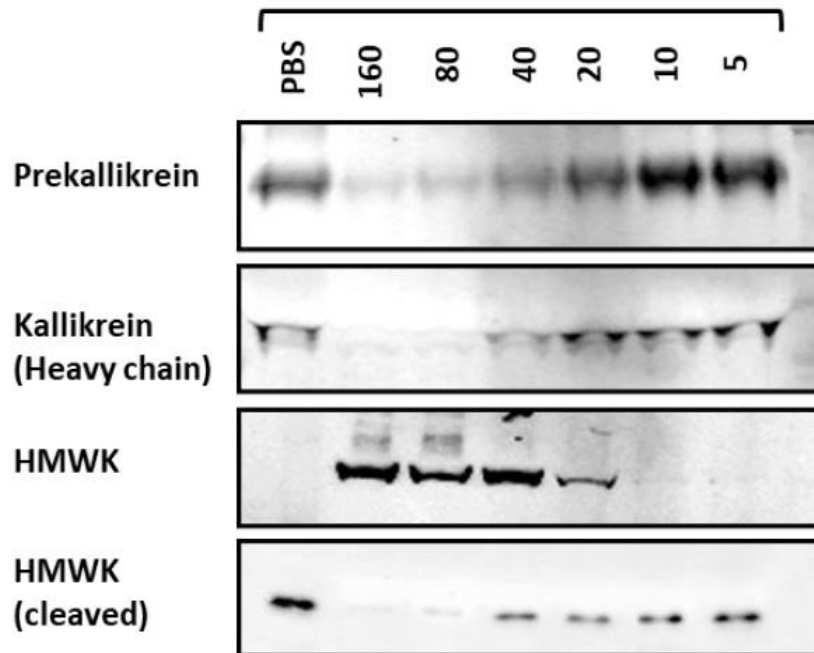


Source: Excerpted from the Applicant's submission
Panel A is basal vascular permeability. Panel B is captopril-induced vascular permeability. Panel C is C1-inhibitor ASO-induced vascular permeability.

Abbreviations: ASO, antisense oligonucleotide; (b) (4); PKK, prekallikrein

Unconjugated PKK ASO mouse surrogate ISIS 482584 (at 10 to 160 mg/kg/week SC for 3 weeks) reduced basal activated high molecular weight kininogen cleavage in the blood of male mice (Figure 36).

Figure 36. Mouse PKK ASO ISIS 482584 Reduces Basal HMWK Cleavage in Blood of Mice
PKK ASO, mg/kg/wk



Source: Excerpted from the Applicant's submission
Abbreviations: ASO, antisense oligonucleotide; HMWK, high molecular weight kininogen; (b) (4); PKK, prekallikrein

Recommendation for Designation of the Established Pharmacological Classification

The recommended EPC for donidalorsen is “prekallikrein-directed antisense oligonucleotide.” Donidalorsen is an ASO that binds to PKK mRNA to cause degradation of PKK mRNA, which reduces PKK protein production. The recommended EPC for donidalorsen is consistent with the EPC of other approved ASO products (both with and without GalNAc).

13.1.1.2. Secondary Pharmacodynamics

The secondary pharmacodynamics of donidalorsen were not evaluated in secondary pharmacology studies as the off-target actions of ASOs are expected to be minimal.

13.1.1.3. Safety Pharmacology

The safety pharmacology of donidalorsen was evaluated in a GLP-compliant study in Cynomolgus monkeys assessing cardiovascular, respiratory, and CNS parameters, and a GLP-compliant in vitro hERG assay.

Evaluation of Cardiovascular, Respiratory, and Central Nervous Systems Following Subcutaneous Administration of ISIS 721744 in Cynomolgus Monkeys (Study Nos. 11752; 721744-AS03)

Male Cynomolgus monkeys (n=4/group) received single SC injections of either the vehicle control (0.9% sodium chloride for injection) or escalating dose of donidalorsen (12 and 30 mg/kg) with a minimum 1-week washout period between administration of the vehicle control and each dose level of donidalorsen. The cardiovascular and respiratory assessments were performed in conscious telemetered monkeys and evaluated arterial blood pressure, heart rate, respiratory rate, core body temperature, and lead II electrocardiogram (ECG) at timepoints that were about 2 hours predose through 24 hours postdose (60, 120, 240, 300, 360, 720, and 1440 minutes); arterial blood gases were measured at predose and 2 hours postdose. The CNS assessment included a Functional Observational Battery (FOB) performed in conscious nontelemetered monkeys at predose and 1, 4, and 24 hours postdose.

Based on cardiovascular and respiratory assessments, there were no test article-related effects in conscious telemetered monkeys on arterial blood pressure parameters (systolic, diastolic, and mean arterial pressure), heart rate, respiratory rate, core body temperature, or ECG parameters (PR interval, QRS duration, QT interval, and RR interval) for up to 24 hours after dosing, or arterial blood gases for up to 2 hours after dosing, with 12 and 30 mg/kg donidalorsen.

Based on CNS assessments, there were no treatment-related effects in conscious nontelemetered monkeys noted by FOB for up to 24 hours after dosing with 12 and 30 mg/kg donidalorsen.

Effect of ISIS 721744 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study Nos. 210830.JTJ; 721744-IS08)

HEK-293 cells were stably transfected with hERG cDNA and treated with donidalorsen (30 and 300µM) at near-physiological temperature to measure the effect on ionic currents by whole-cell

patch-clamp technique. Donidalorsen inhibited hERG current by 0.5% and 1.9% at 30 μ M and 300 μ M, respectively (not statistically significant) versus 2.4% for the vehicle control. The IC₅₀ for the inhibitory effect of donidalorsen on hERG potassium current was not calculated but was estimated to be greater than 300 μ M. The positive control article (60nM terfenadine) inhibited hERG potassium current by 85.6%. In general, oligonucleotides are not thought to interact with the hERG channel.

13.1.2. Absorption, Distribution, Metabolism, Excretion/Pharmacokinetics

The absorption and distribution, PK, and toxicokinetic (TK) profiles of donidalorsen were evaluated both in vitro and in vivo (mice and monkeys).

13.1.2.1. Absorption

The plasma TK profile of SC administration of donidalorsen was evaluated in a GLP-compliant 13-week interim/39-week repeat-dose toxicity study in Cynomolgus monkeys with a 26-week treatment-free recovery period.

A 39-Week Repeat Dose Toxicity Study of ISIS 721744 in Cynomolgus Monkeys With a 13-Week Interim Necropsy and 26-Week Recovery (Study Nos. 727-081; 721744-AS02)

Cynomolgus monkeys received SC doses of vehicle control (0.9% sodium chloride for injection) or donidalorsen (2, 6, 12, or 30 mg/kg/dose with loading doses on Days 1, 5, 9, and 14 and once weekly thereafter) for 13-week interim (monkeys at 30 mg/kg/dose were dosed only until the interim sacrifice) or 39-weeks followed by a 26-week treatment-free recovery period. Validated hybridization electrochemiluminescence (ECL) or high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) methodologies were used to measure total full-length donidalorsen (including fully conjugated, partially conjugated, and unconjugated donidalorsen) in plasma.

Donidalorsen was rapidly absorbed into systemic circulation with median time to maximum concentration (T_{max}) from 1 to 4 hours across dose levels after single-dose (Day 1) and repeat-dose (Days 91 and 273) administration. Mean C_{max} and AUC_{0-48hr} increased between dose levels. After the first dose (Day 1), the 15th dose (Day 91), and the 41st dose (Day 273), AUC_{0-48hr} increased in a greater than dose proportional manner between doses. There was some accumulation of drug at the 12 mg/kg/dose, based on increased AUC_{0-48hr} on Day 273 versus Day 91. There were no clear differences in donidalorsen plasma TK between males and females. The majority of total fully conjugated full-length donidalorsen was cleared within hours from plasma, with a mean residence time (MRT)_{0-48hr} of about 3 to 10 hours.

13.1.2.2. Distribution

The distribution of donidalorsen was evaluated in vitro for plasma protein binding in human, monkey, and mouse plasma, and in vivo into the kidney cortex and liver as part of the GLP-compliant 13-week interim/39-week repeat-dose toxicity study in Cynomolgus monkeys with a 26-week treatment-free recovery period.

In Vitro Evaluation of the Extent of Plasma Protein Binding of ISIS 721744 in Human, Monkey, and Mouse Plasma by Ultrafiltration (Study Nos. IIS-M4-298; 721744-IS03)

The in vitro protein binding of donidalorsen at 0.5, 5, and 150 µg/mL was evaluated in the whole plasma from human, Cynomolgus monkey, and mouse by ultrafiltration methods and a nuclease-dependent hybridization ELISA detection method. Donidalorsen was highly bound ($\geq 96.81\%$) to whole plasma proteins in human, monkey, and mouse at all three concentrations evaluated. The extent of plasma protein binding of donidalorsen was similar across all species.

A 39-Week Repeat Dose Toxicity Study of ISIS 721744 in Cynomolgus Monkeys With a 13-Week Interim Necropsy and 26-Week Recovery (Study Nos. 727-081; 721744-AS02)

Cynomolgus monkeys received SC doses of vehicle control (0.9% sodium chloride for injection) or donidalorsen (2, 6, 12, or 30 mg/kg/dose with loading doses on Days 1, 5, 9, and 14, and once weekly thereafter) for 13-week interim (monkeys at 30 mg/kg/dose were dosed only until the interim sacrifice) or 39-weeks followed by a 26-week treatment-free recovery period. Validated hybridization ECL or HPLC-MS/MS methodologies were used to measure unconjugated donidalorsen in kidney cortex and liver.

After SC administration, donidalorsen distributed to kidney cortex and liver in mostly similar concentrations (less than 2-fold difference in concentrations between tissues). After the 15th dose (Day 91) and the 41st dose (Day 273), the concentration of unconjugated donidalorsen in kidney cortex and liver was dose-dependent across doses. The Applicant claims steady-state tissue concentration (kidney cortex and liver) based on comparable mean tissue concentrations on Days 9, 93, and 275. There were no apparent differences in unconjugated donidalorsen tissue concentrations (kidney cortex or liver) between males and females.

13.1.3. Toxicology

The potential toxicity of donidalorsen was evaluated in GLP-compliant repeat-dose toxicity (13-week and 26-week in mice and 13-week interim/39-week in monkeys), genotoxicity (in vitro Ames assay, in vitro chromosome aberration assay, and in vivo bone marrow micronucleus assay in mice), carcinogenicity (6-month in Tg.rasH2 mice), and reproductive and developmental toxicity (combined fertility and development in mice and PPND in mice) studies. The main nonclinical species used in the toxicology studies were monkeys and mice. Donidalorsen does not align perfectly to the mouse PKK gene sequence and is not pharmacologically active in the mouse. As such, the toxicity studies in mice evaluated donidalorsen and the conjugated PKK ASO mouse surrogate ISIS 722059 (with the same GalNAc-conjugated 2'-MOE ASO chemical backbone structure as donidalorsen) to assess both potential off-target and on-target effects, respectively. The human sequence targeted by donidalorsen has a single-base sequence mismatch

within the binding site of monkey PKK mRNA (position 19). Despite this mismatch, donidalorsen demonstrated dose-dependent inhibition of liver target mRNA expression and reductions in PKK protein and activity levels in peripheral circulation of Cynomolgus monkeys, which supports the use of the monkey as a pharmacologically relevant species for evaluation of on-target effects (i.e., effects related to PKK reduction). There were no single-dose toxicity studies with donidalorsen.

13.1.3.1. Repeat-Dose Toxicity

The pivotal GLP-compliant repeat-dose toxicity studies of longest treatment duration with donidalorsen included a 26-week study in mice and a 13-week interim/39-week study in monkeys.

A 39-Week Repeat Dose Toxicity Study of ISIS 721744 in Cynomolgus Monkeys With a 13-Week Interim Necropsy and 26-Week Recovery (Study Nos. 727-081; 721744-AS02)

Key Study Findings

- Cynomolgus monkeys received SC doses of vehicle control (0.9% sodium chloride for injection) or donidalorsen (2, 6, 12, or 30 mg/kg/dose with loading doses on Days 1, 5, 9, and 14, and then once weekly thereafter) with a 13-week interim sacrifice (monkeys at 30 mg/kg/dose were dosed only until the interim sacrifice) or 39 weeks followed by a 26-week treatment-free recovery period.
- There were no deaths during the study.
- Hematology changes included thrombocytopenia (defined as platelet counts <150000 cells/mcL) that was considered test article-related and adverse in one male at 30 mg/kg/dose and one female at 12 mg/kg/dose. The male animal had marked decreases in platelet counts compared to individual pretest 2 values on Days 80 and 87 (22 and 30 ×10³/mcL, respectively). The female animal had marked decreases in platelet counts relative to individual pretest 2 values on Days 65, 70, 74, 79, and 87 (ranging from <10000 cells/mcL to 66×10³/mcL). Thrombocytopenia was not seen in these two animals during the respective recovery periods. The platelet decreases in these animals were accompanied by mild to marked decreases in red cell mass with mild to moderate increases in reticulocyte counts and/or increased polychromasia (of note, the 12 mg/kg/dose female had mild SC tissue necrosis with fibrin and hemorrhage at injection site 4 and both the 12 mg/kg/dose female and the 30 mg/kg/dose male had red nasal discharge, red skin discoloration, bruising, and/or petechial hemorrhage at various body sites, which may account for decrease in red cell mass and increase in reticulocyte counts).
- Coagulation changes included minimal prolongations in mean aPTT time in males and females at 30 mg/kg/dose relative to pretest on Day 1 (4 hours postdose) and Day 91 (4 hours postdose). These changes were not seen by 24 hours postdose on both Day 1 and Day 91.
- Clinical chemistry changes included increases in C-reactive protein in females at 12 mg/kg/dose on Day 65 through Day 275 and females at 30 mg/kg/dose on Day 65 and Day 93 (up to a greater than 600% change or more than 7-fold increase relative to control

and pretest 2 values). Increases in C-reactive protein was not seen at the recovery period time points.

- Complement Bb levels were slightly elevated for 12 mg/kg/dose males on Days 182 and 273, 12 mg/kg/dose females on Day 273, and at 30 mg/kg/dose on Day 91.
- Organ weight changes included effects on liver, kidney, and mesenteric lymph nodes. Increases in liver (with gallbladder) weight were noted in males and females at the time of the interim sacrifice on Day 93 (seen at 12 and 30 mg/kg/dose in males and at 6, 12, and 30 mg/kg/dose in females) and at the time of the terminal sacrifice on Day 275 (seen in all groups for males and at 6 and 12 mg/kg/dose in females). Increases in mesenteric lymph node weight were noted in females at 12 and 30 mg/kg/dose at the time of interim sacrifice (Day 93). Increases in kidney weight were noted in males and females at 12 mg/kg/dose at the time of the terminal sacrifice on Day 275.
- Histopathology examination identified test article-related findings in the kidneys, liver, lymph nodes (mandibular and mesenteric), and injection sites. In the kidneys, findings included accumulation of basophilic granules in tubular epithelial cells (at all doses). Liver findings included hepatocyte hypertrophy at the time of terminal necropsy (≥ 6 mg/kg/dose), accumulation of basophilic granules in hepatocytes (≥ 6 mg/kg/dose at interim sacrifice and ≥ 2 mg/kg/dose at terminal sacrifice), and accumulation of basophilic granules in Kupffer cells (≥ 6 mg/kg/dose). Mesenteric lymph node findings included accumulation of basophilic granules in macrophages (at all doses). Injections site findings at the time of interim and terminal sacrifices (mostly at all doses with dose-related incidence and/or severity), included: (1) basophilic granules; (2) macrophages; (3) fibrosis; (4) mixed leukocyte infiltration; (5) mononuclear cell infiltration; (6) mixed cell infiltration/inflammation; (7) perivascular/vascular inflammation; (8) myofiber degeneration/regeneration (terminal sacrifice only); (9) hemorrhage (terminal sacrifice only); and (10) necrosis (terminal sacrifice only). Accumulation of basophilic granules in macrophages (tissue histiocytes) was seen in numerous tissues at the interim and terminal sacrifices (seen primarily at ≥ 6 mg/kg/dose). Perivascular/vascular inflammation (with sporadic occurrence) was noted in the liver (one male at 12 mg/kg/dose on Day 93), heart (one male at 6 mg/kg/dose, one male at 12 mg/kg/dose, and one female at 30 mg/kg/dose on Day 93), injection sites (males at 6, 12, and 30 mg/kg/dose and females at 12 mg/kg/dose on Day 93), and vagina (one female at 30 mg/kg/dose on Day 93 and one female at 12 mg/kg/dose on Day 275).
- There were dose-dependent decreases in liver PKK mRNA expression and dose-dependent and time-dependent decreases in PKK plasma protein after treatment with donidalorsen ≥ 2 mg/kg/dose at the interim and/or terminal sacrifices. Both liver PKK mRNA expression and PKK plasma protein increased by the end of the recovery periods. This showed pharmacological activity of donidalorsen in Cynomolgus monkeys.
- Donidalorsen was rapidly absorbed into the systemic circulation with median T_{max} at 1 to 4 hours postdose. Mean C_{max} and AUC_{0-48hr} were dose-dependent and greater than dose-proportionally between doses. The majority of total fully conjugated full-length ASO was cleared within hours from plasma, with a MRT_{0-48hr} of about 3 to 10 hours. Tissue concentrations of unconjugated donidalorsen in liver or kidney increased in a dose-dependent

manner. The Applicant claims steady-state tissue concentrations were achieved based on comparable mean tissue concentrations on Days 9, 93, and 275.

- The no observed adverse effect level (NOAEL) was 6 mg/kg/dose based on the following:
 - Basophilic granules seen by histopathology throughout the body appeared consistent with oligonucleotide uptake and in the absence of correlating histopathology were not considered adverse.
 - The NOAEL was based on thrombocytopenia seen in two monkeys at 12 mg/kg/dose and 30 mg/kg/dose.
 - The NOAEL corresponds to a $C_{\max}$ of 6.57 $\mu\text{g/mL}$ and an $\text{AUC}_{0-48\text{hr}}$ of 37.6 $\mu\text{g}\cdot\text{hr/mL}$ on Day 273.

A 26-Week Repeat Dose Toxicity Study of ISIS 721744 in Mice With a 13-Week Recovery (Study Nos. 8405032; 721744-AS04)

Key Study Findings

- Mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection), donidalorsen (1, 4, or 12 mg/kg/dose), or mouse surrogate ISIS 722059 (4 mg/kg/dose) Q2W for 26 weeks with a 13-week treatment-free recovery period (vehicle control and 12 mg/kg/dose of donidalorsen only).
- There were no unscheduled deaths during the study that were considered related to treatment with donidalorsen or mouse surrogate ISIS 722059.
- Changes in hematology parameters at the end of the 26-week Treatment Period included increases in white blood cells, neutrophils, lymphocytes, and/or monocytes in males and/or females at 1, 4, and/or 12 mg/kg/dose donidalorsen and at 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period, increases in hematology parameters were no longer seen at the 12 mg/kg/dose of donidalorsen.
- Changes in clinical chemistry parameters at the end of the 26-week Treatment Period included increases in ALT, AST, and/or ALP in males and/or females at 1, 4, and/or 12 mg/kg/dose of donidalorsen. Effects on clinical chemistry parameters (including ALT, AST, and ALP) were less clear at 4 mg/kg/dose of ISIS 722059. At the end of the 13-week recovery period, increases in ALT and/or AST were still seen in males and/or females at 12 mg/kg/dose of donidalorsen, while increases in ALP were no longer seen. The toxicological significance of increases in ALT and AST were unclear as histopathology findings in the liver appeared to be partially reversible and levels of unconjugated donidalorsen had declined below the lower limit of quantitation.
- Gross pathology findings after the 26-week Treatment Period with donidalorsen were seen in the liver (discoloration in males and/or females at 1, 4, and/or 12 mg/kg/dose donidalorsen and enlargement in males at 4 mg/kg/dose donidalorsen) and inguinal lymph nodes (enlarged in males at 1 and 4 mg/kg/dose donidalorsen). There were no clear gross pathology findings seen after the 26-week Treatment Period with mouse surrogate ISIS 722059. Gross pathology findings after the 13-week recovery period at 12 mg/kg/dose donidalorsen were noted in the liver (discoloration in males) and subcutaneous injection site (discoloration in males).

- Organ weight changes after the 26-week Treatment Period with donidalorsen included increases in liver weights in males and/or females at 4 and/or 12 mg/kg/dose and increases in spleen weights in males at 12 mg/kg/dose. There were no organ weight changes after the 26-week Treatment Period with 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period for 12 mg/kg/dose donidalorsen, liver weights were still increased in males and/or females.
- Histopathology findings after the 26-week Treatment Period with donidalorsen were seen in the liver, skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen. Histopathology findings after the 26-week treatment with mouse surrogate ISIS 722059 were seen in the skin/subcutis, SC dose sites, spleen, and testis.
 - Liver: Minimal or slight centrilobular hepatocellular hypertrophy was noted in males at 1, 4, and 12 mg/kg/dose donidalorsen and in one female (moderate) at 12 mg/kg/dose donidalorsen. This finding correlated with increased mean liver weights at 4 and 12 mg/kg/dose donidalorsen and increased liver enzymes (AST, ALT, and/or ALP). Despite a background incidence of hepatocyte vacuolation in vehicle control males, there was an increase in the severity (slight to marked) of hepatocyte vacuolation in males at 4 and 12 mg/kg/dose donidalorsen, which correlated with gross pathology findings of liver discoloration at all dose levels. These findings were consistent with uptake of donidalorsen.
 - Skin/subcutis: Increased incidences and/or severity of infiltrates of mononuclear cells, mixed cells, and/or vacuolated macrophages in the skin/subcutis were noted in males and/or females at 4 and/or 12 mg/kg/dose donidalorsen and/or 4 mg/kg/dose ISIS 722059. Increased incidences and/or severity of vacuolated fibrocytes in the skin/subcutis were noted in males and/or females at 12 mg/kg/dose donidalorsen and 4 mg/kg/dose ISIS 722059. Slight to moderate inflammation in the skin was noted in females at 4 and 12 mg/kg/dose donidalorsen. These findings in the skin/subcutis correlated with increased leukocyte counts.
 - Subcutaneous dose sites: Increased incidences and/or severity of infiltrates of vacuolated macrophages, mononuclear cells, and mixed cells, as well as fibrocyte vacuolation at the SC injection sites were noted in males and/or females at 1, 4, and/or 12 mg/kg/dose donidalorsen and/or 4 mg/kg/dose ISIS 722059. These findings at the SC injection sites correlated with increased leukocyte counts.
 - Inguinal lymph nodes: Increased incidences and severities (minimal to marked) of increased lymphocytes in males at 1, 4, and/or 12 mg/kg/dose donidalorsen and one female at 12 mg/kg/dose donidalorsen were noted in the inguinal lymph node. This finding in the inguinal lymph node correlated with increased leukocyte counts. Amyloid in the inguinal lymph node was noted in one male at 4 mg/kg/dose ISIS 722059.
 - Spleen: Increased incidence and/or severity of extramedullary hematopoiesis in the spleen was noted in males and/or females at 1, 4 and/or 12 mg/kg/dose donidalorsen and 4 mg/kg/dose ISIS 722059 and correlated with increased mean spleen weight in males administered 12 mg/kg/dose of donidalorsen.
 - Testis: Moderate interstitial cell hyperplasia, minimal sperm stasis, and minimal vacuolation were noted in the testis of males at 4 mg/kg/dose ISIS 722059.

- Histopathology findings after the 13-week recovery period at 12 mg/kg/dose donidalorsen were still noted in the liver as hepatocellular vacuolation in males and females, which correlated with increased liver weights. Histopathology findings in the skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen at 12 mg/kg/dose donidalorsen were not seen at the end of the recovery period.
- Unconjugated donidalorsen was measurable in a dose-dependent manner in the kidney (greater than dose proportional) and liver (dose proportional) after 26 weeks of treatment with donidalorsen. The mean concentrations of unconjugated donidalorsen were similar in the kidney and liver except at 12 mg/kg/dose where mean unconjugated donidalorsen concentrations were greater in the kidney than in the liver. Mean unconjugated donidalorsen concentrations were higher in females than in males in the kidney but similar in the liver. After the 13-week recovery period at 12 mg/kg/dose donidalorsen, mean unconjugated donidalorsen concentrations were below or near the lower limit of quantitation of 0.107 µg/g, suggesting complete clearance of donidalorsen.
- Mouse PKK mRNA in mouse liver was reduced by 77% (statistically significant) compared to vehicle control in mice treated with mouse surrogate ISIS 722059 at 4 mg/kg/dose for 26 weeks.
- The NOAEL was 12 mg/kg/dose donidalorsen, as test article-related findings were considered related to the oligonucleotide uptake.

13.1.3.2. Genotoxicity

The genotoxicity of donidalorsen was evaluated in a complete battery of GLP-compliant genotoxicity studies (i.e., in vitro Ames assay, in vitro chromosome aberration assay in CHL cells, and in vivo bone marrow micronucleus assay in mice).

ISIS 721744: Bacterial Reverse Mutation Assay (Study Nos. G115138; 721744-IS01)

The in vitro Ames assay, using the plate incorporation method, evaluated vehicle, positive controls, and donidalorsen (15 to 5000 µg/plate) in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2uvrA tester strains (with or without 10% S9 mix for metabolic activation). There was no increase in the number of revertant colonies in any tester strain, at any donidalorsen concentration, with or without metabolic activation. There was no toxicity up to highest donidalorsen concentration, in any tester strain, with or without metabolic activation. Donidalorsen was negative for mutagenicity up to 5000 µg/plate ± S9 metabolic activation.

ISIS 721744: In Vitro Chromosome Aberration Assay in CHL Cells (Study Nos. G115139; 721744-IS02)

The in vitro chromosome aberration assay evaluated vehicle, positive controls, and donidalorsen at 62.5 to 500 µg/mL in CHL cells for 6 hours (with or without 2% S9 mix for metabolic activation) and for 22 hours (without S9 mix). No cytotoxicity was noted at up to 500 µg/mL after 6 hours with or without 2% S9 mix and 22 hours without S9 mix. There was no significant increase in the number of metaphases with structural or numerical aberrations up to 500 µg/mL

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Dawnzera (donidalorsen)

after 6 hours (with or without 2% S9 mix) and 22 hours (without S9 mix). Donidalorsen was negative for clastogenicity up to 500 µg/mL in cultured CHL cells.

Mammalian Micronucleus Assay in Bone Marrow Erythrocytes Following Subcutaneous Injection of ISIS 721744 to ICR Mice (Study Nos. G120005; 721744-AS05)

The in vivo mammalian micronucleus assay evaluated vehicle, positive control, and donidalorsen (500, 1000, and 2000 mg/kg SC) administered on Days 1 and 3 (48 hours postdose on Day 1) to ICR mice. Mice were sacrificed on Day 4 (24 hours postdose on Day 3) for bone marrow collection. There was no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes at any dose of donidalorsen tested compared to the vehicle control (negative control) group. Donidalorsen was negative in the in vivo bone marrow micronucleus assay in mice.

13.1.3.3. Carcinogenicity

The SPAs for the proposed protocols for the 6-month carcinogenicity study in hemizygous transgenic (Tg.rasH2) mice and the 2-year carcinogenicity study in rats included recommendations on dose selection based on a non GLP-compliant 14-week DRF study in Tg.rasH2 mice and 16-week DRF study in Sprague Dawley rats, respectively.

A 14-Week Repeat Dose Toxicity Study of ISIS 721744 and ISIS 722059 IN CBYB6F1-TG(HRAS)2JIC Hemizygous Transgenic Mice (Study No. 721744-AS07P)

Tg.rasH2 mice received SC injections of vehicle control (0.9% sodium chloride for injection), donidalorsen (6, 18, or 36 mg/kg/dose), or mouse surrogate ISIS 722059 (6 mg/kg/dose) Q2W. Animal numbers were noted to be low (n=6/sex/group) and the histopathology examination was limited (i.e., sternum bone marrow and bone, heart, injection sites, kidneys, liver, lung, spleen, testes, thymus, gross lesions, and tissue masses). Donidalorsen resulted in dose-dependent increases in ALP (up to 1.6-fold over control), AST (up to 5.4-fold over control), and ALT (up to 5.1-fold over control) in males at all dose levels. These increases correlated with a dose-dependent increase of minimal karyocytomegaly in the liver in males at ≥ 6 mg/kg Q2W. In females, only minimal and non-dose-dependent increases in liver enzymes (up to 1.9-fold over control) were seen and there were no microscopic correlates in the liver. For male mice, the high dose selection of 20 mg/kg/dose donidalorsen was based upon incidences of karyocytomegaly and increases of ALT, AST, and ALP at ≥ 18 mg/kg/dose in the 14-week DRF study. For female mice, there were no histopathological findings in the liver and there were minimal non-dose-related elevations of ALT, AST, and ALP in the 14-week DRF study. No dose-limiting toxicity was identified for female mice in the 14-week DRF study. To evaluate pharmacological effects in the 6-month carcinogenicity study, male and female mice will receive the mouse surrogate, ISIS 722059, at a dose of 10 mg/kg/dose, although only a single dose level of 6 mg/kg Q2W was evaluated in the 14-week DRF study. A MTD for ISIS 722059 was not determined. At 10 mg/kg Q2W, the mouse surrogate ISIS 722059, is expected to achieve $\sim >80\%$ reduction in liver PKK mRNA expression.

The Executive Carcinogenicity Assessment Committee (ECAC) concurred with doses of 0 (0.9% sodium chloride for injection), 5, 10, and 20 mg/kg Q2W via SC injection in male mice with the

high dose based on a MTD (i.e., incidences of karyocytomegaly and increases of ALT, AST, and ALP in male mice at higher doses in the 14-week DRF study). The mid and low doses are based on adequate dose spacing. For female mice, the Committee could not recommend doses for the proposed study, as no acceptable basis for dose selection could be identified. If the Applicant would like ECAC concurrence for dose selection, a new DRF study should be conducted to identify an acceptable high dose as outlined in the ICH guidances for industry, *S1C(R2) Dose Selection for Carcinogenicity Studies of Pharmaceuticals* ([September 2008](#)) or *S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals* ([November 2022](#)).

A 16-Week Repeat-Dose Toxicity Study of ISIS 721744 in Sprague Dawley Rats (Study Nos. 721744-AS08P)

Sprague Dawley rats received SC injections of vehicle control (0.9% sodium chloride for injection) or donidalorsen (10, 30, or 90 mg/kg) Q2W with one additional loading dose on Day 8. Animal numbers were noted to be low (vehicle control =3/sex/group; test article-treated groups =5/sex/group) and the histopathology examination was limited (e.g., gross lesions, adrenal glands, bone marrow, heart, injection sites, kidneys, liver, lung, lymph nodes, spleen, and thymus). Decreased body weight gains (>10% from control) were seen in males at ≥ 30 mg/kg/dose and females at ≥ 10 mg/kg/dose. In the kidneys, histopathology findings in males and females at ≥ 10 mg/kg/dose included minimal to marked basophilic granules in the proximal convoluted tubule, minimal to mild accumulation of eosinophilic (hyaline) droplets in the proximal convoluted tubule, and minimal to mild mononuclear interstitial infiltrates.

The Committee concurred with the Applicant's proposed doses of 0 (0.9% sodium chloride for injection), 1.5, and 5 mg/kg Q2W via SC injection for both male and female rats. The high dose was identified as the MTD based on the decrease in body weight gain (i.e., >10% from control in females at ≥ 10 mg/kg Q2W) and also class experience with ASO, which suggests that the observed histopathology lesions in the kidneys (seen in male and female rats at ≥ 10 mg/kg Q2W) can progress with longer treatment duration and adversely impact survival in a 2-year study. The low dose for both male and female rats was approximately one-third of the high dose.

13.1.3.4. Reproductive and Developmental Toxicity

The potential reproductive and developmental toxicity of donidalorsen were evaluated in GLP-compliant combined fertility and developmental toxicity study in mice with donidalorsen and mouse surrogate ISIS 722059, a combined fertility and developmental toxicity study in mice with the unconjugated parent compound of donidalorsen (ISIS 546254) and the corresponding unconjugated mouse surrogate (ISIS 482584), and an EFD study in rabbits with the unconjugated parent compound of donidalorsen (ISIS 546254).

A Combined Fertility and Developmental Toxicity Study of ISIS 721744 in Mice Via Subcutaneous Administration (Study Nos. 727-134; 721744-AS06)**Key Study Findings**

- Mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection), donidalorsen (1, 4, or 10 mg/kg/week), or mouse surrogate ISIS 722059 (4 mg/kg/week). Males were dosed once weekly during the pre-mating (beginning 28 days prior to mating), mating, and postmating phases (10 weeks total). Females were dosed beginning 14 days prior to mating. Females were dosed weekly (Days 1, 8, 15, and 22, as needed) during the pre-mating phase and through the mating phase until positive evidence of copulation was observed, and every other day during the gestation period from Gestation Day (GD) 0 to GD 16 (i.e., GDs 0, 2, 4, 6, 8, 10, 12, 14, and 16). Unmated females continued to be dosed weekly until termination (following the last GD 18 necropsies).
- There were no estrous cycle changes (i.e., mean number of cycles and mean cycle length) related to treatment with donidalorsen or mouse surrogate ISIS 722059.
- Percent abnormal sperm was increased at 10 mg/kg/week donidalorsen (15.6%, statistically significant) and 4 mg/kg/week ISIS 722059 (13.4%), compared to the vehicle control (10.1%), and outside of the conducting laboratory historical control ranges of 3.4% to 10.2%. It did not appear that an increase in percent abnormal sperm affected pregnancy indices at 10 mg/kg/week donidalorsen or 4 mg/kg/week ISIS 722059.
- There were no effects of donidalorsen or mouse surrogate ISIS 722059 on male or female mating performance; the number of corpora lutea, implantations, preimplantation and postimplantation losses, or live fetuses; fetal body weights; or fetal external, visceral, or skeletal variations or malformations (i.e., no fetal anomalies) in mice.
- Unconjugated donidalorsen concentrations in the parental liver of males and females increased with increasing dose of drug. Unconjugated donidalorsen concentrations in the fetal liver were below the limit of quantitation in all donidalorsen-treated groups. Notably, unconjugated donidalorsen concentrations in the placenta (up to 0.31 µg/g) and testes (up to 1.61 µg/g) were much lower than in the liver (i.e., 3 to 43 µg/g for 1 to 10 mg/kg/week donidalorsen in F0 females on GD 18 and 7 to 47 µg/g for 1 to 10 mg/kg/week for males on Day 73). It was unclear if donidalorsen or unconjugated donidalorsen crossed the placenta to reach fetal tissues.
- Treatment of F0 male and female mice with mouse surrogate ISIS 722059 at 4 mg/kg/week statistically decreased liver mRNA expression of KLKB1/PKK by 80% and 75%, respectively.
- The NOAEL for donidalorsen on male and female fertility and mating performance, as well as developmental toxicity, was the high dose of 10 mg/kg/week.

A Combined Fertility and Developmental Toxicity Study of ISIS 546254 and ISIS 482584 Administered by Subcutaneous Injection in CD-1 Mice (Study Nos. (b) (4) 090.60; 546254-AS04)

Key Study Findings

- CD-1 mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection), ISIS 546254 (10.5, 42, or 84 mg/kg/week), or mouse surrogate ISIS 482584 (84 mg/kg/week). As described in the study report, animals were dosed every other day and received three or four doses per week; thus, the weekly dose (mg/kg/week) was equivalent to 3.5 times the single mg/kg/dose. Males were dosed every other day during the pre mating (beginning 4 weeks prior to mating), mating, and postmating phases (10 weeks total). Females were dosed every other day for two weeks prior to mating, during the mating phase, and throughout GD 15 (encompassing the period of organogenesis).
- There was one unscheduled death during the study, which was considered incidental and unrelated to treatment with mouse surrogate ISIS 482584. All other animals survived to scheduled termination.
- Body weight decreases were noted in females on GD 18 at 10.5 and 84 mg/kg/week ISIS 546254 and 84 mg/kg/week ISIS 482584 (less than 10% difference from vehicle control). The decreases were not considered adverse.
- There were no effects of ISIS 546254 or mouse surrogate ISIS 482584 on estrous cyclicity.
- There were no effects of ISIS 546254 or mouse surrogate ISIS 482584 on fertility (mating index) or fecundity (pregnancy index).
- At the time of Cesarean section, mean gravid uterus weights were decreased at 10.5, 42, and 84 mg/kg/week ISIS 546254 (-11%, -5%, and -7% difference from vehicle control respectively; not statistically significant) and at 84 mg/kg/week ISIS 482584 (-24% difference from vehicle control; statistically significant).
- The number of live fetuses was decreased at 10.5, 42, and 84 mg/kg/week ISIS 546254 (12 live fetuses in each group; statistically significant only at 10.5 mg/kg/week ISIS 546254) and at 84 mg/kg/week ISIS 482584 (11 live fetuses; statistically significant) compared to the vehicle control (14 live fetuses). This is not likely biologically significant. In addition, the fetal viability percentage at 84 mg/kg/week ISIS 482584 was statistically decreased compared to the vehicle control (86% versus 96%).
- There were no effects of ISIS 546254 on mean fetal weight (male or female) although at 84 mg/kg/week ISIS 482584 mean fetal weight was decreased for males and females (-11% and -10% difference from vehicle control, respectively; statistically significant in females only).
- The number of early resorptions was increased at 10.5, 42, and 84 mg/kg/week ISIS 546254 (one in each group; not statistically significant) and at 84 mg/kg/week ISIS 482584 (one; statistically significant) compared to the vehicle control (zero). This is not likely biologically significant. The percent early resorptions/animal was increased at 10.5, 42, and 84 mg/kg/week ISIS 546254 and at 84 mg/kg/week ISIS 482584 compared to the vehicle

control. The embryo-fetal mortality index was increased at 10.5, 42, and 84 mg/kg/week ISIS 546254 and at 84 mg/kg/week ISIS 482584 compared to the vehicle control. As described in the study report, although these changes were noticeable and statistically significant in some groups, they were consistent with what is noted in the control animals in these types of studies, therefore, a clear relationship to either test article cannot be established.

- Overall, there were no fetal external findings, visceral variations or malformations, or skeletal variations or malformations attributed to treatment with ISIS 546254 or mouse surrogate ISIS 482584 in mice.
- There were no effects of ISIS 546254 or mouse surrogate ISIS 482584 on sperm motility, morphology, or sperm count.
- ISIS 546254 concentrations in the parental liver of vehicle control males and females were below the limit of quantitation. ISIS 546254 concentrations in the parental liver of males and females increased with ISIS 546254 dose with higher concentrations in male mice than female mice. ISIS 546254 concentrations in the fetal liver were below the limit of quantitation in all ISIS 546254-treated groups.
- ISIS 546254 concentrations in the placenta were below the limit of quantitation, 6.73, and 22.3 µg/g with doses of 10.5, 42, and 84 mg/kg/week ISIS 546254, respectively. Notably, ISIS 546254 concentrations in the placenta were lower than in the liver (52 to 612 µg/g for 10.5 to 84 mg/kg/week ISIS 546254 in males and females).
- Treatment of male and female mice with mouse surrogate ISIS 482584 at 84 mg/kg/week statistically decreased liver mRNA expression of KLKB1/PKK by 94% and 87%, respectively.
- Overall, SC administration of ISIS 546254 (up to 84 mg/kg/week) or mouse surrogate ISIS 482584 (84 mg/kg/week) did not affect fertility, pregnancy, or embryofetal development in mice.

An Assessment of Effects of ISIS 546254 on Embryo-Fetal Development in New Zealand White Rabbits (Study Nos. (b) (4) 090.61; 546254-AS05)

It was agreed upon at the time of the EOP2 meeting that an EFD study in rabbits with donidalorsen would not be needed. This was based on the availability of an EFD study in rabbits with ISIS 546254 (unconjugated parent compound of ISIS donidalorsen, which has the same sequence as donidalorsen).

Key Study Findings

- Pregnant New Zealand white rabbits were treated by SC administration with vehicle control (0.9% sodium chloride for injection) or ISIS 546254 (12.5, 25, or 75 mg/kg/week) every other day from GD 6 to GD 18 (i.e., GDs 6, 8, 10, 12, 14, 16, and 18) encompassing the period of organogenesis. As stated in the study report, animals were dosed every other day, thus, the weekly dose (mg/kg/week) was equivalent to 3.5 times the single mg/kg dose.
- There were no unscheduled deaths during the study.

- ISIS 546254 concentrations in the maternal liver of vehicle control females on GD 28 were below the limit of quantitation. ISIS 546254 concentrations increased in the maternal liver of females on GD 20 (TK animals) and GD 28 with increase in ISIS 546254 dose, with concentrations on GD 28 about half of the concentrations on GD 20 (TK animals). ISIS 546254 concentrations in the fetal liver were below the limit of quantitation in all ISIS 546254-treated groups. ISIS 546254 concentrations in the placenta were below the limit of quantitation at 12.5 mg/kg/week ISIS 546254 and at <10% of maternal exposures at 25 and 75 mg/kg/week ISIS 546254.
- There was no effect of ISIS 546254 on fecundity as the fecundity index was similar between 12.5, 25, and 75 mg/kg/week (88%, 100%, and 84%, respectively) and the vehicle control (91%).
- There were no effects of ISIS 546254 on gravid uterine weight, the number of corpora lutea, the number of implantations, the implantation index, percent of early or late resorptions, live fetuses, the sex ratio, or fetal viability.
- Mean fetal weight appeared to decrease at 75 mg/kg/week for males (-12.3% difference from vehicle control; statistically significant), females (-10.4% difference from vehicle control), and combined (-11.6% difference from vehicle control; statistically significant). The embryo-fetal mortality index at 12.5, 25, and 75 mg/kg/week (5.0%, 5.7%, and 6.7%, respectively) was increased (not statistically significant) compared to the vehicle control (1.3%). The percent of dead fetuses/implantation site at 12.5, 25, and 75 mg/kg/week (1.3%, 0.3%, and 5.8%, respectively) was increased (statistically significant at 75 mg/kg/week only) compared to the vehicle control (0%). As stated in the study report, although these changes were noticeable and statistically significant at 75 mg/kg/week, they were consistent with what is noted in the vehicle control animals in these types of studies, therefore, a clear relationship to test article cannot be established.
- Overall, there were no fetal external findings, visceral variations or malformations, or skeletal variations or malformations attributed to treatment with SC administration of ISIS 546254 (up to 75 mg/kg/week) in rabbits.

13.2. Individual Reviews of Studies Submitted With the New Drug Application

Pivotal and supporting pharmacology (primary), absorption, distribution, metabolism, and excretion (ADME), toxicology (carcinogenicity, reproductive and developmental toxicity, and other toxicity), and extractables and leachables studies submitted with NDA 219407 and not previously submitted with IND 142564 have been reviewed and evaluated under the NDA and are summarized here.

13.2.1. Pharmacology

13.2.1.1. Primary Pharmacodynamics

The primary pharmacodynamics of donidalorsen were evaluated in an in vivo primary pharmacology study in human KLKB1/PKK transgenic mice.

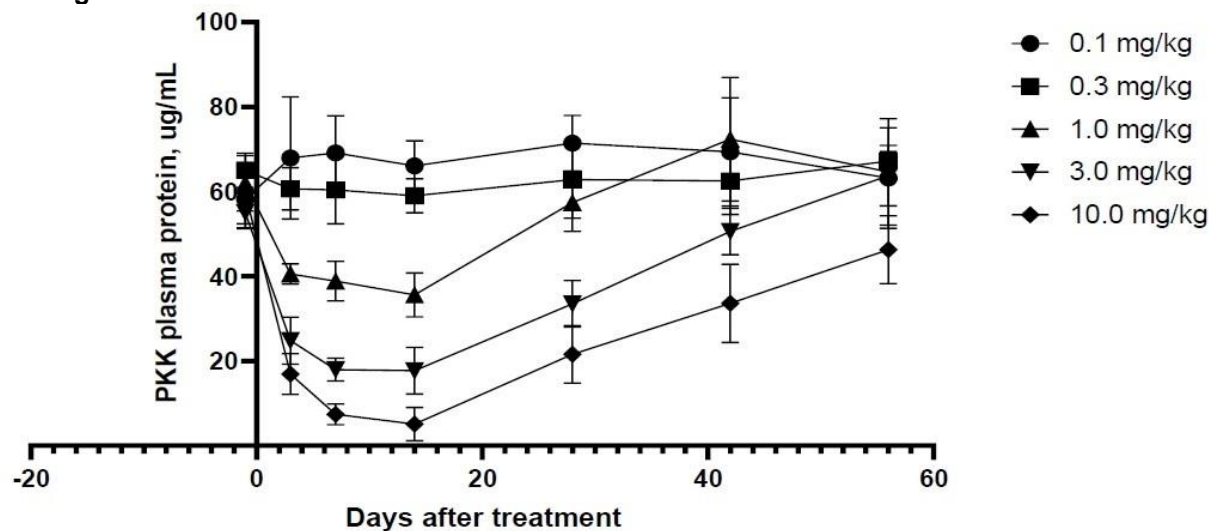
In Vivo Primary Pharmacology Studies

In Vivo Evaluation of ISIS 721744 Activity in Human KLKB1/PKK Transgenic Mice- Analysis of Plasma PKK Protein Levels (ELN 23-100d)

The effects on human PKK plasma protein levels were evaluated following single dose SC administration of donidalorsen (0.1, 0.3, 1, 3, and 10 mg/kg) to transgenic mice expressing the human KLKB1/PKK gene. A single dose of donidalorsen reduced plasma PKK protein in human KLKB1/PKK transgenic mice in a dose- and time-dependent manner (Figure 37). Plasma PKK protein levels were reduced up to a maximum of about 91% on Day 14 after treatment with 10 mg/kg donidalorsen. After Day 14, plasma PKK protein levels gradually increased to baseline levels and were restored by Day 42 or Day 56 at 1 mg/kg or 3 mg/kg donidalorsen, respectively. At Day 56, plasma PKK protein levels at 10 mg/kg were still 20% below baseline levels.

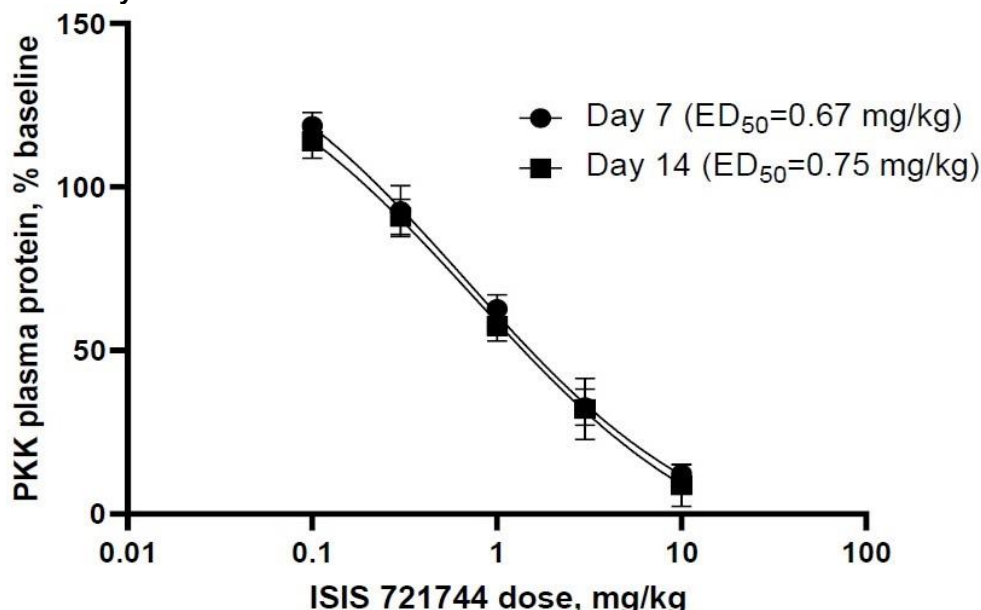
Based on nonlinear curve fit analysis, the median effective dose 50 (ED₅₀) at Days 7 and 14 after treatment with donidalorsen was 0.67 and 0.75 mg/kg, respectively (Figure 38).

Figure 37. Dose- and Time-Dependent Reduction of Plasma PKK Protein in Human KLKB1/PKK Transgenic Mice Treated With Donidalorsen



Source: Excerpted from the Applicant's submission
Abbreviations: KLKB1, gene that encodes prekallikrein; PKK, prekallikrein

Figure 38. Dose-Dependent Reduction of Plasma PKK Protein in Human KLKB1/PKK Transgenic Mice at Days 7 and 14 After Treatment With Donidalorsen



Source: Excerpted from the Applicant's submission

Abbreviations: ED₅₀, median effective dose 50; KLKB1, gene that encodes prekallikrein; PKK, prekallikrein

13.2.2. Absorption, Distribution, Metabolism, Excretion/Pharmacokinetics

The ADME, PK, and TK profiles of donidalorsen were evaluated both in vitro and in vivo (mice and monkeys).

13.2.2.1. Absorption

The plasma pharmacokinetics of donidalorsen was evaluated in a GLP-compliant single-dose SC ADME study in rats with radiolabeled [3H]donidalorsen and a GLP-compliant 6-week repeat-dose SC study in mice.

ADME of [3H]ISIS 721744 Following a Single Subcutaneous Dose Administration to Sprague Dawley Rats (Study Nos. IPI/06; 721744-APK02)

Nonpigmented Sprague Dawley rats were treated with single SC doses of [3H]donidalorsen (2 or 10 mg/kg).

Following a single SC dose of [3H]donidalorsen at 2 mg/kg to male nonpigmented Sprague Dawley rats, C_{max} of 0.375 and 0.538 µg equivalent/mL were reached in the blood and plasma, respectively, at 1 hour for both, after which radioactivity declined in a biphasic manner. The blood:plasma ratio calculated from AUC_{0-∞} was 0.76. C_{max} (µg equivalent/g) of 24.40 at 24 hours and 14.89 at 168 hours were reached in the liver and kidney, respectively. Most tissues achieved concentrations similar to or slightly above the blood radioactivity concentrations. Tissue:blood ratios at 24 hours were highest in the liver and kidney (1877 and 850, respectively) consistent

NDA 219407

Dawnzera (donidalorsen)

with uptake of donidalorsen. The thyroid gland, pancreas, large and small intestine, spleen, lymph node, and bone marrow had tissue: blood ratios between 24.2 to 69.2 at 24 hours.

Following a single SC dose of [3H]donidalorsen at 2 mg/kg to male nonpigmented Sprague Dawley rats, the mean cumulative recovery (0 to Day 56) of radioactivity in excreta was 76.8% (17.14% from urine +57.46% from feces +2.16% from cage wash). Residual radioactivity in the carcass on Day 56 accounted for 5.84% of the administered dose resulting in an overall recovery of 82.6%.

Following a single SC dose of [3H]donidalorsen at 10 mg/kg to male and female nonpigmented rats, radioactivity was distributed to tissues by the first sampling time at 2 hours and reached maximal concentrations within 2 to 48 hours postdose with highest concentrations of radioactivity in the kidney, liver, intestinal mucosa, salivary gland, bone marrow, lymph nodes, and splenic red pulp. Concentrations of radioactivity in most organs/tissues decreased by the end of the sampling period at 336 hours, except for the kidney where radioactivity levels remained at similar levels throughout the sampling period.

Recovery of radioactivity in urine and feces from nonpigmented rats following a single SC dose of [3H]donidalorsen at 10 mg/kg was 0.35% and 1.37% of dose, respectively for males and 0.30% and 1.31% of dose, respectively for females.

A 6-Week Subcutaneous Repeat-Dose Pharmacokinetic Study of ISIS 721744 in CD-1 Mice (Study Nos. G122111; 721744-APK03)

Male ICR mice were treated by SC administration with donidalorsen (10 or 20 mg/kg/week once weekly) for 6 weeks (Days 1, 8, 15, 22, 29, 36, and 43) to determine the PK profile and distribution of donidalorsen to kidney and liver. As described in the study report, concentrations of total full-length donidalorsen, including fully conjugated, partially conjugated (minus 1-, 2-, and 3-GalNAc sugars), and unconjugated donidalorsen (ISIS 780124), were determined in mouse plasma by a validated hybridization ECL assay, while concentrations of unconjugated donidalorsen in kidney and liver samples were analyzed by a validated HPLC-MS/MS bioanalytical method.

Median T_{max} was 0.5 hours on Days 1 and 43 at 10 and 20 mg/kg donidalorsen. Mean C_{max} and AUC increased in a slightly greater than dose proportional manner on Days 1 and 43. There was lack of accumulation after repeat dosing.

Concentrations of unconjugated donidalorsen were higher in the kidney than in the liver. Concentrations of unconjugated donidalorsen in the liver were similar at 10 and 20 mg/kg/week possibly due to saturation of tissue uptake at these dose levels.

13.2.2.2. Distribution

The distribution of donidalorsen was evaluated in vitro for the whole blood and plasma concentration ratio of donidalorsen in human, monkey, and rat.

In Vitro Evaluation of Whole Blood and Plasma Concentration Ratio of ISIS 721744 (Donidalorsen) in Human, Monkey, and Rat (Study Nos. IIS-P2-008; 721744-IS11)

The in vitro mean blood to plasma ratio of donidalorsen (tested neat, 0.01, 0.03, 0.1, 0.3, 1, and 2 µg/mL in human whole blood and neat, 0.1, 0.3, 1, 3, 10, 30, 100, and 200 µg/mL in monkey and rat whole blood) was between 0.630 and 0.824 in human, 0.468 and 0.566 in monkey, and 0.501 and 0.661 in rat, suggesting that donidalorsen distributes primarily in the plasma compartment across the concentrations tested in all three species.

13.2.2.3. Metabolism

The metabolism of donidalorsen was evaluated after single SC administration of [3H]donidalorsen to rats, as well as from select urine samples from studies in mice, monkeys, and humans. Further, the metabolism of donidalorsen linker-related metabolites was evaluated in select plasma, urine, and fecal samples from studies in mice, monkeys, and humans, as well as from liver and kidney samples from rat.

Metabolite Profiling and Identification Studies Following a Single Subcutaneous Dose Administration of [3H]ISIS 721744 to Male and Female Rats (Study Nos. IPI/07; 721744-APK02)

This study evaluated the metabolic profile of donidalorsen in rat whole blood and plasma, liver and kidney tissues, and in the excreta (urine and feces) by liquid chromatography mass spectrometry (LC-MS) after single SC administration of [3H]donidalorsen at 10 mg/kg to male and female nonpigmented rats.

Donidalorsen was seen in the liver, kidney, plasma, and whole blood. Donidalorsen was the major component at 2 hours in plasma and blood but was not detected at 24 hours in plasma and blood. Unconjugated donidalorsen (i.e., ISIS 780124) was the major component in liver (2 and 24 hours) and kidney (24 hours). Two major components were present in the kidney at 2 hours: unconjugated donidalorsen (i.e., ISIS 780124) and donidalorsen minus 3 GalNAc sugars (i.e., ISIS 721744 -3 GalNAc). Two major components were detected in feces samples at 0 to 24 hours: ISIS 780124 3'N-18 and ISIS 780124 3'N-17. Several ISIS 780124 shortmers were identified as major components in urine at 0 to 24 hours, from ISIS 780124 3'N-10 to ISIS 780124 3'N-14. As described in the study report, all identified metabolites were consistent with nuclease-mediated metabolism to chain shortened oligonucleotides with full, partial, or without GalNAc or linker-conjugation.

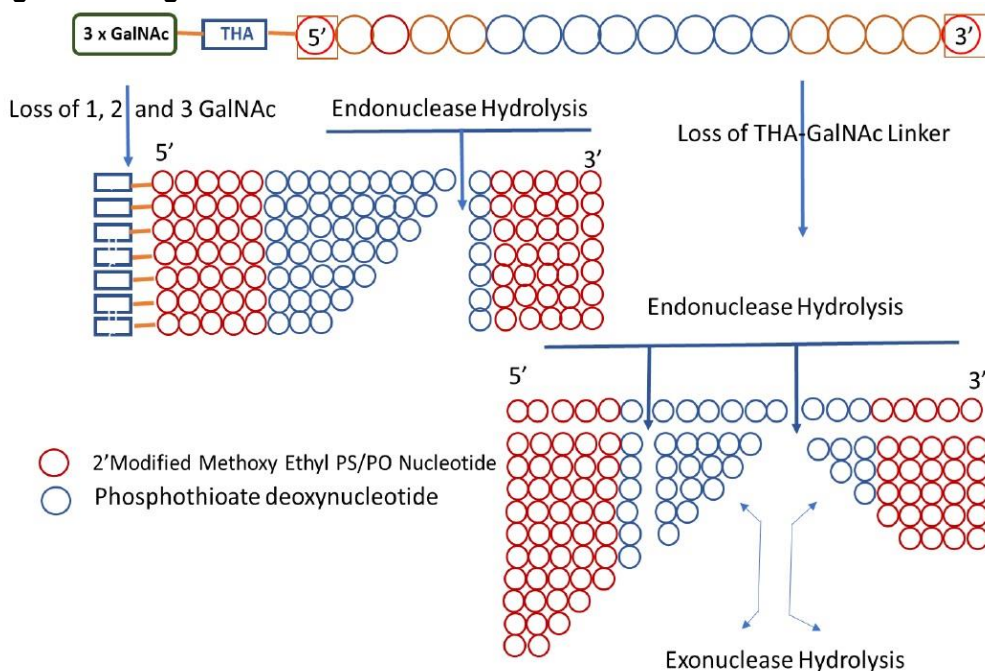
Metabolic Profiling and Identification of ISIS 721744 Oligonucleotide-Related Metabolites by HPLC-High-Resolution Mass Spectrometer in Select Urine Samples From Mice, Monkeys, and Humans (Study Nos. 727-085, 727-081, ISIS 721744-CS1; 721744-IS10)

This study identified donidalorsen oligonucleotide containing metabolites (full-length or chain-shortened) from urine samples at steady state from mice, monkeys, and humans treated with multiple SC doses of donidalorsen (i.e., 10 mg/kg from 13-week repeat-dose toxicity study in mice [Study No. 721744-AS01]; 30 mg/kg from 39-week repeat-dose toxicity study in monkeys [Study No. 721744-AS02]; and 80 mg from phase 1 trial in humans with dosing every 4 weeks for four total doses [Study No. ISIS 721744-CS1]) using liquid chromatography with a tandem ultraviolet detector and high-resolution mass spectrometer method.

As described in the study report, the oligonucleotide portions of conjugated ASO molecules are metabolized by hydrolysis of the phosphodiester bonds via endonuclease and/or exonuclease (either 5' and/or 3') enzymes. Metabolism of the GalNAc-conjugation component of donidalorsen typically occurs by a sequential loss of the three GalNAc sugar moieties (-1, -2, and -3 GalNAc), followed by the loss of a portion, or all the remaining THA linker ([Shemesh et al. 2016](#); [Weidolf et al. 2021](#)). Complete loss of the THA linker results in the formation of ISIS 780124, the unconjugated version of donidalorsen. The metabolism of the two portions of donidalorsen (oligonucleotide and GalNAc) may occur simultaneously resulting in many potential metabolites with multiple potential metabolic pathways responsible for a single metabolite compound.

A diagram of the proposed putative metabolism of donidalorsen is shown in [Figure 39](#).

Figure 39. Diagram of Putative Metabolism of Donidalorsen



Source: Study No. 721744-IS10, Figure 1

Source: Excerpted from the Applicant's submission

Abbreviations: GalNAc, *N*-acetyl galactosamine; PO, phosphodiester; PS, phosphorothioate; THA, tris(hexylamino)

Mouse Urine

Donidalorsen was the major component (about 53% of total oligonucleotide signal) in mouse urine samples when administered at 10 mg/kg. As described by the Applicant, other oligonucleotide metabolites included the full-length ASO with partial conjugations, fully and partially conjugated donidalorsen with loss of nucleotides from the 3'-end (referred to as 3'-deletion chain-shortened metabolites), and unconjugated donidalorsen with loss of nucleotides from the 5'-end (referred to as 5'-deletion chain-shortened metabolites).

Monkey Urine

Donidalorsen was the major component (about 41% of total oligonucleotide signal), followed by ISIS 780124 (unconjugated version of donidalorsen; about 14% of total oligonucleotide signal) in the monkey urine samples when administered at 30 mg/kg. As described by the Applicant, other identified metabolites were 3'-deletion chain-shortened metabolites of donidalorsen (with GalNAc sugar moieties and THA linker attached), as well as metabolites of unconjugated donidalorsen with loss of nucleotides from the 5'-end.

Human Urine

ISIS 780124 (unconjugated version of donidalorsen) was the major component (about 38% of total oligonucleotide signal) in human urine samples when administered at 80 mg/kg.

Donidalorsen was not detected. As described by the Applicant, the other most abundant metabolites were from unconjugated donidalorsen with loss of nucleotides from the 5'-end. However, 3'-deletion chain-shortened metabolites of donidalorsen (with GalNAc sugar moieties and THA linker attached) were also seen.

There were no unique metabolites found in humans compared to mice or monkeys.

Profiling, Identification, and Quantitation of ISIS 721744 Linker-Related Metabolites by HPLC-MS/MS in Select Plasma, Urine, and Fecal Samples From Mice, Monkeys, and Humans (Study No. 721744-IS12)

This study identified linker-only-related metabolites of donidalorsen (no full-length or chain-shortened oligonucleotide components) from plasma (monkey and human), urine (mouse, monkey, and human), and feces (monkey) after SC dosing of donidalorsen (i.e., 2 and/or 10 mg/kg from 13-week repeat-dose toxicity study in mice [Study No. 721744-AS01]; 2 and/or 30 mg/kg from 39-week repeat-dose toxicity study in monkeys [Study No. 721744-AS02]; and 80 mg from phase 1 trial in humans with dosing every 4 weeks for four total doses [Study No. ISIS 721744-CS1]) by a HPLC-MS/MS method. Eleven total linker metabolites (putative linker metabolites M4 to M14) were monitored in these studies.

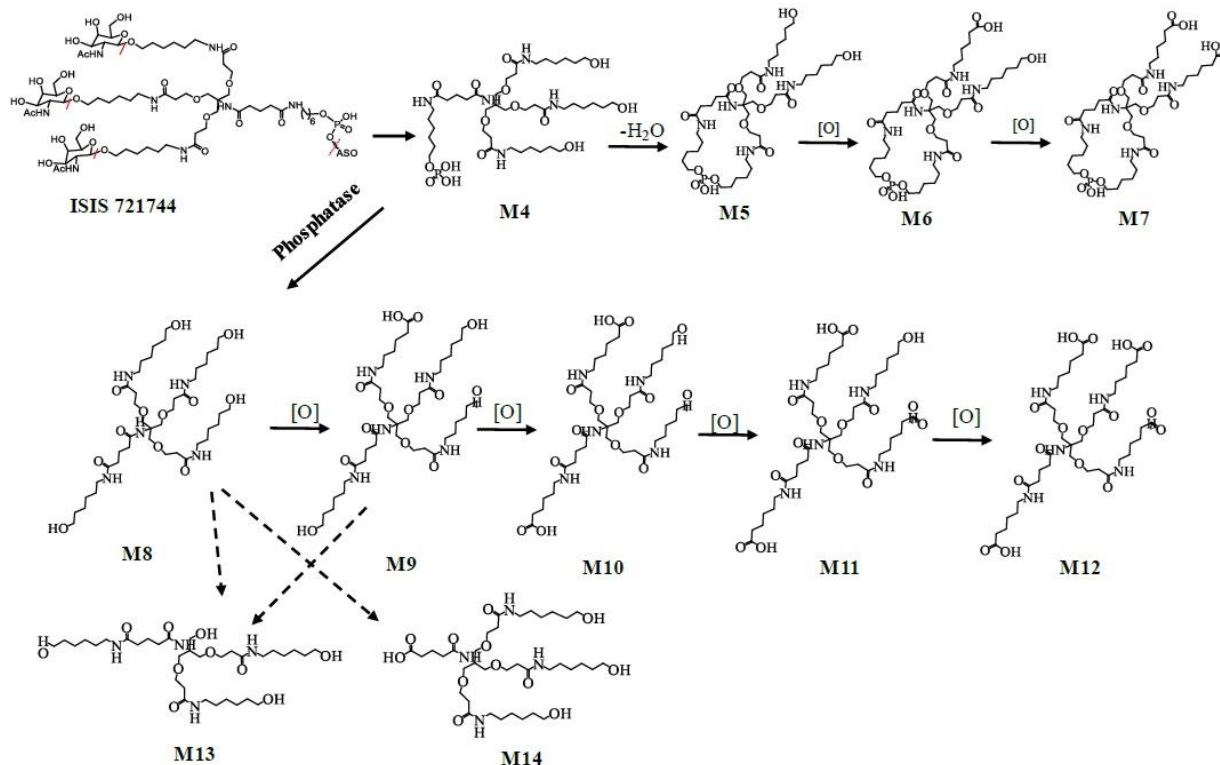
Six linker metabolites were detected in monkey plasma (M5 and M8 to M12) and three linker metabolites were detected in human plasma (M5, M8, and M12).

Ten linker metabolites (M5 to M14) were similarly detected in mouse, monkey, and human urine. The linker metabolites with the highest peak area ratios were M8, M5, and M12, which were consistent across the three species.

Ten linker metabolites (M5 to M14) were detected in monkey feces. The linker metabolites with the highest peak area ratios were M8, M10, M11, and M5.

As described by the Applicant, the THA-linker-related metabolism is mostly similar across species with oxidative metabolites being formed and eliminated through urine and bile. The overall metabolic pathways for the THA-linker-related metabolites of donidalorsen involve hydrolysis of GalNAc sugars and oligonucleotide moieties to form M4 metabolite, followed by internal cyclization of M4 to form cyclized linker M5 metabolite which would then undergo mono- and di-oxidation to form M6 and M7 metabolites. Dephosphorylation of M4 metabolite resulted in the formation of M8 metabolite followed by sequential oxidation at each branching arm of M8 metabolite to form mono-, di-, tri-, and tetra-oxidation metabolites (M9 to M12 metabolites). Additional metabolites resulting from dealkylation (M13 metabolite), or deamination (M14 metabolite) were also detected. The proposed pathway for the metabolism of linker-related moieties of donidalorsen is shown in [Figure 40 \(Shemesh et al. 2016\)](#).

Figure 40. Proposed Biotransformation Pathway for the Linker-Related Metabolism of Donidalorsen



Source: Excerpted from the Applicant's submission

Method Development, Qualification and Sample Analysis of a Bioanalytical Method for the Determination of M8 in Liver and Kidney Samples Generated From Rat Using LC-MS/MS (Study Nos. IPI/08; 721744-IS17)

This study developed a liquid chromatographic-tandem mass spectrometric (UPLCMS/MS) bioanalytical method to determine the donidalorsen THA linker-related metabolite M8 (chemical formula = C₄₂H₈₁N₅O₁₂; molecular weight = 847.5882) in liver and kidney samples from rats treated with single SC dose levels of donidalorsen at 2 and 10 mg/kg (Study Nos. IPI/06 and IPI/07; Study No. 721744-APK02).

Mean M8 concentrations were below the limit of quantitation in liver and kidney from rats treated with a single SC dose level of donidalorsen at 2 mg/kg. Mean M8 concentrations from rats treated with a single SC dose level of donidalorsen at 10 mg/kg were 211 ng/g at the 2-hour timepoint and below the limit of quantitation at the 24-hour timepoint in the liver, and 526 ng/g at the 2-hour timepoint and 317 ng/g at the 24-hour timepoint in the kidney.

13.2.3. Toxicology

The potential carcinogenic toxicity of donidalorsen and mouse surrogate ISIS 722059 were evaluated in a 6-month study in Tg.rasH2 mice. A 2-year carcinogenicity study in rats is ongoing. At the time of the pre-NDA meeting, the Division agreed that it would be acceptable to

submit a summary of the in-life data for the 2-year carcinogenicity study in rats at the time of the NDA submission and to submit the final report as a PMR.

13.2.3.1. Carcinogenicity

A 6-Month Study of ISIS 721744 by Subcutaneous Bolus Injection in CByB6F1-Tg(HRAS)^{2Jic} Hemizygous Transgenic Mouse (Study Nos. 727-140; 721744-AS07)

Key Study Findings

- Tg.rasH2 mice (n=25/sex/group) were administered vehicle control (0.9% sodium chloride for injection), donidalorsen (5, 10, or 20 mg/kg/dose for males and 10, 20, or 60 mg/kg/dose for females), or mouse surrogate ISIS 722059 (10 mg/kg/dose) Q2W for up to 6 months by SC injection. A positive control group was included in which Tg.rasH2 mice (10/sex) were administered urethane (500 mg/kg/dose) via intraperitoneal injection on Days 1, 3, and 5. The mouse was not a pharmacologically relevant species with donidalorsen, and this study evaluated off-target effects. The mouse surrogate ISIS 722059 was included to assess inhibition of PKK and potential on-target effects.
- There was no statistically significant increase or decrease in mortality dose response across the vehicle control and donidalorsen-treated groups, in either male or female mice. The pairwise comparisons showed a statistically significant decrease in mortality in the male 5 mg/kg/dose group (low dose) compared to the vehicle control group (p=0.0160). The pairwise comparisons showed no statistically significant increase or decrease in mortality between the mouse surrogate ISIS 722059 group and the vehicle control group, in either male or female mice.
- There were no donidalorsen-related or mouse surrogate ISIS 722059-related effects on body weights.
- Effects on clinical chemistry included increases in AST, ALT, and glutamate dehydrogenase in males at 10 and 20 mg/kg/dose donidalorsen and females at 60 mg/kg/dose donidalorsen, which correlated with histopathology findings in the liver.
- A MTD was achieved at the high dose of 20 mg/kg Q2W in males based on histopathology findings in the liver and elevations of glutamate dehydrogenase. The high dose of 60 mg/kg Q2W in females did not meet the criteria for an MTD.
- Gross pathology findings included liver mass in one male at 5 mg/kg/dose donidalorsen and one male at 20 mg/kg/dose donidalorsen, which correlated with neoplastic histopathology findings of hepatocellular adenoma in the liver of both mice.
- Neoplastic histopathology findings included hepatocellular adenoma in the liver in 1/25 males at 5 mg/kg/dose donidalorsen, 2/25 males at 20 mg/kg/dose donidalorsen, and 1/25 males at 10 mg/kg/dose ISIS 722059. The hepatocellular adenoma seen in the males at 5 and 20 mg/kg/dose donidalorsen were accompanied by increases in AST and ALT, gross pathology findings in the liver, and non-neoplastic histopathology findings in the liver of microvesicular hepatocellular vacuolation, basophilic focus of cellular alteration, hepatocellular single cell necrosis, and/or basophilic pigmented macrophage. There were no

correlating gross pathology or non-neoplastic histopathology findings for the male at 10 mg/kg/dose ISIS 722059.

- Non-neoplastic histopathology findings were noted in the liver (microvesicular hepatocellular vacuolation, basophilic pigmented macrophages, hepatocellular single cell necrosis, karyocytomegaly, and/or basophilic focus of cellular alteration in males at 5, 10, and/or 20 mg/kg/dose donidalorsen, and/or females at 10, 20, and/or 60 mg/kg/dose donidalorsen, and/or males and/or females at 10 mg/kg/dose ISIS 722059); kidney (epithelial vacuolation in females at 60 mg/kg/dose donidalorsen, and epithelial basophilic granules in males at 20 mg/kg/dose donidalorsen and females at 20 and 60 mg/kg/dose donidalorsen); injection site (macrophage granular vacuolation in males and females at all donidalorsen doses, and in males and females at 10 mg/kg/dose ISIS 722059); urinary bladder (urothelial vacuolation in males at 10 and 20 mg/kg/dose donidalorsen and females at 20 and 60 mg/kg/dose donidalorsen); and the axillary and/or mesenteric lymph nodes (macrophage vacuolation in females at 60 mg/kg/dose donidalorsen). The histopathology findings were attributed to or a consequence of oligonucleotide uptake.
- The positive control (urethane) group confirmed the carcinogenic responsiveness of the Tg.rasH2 model. Urethane treatment resulted in a decrease in body weight in both male and female mice and a statistically significant increase in unscheduled deaths (i.e., decrease in survival) compared to the vehicle control group in both male and female mice. Neoplastic findings included hemangiosarcoma (whole body in male and female mice), malignant lymphoma (hemolymphoreticular tissue in male mice), and bronchioloalveolar adenoma and carcinoma (lungs in male and female mice). These findings are consistent with known effects of urethane administration ([National Toxicology Program 2021](#)).
- Toxicokinetic parameters were not evaluated.
- Donidalorsen concentrations in the kidney and liver of males and females were mostly increased with increasing donidalorsen dose following 6-months of treatment.
- Liver PKK mRNA expression was statistically reduced in male and female mice treated with 10 mg/kg/dose ISIS 722059 Q2W for 26 weeks by 94% and 79%, respectively, compared to controls.
- The ECAC concurred with the doses for the study in male mice but could not recommend doses for female mice. The Applicant did not conduct a new DRF study to identify an acceptable high dose or seek ECAC concurrence for their final dose selection in female mice. The ECAC concluded that the carcinogenicity study was adequate for male mice but inadequate for female mice because a MTD was not achieved and no other valid basis to support the high dose was discernable.

13.2.3.2. Reproductive and Developmental Toxicity

The potential reproductive and developmental toxicity of donidalorsen was evaluated in a GLP-compliant PPND study in mice with donidalorsen and mouse surrogate ISIS 722059.

Study for Toxic Effects of ISIS 721744 on Pre- and Postnatal Development, Including Maternal Function in Mice (Study Nos. 727-142; 721744-AS09)

Key Study Findings

- F0 female mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection), donidalorsen (5, 10, or 20 mg/kg/week), or mouse surrogate ISIS 722059 (5 mg/kg/week) every other day from GD 6 to GD 18 (GDs 6, 8, 10, 12, 14, 16, and 18) and weekly from Lactation Day (LD) 1 to LD 20 (LDs 1, 7, 13, and 20).
- There were no deaths in any of the F0 females attributed to treatment with donidalorsen or mouse surrogate ISIS 722059.
- AST (3× and 2× of control, respectively) and ALT (5× and 4× of control, respectively) were increased in F0 females on LD 22 at 10 and 20 mg/kg/week donidalorsen that was consistent with uptake into the liver.
- Mean donidalorsen concentrations in milk from F0 females was below the limit of quantitation for the vehicle control and 5 mg/kg/week donidalorsen groups, and 7.63 and 24.63 ng/mL at 10 and 20 mg/kg/week donidalorsen, respectively.
- There were no effects of donidalorsen or mouse surrogate ISIS 722059 on parturition parameters (gestation index, live newborn pups/litter, live birth index, uterine implantation sites, and gestation length).
- Kidney, liver, and/or spleen weights (absolute and/or relative to terminal body weight) on LD 22 were increased in F0 females treated with 5, 10, and/or 20 mg/kg/week donidalorsen. Increases of kidney and liver weights were consistent with uptake of donidalorsen.
- Unconjugated donidalorsen concentrations in the kidney and liver of F0 females on LD 22 increased with donidalorsen dose, which was consistent with uptake of donidalorsen.
- Treatment of F0 females with mouse surrogate 5 mg/kg/week ISIS 722059 statistically decreased liver mRNA expression of KLKB1/PKK by 82% on LD 22.
- There were no effects of donidalorsen or mouse surrogate ISIS 722059 on F1 pups (preweaning) based on evaluation of survival, clinical signs, body weights, sex ratios, physical development, and gross pathology.
- There were no effects of donidalorsen or mouse surrogate ISIS 722059 on the F1 generation based on evaluation of survival, clinical signs, sexual maturation, body weights, food consumption, behavioral evaluations (motor activity, Morris Water Maze, acoustic startle response), estrous cyclicity, mating/reproductive/fertility performance indices, GD 13 corpora lutea/uterine implantation data, organ weights, and gross pathology evaluations.
- The NOAEL for maternal toxicity and the PPND study was the high dose of 20 mg/kg/week donidalorsen.

13.2.3.3. Other Toxicity Studies

The potential toxicity of donidalorsen-related impurities was evaluated in a GLP-compliant 13-week repeat-dose SC toxicity study in mice with donidalorsen and associated test article mixtures (TAMs).

13.2.3.3.1. Impurities

In the 13-week repeat-dose SC toxicity and impurity qualification study, ICR mice were treated with vehicle control (0.9% sodium chloride for injection/solution [saline]) or (b) (4) mg/kg total oligonucleotide dose level of donidalorsen, TAM 1, or TAM 2 Q2W. The two TAMs were made up of donidalorsen and associated impurities (i.e., (b) (4) impurities for TAM 1 and (b) (4) impurity for TAM 2).

Key Study Findings

- There were no unscheduled deaths during the study.
- Red blood cell values were minimally decreased only in females at (b) (4) mg/kg/dose TAM 2.
- BUN values were decreased in males and females at (b) (4) mg/kg/dose TAM 2; AST values were increased only in males at (b) (4) mg/kg/dose donidalorsen, TAM 1, and TAM 2; ALT values were increased only in males at (b) (4) mg/kg/dose donidalorsen, TAM 1, and TAM 2; and triglycerides were decreased in females at (b) (4) mg/kg/dose donidalorsen and males at (b) (4) mg/kg/dose TAM 2.
- Liver weights (absolute and/or relative to body weight) were increased only in males at (b) (4) mg/kg/dose donidalorsen and TAM 1, and (b) (4) mg/kg/dose TAM 2.
- Histopathology findings attributed to treatment with donidalorsen, TAM 1, and/or TAM 2 were noted in the liver and injection site.
 - Liver: Minimal to slight mononuclear cell infiltration in males and/or females at (b) (4) mg/kg/dose donidalorsen, TAM 1, and/or TAM 2. Minimal karyocytomegaly in males only at (b) (4) mg/kg/dose donidalorsen, TAM 1, and TAM 2, which correlated with minimally increased ALT and AST and increased liver weight in males. Liver karyocytomegaly was attributed to uptake and accumulation of ASOs into the liver.
 - Injection Site: Minimal to slight vacuolated granular macrophages in males and females at (b) (4) mg/kg/dose donidalorsen, (b) (4) mg/kg/dose TAM 1, and (b) (4) mg/kg/dose TAM 2. Injection site findings were attributed to uptake and accumulation of ASOs into the macrophages.
- Mean concentrations of unconjugated donidalorsen in the mouse liver on Day 87 were comparable for donidalorsen-, TAM 1-, and TAM 2-treated groups.

In summary, the findings in TAM 1- and TAM 2-treated groups were similar to those in donidalorsen-treated groups, which included increases in AST and ALT, increases in liver weights, and histopathology findings of mononuclear cell infiltration and karyocytomegaly in the liver and vacuolated granular macrophages at the injection site. Overall, there were no

meaningful differences between the findings in either of the TAM groups (donidalorsen + associated impurities) compared to the donidalorsen group alone, and it did not appear that the added impurities affected the toxicity profile of donidalorsen.

[Table 34](#) shows the impurity qualification based on the 13-week repeat-dose toxicity study of donidalorsen and associated TAMs in mice.

Table 34. Impurity Qualification Based on 13-Week Repeat-Dose Toxicity Study of Donidalorsen and Associated TAMs in Mice

Impurity	Composition (%) ^a	
	Test Article 1	Test Article 2
(b) (4)		

Source: Excerpted from the Applicant's submission

Abbreviations: (b) (4); TAM; test article mixture

In addition, the pivotal GLP-compliant 39-week repeat-dose toxicity study of donidalorsen (0, 2, 6, 12, and 30 mg/kg/week) in *Cynomolgus* monkeys with a 13-week interim sacrifice and 26-week treatment-free recovery period was relied upon for impurity qualifications. The impurity levels seen in the toxicology batch at the NOAEL of 6 mg/kg/week provide support for the proposed impurity limits for the clinical batch. The safety margins for the donidalorsen specified oligonucleotide impurities based on comparing the proposed acceptance criterion at the proposed clinical dose to the impurity levels in the toxicology lot are shown in [Table 35](#) and [Table 36](#) based on body weight and body surface area, respectively.

Table 35. Safety Margins for Donidalorsen Specified Oligonucleotide Impurities Based on 39-Week Monkey Study Using Body Weight (mg/kg)

(b) (4) Impurity	Drug Substance Acceptance Criterion - Clinical	Maximum Accepted Impurity in 80 mg Q4W (2.7 mg/kg Q4W) Dawnzera ^a	Impurity Levels in Toxicology Lot # CA721744-001 ^b	Amount of Toxicology Lot Impurity at 6 mg/kg/Week NOAEL From 39-Week Monkey Study	Safety Margin ^c
					1.2x
					4.8x
					1.3x
					5.1x
					<0.1x
					<0.6x
					0.7x
					2.7x
					0.5x
					2.1x
					0.4x
					1.8x
					1.1x
					4.3x
					1.6x
					6.5x
					0.6x
					2.3x
					0.9x
					3.6x
					0.6x
					2.5x

Source: Table generated by reviewer

^a The recommended dosage of Dawnzera (donidalorsen) is SC administration of 80 mg Q4W or Q8W in adult and pediatric patients 12 years of age and older. For calculation purposes, the maximum clinical dose of 2.7 mg/kg Q4W is determined by dividing 80 mg by the average body weight of 29.5 kg for the adolescent patient population.

^b Donidalorsen toxicology lot # CA721744-001 was used in the 39-week monkey study.

^c In the 39-week monkey study, the monkeys were dosed more frequently (i.e., loading doses on Days 1, 5, 9, and 14, and once weekly thereafter) than is proposed in the clinical population (i.e., Q4W or Q8W). The top safety margin does not account for the difference in dosing frequency between the toxicity study and the proposed clinical population while the second safety margin accounts for the difference (about 4x).

^d Per the Applicant, the limit for (b) (4) is at the proposed qualification threshold of 1.5% so no toxicology assessment is required.

Abbreviations: (b) (4); ND, not determined; NMT, no more than; NOAEL, no observed adverse effect level; (b) (4) donidalorsen sodium; (b) (4) donidalorsen sodium; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous

Table 36. Safety Margins for Donidalorsen Specified Oligonucleotide Impurities Based on 39-Week Monkey Study Using Body Surface Area (mg/m²)

Oligonucleotide Impurity	Drug Substance Acceptance Criterion - Clinical	Maximum Accepted Impurity in 80 mg Q4W (2.7 mg/kg Q4W) Dawnzera ^a	Impurity Levels in Toxicology Lot # CA721744-001 ^b	Amount of Toxicology Lot Impurity at 1.94 mg/kg/Week HED NOAEL From 39-Week Monkey Study ^c	Safety Margin ^d
				(b) (4)	0.4x
					1.6x
					0.4x
					1.6x
					<0.05x
					<0.2x
					0.2x
					0.9x
					0.2x
					0.7x
					0.1x
					0.6x
					0.3x
					1.4x
					0.5x
					2.1x
					0.2x
					0.7x
					0.3x
					1.1x
					0.2x
					0.8x

Source: Table generated by reviewer

^a The recommended dosage of Dawnzera (donidalorsen) is SC administration of 80 mg Q4W or Q8W in adult and pediatric patients 12 years of age and older. For calculation purposes, the maximum clinical dose of 2.7 mg/kg Q4W is determined by dividing 80 mg by the average body weight of 29.5 kg for the adolescent patient population.

^b Donidalorsen toxicology lot # CA721744-001 was used in the 39-week monkey study.

^c The NOAEL of 6 mg/kg/week from the 39-week monkey study was divided by a scaling factor of 3.1 to convert based on body surface area (mg/m²) to a HED of 1.94 mg/kg/week.

^d In the 39-week monkey study, the monkeys were dosed more frequently (i.e., loading doses on Days 1, 5, 9, and 14, and once weekly thereafter) than is proposed in the clinical population (i.e., Q4W or Q8W). The top safety margin does not account for the difference in dosing frequency between the toxicity study and the proposed clinical population while the second safety margin accounts for the difference (about 4x).

^e Per the Applicant, the limit for (b) (4) is at the proposed qualification threshold of 1.5% so no toxicology assessment is required.

Abbreviations (b) (4) HED, human equivalent dose; ND, not determined; NMT, no more than; NOAEL, no observed adverse effect level; (b) (4)

(b) (4) donidalorsen sodium; (b) (4) donidalorsen sodium; Q4W, every 4 weeks; Q8W, every 8 weeks;

SC, subcutaneous

Based on [Capaldi et al. \(2017\)](#), one approach for evaluating toxicology coverage for impurities is to multiply the amount of impurity present in the drug substance by the NOAEL and divide the product by the clinical dose (i.e., 2.7 mg/kg Q4W [80 mg Q4W considering an average patient weight of 29.5 kg for the adolescent patient population]). Refer to [Table 37](#) for toxicology coverage based on this approach, which is considered acceptable.

Table 37. Impurity Qualification Based on a 39-Week Repeat-Dose Toxicity Study of Donidalorsen With a 13-Week Interim Necropsy in Monkeys

Oligonucleotide Impurity	721744-AS02	
	Amount in CA721744-001 (%)	Coverage (% of clinical dose) ^a
(b) (4)		

^a Coverage = Impurity level × NOAEL determined in tox study (24 mg/kg/month) / clinical dose (80 mg/month = 2.7 mg/kg/month); calculated values are rounded to the nearest 0.1%

Source: Excerpted from the Applicant's submission

Abbreviations (b) (4) NOAEL, no observed adverse effect level; (b) (4)
 (b) (4) donidalorsen sodium; (b) (4) donidalorsen sodium; tox, toxicity

Considering the impurity qualification based on the 13-week repeat-dose toxicity study and associated TAMs in mice and the 39-week toxicity study in monkeys, the proposed impurity specifications are acceptable, and there are no outstanding nonclinical safety concerns.

13.2.3.4. Extractables and Leachables

The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The drug product is packaged in a staked needle glass PFS closed with a (b) (4) rubber plunger stopper. The PFS is assembled into an AI for the final drug product presentation. The AI delivers a single dose of 80 mg donidalorsen in 0.8 mL (100 mg/mL). Extractables and leachables studies were conducted to assess the donidalorsen drug product container closure system, which were evaluated (see Nonclinical Primary Review dated July 25, 2025). There were no nonclinical safety concerns with the potential leachables from the primary container closure system of donidalorsen.

Safety Margins

The NOAELs from the nonclinical repeat-dose toxicity and reproductive and developmental toxicity studies provide adequate safety margins for the maximum recommended human dose of donidalorsen in adult and pediatric patients 12 years of age and older of 80 mg administered SC Q4W ([Table 38](#) and [Table 39](#)).

Table 38. Human Drug Exposure for Donidalorsen at MRHD of 80 mg Q4W

MRHD	C_{max,ss} (ng/mL)	AUC_{T,ss} (ng*h/mL)
80 mg donidalorsen Q4W SC	417	5240

Source: Table generated by reviewer

Abbreviations: AUC_{T,ss}, area under the concentration time curve over the dosing interval at steady state; C_{max,ss}, maximum plasma concentration at steady state; MRHD, maximum recommended human dose; Q4W, every 4 weeks; SC, subcutaneous

Table 39. Animal to Human Exposure Margins for Donidalorsen Based on Repeat-Dose and Reproductive and Developmental Toxicity Studies

Animal Study	Animal NOAEL	Animal C _{max} ^a	Animal AUC ^a	HED	C _{max} Margins ^{a,b,c}	AUC Margins ^{a,b,c}	BSA (mg/m ²) Margins ^d	Body Weight (mg/kg) Margins ^d	
Monkey 39-Week SC	6 mg/kg/week	6.57 µg/mL	37.6 µg*hr/m	1.94 mg/kg	16x	7x			
		or	or						
		6570 ng/mL (Day 273)	37600 ng*hr/mL (Day 273 AUC _{0-48hr})	116 mg adult				1.5x	4.6x
				58 mg 12-year-old				5.8x	18.5x
							0.7x	2.2x	
							2.9x	8.9x	
		-	-	0.98 mg/kg	-	-			
Mouse 26-Week SC	12 mg/kg Q2W			59 mg adult			0.7x	9.2x	
				29 mg 12-year-old			1.5x	18.5x	
							0.4x	4.4x	
							0.7x	8.9x	
Mouse Combined Male and Female Fertility and EFD SC	10 mg/kg/week			0.81 mg/kg	-	-			
				49 mg adult			0.6x	7.7x	
				24 mg 12-year-old			2.4x	30.8x	
							0.3x	3.7x	
							1.2x	14.8x	

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Dawnzera (donidalorsen)

Animal Study	Animal NOAEL	Animal C _{max} ^a	Animal AUC ^a	HED	C _{max} Margins ^{a,b,c}	AUC Margins ^{a,b,c}	BSA (mg/m ²) Margins ^d	Body Weight (mg/kg) Margins ^d
Mouse PPND		-	-	1.63 mg/kg	-	-		
Maternal; Offspring Development SC	20 mg/kg/week			98 mg adult			1.2x 4.9x	15.4x 61.5x
			49 mg 12-year-old				0.6x 2.4x	7.4x 29.6x

Source: Table generated by reviewer

^a Mouse studies did not have toxicokinetic data, therefore exposure margins for C_{max} and AUC were not calculated.

^b Following clinical dosing of 80 mg donidalorsen Q4W, geometric mean for C_{max,ss} (maximum plasma concentration at steady state) was 417 ng/mL and AUC_{t,ss} (area under the plasma concentration time curve over the dosing interval at steady state) was 5240 ng*hr/mL.

^c The margins between the animal and clinical exposures were not adjusted to account for differences between the dosing frequencies to consider the PK/TK of donidalorsen (i.e., fast clearance from plasma and distribution to tissues).

^d The BSA and body weight margins are calculated based on the maximum recommended dosage of donidalorsen of 80 mg (i.e., 1.3 mg/kg for a 60 kg adult and 2.7 mg/kg for a 30 kg 12-year-old) Q4W. The margins shown are nonadjusted and adjusted margins, respectively (i.e., do not account for and do account for, respectively, the more frequent dosing frequency in the animal studies compared to the recommended clinical dose).

Abbreviations: AUC, area under the concentration-time curve; BSA, body surface area; C_{max}, maximum plasma concentration; EFD, embryo-fetal development; HED, human equivalent dose; NOAEL, no observed adverse effect level; PK, pharmacokinetic; PPND, pre- and postnatal development; Q2W, every 2 weeks; Q4W, every 4 weeks; SC, subcutaneous; TK, toxicokinetic

14. Clinical Pharmacology

14.1. In Vitro Studies

14.1.1. Metabolism and Excretion of the Antisense Oligonucleotide Moiety of Donidalorsen (Studies ISIS 721744-IS10 and ISIS 721744-APK02)

Donidalorsen contains a 2'-O-methoxyethyl (MOE)-modified chimeric gapmer ASO with a mixed backbone of phosphodiester (PS) and phosphorothioate (PO) internucleotide linkages. These have been used in previously approved GalNAc-ASO products, such as Eplontersen (NDA 217388), Nusinersen (NDA 209531), Tegsedi (NDA 211172), and Tofersen (NDA 215887). The ASO moiety of donidalorsen is expected to share the same metabolic pathway as other approved GalNAc-conjugated ASO moieties with similar modifications in the oligonucleotide backbone. Therefore, the ASO moiety is majorly metabolized by endo- and exonucleases in tissues to shortmer oligonucleotide metabolites and minimally modulated by major cytochrome P450 (CYP) enzymes ([AstraZeneca 2023](#)).

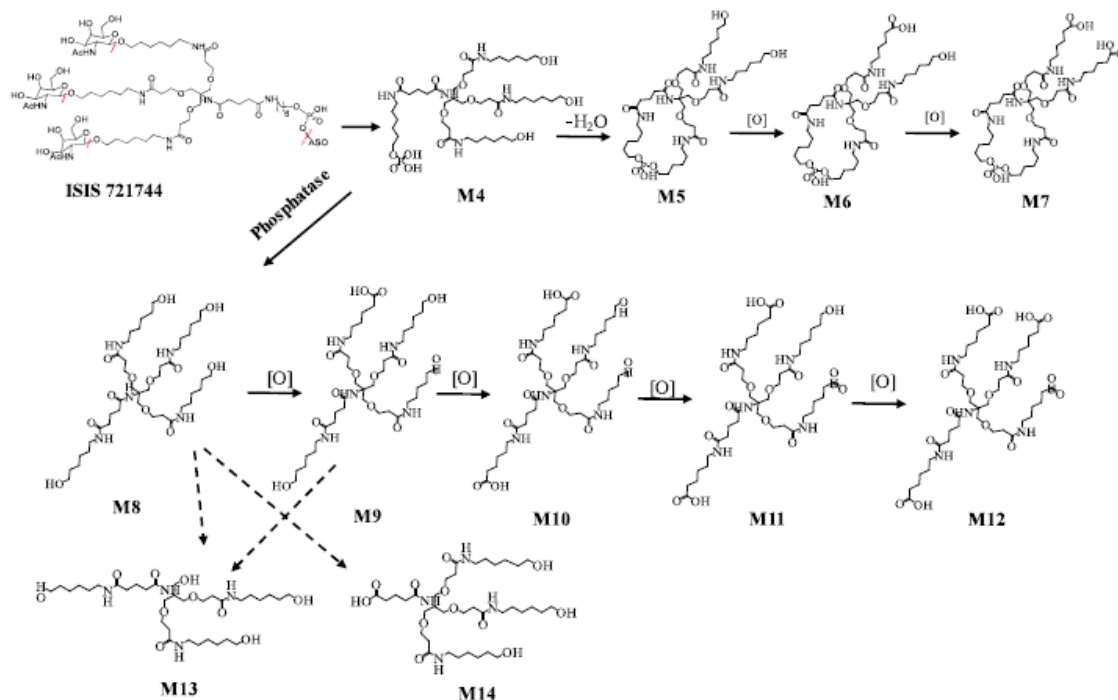
The metabolic profile of ASO related metabolites was evaluated in human plasma and urine samples collected following four doses of donidalorsen-4 in Trial ISIS721744-CS1 (see Section [14.2](#) for more details). Shortmer oligonucleotide metabolites were not detected in plasma 2 hours postdose. Total full length donidalorsen (fully conjugated + partially conjugated + unconjugated) in urine represents <1% of administered dose collected within 24 hours after the fourth dose administered every 4 weeks. The metabolic profiling results for human urine showed that various shortmer metabolites together constitute more than 50% of all drug-related species detected in urine, while the full-length unconjugated donidalorsen constitutes about 38% of all drug-related species. Although shortmer metabolites appear to have a slightly higher relative abundance compared to full length donidalorsen in urine based on the metabolic profiling results, the percent of the dose excreted as shortmer metabolites in urine is not likely to exceed 30% of the dose. This is primarily as full-length donidalorsen represented <1% of the dose in human urine while constituting 38% of all drug-related species detected in urine over 24 hours postdose. Overall, the fragmented ASO metabolites were slowly formed and were subsequently excreted in the urine. The renal elimination of ASO and its related metabolites is considered a minor route.

14.1.2. Metabolism and Excretion of Linker and Its Related Metabolites (Studies ISIS 721744-IS12 and ISIS 721744-IS16)

The GalNAc moiety is covalently conjugated to the ASO portion of donidalorsen via a THA linker. The GalNAc conjugate undergoes hydrolysis once distributed to the tissue and is detached from the parent drug. The GalNAc sugar undergoes endogenous carbohydrate catabolism, while the THA linker undergoes extensive oxidative metabolism based on a study in animals (monkey

and rats) (Shemesh et al. 2016). The proposed metabolic pathway for the GalNAc-linker moiety is shown in Figure 41.

Figure 41. Proposed Metabolic Pathway for Linker Portion of Donidalorsen



Source: Report 721744-IS12

The metabolic profile of the linker portion was assessed in plasma, urine, and feces samples across species (monkey, mice, and human). In total, 11 GalNAc-linker-related metabolites were detected from various specimens (i.e., urine, plasma, and feces) across species and the metabolic profiles were generally consistent across species. Among these metabolites, metabolite M4, the THA linker, is formed by hydrolysis of the GalNAc sugars and hydrolysis from the ASO moiety. Metabolite M8 is formed by hydrolysis of the phosphate moiety from the linker M4. M8 can be further metabolized by oxidation to form other metabolites (M9 to M12).

Of the 11 linker-related metabolites, the 3 metabolites with the highest peak area ratio in human plasma and urine were M5, M8, and M12. Concentrations of major THA-related metabolites M8 and M12 were quantified in human plasma and urine samples collected following four doses of donidalorsen-4 from Trial ISIS721744-CS1. The concentrations of THA-related metabolites were assessed in human plasma at hours 2 and 24, as well as in human urine over 0 to 24 hours. Data are summarized in Table 40.

Table 40. Median (Range) Concentration (nM) of M8 and M12 in Human Plasma and Urine

Sample	Trial Day	Timepoint	M8 (nM)	M12 (nM)
Plasma (N=6)	85	2 h	1.16 (0.812, 2.46)	0.980 (0.771, 2.13)
		24 h	0.529 (0, 0.604)	1.15 (0, 1.73)
Urine (N=14)	85	0-24 h	301 (220, 402)	97.3 (81.0, 159)

Source: Adapted from Table 1 and 5, Report 721744-IS12

Abbreviations: h, hour; M, metabolite; N, number

In human plasma, mean concentrations of M8 and M12 were <2% of those determined for parent drug. In human urine collected over 24 hours, ≥ 100 -fold higher concentrations of M8 and M12 were observed compared to their plasma concentrations (Table 40). However, the amount of M8 and M12 excreted in the urine over 24 hours only accounted for 6.4% and 2.2% of the total administered dose, respectively.

In vitro cytochrome P450 phenotyping for linker metabolite M8 was conducted in human liver microsomes and recombinant CYP enzymes (rCYP1A2, 2B6, 2C8, 2C9, 2D6, 3A4). After 60 minutes incubation with M8 at concentrations of 1 μ M and 3 μ M in human liver microsomes, there was little NADPH-dependent consumption of M8. Following incubation with recombinant CYP enzymes, M8 remained stable with most recombinant enzymes except rCYP3A4, where an approximate 30% reduction in M8 was observed after 60 minutes. In a repeat study with rCYP3A4 using 10 μ M M8, oxidative metabolites were detected with a low signal to noise ratio. The study suggests that CYP3A4 may contribute to the metabolism of M8. However, M8 is not considered a major metabolite as M8 demonstrated a low abundance in human plasma (1.3% relative to parent drug) and urine (6.4% of administered dose).

14.1.3. Plasma Protein Binding and Distribution (Studies ISIS 721744-IS03, ISIS 721744-IS05, and 721744-IS11)

The extent of plasma protein binding of donidalorsen was evaluated at three nominal concentrations of 0.5, 5, and 150 μ g/mL in human plasma. The degree of donidalorsen bound to plasma proteins was similar across all concentrations and was $\geq 98\%$.

The potential interaction of donidalorsen with warfarin and ibuprofen due to plasma protein binding was assessed in vitro using an ultrafiltration method. The plasma protein binding of donidalorsen at 1 μ g/mL was similar irrespective of the presence of warfarin (30 μ g/mL) and ibuprofen (300 μ g/mL), while the plasma protein binding of warfarin at 3 μ g/mL or ibuprofen at 30 μ g/mL was also similar irrespective of the presence of donidalorsen (10 μ g/mL).

The blood-to-plasma ratio was assessed at six concentrations (0, 0.01, 0.03, 0.1, 0.3, 1, and 2 μ g/mL) in human whole blood incubated over approximately 1 hour. At all evaluated concentrations, the blood-to-plasma distribution ratio of donidalorsen was between 0.63 to 0.82, suggesting that donidalorsen mostly distributes in the plasma compartment.

14.1.4. Drug-Drug Interaction Potential for Donidalorsen (Studies ISIS 721744-IS06, ISIS 721744-IS07, and ISIS 721744-IS09)

An in vitro study was conducted with cryopreserved human primary hepatocytes to assess the potential of donidalorsen to induce CYP1A2, CYP2B6, and CYP3A4. The study demonstrated that donidalorsen did not cause induction of mRNA of these CYP enzymes in all three donors up to 30 μ M after 48 hours of incubation. The concentration of 30 μ M is around 43-fold higher than

peak donidalorsen plasma concentration (around 0.7 μ M) and is also higher than the Applicant's estimated liver concentration of donidalorsen (<10 μ M) following administration of 80 mg Q4W.

An in vitro study was conducted with cryopreserved human primary hepatocytes to evaluate cellular uptake of donidalorsen and the inhibition potential of donidalorsen on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. The uptake of donidalorsen into hepatocytes at 1, 10, and 50 μ M were up to 4.73 μ M, 12.3 μ M, and 43.3 μ M at 120 minutes, suggesting high cellular uptake during the 120-minute incubation period. The inhibition study results indicated that donidalorsen did not inhibit these CYP enzymes in human hepatocytes at concentrations up to 50 μ M.

An in vitro study was conducted with transporter-transfected cells (Madin-Darby Canine Kidney [MDCK-II]) compared to control cells for solute carrier family and adenosine triphosphate binding cassette transporters or BSEP vesicles made from *Spodoptera frugiperda* 9 membrane, to assess the potential of donidalorsen as an inhibitor or a substrate of major transporters. At a concentration of 100 μ M, donidalorsen is not considered as an inhibitor of human organic anion transporter (OAT) 1, OAT3, organic cation transporter (OCT) 1, OCT2, organic anion transporting polypeptide (OATP) 1B1, OATP1B3, MATE1, MATE2-K, Breast Cancer Resistance Protein (BCRP), P-glycoprotein (P-gp), and BSEP transporters. Donidalorsen is also not a substrate for OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, and BSEP under the study conditions.

When assessing donidalorsen (10 μ M) as a substrate of P-gp and BCRP, the concentration of donidalorsen in all the receiver wells for apical to basolateral flux (absorption) were below lower limit of quantification (LLOQ = 1nM). Some of the receiver wells for basolateral to apical flux (efflux) were below LLOQ, while some had concentrations slightly above the LLOQ ranging from 1.1 to 2.7nM. The efflux ratio in BCRP or P-gp transfected MDCK-II cells cannot be determined. The results in control cells (without BCRP or P-gp) were also similar. The potential of donidalorsen as a substrate of BCRP and P-gp is low.

14.1.5. Drug-Drug Interaction Potential for Linker Related Metabolite M8 (Studies ISIS 721744-IS13, ISIS 721744-IS14, and ISIS 721744-IS15)

An in vitro study was conducted with human hepatic microsomal CYP enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and CYP3A4/5) to assess the potential of major linker-related inactive metabolite M8 to act as an inhibitor of human CYP enzymes. Detectable inhibition was only observed for CYP2B6, 2C9, 2C19, and CYP3A4 (with midazolam or testosterone as probe substrates) at concentrations up to 100 μ M. In all cases, IC₅₀ values were >100 μ M. No shift in IC₅₀ was noted when enzymes were pre-incubated with M8 in the absence or presence of NADPH.

An in vitro study was conducted in cryopreserved human hepatocytes to assess the induction potential of M8 on CYP1A2, CYP2B6, and CYP3A4 following 48 hours incubation. M8 did not induce CYP1A2, CYP2B6, or CYP3A4 mRNA at concentrations up to 100 μ M across three donors.

An in vitro study was conducted to assess M8 as an inhibitor or a substrate of human OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, BCRP, P-gp, and BSEP. The assay used MDCK-II cells expressing the transporter of interest or a control vector, except for the BSEP assay, which used *Spodoptera frugiperda* 9 membrane vesicles expressing human BSEP.

M8 was not found to be a substrate for OAT1, OAT3, OCT1, OCT2, OATP1B1, MATE1, MATE2-K, BCRP, and P-gp under the study conditions. However, M8 is a substrate for OATP1B3 and BSEP with >2-fold of uptake of M8 observed. As M8 is low in abundance in systemic circulation (1.3% relative to parent drug), the inhibition of BSEP or OATP1B3 is not likely to result in clinically meaningful changes in M8 systemic concentrations. However, the impact on M8 concentrations in the liver is unclear.

At an M8 concentration of 100 μ M (about 300-fold higher than concentrations observed in human urine collected over 24 hours post dose [mean concentration 299nM]), 62.9% inhibition was observed for MATE1 (IC₅₀ of 62.7 μ M). For the remaining transporters evaluated, the mean percent inhibition ranged from 6.30% (OAT3) to 46.1% (BSEP). Therefore, M8 is not considered as an inhibitor of OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE2-K, BCRP, P-gp, and BSEP at 100 μ M. As M8 demonstrates a low abundance in human plasma and is minorly excreted in urine (6.4% of total dose over 24 hours), the potential of M8 to inhibit MATE1 in vivo is low.

14.2. In Vivo Studies

Donidalorsen was evaluated in four completed clinical trials (CS1, CS2, CS5, and CS9) and two ongoing clinical trials (CS3 and CS7). A summary of clinical trials for the donidalorsen clinical pharmacology assessment is presented in [Table 41](#).

Table 41. Complete and Ongoing Clinical Trials for Donidalorsen Clinical Pharmacology Assessment

Study Number	Study Design	Study Population	Dosing Regimen, Route, and Duration of Administration	Total Number of Subjects
ISIS 721744-CS1	Phase 1, single site, randomized, double-blind, placebo-controlled, multiple-dose, and dose-escalation study	Healthy participants	Cohorts A, B, C, and D received individual SC doses of donidalorsen 20, 40, 60, and 80 mg, respectively. Participants received SC doses of study drug (donidalorsen or placebo) once every 4 weeks for a total of 12 weeks (4 total doses).	n = 32 (8 participants per cohort, randomized 3:1 donidalorsen: placebo)
ISIS 721744-CS2	Phase 2a study conducted in 2 parts Part A: randomized, double-blind, placebo-controlled; Part B: open-label	Part A: Patients with HAE-1 or HAE-2 Part B: Patients with nC1-INH-HAE (formerly Type III)	Part A and Part B: Patients received SC dose of donidalorsen 80 mg once every 4 weeks during the 12-week Treatment Period (on Day 1, 29, 57, and 85).	Part A: n = 20 donidalorsen = 14 placebo = 6 Part B: n = 3 PK subgroup: n = 4 (Exploratory Objective described in Section 2.2.2)
ISIS 721744-CS3 Date of cutoff: 26 Feb 2024	Phase 2 multicenter, OLE study of ISIS 721744-CS2	Patients with HAE who had rolled over from ISIS 721744-CS2	Patients received SC dose of donidalorsen 80 mg once every 4 weeks or every 8 weeks if attack-free for ≥ 12 weeks, or donidalorsen 100 mg SC dose every 4 weeks (if not attack-free at the start of OLE) for at least 3 months.	n = 20 (17 patients with HAE-1/HAE-2 [5 of which had received placebo in the Index study] and 3 patients with nC1-INH-HAE [formerly Type III])
ISIS 721744-CS5	Phase 3, multicenter, randomized, double-blind, placebo-controlled study	Patients with HAE-1 or HAE-2	Patients received SC dose of donidalorsen 80 mg or placebo once every 4 weeks (Cohort A) or once every 8 weeks (Cohort B) for a total of 24 weeks.	n = 91 total Placebo = 22 Cohort A (donidalorsen 80 mg once every 4 weeks): n = 61 Cohort B (donidalorsen 80 mg once every 8 weeks): n = 30

ISIS 721744-CS7 Date of cutoff: 28 Feb 2024	Phase 3, multicenter, OLE study who rolled over from ISIS 721744-CS5	Patients with HAE-1 or HAE-2 who rolled over from ISIS 721744-CS5 (OLE Patients) or who were previously maintained on HAE prophylactic therapy (Switch Patients)	OLE Patients: Patients who rolled over from Cohort A in ISIS 721744-CS5 received SC dose of donidalorsen 80 mg every 4 weeks beginning 28 ± 3 days after the last dose in the Index study. Patients who rolled over from Cohort B in ISIS 721744-CS5 received SC dose of donidalorsen 80 mg every 8 weeks beginning 56 ± 3 days after the last dose in the Index study unless they were not attack-free for ≥ 8 weeks (in which case, patients received donidalorsen 80 mg every 4 weeks). Switch Patients: patients received SC dose of donidalorsen 80 mg every 4 weeks.	OLE Patients: n = 83 (69 patients from the Index/OLE donidalorsen-4 80 mg group [contained the Index donidalorsen-4, Index donidalorsen-8 and the Index pooled placebo groups] and 14 patients in the Index/OLE donidalorsen-8 80 mg group) Switch Patients: n = 64 (31 patients lanadelumab, 11 patients berotralstat, and 22 patients C1-esterase inhibitor)
ISIS 721744-CS9	Phase 1, single dose, single-site, randomized, open-label, 2-period, 2-sequence, 2-treatment crossover, BE study	Healthy participants	Sequence 1 (Treatment Period 1: Autoinjector and Treatment Period 2: vial): participants received SC dose of donidalorsen 80 mg once at the beginning of the Treatment Period in the assigned dose sequence. Sequence 2 (Treatment Period 1: vial and Treatment Period 2: autoinjector): participants received SC dose donidalorsen 80 mg once at the beginning of the Treatment Period in the assigned sequence.	n = 78 participants randomized in a 1:1 ratio to Sequence 1 and Sequence 2

Source: 2.7.2 Summary of Clinical Pharmacology Studies, Table 3

Trials ISIS 721744-CS1, ISIS 721744-CS2, ISIS 721744-CS3, ISIS 721744-CS5, ISIS 721744-CS7, and ISIS 721744-CS9 are abbreviated as CS1, CS2, CS3, CS5, CS7, and CS9 in this review.

Abbreviations: BE, bioequivalence; donidalorsen-4, treatment group referring to those subjects who received donidalorsen 80 mg every 4 weeks; donidalorsen-8, treatment group referring to those subjects who received donidalorsen 80 mg every 8 weeks; HAE, hereditary angioedema; HAE-1, hereditary angioedema type I; HAE-2, hereditary angioedema type II; ISIS 721744, donidalorsen; n, number of subjects; nC1-INH-HAE (Type III), hereditary angioedema formerly type III; OLE, open-label extension; PK, pharmacokinetic; SC, subcutaneous

14.2.1. Trial CS1

Trial Design

CS1 was a phase 1, double-blind, randomized, placebo-controlled, dose-escalation trial conducted in healthy adult subjects. The objective of the trial was to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of donidalorsen following multiple doses of donidalorsen administered subcutaneously. Eight subjects (six active and two placebo) each were randomized to the following four cohorts:

- Cohort A: 20 mg SC every 4 weeks for 12 weeks (total: 4 doses)
- Cohort B: 40 mg SC every 4 weeks for 12 weeks (total: 4 doses)
- Cohort C: 60 mg SC every 4 weeks for 12 weeks (total: 4 doses)
- Cohort D: 80 mg SC every 4 weeks for 12 weeks (total: 4 doses)

SC doses were administered using a 100 mg/mL solution in a single use vial. Doses were administered on alternating sites (arm for first and third dose, and abdomen for second and fourth dose). One subject ((b) (6)) received the second dose in abdomen and third dose in thigh.

PK, PD, and ADA Sampling Time

Blood samples for assessing plasma donidalorsen concentration was collected at Day 1 (pre-first dose and 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12-hour postdose), Day 2 (24-hour post-first dose), Day 3 (48-hour postdose), Day 8 (anytime), and Day 15 (anytime) as well as at Day 85 (pre-fourth dose and 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12-hour postdose), and Day 86 (24-hour post-fourth dose).

Urine samples for assessing donidalorsen concentrations were collected over 24 hours after the first (Day 1) and fourth dose (Day 85).

Blood samples for assessing plasma PKK samples were collected at Screening, Day 1 (predose), Day 15, Day 29 (predose), Day 43, Day 57 (predose), Day 71, Day 85 (predose), Day 92, Day 99, Day 141, and Day 176.

Blood samples for assessing ADAs were collected at Day 1 (predose), Day 15, Day 29 (predose), Day 92, and Day 176.

PK Results

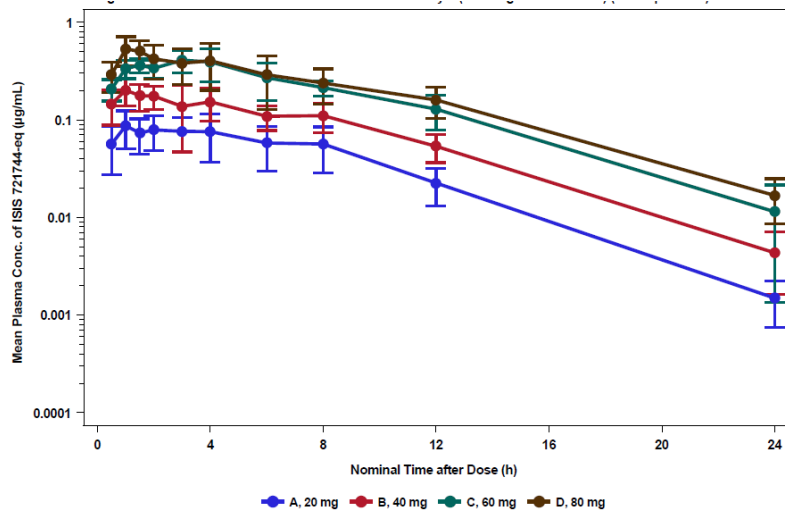
A total of 32 subjects were randomized and completed the trial. The mean (SD) age was 56 (9) years. The mean (SD) body weight was 80.6 (12.8) kg. The mean body weight is consistent across treatment groups.

The plasma donidalorsen concentrations were analyzed based on full-length ASO concentration, which included fully conjugated, partially conjugated (i.e., donidalorsen with 1, 2, or 3 GalNAc sugar deletions), and unconjugated donidalorsen. See Section [14.3](#) for details about the bioanalytical assay.

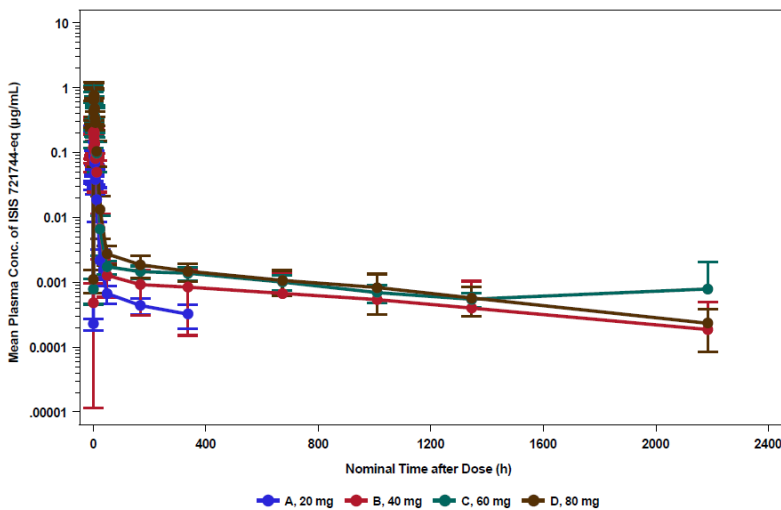
PK profiles and a summary of PK parameters following both single dose (Day 1) and multiple doses (Day 85) are shown in [Figure 42](#) and [Table 42](#). The plasma PK profiles were similar between Day 1 and Day 85 and minimal accumulation was observed in mean plasma C_{max} and AUC_{0-24h} following four doses of donidalorsen. A long terminal half-life was noted. However, the plasma concentration at terminal phase was 1000-fold lower than C_{max} . The effective half-life of donidalorsen in plasma, reflecting tissue distribution, is expected to be much shorter than the terminal half-life.

Figure 42. Mean (SD) Plasma Concentration of Donidalorsen Over Time by Dose on Day 1 (Post-First Dose) and Day 85 (Post-Fourth Dose)

A) Day 1 (Post-First Dose)



B) Day 85 (Post-Fourth Dose)



Source: Figure 14.2.2.2-2 and Figure 14.2.2.2-6, CSR CS1

Abbreviations: Conc., concentration; CSR, clinical study report; h, hour; SD, standard deviation

Table 42. Summary of Geometric Mean (CV%) Plasma Donidalorsen PK Parameters

Cohort	Dose (mg)	Profile Day	N	C _{max} (µg/mL)	T _{max} (h)	AUC _{0-24h} (µg·h/mL)	AUC _τ (µg·h/mL)	CL _{ss} /F (L/h)	Vz/F (L)	t _{1/2z} ^a (day)
A	20	1	6	0.0845 (58.4)	1.52 (1.00, 4.00)	0.685 (66.5)	NC	NC	NC	NC
		85	6	0.124 (60.0)	1.00 (1.00, 2.00)	0.757 (24.8)	0.975 (23.5)	20.5 (23.5)	NC	NC
B	40	1	6	0.200 (36.7)	1.02 (1.00, 2.00)	1.60 (28.3)	NC	NC	NC	NC
		85	6	0.196 (71.8)	1.75 (1.00, 6.00)	1.50 (36.1)	2.04 (42.0)	19.6 (42.0)	22088 (24.7) ^b	36.9 (37.4) ^b
C	60	1	6	0.449 (22.2)	3.03 (1.00, 4.00)	3.58 (23.5)	NC	NC	NC	NC
		85	6	0.693 (56.1)	2.50 (0.500, 8.00)	4.61 (34.0)	5.50 (30.7)	10.9 (30.7)	13042 (15.7) ^c	30.6 (11.8) ^c
D	80	1	6	0.592 (38.8)	1.13 (1.00, 4.00)	4.22 (19.7)	NC	NC	NC	NC
		85	6	0.718 (57.2)	2.50 (1.50, 6.00)	4.91 (25.9)	6.03 (24.6)	13.3 (24.6)	13353 (30.7)	29.1 (15.2)

Note: Data presented are geometric mean (geometric %CV) except T_{max}, which is presented as median (minimum, maximum).

N = number of subjects; NC = not calculated

τ = 672 hr

a t_{1/2z} was not reported or calculated for subjects with adjusted r-squared < 0.8, or T_{last} < 670 hr or if less than 3 time points were quantifiable in the elimination phase.

b N = 5

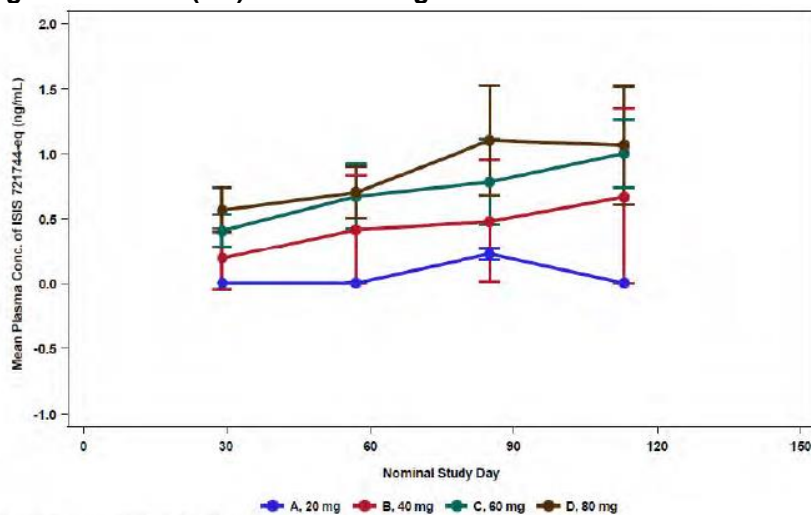
c N = 4

Source: Table 10, CSR CS1

Abbreviations: AUC_{0-24h}, area under the concentration-time curve from 0 to 24 hours; AUC_τ, area under the plasma concentration-time curve during a dosage interval (τ); CL_{ss}/F, apparent total body clearance after oral administration (at steady state); C_{max}, maximum plasma concentration; CSR, clinical study report; CV, coefficient of variation; PK, pharmacokinetic; τ, dosage interval; t_{1/2z}, terminal half-life; T_{last}, time to last measurable concentration; T_{max}, time to maximum concentration; Vz/F, apparent volume of distribution

The trough concentrations during the Treatment Period following multiple doses are shown in [Figure 43](#). Following administration of donidalorsen-4, steady state was reached by Day 85 (after the fourth dose). The mean C_{trough} on Day 85 was 1.1 ng/mL, which is approximately 2-fold higher than that on Day 29 (0.6 ng/mL).

Figure 43. Mean (SD) Plasma Trough Concentrations of Donidalorsen Over Time



Error bars represent standard deviation (N=6).

Source: Figure 4, CSR CS1

ISIS 721744eq = donidalorsen.

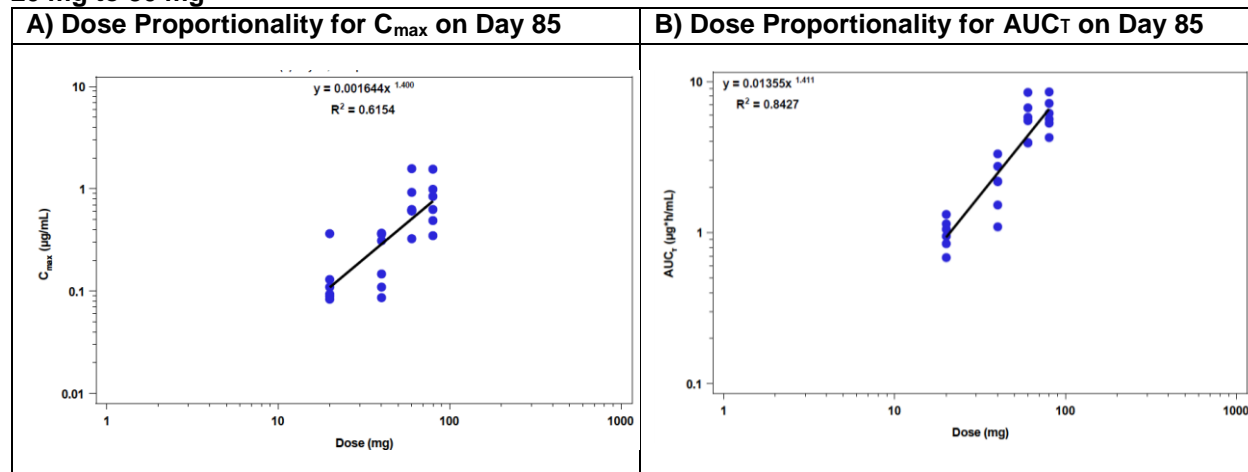
Abbreviations: Conc., concentration; CSR, clinical study report; N, number; SD, standard deviation

Dose Proportionality

Based on the noncompartmental analysis, the plasma exposure (C_{max} or AUC_{tau}) on Day 85 increased dose proportionally between 20 and 40 mg, more than dose proportionally between

40 mg and 60 mg, and less than dose proportionally between 60 mg and 80 mg. Overall, the AUC_{tau} on Day 85 was more than dose-proportional over the range of 20 to 80 mg. However, the dose proportionality for C_{max} is inconclusive given high variability ([Figure 44](#)).

Figure 44. Analyses of Dose Proportionality Using Log-Transformed Data at Doses Ranging From 20 mg to 80 mg



Source: Figure 14.2.2.8 and Figure 14.2.2.10, CSR CS1

Abbreviations: AUC_{τ} , area under the plasma concentration-time curve during a dosage interval; C_{max} , maximum plasma concentration; CSR, clinical study report

Metabolites in Plasma

In 2-hour postdose samples collected on Day 85, the most abundant oligonucleotide (>90%) in human plasma was the intact donidalorsen (full length ASO with a THA-GalNAc cluster) ([Table 43](#)). No oligonucleotide chain shortened metabolite was identified in the plasma after 2 hours postdose, which roughly corresponds to the time of peak donidalorsen concentration.

Table 43. Metabolite Profile in Human Plasma Pre- and Two-Hour Postdose on Day 85 for Donidalorsen 80 mg

Cohort	Dose	Study Day	Time Point	Analyte	N	Analyte Conc. (nM)	Percent Abundance (%)
D	80 mg	85	Pre-dose	ISIS 721744	6	BLQ	NC
				ISIS 721744-1GalNAc	6	BLQ	NC
				ISIS 721744-2GalNAc	6	BLQ	NC
				ISIS-721744-3GalNAc	6	BLQ	NC
				Unconjugated ISIS-721744	6	BLQ	NC
				Total Full-Length	6	BLQ	NC
			2 hr	ISIS 721744	6	70 ± 49	94.5 ± 2.01
				ISIS 721744-1GalNAc	6	2.44 ± 0.941	3.70 ± 0.79
				ISIS 721744-2GalNAc	6 ^a	1.13 ± 0.733	1.18 ± 1.05
				ISIS-721744-3GalNAc	6 ^b	0.375 ± 0.585	0.585 ± 0.948
				Unconjugated ISIS-721744	6	BLQ	NA
				Total Full-Length	6	74.1 ± 45.8	100 ± 0

BLQ = below the lower limit of quantitation

LLOQ = 1.00 nM

N = number of samples

NA = not applicable

NC = not calculated

Data presented are mean ± standard deviation

^{a,b} 2 and 4 samples, respectively, BLQ and were set to 0 for descriptive statistics

Source: Table 11, CSR CS1

Abbreviations: CSR, clinical study report; GalNAc, N-acetylgalactosamine; hr, hour

Urine Excretion

Urinary excretion of total full-length ASO within 24 hours represented a small fraction (<1%) of the administered dose and was dose-dependent. Urinary excretion after multiple doses was higher than after a single dose but still represents a small fraction of the administered dose ([Table 44](#)).

Table 44. Summary of Urine Excretion Following Single and Multiple Doses of Donidalorsen in Healthy Subjects

Cohort	Dose (mg)	Study Day	N	Ae _{0-24h} (µg)	%Dose Excreted (%)	CL _r ^a (mL/h)
A	20	1	4 ^{b,c}	9.71 (40.3)	0.0486 (40.3)	11.9 (39.6)
		85	5 ^c	22.4 (62.3)	0.112 (62.3)	31.5 (42.3)
B	40	1	6	10.7 (144)	0.0267 (144)	6.65 (136)
		85	5 ^c	32.7 (94.7)	0.0818 (94.7)	22.8 (110)
C	60	1	3 ^c	20.6 (147)	0.0344 (147)	5.13 (145)
		85	5 ^c	80.9 (40.3)	0.135 (40.3)	16.4 (34.8)
D	80	1	5 ^c	52.0 (292)	0.0650 (292)	12.3 (305)
		85	5 ^c	138 (64.9)	0.172 (64.9)	27.9 (56.6)

Ae_{0-24h} = the amount excreted in the urine following a 0 to 24-hour collection; BLQ = below the lower limit of quantification (LLOQ), where LLOQ = 1.301 ng/mL (or 0.001301 µg/mL); CL_r = renal clearance (over a 24-hour period); N = number of subjects

Note: Data presented are geometric mean (geometric %CV)

^a CL_r is calculated by Ae_{0-24h}/AUC_{0-24h}

^b Urine concentration for 1 subject in Cohort A (b) (6) was BLQ (with measurable ISIS 721744 plasma concentrations) thus all parameters were calculated to be “0” and were excluded for the purpose of calculating descriptive statistics.

^c Duration of urine collection was less than 18 hours for 1 subject in Cohort A (b) (6), 1 subject in Cohort B (b) (6), 3 subjects in Cohort C (b) (6) on Day 1 and only (b) (6) on Day 85) and 2 subjects in Cohort D (b) (6) on Day 1 and (b) (6) on Day 85) and were excluded for the purpose of calculating descriptive statistics.

Source: Table 12, clinical study report CS1

Note that the long-term stability at –80 °C of donidalorsen in urine samples was evaluated for up to 23 days, which may not be sufficient to support the stability of the storage duration for urine samples in this trial (urine samples were analyzed between November 19, 2018, and January 8, 2019, which was in a 60-day span). The data should be interpreted with caution. See Section 14.3 for details. Despite this, the urinary excretion results for donidalorsen were consistent with other GalNAc conjugated antisense oligonucleotide products that shared the same linker (i.e., eplontersen), which also demonstrated minimal excretion (<1% of administered dose) of total full-length antisense oligonucleotide in urine over 24 hours following both single and multiple doses. Notably, in the case of eplontersen, urine sample stability was adequately supported.¹⁰

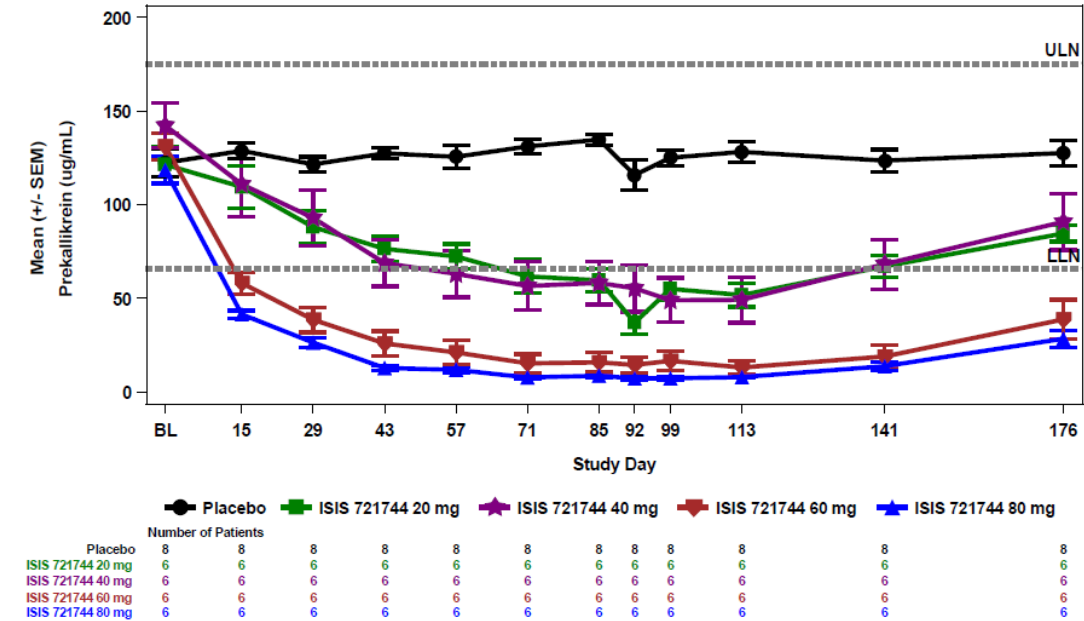
PD Results

The absolute PKK concentration and mean percent change in PKK from baseline in healthy subjects are shown in Figure 45 and Figure 46, respectively. The dose response in PKK reduction was observed starting 15 days after the first dose, with a greater percent reduction from baseline observed at higher doses. The nadir for PKK reduction was generally reached between Day 71 and Day 99 for all doses. The mean percent reduction at Day 99 was –52.9% with 20 mg,

¹⁰ See FDA Summary Review for Wania (eplontersen NDA 217388), dated December 20, 2023, available at <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm>.

-65.4% with 40 mg, -87.1% with 60 mg, and -93.6% with 80 mg (Table 45). For both the 60 mg and 80 mg treatment groups, mean PKK plasma concentrations did not return to baseline for 13 weeks after the last dose.

Figure 45. Mean (SEM) Plasma PKK Concentration Over Time

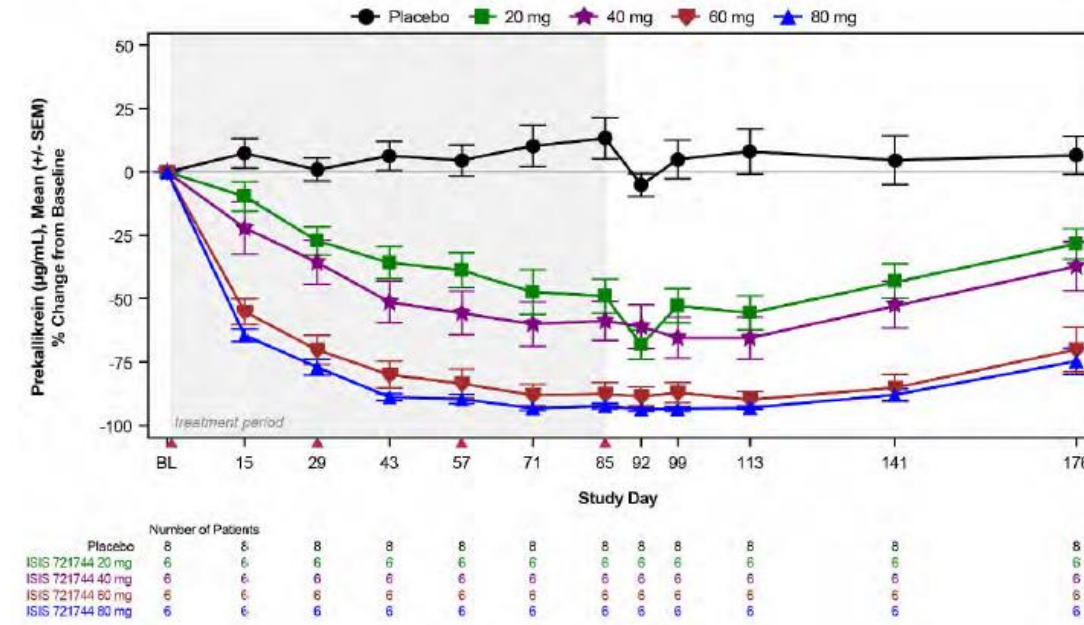


Source: Figure 14.2.1.1-1, CSR CS1

Doses were administered at Day 1 (BL), Day 29, Day 57, and Day 85

Abbreviations: BL, baseline; CSR, clinical study report; PKK, prekallikrein; SEM, standard error of the mean; x, upper limit of normal

Figure 46. Mean (SEM) Percent Change of Plasma PKK From Baseline Over Time



Source: Figure 14.2.1.1-3, CSR CS1

Doses were administered at Day 1 (BL), Day 29, Day 57, and Day 85.

Abbreviations: BL, baseline; CSR, clinical study report; PKK, prekallikrein; SEM, standard error of the mean

Table 45. Change From Baseline for Plasma PKK Concentration on Day 15 and Day 99

Timepoint	Placebo (N=8)	20 mg (N=6)	40 mg (N=6)	60 mg (N=6)	80 mg (N=6)
Baseline					
Mean (SD) value (µg/mL) (µg/mL)	122.28 (20.70)	121.33 (23.96)	142.21 (29.22)	131.13 (17.51)	118.50 (17.64)
Day 15					
Mean (SD) value (µg/mL) (µg/mL)	128.70 (12.05)	109.45 (27.55)	110.84 (41.90)	58.16 (14.17)	41.46 (4.86)
Mean (SD) percent change from baseline (%)	7.3 (16.4)	-9.7 (14.2)	-22.1 (25.3)	-55.2 (12.5)	-64.5 (6.0)
Day 99					
Mean (SD) value (µg/mL) (µg/mL)	125.00 (11.93)	55.04 (14.63)	49.02 (29.13)	16.66 (12.30)	7.32 (1.93)
Mean (SD) percent change from baseline (%)	5.0 (21.6)	-52.9 (16.5)	-65.4 (19.8)	-87.1 (9.7)	-93.6 (2.3)

Source: Adapted from Table 14, CSR CS1

Abbreviations: CSR, clinical study report; N, number of subjects; PKK, prekallikrein; SD, standard deviation

Immunogenicity Results

In total, 4 out of 26 subjects (16.7%) who received donidalorsen tested positive for ADA. Of these four subjects, two received 40 mg donidalorsen, and two received 60 mg donidalorsen. Given the small sample size, the impact of immunogenicity on pharmacokinetics and pharmacodynamics was inconclusive.

14.2.2. Trial CS9

Trial Design

Trial CS9 was a phase 1, single dose, single site, randomized, open-label, 2-period, 2-sequence, 2-treatment, crossover, bioequivalence trial evaluated at one dose level (donidalorsen 80 mg) in adult healthy subjects. Participants entered an up to 28-day Screening Period. Following Screening, 78 eligible participants were randomized in a 1:1 ratio to one of two treatment sequences. Subjects received donidalorsen 80 mg administered via subcutaneous injection (abdomen only) using either an AI or a single-use vial (syringe with manual injection): Sequence 1 (Treatment Period 1: AI and Treatment Period 2: vial) or Sequence 2 (Treatment Period 1: vial and Treatment Period 2: AI). Participants were administered donidalorsen 80 mg once at the beginning of each Treatment Period. Treatment Period 2 dosing began at least 28 days but no more than 42 days after Treatment Period 1 dosing.

The drug products used in this trial included a vial product that contained 100 mg/mL donidalorsen (0.8 mL per vial) and an AI that contained 100 mg/mL with a deliverable volume of 0.8 mL. The AI is the proposed to-be-marketed (TBM) product.

PK, PD, and ADA Sampling Time

PK blood samples were collected on Day 1 of each Treatment Period at predose (0 hour) and 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 168, and 336 hour postdose. Samples were also collected in the Post-Treatment Follow-Up Period at Day 28, 56, and 91/early termination following last dose of trial drug administration (Treatment Period 2).

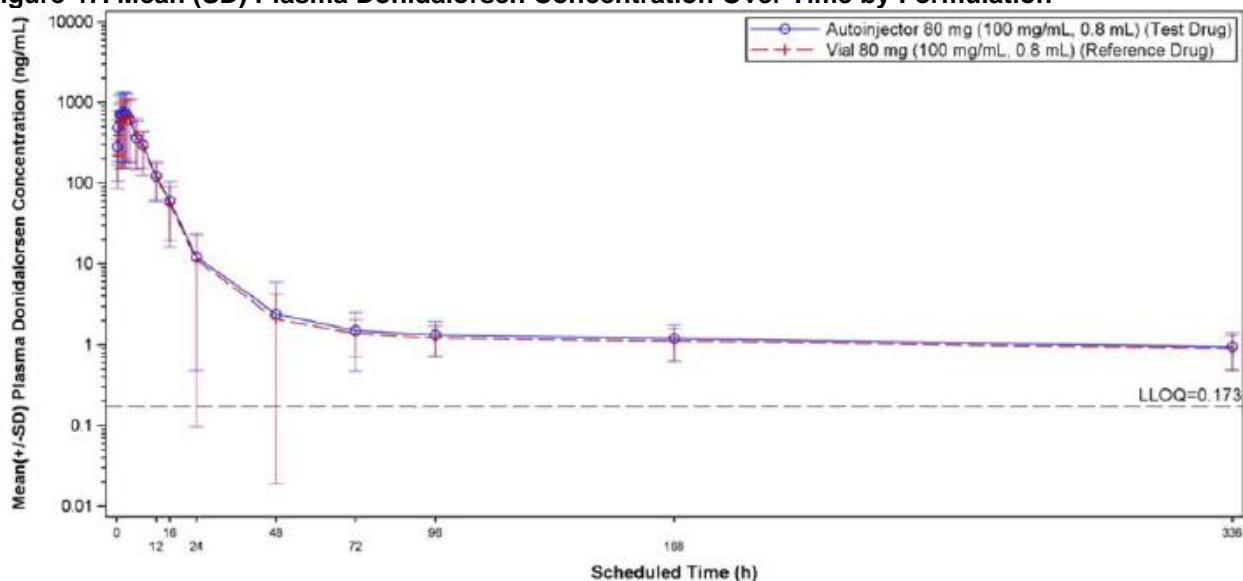
PD blood samples were collected within 1 hour prior to dosing on Day 1 during both Treatment Period 1 and 2. In addition, blood samples were collected on Day 4, Day 8, and Day 15 for Treatment Period 1.

ADA samples were not collected.

PK Results

A total of 78 subjects were randomized and dosed in the trial. Among them, 76 subjects completed the trial and two subjects (ID: (b) (6) and (b) (6)) discontinued the treatment early due to ineligibility. The mean (SD) baseline body weight for randomized subjects was 77.6 (12.9) kg and mean (SD) age was 43.1 (12.6) years. The mean body weight is consistent across treatment groups. The mean plasma concentration time profile by formulation and bioequivalence analysis are shown [Figure 47](#) and [Table 46](#), respectively. Administration of donidalorsen via AI yielded comparable C_{max} and AUC_{0-336h} as compared with the vial product. For both parameters, the geometric mean ratio point estimates and 90% confidence intervals were contained within the 80% to 125% range.

Figure 47. Mean (SD) Plasma Donidalorsen Concentration Over Time by Formulation



Source: Figure 14.2.1.4 CSR CS9

Abbreviations: CSR, clinical study report; h, hour; LLOQ, lower limit of quantification; SD, standard deviation

Table 46. Bioequivalence Analysis of Plasma Donidalorsen PK Parameters

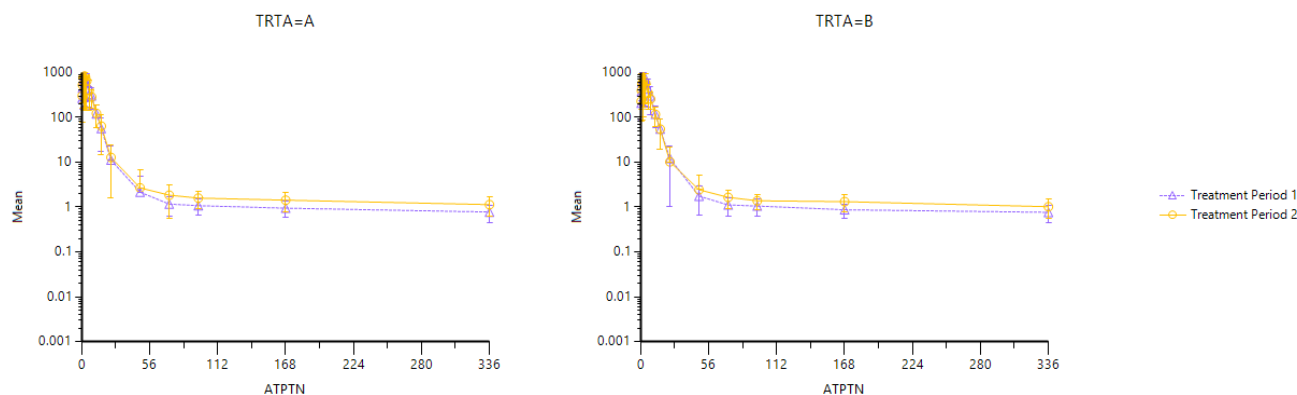
PK Parameter	PK Unit	Autoinjector		Vial		Ratio of Adj Geom Means* (Autoinjector/Vial) (%)	90% CI for Ratio** (%)
		n	Adj Geom Mean*	n	Adj Geom Mean*		
AUC _{0-336h}	h*ng/mL	76	5525	76	5162	107.0	(103.5, 110.6)
C _{max}	ng/mL	76	732	76	649	112.8	(105.0, 121.0)

Autoinjector = Donidalorsen 80 mg (100 mg/mL, 0.8 mL) in a single-use autoinjector; Vial = Donidalorsen 80 mg (100 mg/mL, 0.8 mL) in a single-use vial.
 Note: A linear mixed model was performed on the ln-transformed PK parameter of the 2 treatments (autoinjector versus vial) including terms for sequence, treatment, and period as fixed effects, and participants nested within a sequence as a random effect. Vial was used as the reference treatment.
 * Adj geom means were the least square means from the mixed model presented after back transformation to the original scale.
 ** The 90% CIs are presented after back transformation to the original scale.
 Adj = adjusted; AUC_{0-336h} = area under the plasma concentration-time curve from time 0 to 336 hours; CI = confidence interval; C_{max} = maximum plasma concentration; Geom = geometric; h = hour(s); ln = natural logarithm; PK = pharmacokinetic(s).

Source: Table 14.2.3.1. CSR CS9
 Abbreviations: CSR, clinical study report; n, number of subjects

The mean plasma concentration time profile by treatment sequence and PK parameters are shown in [Figure 48](#) and [Table 47](#), respectively. For the AI, a slightly greater geometric mean C_{max} and AUC_{0-336h} were noted in Treatment Period 2 compared to Treatment Period 1. Meanwhile, for the vial product, PK parameters were slightly lower in Treatment Period 2 as compared to those in Treatment Period 1. Overall, the washout period (at least 28 days) between the two treatment periods appears sufficient to mitigate the potential carryover effect on pharmacokinetics.

Figure 48. Mean (SD) Plasma Donidalorsen Concentration (ng/mL) Over Time (Hour) by Treatment Periods



Source: Reviewer-generated graph based on adpc.xpt for CS9
 Treatment A = autoinjector; Treatment B = vial.
 Abbreviations: ATPTN, Analysis Timepoint; SD, standard deviation; TRTA, actual treatment

Table 47. Geometric Mean (CV%) Plasma Donidalorsen PK Parameters by Treatment Periods

Period	Autoinjector			Vial		
	C _{max} (ng/mL) (N=39)	T _{max} (h) ^a (N=39)	AUC _{0-336h} (ng*h/mL) (N=39)	C _{max} (ng/mL) (N=39)	T _{max} (h) ^a (N=37)	AUC _{0-336h} (ng*h/mL) (N=39)
Period 1	701.9 (60.3) (N=39)	2 (0.25, 8) (N=39)	5430.9 (37.6) (N=39)	694.6 (66.3) (N=39)	4 (0.5, 8) (N=37)	5565.6 (41.0) (N=39)
Period 2	758.9 (76.0) (N=39)	2 (0.5, 8) (N=39)	6087.4 (43.1) (N=39)	606.1 (75.6) (N=37)	3 (0.25, 8) (N=37)	5179.6 (38.5) (N=37)
Combined	729.8 (67.9) (N=78)	2 (0.25, 8) (N=78)	5749.8 (40.6) (N=78)	650.0 (70.8) (N=76)	3 (0.25, 8) (N=76)	5374.2 (39.7) (N=76)

Source: Reviewer's analysis based on adpc.xpt for CS9

^a Median (range).

Abbreviations: AUC_{0-336h}, area under the concentration-time curve from 0 to 336 hours; C_{max}, maximum plasma concentration; CV, coefficient of variation; PK, pharmacokinetic; T_{max}, time to maximum concentration

[Table 48](#) shows bioequivalence analysis conducted only using data from Treatment Period 1. Based on Treatment Period 1 data, the vial and AI drug products had similar C_{max} and AUC_{0-336h}, with geometric mean ratio values close to 100%. The results were overall similar compared to analysis using data from both treatment periods ([Table 46](#)). The slightly higher C_{max} observed for the AI based on all data from both treatment periods appears to have been driven by the higher exposure observed from subjects who received the AI in Treatment Period 2.

Table 48. Bioequivalence Analysis for Plasma Donidalorsen Concentrations Between Autoinjector and Vial Based on Treatment Period 1

PK Parameter	Ratio (Autoinjector/Vial) (%)	90% Confidence Interval
C _{max}	101.1	(81.2, 125.8)
AUC _{0-336 h}	97.6	(84.6, 112.6)

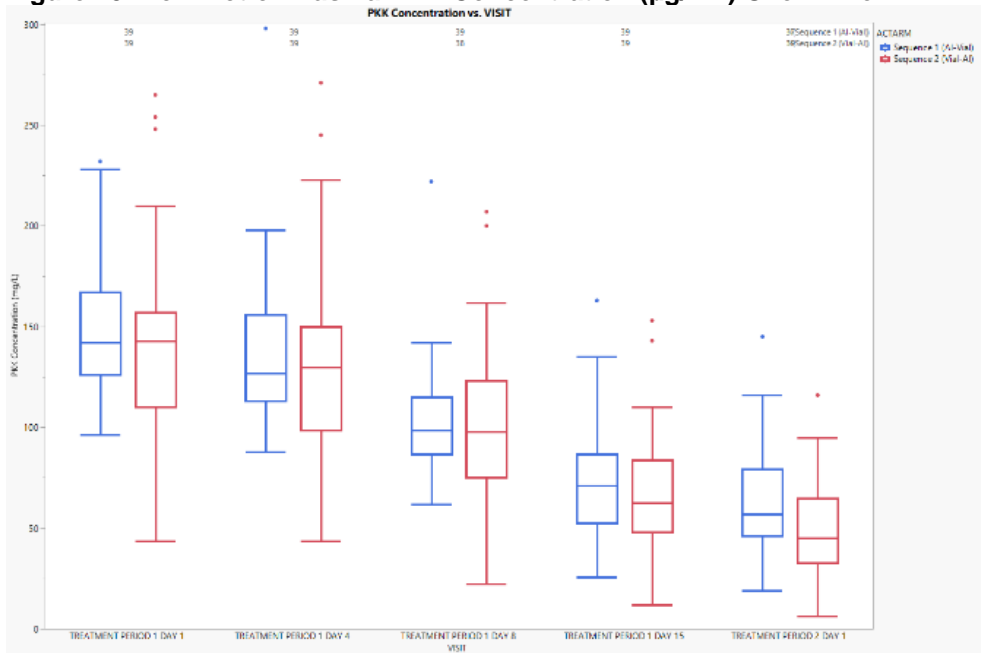
Source: Reviewer's analysis based on adpc.xpt for CS9

Abbreviations: AUC_{0-336h}, area under the concentration-time curve from 0 to 336 hours; C_{max}, maximum plasma concentration; PK, pharmacokinetic

PD Results

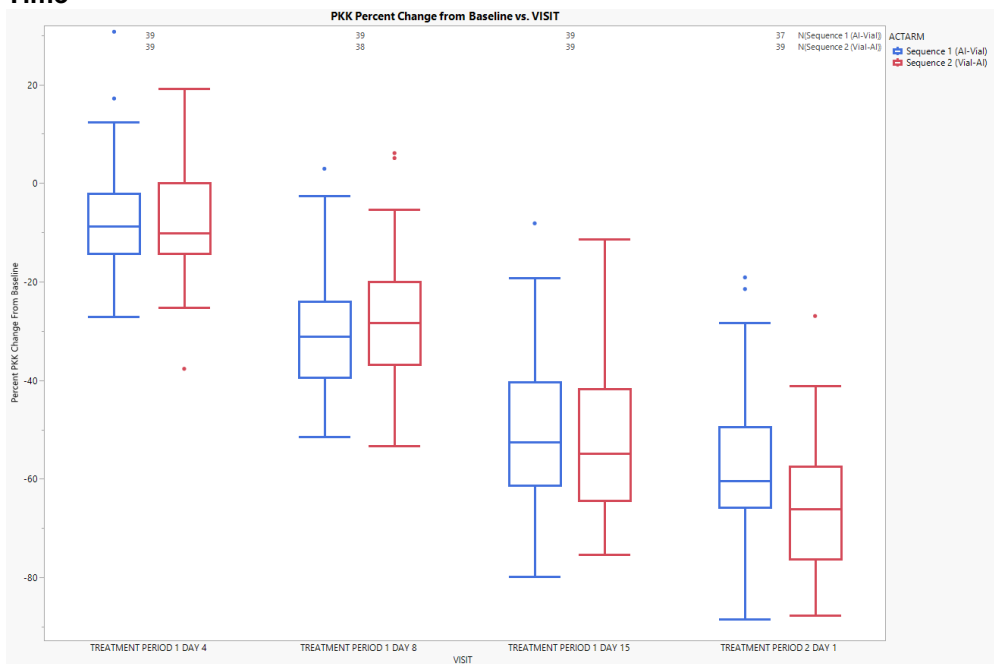
Box plots for PKK plasma concentrations and percent change from baseline in PKK concentrations are shown in [Figure 49](#) and [Figure 50](#), respectively. Similar to other GalNAc-conjugated ASO products, donidalorsen demonstrates a prolonged PD effect. As a continuous decline in PKK concentrations was observed up to Treatment Period 2 (i.e., at least 28 days apart from Treatment Period 1), it is reasonable to only assess postdose pharmacodynamics in Treatment Period 1. The range of PKK concentrations and percent change from baseline in PKK concentrations were similar between the two drug products.

Figure 49. Box Plot of Plasma PKK Concentration ($\mu\text{g}/\text{mL}$) Over Time



Source: Reviewer's analysis based on adpd.xpt for CS9
 Blue = Sequence 1 (received autoinjector in Period 1).
 Red = Sequence 2 (received vial in Period 1).
 Treatment Period 2 Day 1 occurred 28 to 42 days following Treatment Period 1 Day 1.
 Abbreviations: PKK, prekallikrein

Figure 50. Box Plot of Percent Change (%) From Baseline for Plasma PKK Concentration Over Time



Source: Reviewer's analysis based on adpd.xpt for CS9
 Blue = Sequence 1 (received autoinjector in Period 1).
 Red = Sequence 2 (received vial in Period 1).
 Treatment Period 2 Day 1 occurred 28 to 42 days following Treatment Period 1 Day 1.
 Abbreviations: PKK, prekallikrein

Table 49. Summary of Mean (SD) Plasma Concentration and Mean (SD) Percent Change From Baseline for PKK Over Time by Formulation

Timepoint	Autoinjector (N=39)	Vial (N=39)
Mean (SD) PKK concentration (mg/L)		
Baseline	147.9 (32.4)	144.2 (45.2)
Day 4	138.0 (37.9)	132.9 (46.8)
Day 8	102.0 (28.6)	104.2 (37.0)
Day 15	73.7 (27.3)	69.3 (28.9)
Pre-Treatment Period 2	62.2 (27.3)	50.8 (24.6)
Mean (SD) percent PKK change from baseline (%)		
Day 4	-6.7 (11.9)	-8.1 (10.8)
Day 8	-30.7 (12.3)	-27.5 (13.8)
Day 15	-49.8 (15.7)	-51.8 (14.8)
Pre-Treatment Period 2	-57.3 (17.6)	-64.7 (13.8)

Source: Adapted from Table 14.2.4.1. CSR CS9

Abbreviations: CSR, clinical study report; PKK, prekallikrein; SD, standard deviation

Immunogenicity Results

Not assessed.

14.2.3. Trial CS2

Trial Design

Trial CS2 was conducted in two parts concurrently with different designs. Part A was randomized, double-blind, and placebo-controlled while Part B was open-label. Subjects were allocated into Part A and B according to type of HAE (HAE Type I or II in Part A and HAE Type III in Part B). In Part A, 20 subjects were randomized 2:1 to receive SC injection of donidalorsen 80 mg or placebo every 4 weeks (weeks 1, 5, 9, and 13). In Part B, three subjects were administered open-label SC injections of donidalorsen-4 and completed the trial. The treatment duration was 12 weeks. After completing the Treatment Period, subjects had the option of enrolling in open-label extension Trial CS3 (refer to Section [14.2.5](#)).

PK, PD, and ADA Sampling Time

PK samples were collected at predose, and 1, 2, 4, and 6 hours postdose on Day 1 and Day 85 in the PK subgroup. For all subjects, predose samples were collected on Day 29, 57, and anytime on Day 15, and during the Post-Treatment Period (Days 113, 141, and 176).

PD samples were collected at predose on Days 1, 29, 57, 85, and anytime on Day 15 and during the Post-Treatment Period (Days 113, 141, and 176).

Immunogenicity samples were collected at predose on Days 1, 29, and 57 and anytime on Day 15 and during the Post-Treatment Period (Day 113 and Day 176).

PK Results

A total of 20 subjects with HAE Type I or II (6 in placebo and 14 in donidalorsen) were randomized in Part A, while 3 subjects with HAE Type III received donidalorsen in Part B. The mean age was and 38.5 (13.9) and 34 (7.9) years for Parts A and B, respectively. The mean (SD) body weight was 83.4 (18.4) and 95.7 (8.2) kg for Parts A and B, respectively.

In a small PK subgroup from Part A (N=4) who had more intensive samples collected postdose on Day 1 and Day 85, the PK parameters are summarized in [Table 50](#).

Table 50. Plasma PK Parameters of Donidalorsen in Part A PK Subgroup

Treatment Group	Dose (mg)	Study Day	N	C _{max} (ug/mL)	T _{max} (h)	AUC _{0-6h} (ug*h/mL)
ISIS 721744	80	1	4	0.282 (35.3)	1.00 (0.833, 1.97)	1.09 (31.6)
		85	4	0.236 (31.6)	0.992 (0.917, 2.00)	1.13 (34.1)

Data presented are geometric mean (geometric %CV) except T_{max}, which is presented as median (minimum, maximum). N = number of subjects; NC = not calculated.

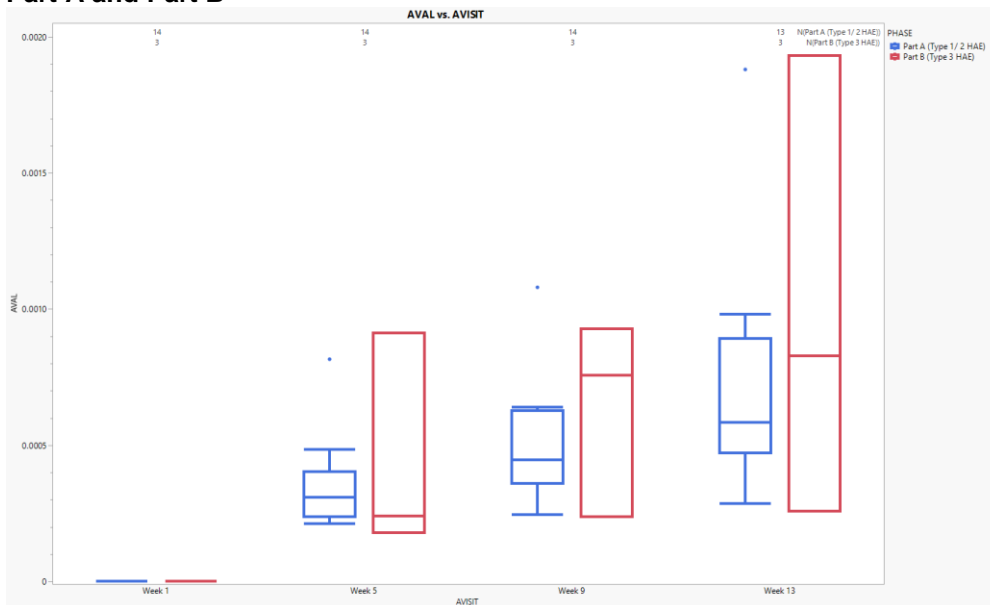
Source: Table 15, CSR CS5

Abbreviations: AUC_{0-6h}, area under the concentration-time curve from 0 to 6 hours; C_{max}, maximum plasma concentration; CSR, clinical study report; h, hour; PK, pharmacokinetic; T_{max}, time to maximum concentration

Similar to results observed in CS1, there was no accumulation following administration of multiple doses and the median T_{max} was observed at around 1 hour postdose. However, the C_{max} in Trial CS2 on Days 1 and 85 was 2- to 3-fold lower compared to that in Trial CS1 following administration of the same dosing regimen (i.e., donidalorsen-4; C_{max} values of 0.592 µg/mL and 0.718 µg/mL on Days 1 and 85, respectively). The Applicant attributed the cross-trial difference in part to different median body weights across the two trials (91 kg for PK subgroup in Trial CS2 versus 75 kg in Trial CS1). However, a definitive conclusion cannot be drawn due to small sample size (N=4) and less intensive postdose sampling in Trial CS2.

No subjects from Part B had intensive PK samples collected. The range of trough concentrations between Part A and Part B was similar during the Treatment Period, as shown in [Figure 51](#). However, the limited sample size from Part B limits interpretation of the data.

Figure 51. Box Plot of Trough Concentrations of Donidalorsen ($\mu\text{g/mL}$) at Weeks 1, 5, 9, and 13 in Part A and Part B



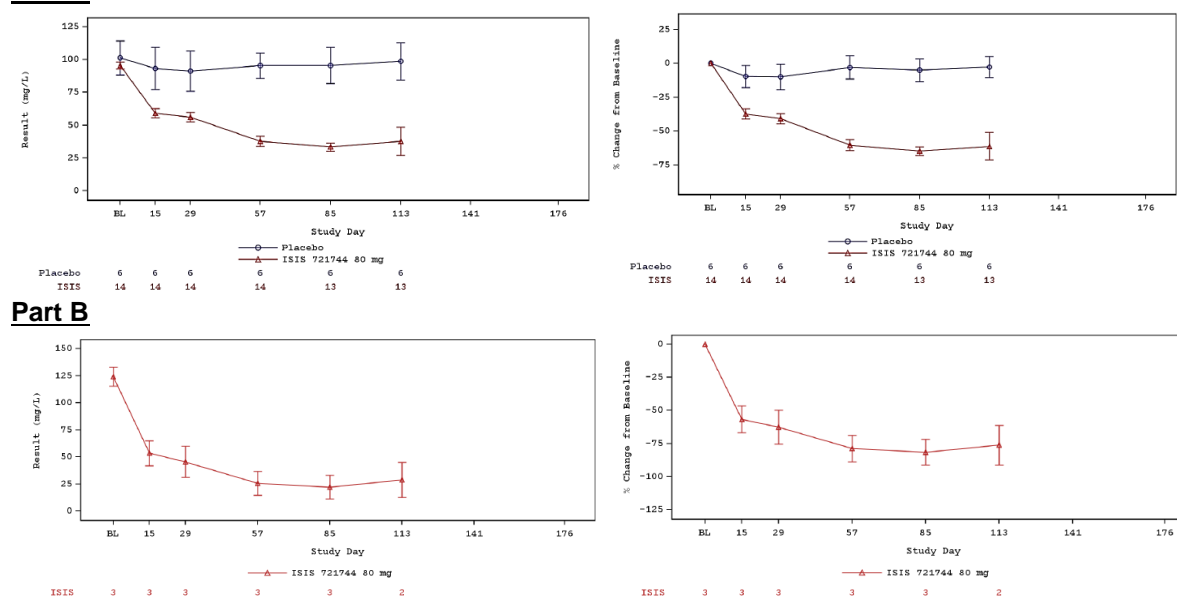
Source: Reviewer-generated graph based on adpc.xpt for CS2
N=14 in Part A and N=3 in Part B.

Abbreviations: AVAL, analysis value; AVISIT, analysis visit; HAE, hereditary angioedema

PD Results

The plasma PKK trough concentrations and percent change from baseline in PKK concentrations over time are presented in [Figure 52](#) and [Table 51](#). PKK trough concentrations in plasma were reduced from baseline following treatment of donidalorsen and reached nadir by Day 57 (Week 9) in both Parts A and B. The mean percent reduction in plasma PKK from baseline at Week 17 was slightly greater in Part B (76%) compared to Part A (61%). The small sample size of subjects (N=2) in Part B with PD data limits the interpretation of the data.

Figure 52. Mean (SE) Plasma PKK Trough Concentrations Absolute Values (Left) and Percent Change From Baseline (Right) in Part A and B



Source: Figure 14.2.1.2.-1 CSR CS2
Abbreviations: CSR, clinical study report; PKK, prekallikrein; SE, standard error

Table 51. Mean (SD) Plasma PKK Trough Concentration Absolute Values and Percent Change From Baseline at Week 9 and 17 in Part A and Part B

Timepoint	Part A		Part B
	Placebo (N=6)	Donidalorsen 80 mg Q4W (N=14)	Donidalorsen 80 mg Q4W (N=3)
PKK trough concentration (µg/mL)			
Baseline	101.2 (31.7)	95.4 (9.7) (N=14)	123.9 (15.1) (N=3)
Week 9	95.5 (23.7)	37.7 (14.6) (N=14)	25.6 (19.3) (N=3)
Week 17	98.6 (34.6)	37.8 (38.7) (N=14)	28.6 (22.8) (N=2)
Percent change from baseline (%)			
Week 9	-2.9 (21.2)	-60.3 (15.5) (N=14)	-78.7 (17.3) (N=3)
Week 17	-2.9 (19.2)	-61.2 (37.0) (N=14)	-76.2 (21.1) (N=2)

Source: Adapted from Table 14.2.1.9.1-1, CSR CS2
Abbreviations: CSR, clinical study report; N, number of subjects; PKK, prekallikrein; Q4W, every 4 weeks; SD, standard deviation

Immunogenicity Results

In subjects treated with donidalorsen (Part A and B), the proportion of the ADA-positive patient rate was 52.9% (9 out of 17 subjects). This included three subjects (17.6%) with pre-existing antibodies (ADA titers for these subjects did not increase more than 4-fold from baseline after treatment) and six subjects (35.3%, including two subjects with HAE Type III) with treatment-emergent ADA. The overall median onset of the ADA response in subjects who tested positive for ADA was Day 15 with a range of 1 to 118 days.

The plasma PKK trough concentrations over time by overall immunogenicity irrespective of baseline ADA titers is shown in [Table 52](#). At Week 17, subjects with positive ADA had lower percent reduction from baseline.

Table 52. Mean (SD) PKK Trough Concentration and Percent Change From Baseline by ADA Status

Timepoint	Donidalorsen 80 mg Q4W From Part A and B (N=17)	
	ADA Positive (N=9)	ADA Negative (N=8)
PKK concentration (µg/mL)		
Baseline	101.6 (10.4) (N=9)	99.1 (20.0) (N=8)
Week 9	39.2 (14.4) (N=9)	31.4 (16.7) (N=8)
Week 17	49.2 (45.9) (N=8)	22.2 (13.9) (N=7)
Percent change from baseline (%)		
Week 9	-60.9 (15.2) (N=9)	-66.6 (19.2) (N=8)
Week 17	-51.4 (43.9) (N=8)	-76.7 (15.5) (N=7)

Source: Adapted from Table 14.3.5.7-1, CSR CS2

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; N, number of subjects; PKK, prekallikrein; Q4W, every 4 weeks; SD, standard deviation

The mean percent reduction in HAE attack rate was greater in ADA-negative subjects than ADA-positive subjects, as shown in [Table 53](#). However, the mean percent reduction in HAE every-4-week attack rate in ADA-positive subjects remained higher than placebo (-13.09%).

Table 53. Mean (SD) HAE Attack Rate Per 4 Weeks Over Time by ADA Status

Time Period	Donidalorsen 80 mg ADA Positive (N=9)	Donidalorsen 80 mg ADA Negative (N=8)
Run-In Period	2.8 (2.2)	3.3 (1.3)
Week 1 to Week 17 (End of On-Treatment Period)	0.8 (1.2)	0.1 (0.1)
Percent change from Run-In Period to Week 1 to End of On-Treatment Period (%)	-76.9 (17.7)	-96.4 (5.5)

Source: Adapted from Table 14.3.5.6-1, CSR CS2

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; HAE, hereditary angioedema; N, number of subjects; SD, standard deviation

Both subjects with HAE Type I or II (Part A, N=14) and subjects with HAE Type III (Part B, N=3) who received donidalorsen were included in this assessment of the impact of ADAs on HAE attack rate. As noted above, two subjects with HAE Type III were ADA-positive. Of note, regardless of ADA status, the mean every-4-week attack rates during the Run-In Period (4.32) and the Week-1-to-Week-17 period (1.52) were higher in the three subjects with HAE Type III compared to those in subjects with HAE Type I or II treated with the same dosing regimen of donidalorsen (Run-In Period: 2.82 and Week-1-to-Week-17 period: 0.23). Thus, the assessment of ADA impact on efficacy may be confounded by different responses to donidalorsen treatment among patients with different types of HAE.

14.2.4. Trial CS5

Trial Design

For full details on the design of Trial CS5, refer to Section [6](#).

A vial presentation (100 mg/mL of donidalorsen, 0.8 mL per vial) was used for administration of donidalorsen in Trial CS5. Alternate injection administration sites (arm, abdomen, and thigh) were used for some subjects during the Treatment Period.

PK, PD, and ADA Sampling Time

Samples for assessing plasma donidalorsen concentrations were collected at predose and 2 hours postdose on Day 1 (Week 1) and Day 141 (Week 21) for the donidalorsen-4 group or Day 113 (Week 17) for the donidalorsen-8 group. Additional samples were collected at predose on Days 29, 57, 85, and 113 (i.e., Week 5, 9, 13, 17) as well as up to 13 weeks post-last dose.

Samples for assessing plasma PKK samples were collected predose at Days 1, 29, 57, 85, 113, 141, and 169 (i.e., Week 1, 5, 9, 13, 17, 21, and 25) as well as up to 13 weeks post-last dose.

ADA samples were collected at Days 1, 29, 85, 141, and 169 (i.e., Week 1, 5, 13, 21, and 25) as well as 4 and 13 weeks post last dose.

PK Results

The 2-hour postdose donidalorsen concentrations were similar between donidalorsen-4 and donidalorsen-8 groups on both Day 1 (Week 1) and Day 141 (Week 21) or Day 113 (Week 17), as reported in [Table 54](#). The geometric mean trough concentrations on Day 113 (Week 17) were around 2-fold higher in the donidalorsen-4 group than in the donidalorsen-8 group, although high PK variability was noted.

Table 54. Geometric Mean (CV%) Donidalorsen Plasma Concentrations (ng/mL) in Q4W and Q8W Treatment Groups

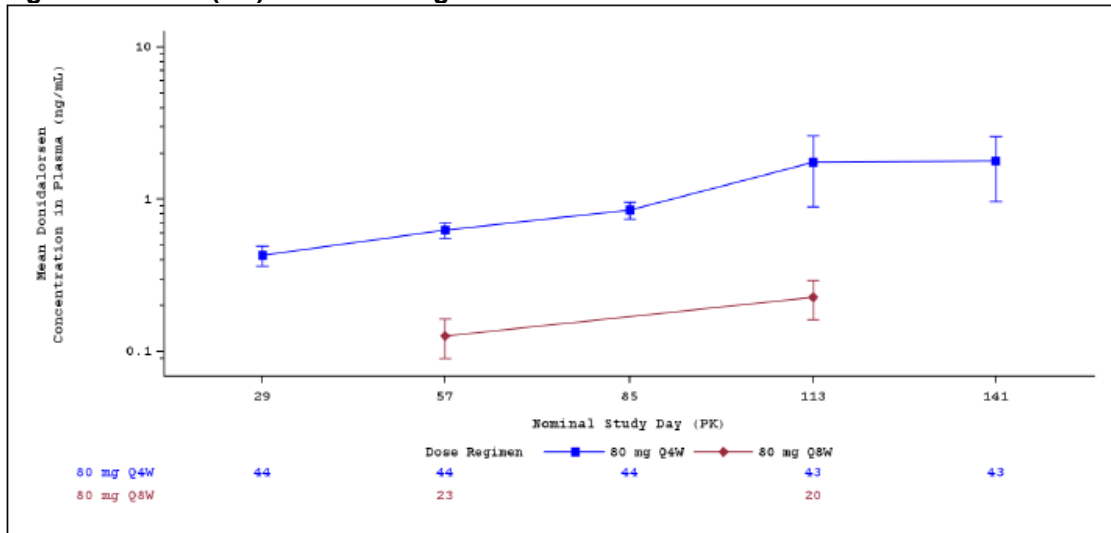
Timepoint	Q4W	Q8W
2 hours postdose concentration (ng/mL)		
Day 1 (Week 1)	388 (108) (N=44)	499 (433) (N=23)
Day 141 (Week 21) for Q4W or Day 113 (Week 17) for Q8W	447 (98.9) (N=43)	639 (584) (N=20)
Trough concentration (ng/mL)		
Day 29 (Week 5) for Q4W or Day 57 (Week 9) for Q8W	0.406 (71.1) (N=44)	0.264 (45.4) (N=23)
Day 113 (Week 17)	0.809 (115) (N=43)	0.394 (60.5) (N=20)

Source: Adapted from Table 23, CSR CS5

Abbreviations: CSR, clinical study report; CV, coefficient of variation, N, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks

The mean trough concentrations of donidalorsen over time in the donidalorsen-4 and donidalorsen-8 groups is shown in [Figure 53](#). The steady-state donidalorsen concentrations for the donidalorsen-4 group was achieved by Day 113 (Week 17). The mean steady-state trough concentration for subjects with HAE was similar to that in healthy subjects as observed in Trial CS1 (mean 1.1 ng/mL).

Figure 53. Mean (SD) Plasma Trough Concentrations of Donidalorsen Over Time

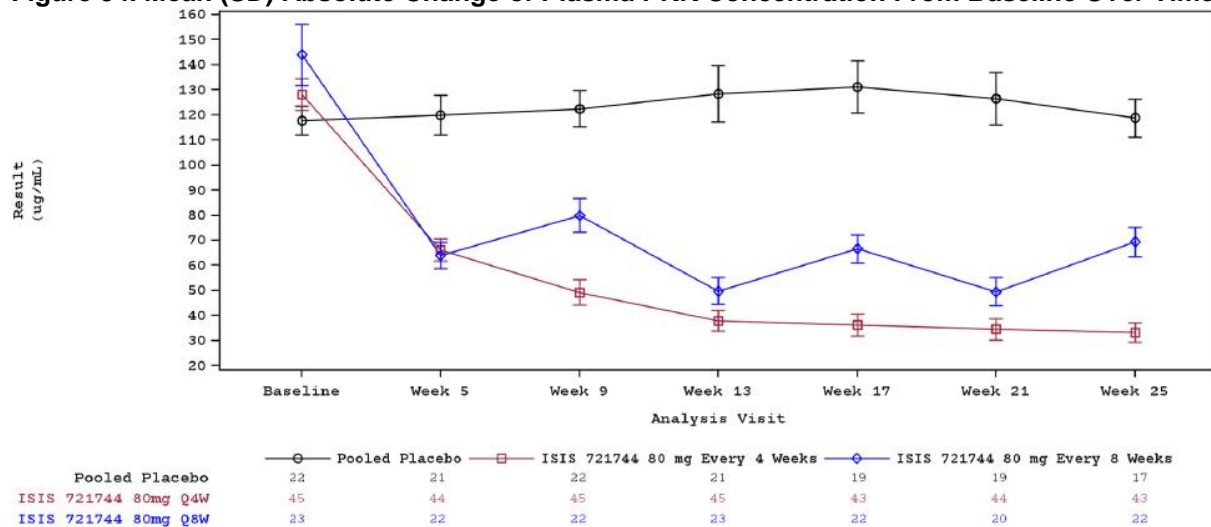


Source: Figure 14.2.4.1-2, CSR CS5
Abbreviations: CSR, clinical study report; PK, pharmacokinetic; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

PD Results

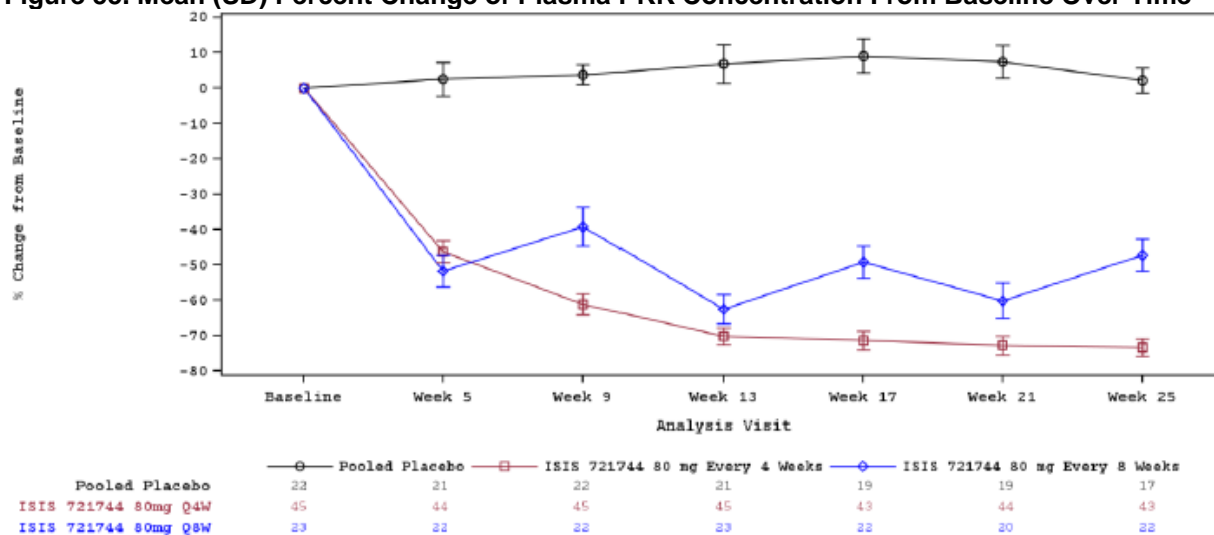
The mean absolute and percent change in PKK plasma concentrations from baseline over time are presented in [Figure 54](#) and [Figure 55](#), respectively. The percent change in plasma PKK concentration from baseline in the donidalorsen-4 group is of higher magnitude and more stable as compared to that observed in the donidalorsen-8 group. Steady state for PKK reduction was reached by Week 13 (Day 85), which is approximately consistent with the time to reach steady state for donidalorsen plasma concentrations (around Day 113).

Figure 54. Mean (SD) Absolute Change of Plasma PKK Concentration From Baseline Over Time



Source: Figure 14.2.9.1. CSR CS5
Abbreviations: CSR, clinical study report; PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

Figure 55. Mean (SD) Percent Change of Plasma PKK Concentration From Baseline Over Time



Source: Figure 14.2.9.1, CSR CS5

Abbreviations: CSR, clinical study report; PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

A decrease in plasma PKK concentration was observed at the first assessment (Week 4). The mean percentage reduction from baseline at Week 4 across both treatment groups was 48%. The mean plasma trough PKK concentrations and percent reduction from Baseline for the donidalorsen-4 and donidalorsen-8 groups at Week 25 were 73.4% and 47.2%, respectively. Both groups demonstrated greater reduction compared to placebo. Subjects in the placebo demonstrated a 2.1% increase from baseline in PKK trough concentrations ([Table 55](#)).

Table 55. Mean (SD) PKK Trough Concentrations and Percent Change From Baseline at Week 25

Timepoint	Pooled Placebo (N=22)	80 mg Q4W (N=45)	80 mg Q8W (N=23)
Baseline ($\mu\text{g/mL}$)	117.84 (27.0)	128.15 (41.9)	143.95 (58.6)
Week 25 ($\mu\text{g/mL}$)	118.7 (31.2)	33.3 (25.3)	69.4 (27.6)
Percent change from baseline at Week 25 (%)	2.1 (14.5)	-73.4 (16.5)	-47.2 (20.9)

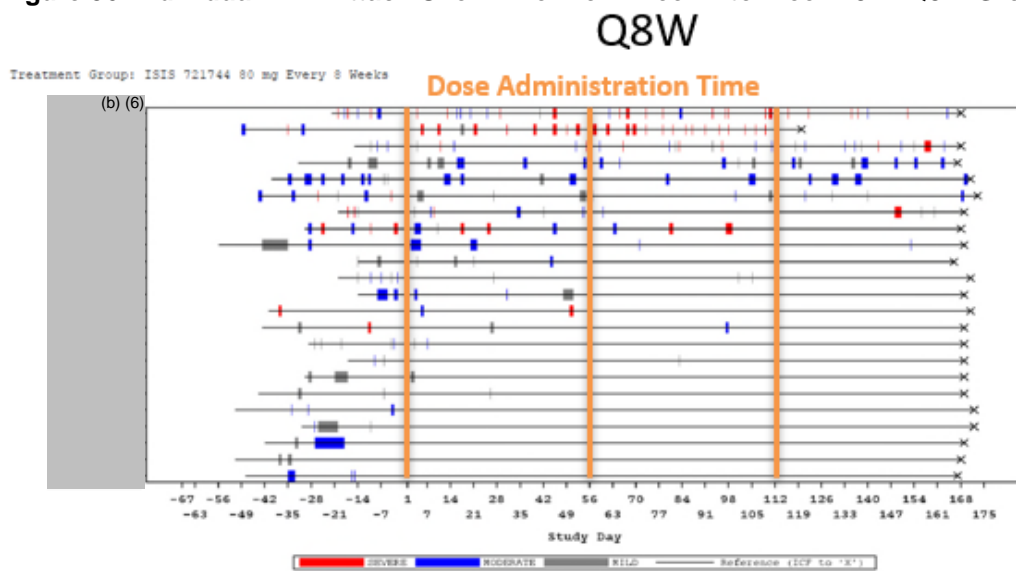
Source: Table 14.2.9.1. CSR CS5

Abbreviations: CSR, clinical study report; N, number of subjects; PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

A slightly lower reduction in PKK concentrations from baseline was noted prior to each subsequent dose in the donidalorsen-8 group (Week 9 and Week 17), which may be attributed to the longer dosing interval.

Nevertheless, the observed fluctuations in PKK trough concentration do not appear to impact the efficacy, as HAE attacks did not increase at trough prior to next dose in the donidalorsen-8 group when trough PKK concentrations were obtained. The HAE attack time profile for the donidalorsen-8 group is shown in [Figure 56](#) and the mean every-4-week HAE attack rate at 4-week intervals is shown in [Table 56](#). A greater HAE attack rate was observed in the donidalorsen-8 group relative to the donidalorsen-4 group from Week 5 onward, which may be attributable to the overall lower reduction in PKK concentrations in the donidalorsen-8 group.

Figure 56. Individual HAE Attack Over Time From Week 1 to Week 25 in Q8W Group, Trial CS5



APPEARS THIS WAY
ON ORIGINAL

Q8W dose administration: Day 1 (week 1), Day 57
(week 9), Day 113 (week 17)

Source: Adapted from Figure 14.2.1.2. CS5

The long vertical orange lines represent dose administration time; shorter vertical lines represent occurrence of HAE attack with different severity: severe (red), moderate (blue) and mild (gray).

Horizontal lines represent participating time in the trial from informed consent form to end of trial or early termination date.

Abbreviations: HAE, hereditary angioedema; Q8W, every 8 weeks

Table 56. Mean (SD) Every-4-Week HAE Attack Rate by 4-Week Intervals Between Weeks 1 to 17, Trial CS5

Time Interval	Q4W (N=45)	Q8W (N=23)
Baseline	3.61 (2.236)	3.18 (2.147)
Week 1 to 5	1.6 (2.14)	1.74 (1.71)
Week 5 to 9	0.84 (2.033)	1.09 (1.4)
Week 9 to 13	0.76 (1.86)	1.13 (2.12)
Week 13 to 17	0.53 (1.502)	1.17 (2.059)

Source: Adapted from Table 14.2.1.4-1 CS5

As Q8W dose was administered on Day 1 (Week 1), Day 57 (Week 9), and Day 113 (Week 17), the trough concentrations for Q8W were obtained during Week 5 to 9 and Week 13 to 17.

Abbreviations: HAE, hereditary angioedema; N, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

Immunogenicity Results

In the donidalorsen-4 group, 12 (26.7%) subjects were positive for ADA, 4 of whom were positive at baseline. In the donidalorsen-8 group, seven (30.4%) subjects were positive for ADA, two of whom (8.7%) were positive at baseline. In total, nine (20%) and five (21.7%) subjects had treatment-emergent ADAs in the donidalorsen-4 and donidalorsen-8 groups, respectively. Of note, subjects with treatment-emergent ADAs included subjects who were ADA-negative at baseline and developed ADAs during the Treatment Period as well as subjects who were ADA-positive at baseline and ADA titers increased during the Treatment Period.

In subjects who developed treatment-emergent ADAs against donidalorsen, the median time to onset of ADA was longer in the donidalorsen-4 group than in the donidalorsen-8 group (147.0 days versus 88.0 days, respectively). However, the median time to reach peak titer was similar in the donidalorsen-4 and donidalorsen-8 groups (147.0 days and 141.0 days, respectively).

The mean donidalorsen concentrations by ADA status is shown in [Table 57](#).

Table 57. Summary Statistics of Plasma Donidalorsen Concentrations in Q4W and Q8W Treatment Groups by ADA Status

Timepoint	Statistic	Q4W		Q8W	
		Negative ADA	TE-ADA	Negative ADA	TE-ADA
2-hour postdose (ng/mL)	N	32	8	14	4
	Mean (SD)	632 (548)	354 (211)	658 (666)	425 (201)
	Median (range)	431 (120, 2190)	314 (101, 635)	338 (199, 2290)	455 (163, 625)
Trough concentration (ng/mL)	N	32	8	14	4
	Mean (SD)	1.0 (0.8)	5.1 (12.5)	0.2 (0.3)	0.2 (0.3)
	Median (range)	0.65 (BLQ, 3.6)	0.55 (0.2, 37.6)	0.183 (BLQ, 1.01)	BLQ (BLQ, 0.692)

Source: Adapted from Table 23, CSR CS5

2-hour postdose concentrations were assessed at Week 21 (Day 141) for Q4W and at Week 17 (Day 113) for Q8W.

Trough concentrations were assessed at Week 17 (Day 113) for both Q4W and Q8W.

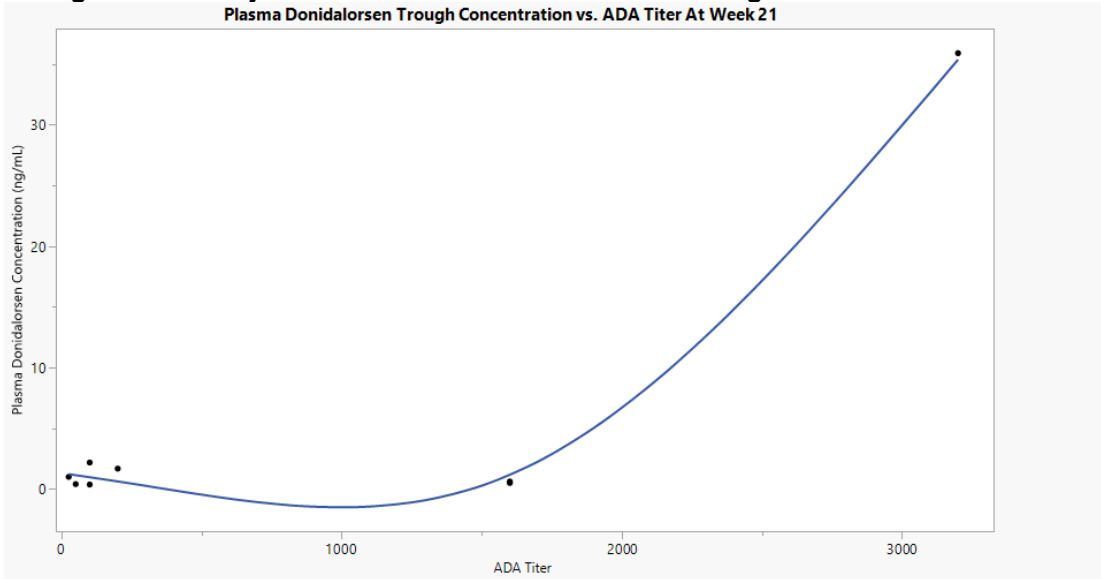
Abbreviations: ADA, antidrug antibody; BLQ, below limit of quantification; CSR, clinical study report; N, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation; TE-ADA, treatment-emergent antidrug antibody

Around 2-fold higher mean $C_{\max,ss}$ (collected 2-hours postdose at Week 21 for donidalorsen-4 and Week 17 for donidalorsen-8) was noted in ADA-negative subjects compared to subjects with treatment-emergent ADAs in the donidalorsen-4 and donidalorsen-8 groups. However, the median $C_{\max,ss}$ values were similar across ADA groups. The greater mean values in the negative ADA subgroup are likely driven by greater maximum values (2190 ng/mL in donidalorsen-4 and 2290 ng/mL in donidalorsen-8) that skew the mean upwards.

In the donidalorsen-4 group, subjects with treatment-emergent ADAs appear to have 5-fold higher mean $C_{\text{trough},ss}$ as compared to ADA-negative subjects, while the mean $C_{\text{trough},ss}$ was similar across ADA groups in the donidalorsen-8 group. The difference in $C_{\text{trough},ss}$ by ADA status in the donidalorsen-4 group was driven by one extreme value from Subject (b) (6) in the ADA-positive subgroup who presented with a $C_{\text{trough},ss}$ at Week 17 of 37.6 ng/mL. Of note, this subject also presented with the highest ADA titer value at Week 21 (3200) among all ADA-positive subjects.

The relationship between donidalorsen plasma C_{trough} and ADA titer was further explored. The plasma C_{trough} values for donidalorsen versus ADA titer values obtained at Week 21 for each individual with treatment-emergent ADAs in the donidalorsen-4 group were plotted in [Figure 57](#). The positive relationship between plasma C_{trough} and ADA titers appears to be driven by one subject (Subject (b) (6)), as hypothesized above. Meanwhile another subject with higher ADA titers (i.e., 1600) had similar C_{trough} compared to that in other subjects with low ADA titers (≤ 200). However, the small sample size of ADA-positive subjects precludes making a definitive conclusion on the impact of ADA titers on donidalorsen C_{trough} .

Figure 57. Individual Plasma Donidalorsen C_{trough} vs. ADA Titers at Week 21 in Treatment-Emergent ADA Subjects Who Received Donidalorsen 80 mg Q4W



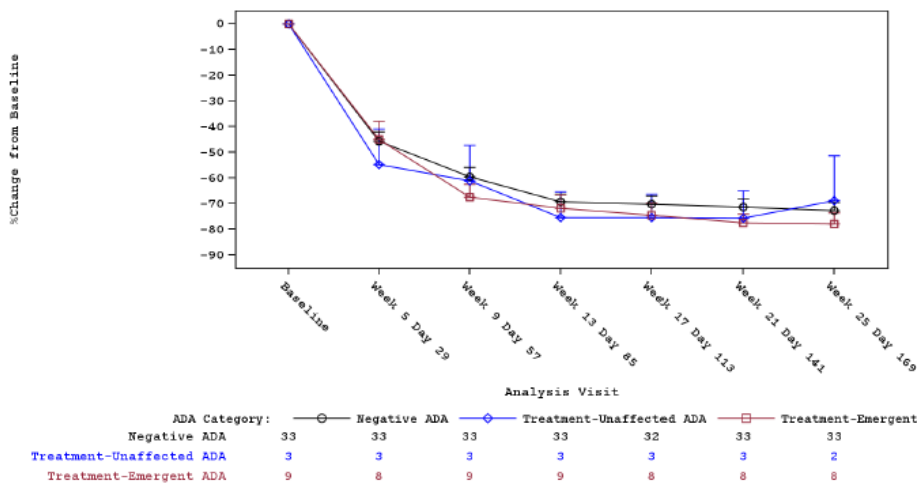
Source: Reviewer-generated graph based on adpc.xpt and adis.xpt for CS5

Abbreviations: ADA, antidrug antibody; C_{trough} , trough concentration; Q4W, every 4 weeks

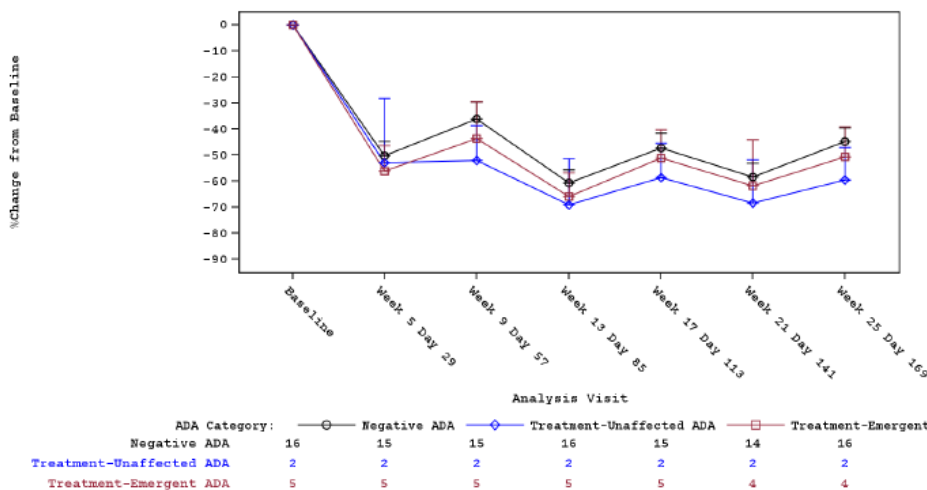
The mean (SEM) percent change from baseline in plasma PKK trough concentrations over time stratified by ADA status is shown in [Figure 58](#). In both the donidalorsen-8 and donidalorsen-4 groups, the change from baseline in PKK was similar between ADA-negative subjects and subjects with treatment-emergent ADAs.

Figure 58. Mean (SEM) Percent Change From Baseline in PKK Concentration Over Time by ADA Status

(A) Donidalorsen-4 Group



(B) Donidalorsen-8 Group



Source: Figure 10, CSR CS5

Treatment-Emergent ADA: Includes both Treatment-Induced and Treatment-Boosted ADA-positive subjects.

Treatment-Induced ADA: ADA developed de novo (seroconversion) following trial drug administration (i.e., formation of ADA any time after the initial drug administration in a subject without pre-existing ADA, i.e., baseline negative ADA).

Treatment-Boosted ADA: Pre-existing ADA that were boosted to a higher level following trial drug administration (i.e., any time after the initial drug administration the ADA titer is greater than the baseline titer by a factor of 8-fold or more).

Treatment-Unaffected ADA: Pre-existing ADA that were not affected (boosted) following trial drug administration (i.e., any time after the initial drug administration the ADA titer is less than 8-fold).

Negative ADA: All evaluated immunogenicity sample results during the Treatment and Post-Treatment Periods were negative and subjects were to have at least one evaluable immunogenicity result collected after the first dose of trial drug. If a subject had only a positive sample at Baseline (Day 1 predose) and was negative at all other time points, the subject had a negative immunogenicity status.

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; PKK, prekallikrein; SEM, standard error of mean

ADA status was not found to affect efficacy in Trial CS5. The mean and median percent reduction from baseline in the every-4-week HAE attack rate were similar between ADA-negative subjects and subjects with treatment-emergent ADAs in the donidalorsen-4 group (Table 58). Although the mean percent reduction in every-4-week HAE rate from baseline was

greater among ADA-negative subjects relative to subjects with treatment-emergent ADAs in the donidalorsen-8 group, the median and range percent reduction in HAE rate were similar (Table 58). Notably, within the donidalorsen-8 group, the baseline HAE attack rate was lower among subjects with treatment-emergent ADAs relative to ADA-negative subjects. The small number of subjects with treatment-emergent ADAs limits the interpretation of the data. Of note, Subject (b) (6) in donidalorsen-4 group who presented with the highest ADA titers at Week 21 (i.e., 3200) had a baseline HAE attack rate of 2.4, which reduced to 0.16 between Week 1 and Week 25.

Table 58. HAE Attack Rate Per 4 Weeks Over Time by Subjects ADA Status

Time Interval	Q4W		Q8W	
	Negative ADA (N=33)	TE-ADA (N=9)	Negative ADA (N=16)	TE-ADA (N=5)
Baseline (Run-In)				
Mean (SD)	3.6 (2.1)	3.5 (2.5)	3.6 (2.3)	1.8 (0.7)
Median (range)	3.3 (0.5, 10)	2.4 (1.3, 9.3)	3.8 (0.7, 8.4)	1.6 (1, 2.7)
Week 1 to Week 25 ^a				
Mean (SD)	0.78 (1.5)	1.5 (3.1)	1.2 (1.4)	1.4 (2.5)
Median (range)	0.3 (0, 6.3)	0.3 (0, 9.6)	0.5 (0, 4.7)	0.3 (0, 5.8)
Percent change from baseline to Week 1 to Week 25 (%)				
Mean (SD)	-82.5 (21.1)	-72.3 (34.7)	-67.5 (41.3)	-30.5 (107.3)
Median (range)	-89.5 (-100, -17.5)	-84.0 (-100, 2.4)	-85.0 (-100, 63.9)	-74.4 (-100, 155.2)

Source: Adapted from Table 47, CSR CS5

^a The Week 1 to Week 25 HAE attack rate for each subject was calculated as number of investigator-confirmed HAE attacks occurring from Week 1 to Week 25 divided by the number of days the subject contributed to the period multiplied by 28.

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; N, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation; TE-ADA, treatment-emergent antidrug antibody

Safety (administration site conditions and treatment-emergent hypersensitivity events) by ADA status is shown in Table 59. A higher proportion of ADA-positive subjects in the donidalorsen-4 group reported these AEs, while no ADA-positive subjects from the donidalorsen-8 group reported these AEs.

Table 59. Adverse Events Rate for Injection Site Reactions and Hypersensitivity by ADA Status, Trial CS5

Adverse Event	Q4W		Q8W	
	ADA Negative (N=33)	TE-ADA (N=9)	ADA Negative (N=16)	TE-ADA (N=5)
General disorder and administration site conditions	N=5 (15.2%)	N=6 (66.7%)	N=2 (12.5%)	N=0 (0%)
Treatment-emergent hypersensitivity event	N=2 (6.1%)	N=2 (33.3%)	N=1 (6.3%)	N=0 (0%)

Source: Adapted from 14.3.5.9-1 and 14.3.5.9-2, CSR CS5

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; N, number of subjects; Q4W, every 4 weeks; Q8W, every 8 weeks; TE-ADA, treatment-emergent antidrug antibody

The injection site reactions (i.e., injection site erythema, injection site discoloration, injection site pain, injection site pruritus, injection site induration, injection site bruising, injection site hematoma, injection site hypersensitivity, injection site swelling, injection site reaction, injection

site urticaria) in the Applicant's adae.xpt were initially reported after the first dose (Day 2) or the second dose (around Day 30). Reports of injection site reactions continued throughout the Treatment Period in most subjects, while the median time to onset of ADAs generally occurred around Week 13 or later. There was thus no strong temporal association between high ADA titers and injection site reactions. ADAs were not found to impact safety. However, given the limited sample size of subjects with treatment-emergent ADAs, a definitive conclusion cannot be drawn.

14.2.5. Trial CS3

Trial Design

Trial CS3 is an ongoing OLE trial of phase 2 double-blind Trial CS2 (referred to as the Index Trial) to evaluate the long-term safety, efficacy, and alternative dosing of donidalorsen. Subjects from Trial CS2 were enrolled into Trial CS3 if they successfully completed qualification procedures and in the opinion of the investigator, the subject would benefit from continued treatment.

The trial consisted of four periods including the Qualification Period, a Treatment Period, an Extended Treatment Period, and a Post-Treatment Period. The Treatment Period was composed of a Fixed Dosing Period and a Flexible Dosing Period during which the donidalorsen dosage for each subject could be changed.

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

- Subjects could continue receiving donidalorsen-4.
- For subjects who were attack free for ≥ 12 weeks after entering this OLE Trial (the Fixed Dosing Period), the investigator could initiate a dose at a reduced frequency of donidalorsen-8. The reduced frequency dosing could begin at any trial visit starting at or after Week 17. If a subject's disease was not adequately controlled on donidalorsen-8, then dosing could return to donidalorsen-4.
- For subjects who were not attack free for ≥ 12 weeks after entering this OLE Trial (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 trial visit starting at or after Week 17. If subjects developed any tolerability or safety issue during treatment, the dose could be reduced back to donidalorsen-4.

At the end of the 52-week Treatment Period (Fixed Dosing + Flexible Dosing), subjects 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.

PK, PD, and ADA Sampling Time

PK, PD, and ADA blood samples were collected at predose at Weeks 1, 5, 9, and 13 in the Fixed Dosing Period, and at predose at Weeks 21, 29, 37, 45, and 53 in the Flexible Dosing Period, and anytime in the Post-Treatment Period (Week 65).

In the Extended Treatment Period Year 2, PK, PD, and ADA blood samples were collected at predose at Weeks 65, 77, 89, 105, and anytime in the Post-Treatment Period (Week 113).

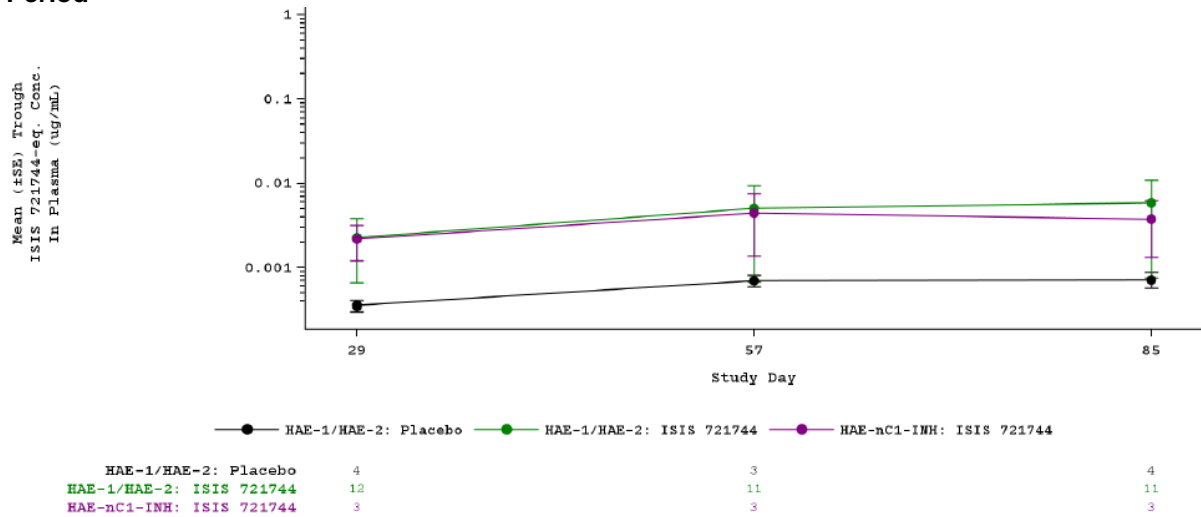
In the Extended Treatment Period Year 3, PK, PD, and ADA blood samples were collected at predose at Weeks 117, 129, 141, 157, and anytime in the Post-Treatment Period (at Week 169).

PK Results

In this OLE trial, 20 subjects were enrolled from Index Trial CS2 and received the trial drug, including 17 subjects with HAE Type I/II (12 from Index Q4W and 5 from Index placebo) and 3 subjects with HAE Type III. This interim CSR describes data collected as of the data cutoff date of February 26, 2024. At the time of data cutoff, 15 subjects completed ≥ 2 years of trial drug, and 14 subjects completed ≥ 3 years of trial drug. During the Flexible Dosing Period, eight subjects with HAE Type I or II changed dosing frequency from donidalorsen-4 during the Fixed Dosing Period to donidalorsen-8. However, three subjects ([REDACTED] (b) (6) [REDACTED]) returned to a donidalorsen-4 dosing frequency due to experiencing an HAE attack. The remaining five subjects remained on a donidalorsen-8 dosing frequency.

The mean plasma trough concentration over time during the Fixed Dosing Period (Week 1 to Week 13 of Trial CS3) is shown in [Figure 59](#). In the Index Placebo group (i.e., did not receive donidalorsen until Trial CS3), the trough concentrations reached steady state by Day 57 (Week 9) following administration of donidalorsen-4. In the Index Q4W group (for both HAE Type I/II and Type III), trough concentrations were relatively stable from the beginning of the Fixed Dosing Period, indicating no significant accumulation of donidalorsen in plasma over time.

Figure 59. Mean (SE) Plasma Donidalorsen Trough Concentrations Over Time in the Fixed Dosing Period



Source: Figure 14.2.2.2-1 CSR CS3

HAE-1/HAE-2:Placebo = HAE-1/HAE-2 Index placebo group, subjects with HAE Type I or HAE Type II treated with placebo during the Index Trial.

HAE-1/HAE-2:ISIS 721744 = HAE Type I or II Index donidalorsen-4 group, subjects with HAE Type I or II treated with donidalorsen 80 mg every 4 weeks during the Index Trial.

HAE-nC1-INH:ISIS 721744 = nC1-INH-HAE group, all subjects with nC1-INH-HAE (i.e., HAE Type III).

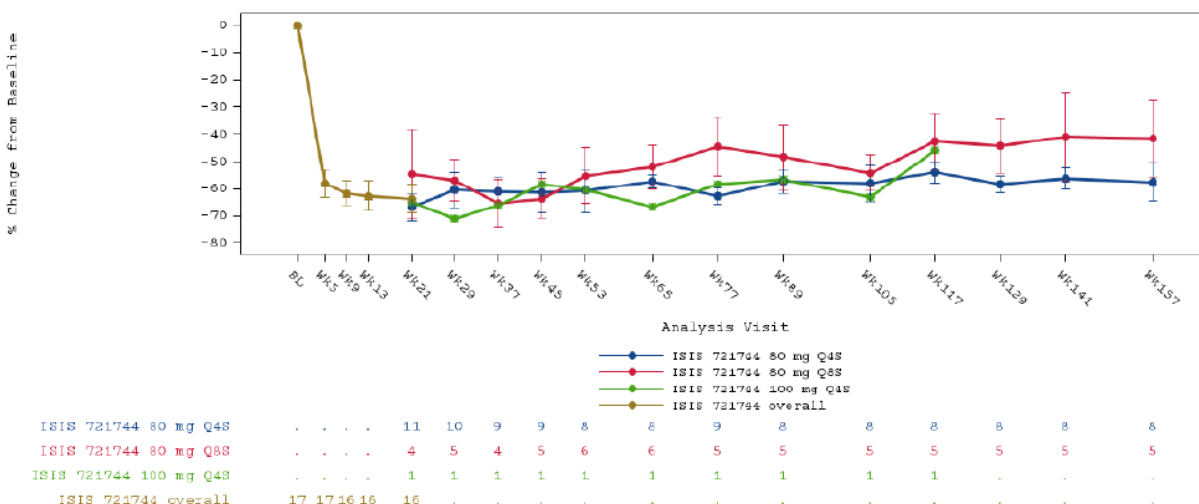
The Fixed Dosing Period was defined as from OLE Trial Day 1 to the earliest date of the following: the day before the Week 17 dose for subjects who were dosed at Week 17, or 28 days after the fourth dose for any subjects who skipped dosing at Week 17, or 28 days after the last dose date if the subject terminated treatment before the fourth dose, or the last date of lost to follow-up, whichever was sooner.

Abbreviations: CSR, clinical study report; HAE, hereditary angioedema; nC1-INH-HAE, hereditary angioedema formerly type III; OLE, open-label extension; SE, standard error

PD Results

Plasma PKK levels over time during both Fixed Dosing Period and Flexible Dosing Period are presented in [Figure 60](#).

Figure 60. Mean (SE) Percent Change From Index Run-In Baseline for Plasma PKK Levels Over Time for HAE Type I/II Subjects



Source: Figure 9, CSR CS3

ISIS 721744 80 mg Q4S = OLE donidalorsen-4 group, subjects treated with donidalorsen 80 mg every 4 weeks during CS3.

ISIS 721744 80 mg Q8S = OLE donidalorsen-8 group, subjects treated with donidalorsen 80 mg every 8 weeks during CS3.

ISIS 721744 100 mg Q4S = OLE donidalorsen-100 mg-4 group, subjects treated with donidalorsen 100 mg every 4 weeks during CS3.

ISIS 721744 overall = Total subjects treated with donidalorsen 80 mg every 4 weeks or every 8 weeks, or donidalorsen 100 mg every 4 weeks during CS3.

The Index Run-In Baseline was defined as the period when subjects were observed prior to any treatment in the Index Trial (the Baseline used for assessments during the Index Trial [CS2]).

Abbreviations: CSR, clinical study report; HAE, hereditary angioedema; PKK, prekallikrein; OLE, open-label extension; SE, standard error

During the Fixed Dosing Period in which all subjects received donidalorsen-4 dosing, the HAE Type I or II Index Placebo groups and HAE Type I or II Index Q4W groups had similar mean percent change in plasma PKK from Index Run-In baseline at Week 13 (−64.6% and −61.9%, respectively). The percent change in plasma PKK levels from baseline was similar to that observed during Index Trial CS2. For the three HAE Type III subjects, the mean percent change from Run-In baseline at Week 13 was −81%, which was also similar to that observed in Trial CS2.

For most visits during the Flexible Dosing Period, the percent reduction from baseline in plasma PKK levels was greater with donidalorsen-4 dosing compared with donidalorsen-8 dosing.

Immunogenicity Results

The ADA incidence rate was 70.6% (12 out of 17 subjects) in the overall HAE Type I/II group (4 from Index Placebo and 8 from Index Q4W), out of which 11 subjects (64.7%) had treatment-emergent ADAs, and 1 subject (5.9%) had treatment-unaffected ADAs (pre-existing ADAs that were not boosted following drug administration). Among subjects with HAE Type III, the incidence rate of treatment-emergent ADAs was 100% (three out of three subjects). ADA-positive subjects who rolled over from Trial CS2 remained ADA-positive in Trial CS3.

14.2.6. Trial CS7

Trial Design

Trial CS7 is an ongoing open-label trial to evaluate the long-term safety and efficacy of donidalorsen in subjects with Type I and II HAE. Two treatment arms were evaluated:

- OLE subjects who rolled over from Trial CS5 (referred to as the Index Trial).
- Donidalorsen-naïve subjects who were previously maintained on other HAE prophylactic therapies (i.e., lanadelumab, berotralstat, or a C1-esterase inhibitor).

The trial consists of four periods, including a Qualification Period for OLE subjects (Week -4 to -1) or a Screening Period for new subjects who switched from other HAE prophylactic therapies (Week -10 to -1), a Treatment Period (Week 1 to 53), an Extended Treatment Period (Week 54 to 157), and a Post-Treatment Period (end of treatment +13 weeks).

For OLE subjects who rolled over from Index Trial CS5 (OLE subjects), subjects who were previously treated with donidalorsen-4 or placebo Q4W in Trial CS5 received donidalorsen-4 beginning 28±3 days after the last dose received in Trial CS5. Subjects who were previously treated with donidalorsen-8 in Trial CS5 received donidalorsen-8 in Trial CS7 beginning 56±3 days after last dose in Trial CS5, unless they were not attack free for ≥8 weeks (Week 17 to 25 of Trial CS5), in which case they received donidalorsen-4. If subjects participated in the Extended Treatment Period (Week 54 to Week 157), they could change dosing frequency (from donidalorsen-8 to donidalorsen-4 or from donidalorsen-4 to donidalorsen-8), as determined by clinical symptoms (per investigator).

For donidalorsen-naïve subjects who switched from other HAE prophylactic treatments (Switch Subjects), subjects were treated with donidalorsen-4 during the Treatment Period and had the option to change dose frequency based on clinical symptoms (per investigator). Switch Subjects 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 taking their HAE prophylactic treatment during the Screening Period. The schedule to switch from other prophylactic treatments to donidalorsen is as follows:

- Lanadelumab: Subject was to take last dose of lanadelumab 14 days ±3 days prior to Day 1.
- Berotralstat: Subject was to continue taking a stable dose of berotralstat for 14 days ±3 days after Day 1.
- C1-esterase inhibitor: Subject was to continue taking a stable dose of C1-esterase inhibitor for 14 days ±3 days after Day 1.

PK, PD, ADA Sampling Time

OLE Subjects

Plasma samples for assessing pharmacokinetics (donidalorsen plasma concentrations), pharmacodynamics (plasma PKK concentrations) and ADA were collected at predose on

Weeks 1, 9, 25, 37, 49, and 53 in the Treatment Period, predose on Weeks 65, 77, 89, and 105 in the Extended Treatment Period, and in the Post-Treatment Period up to 13 weeks after the last dose.

Switch Subjects

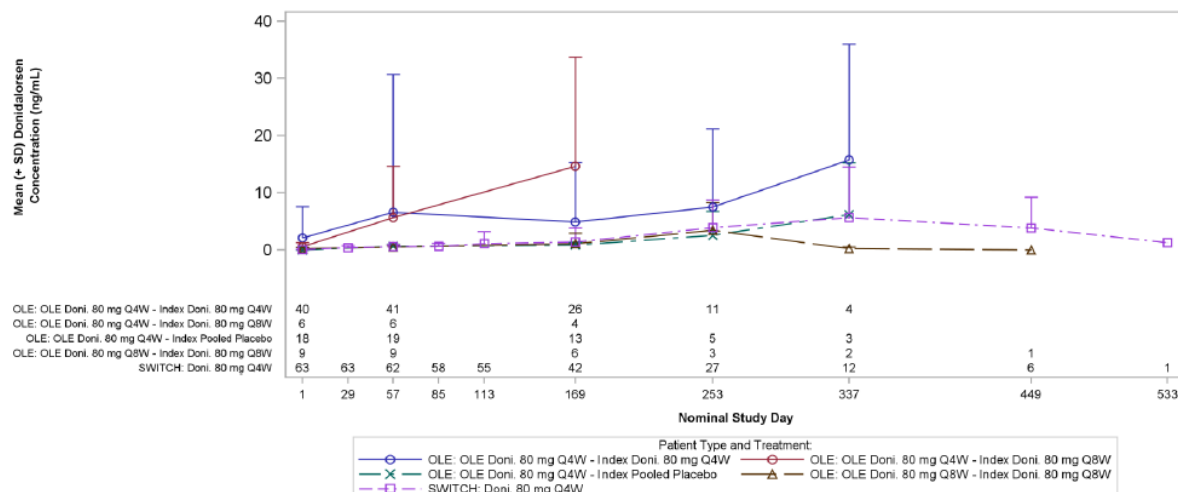
Plasma samples for assessing pharmacokinetics (donidalorsen plasma concentrations), pharmacodynamics (plasma PKK concentrations) and ADA were collected at predose on Weeks 1, 5, 9, 13, 17, 25, 37, 49, and 53 in the Treatment Period, predose on Weeks 65, 77, 89, and 105 in the Extended Treatment Period, and in the Post-Treatment Period up to 13 weeks after the last dose.

PK Results

As of the cutoff date of the interim report (February, 28, 2024), a total of 83 subjects rolled over from Trial CS5 (44 from Trial CS5 donidalorsen-4 group, 14 from Trial CS5 donidalorsen-8 group, and 19 from Trial CS5 placebo), while 65 donidalorsen-naive subjects who switched from other prophylactic treatments were also enrolled (32 from lanadelumab, 11 from berotralstat, 22 from C1-esterase inhibitor). As of the cutoff date, the majority of OLE subjects did not complete the 52-week Treatment Period with a current median exposure duration of 227 days.

The mean donidalorsen plasma trough concentrations over time in each group is shown in [Figure 61](#).

Figure 61. Mean (SD) Donidalorsen Plasma Trough Concentrations (ng/mL) Over Time, Trial CS7



Source: CSR CS7, Figure 14.2.3.3.3

Abbreviations: CSR, clinical study report; Doni., donidalorsen; OLE, open-label extension; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

For OLE subjects, subjects who received donidalorsen-8 in Trial CS5 and then received donidalorsen-4 in Trial CS7 had increased donidalorsen trough concentrations during Trial CS7, which was expected due to the increase in dosing frequency. Subjects who received donidalorsen-8 during Trial CS5 and remained on a donidalorsen-8 frequency during Trial CS7 had relatively stable trough concentrations over time. Meanwhile, subjects who received

donidalorsen-4 during Trial CS5 and remained on a donidalorsen-4 frequency in Trial CS7 showed a trend of increasing trough concentrations.

For Switch Subjects who were Naïve to donidalorsen treatment and received donidalorsen-4, the mean trough concentrations at steady state were slightly lower than those in OLE subjects who remained on a donidalorsen-4 regimen. Despite the lower plasma trough concentrations, the change from baseline in plasma PKK trough concentrations was similar among Switch Subjects and OLE subjects who received donidalorsen-4 in Trial CS5. See PD results for more details.

PD Results

OLE Subjects

For OLE subjects, the plasma PKK trough concentrations in Trial CS7 compared to baseline levels prior to receiving treatment in Trial CS5 is reported in [Table 60](#). PD results in Trial CS7 were comparable to those observed in Trial CS5 in which subjects who received donidalorsen-4 had greater reductions from baseline in plasma trough PKK relative to subjects who received donidalorsen donidalorsen-8.

Table 60. Mean Plasma PKK Trough Concentrations and Percent Change From Baseline (Run-In Trial CS5), Trial CS7

Metric and Timepoint	Q4W in CS7			Q8W in CS7
	Q4W in CS5 (N=44)	Q8W in CS5 (N=6)	Placebo in CS5 (N=19)	Q8W in CS5 (N=14)
Mean (SD) plasma PKK trough concentrations (µg/mL)				
Baseline (index Run-In)	128.63 (42.3)	141.38 (43.6)	121.11 (27.6)	144.5 (69.3)
CS7 Week 9 (predose)	28.7 (18.5)	30.3 (18.9)	31.8 (18.7)	66.7 (29.6)
CS7 Week 25 (predose)	33.3 (17.8) (n=25)	25.9 (12.7) (N=4)	25.7 (15.0) (N=12)	78.4 (19.4) (n=9)
Mean (SD) percent change of plasma PKK from baseline (%)				
CS7 Week 9	-76.40 (16.0)	-79.4 (9.1)	-65.9 (17.3)	-45.1 (28.0) (N=13)
CS7 Week 25	-71.5 (15.4) (N=25)	-80.9 (8.5) (N=4)	-78.6 (12.4) (N=12)	-36.1 (29.6) (N=9)

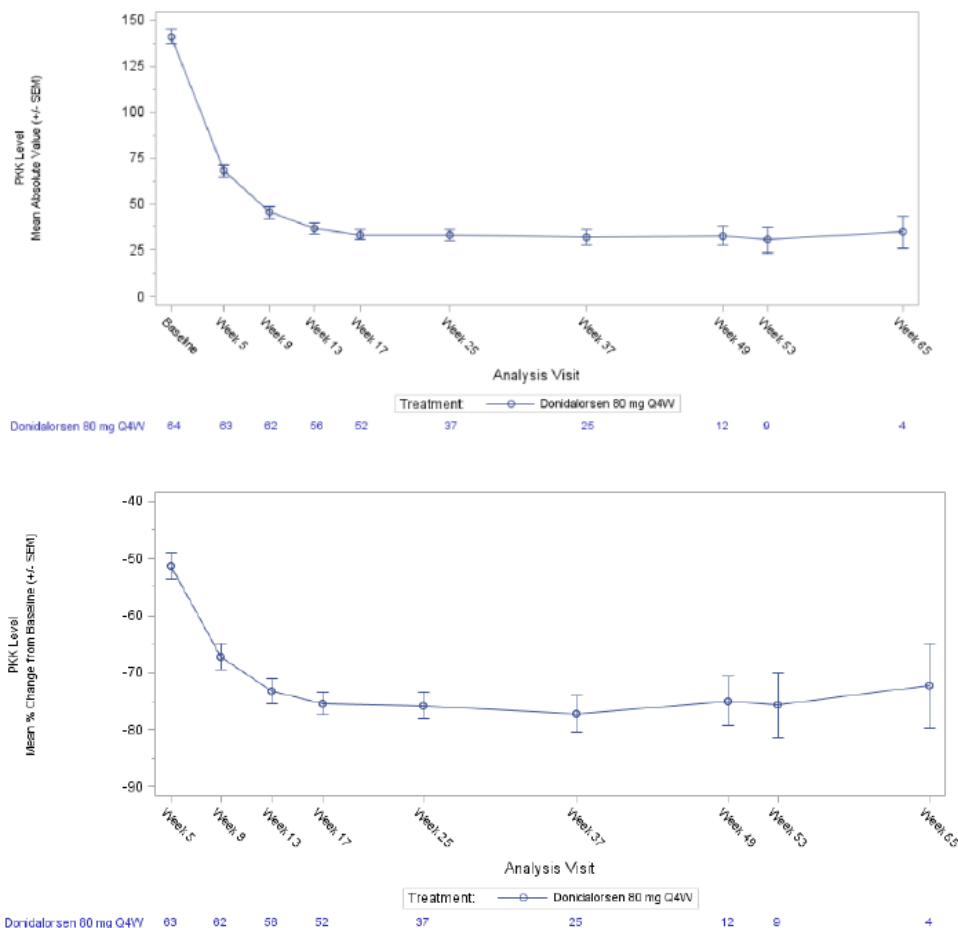
Source: Adapted from Table 14.2.2.7.1. CSR CS7

Abbreviations: CSR, clinical study report; N, number of subjects; PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

Switch Subjects

The trough plasma PKK concentrations over time are shown in [Figure 62](#). The mean baseline PKK plasma concentration in Switch Subjects was 141.32 µg/mL, which was similar to those reported during the Trial CS5 Run-In Period for subjects who rolled over from Trial CS5. PKK levels dropped by the first assessment time point at Week 5, reached nadir by Week 17, and thereafter remained stable. At Week 17, the mean PKK reduction from baseline at Week 17 was 75.4%. This magnitude reduction is comparable to that observed among subjects who received donidalorsen-4 during Trial CS5.

Figure 62. Mean (SE) PKK Trough Concentrations (Top) and Percent Change From Baseline (Bottom) Over Time for Switch Subjects



Source: Figure 14.2.2.3.2, CSR CS7

Abbreviations: CSR, clinical study report; PKK, prekallikrein; Q4W, every 4 weeks; SE, standard error; SEM, standard error of the mean

Immunogenicity Results

OLE Subjects

In OLE subjects treated with donidalorsen-4 during Trial CS7, 25/69 subjects (36.2%) had treatment-emergent ADAs, including subjects who were ADA-negative at Trial CS5 baseline and developed ADAs during the treatment (i.e., treatment induced) and subjects who were ADA-positive at baseline and ADA titers increased during treatment (i.e., treatment boosted). Among subjects treated with donidalorsen-4 (N=69), there were 16/44 (36.4%), 3/6 (50.0%), and 6/19 (31.6%) subjects positive for treatment-emergent ADAs in the Index/OLE Q4W, Index Q8W/OLE Q4W, and Index placebo/OLE Q4W groups, respectively. In the Index/OLE Q8W group, 3/14 subjects (21.4%) had treatment-emergent ADAs. All subjects who were reported to have treatment-emergent ADAs in Trial CS5 had the same status in Trial CS7.

For subjects who continued taking the same dosing regimen in both Trial CS5 and Trial CS7 (i.e., Index Q4W/OLE Q4W and Index Q8W/OLE Q8W), the summary of plasma donidalorsen trough concentrations stratified by ADA status are reported in [Table 61](#).

Table 61. Summary of Donidalorsen Trough Concentrations (ng/mL) by Treatment Group and ADA Status, Trial CS7

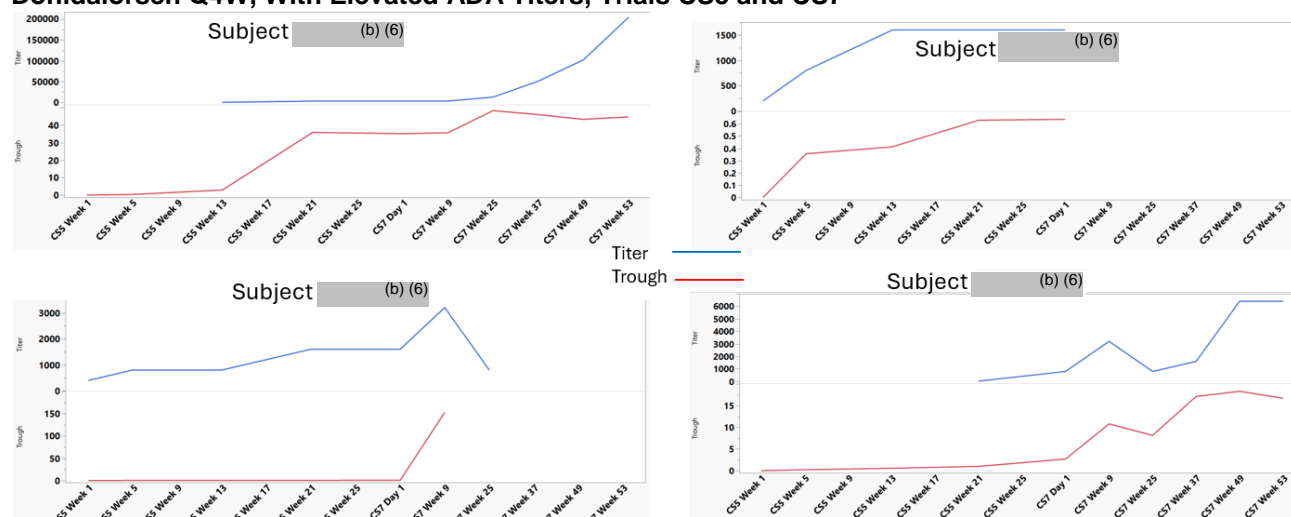
Timepoint	Statistic	Index Q4W/OLE Q4W (N=44)		Index Q8W/OLE Q8W (N=14)	
		Negative ADA	Treatment- Emergent ADA	Negative ADA	Treatment- Emergent ADA
CS7 Week 1	N	24	14	6	3
	Geometric mean (CV%)	0.78 (63.9)	1.63 (176.7)	0.33 (47.12)	0.289 (83.32)
	Median (range)	0.83 (0.27, 2.32)	1.96 (0.36, 35.2)	0.098 (0.00, 0.43)	0.193 (0.19, 0.67)
CS7 Week 9	N	26	14	6	3
	Geometric mean (CV%)	0.98 (64.57)	3.91 (410.0)	0.37 (61.57)	0.62 (84.29)
	Median (range)	1.05 (0.24, 2.22)	2.32 (0.371, 153.0)	0.31 (0.00, 0.77)	0.89 (0.27, 0.99)
CS7 Week 25	N	16	8	4	2
	Geometric mean (CV%)	0.95 (90.33)	4.46 (450.27)	0.34 (34.91)	1.43 (425.21)
	Median (range)	0.98 (0.27, 4.26)	7.31 (0.45, 48.4)	0.31 (0.11, 0.42)	2.62 (0.43, 4.82)

Source: Table 14.2.3.5.1. CS7 CSR

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; CV, coefficient of variation; N, number of subjects; OLE, open-label extension, Q4W, every 4 weeks, Q8W, every 8 weeks

A trend of increasing geometric mean and median $C_{trough,ss}$ was noted in ADA-positive subjects who continued taking donidalorsen throughout Trial CS5 and Trial CS7. Four subjects who received donidalorsen-4 in both Trial CS5 and Trial CS7 had ADA titers rising to >1600 and also had a notable increase in $C_{trough,ss}$ during Trial CS7, including Subject (b) (6) who already had high ADA titer and high C_{trough} in Trial CS5 ($C_{trough,ss}$ at Week 17 was 37.6 ng/mL and ADA titer value at Week 21 was 3200 in Trial CS5). The time-profiles of ADA titers and $C_{trough,ss}$ for these four subjects are shown in [Figure 63](#).

Figure 63. Time Profiles For ADA Titers and Plasma C_{trough} (ng/mL) for Subjects Receiving Donidalorsen Q4W, With Elevated ADA Titers, Trials CS5 and CS7



Source: Clinical pharmacology reviewer's analysis based on adpc.xpt and adis.xpt for CS5 and CS7
Abbreviations: ADA, antidrug antibody; C_{trough}, trough concentration; Q4W, every 4 weeks

As most OLE subjects had PKK plasma trough concentrations at Week 25 available in the interim report, a summary of mean trough plasma PKK levels and percent change from baseline for OLE subjects up to 25 weeks and stratified by ADA status is shown in [Table 62](#). Within each dosing frequency group, the percent change in PKK from Trial CS5 baseline were similar irrespective of ADA status. Greater reductions in PKK were observed among subjects who received donidalorsen-4 relative to subjects who received donidalorsen donidalorsen-8.

Table 62. Mean (SD) Trough Plasma PKK Concentrations and Percent Change From Baseline in OLE Subjects

Timepoints	OLE Q4W ^a		OLE Q8W	
	ADA Negative	Treatment-Emergent ADA	ADA Negative	Treatment-Emergent ADA
PKK concentration (µg/mL)				
Run-In baseline in CS5	120.7 (41.7) (N=38)	135.0 (35.5) (N=25)	157.5 (79.1) (N=10)	111.0 (7.6) (N=3)
Week 9	31.0 (19.3) (N=38)	34.3 (18.4) (N=23)	74.08 (24.6) (N=9)	43.8 (42.7) (N=3)
Week 25	32.0 (18.4) (N=22)	28.1 (14.0) (N=15)	78.9 (18.0) (N=7)	76.4 (32.5) (N=2)
Percent change from CS5 baseline (%)				
Week 9	-72.1 (18.9) (N=38)	-74.3 (12.5) (N=23)	-40.2 (27.0) (N=9)	-61.7 (35.3) (N=3)
Week 25	-71.6 (16.0) (N=22)	-77.7 (11.1) (N=15)	-36.7 (32.5) (N=7)	-34.1 (25.8) (N=2)

Source: Adapted from Table 14.2.3.4.1, CSR CS7

^a Included subjects who received either same dosing frequency (Q4W) or different dosing frequency (Q8W) or placebo in CS5.

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; N, number of subjects; OLE, open-label extension, PKK, prekallikrein; Q4W, every 4 weeks, Q8W, every 8 weeks; SD, standard deviation

The HAE attack rate by ADA status is summarized in [Table 63](#). Overall, ADAs did not appear to have an impact on HAE attack rate change from index Run-In baseline. It was noted that a lower

percent HAE attack reduction was observed in the Index Q8W/OLE Q4W treatment group among subjects with treatment-emergent ADAs as compared to that in ADA-negative subjects. A definitive conclusion cannot be made due to the small sample size. Subjects who received the same dosing regimens in both the Index and OLE trials (i.e., Index Q4W/OLE Q4W and Index Q8W/OLE Q8W groups) had comparable HAE attack rate reduction irrespective of ADA status.

Table 63. Mean (SD) HAE Attack Rate Per 4 Weeks and Comparison With Index (Trial CS5) Run-In Baseline by ADA Status in OLE Subjects

Time Period	Index Placebo/OLE Q4W	Index Q4W/OLE Q4W	Index Q8W/OLE Q4W	Index Q8W/OLE Q8W
ADA-negative				
Run-In baseline in CS5	3.1 (2.3) (N=10)	3.4 (2.0) (N=26)	6.6 (1.4) (N=2)	3.3 (2.3) (N=10)
Week 1 to Week 53 ^a	0.2 (0.3) (N=10)	0.1 (0.1) (N=26)	1.4 (1.4) (N=2)	0.1 (0.1) (N=10)
Percent change from CS5 baseline to Week 1 to Week 53	-93.3 (8.9) (N=10)	-97.7 (4.5) (N=26)	-80.8 (16.0) (N=2)	-91.2 (17.8) (N=10)
Treatment-emergent ADA				
Run-In baseline in CS5	2.9 (1.0) (N=6)	3.9 (2.5) (N=16)	2.37 (1.4) (N=3)	1.9 (0.7) (N=3)
Week 1 to Week 53 ^a	0.14 (0.3) (N=6)	0.3 (0.4) (N=16)	(0.8) (N=3)	0.2 (0.1) (N=3)
Percent change from CS5 baseline to Week 1 to Week 53	-96.8 (5.8) (N=6)	-92.4 (10.7) (N=16)	-59.9 (33.3) (N=3)	-92.0 (7.5) (N=3)

Source: Adapted from Table 14.2.3.6.1, CSR CS7

^a The Week 1 to Week 53 HAE attack rate is calculated as the number of HAE attacks occurring from Week 1 to Week 53 divided by the number of days the subject contributed to the period multiplied by 28 days.

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; HAE, hereditary angioedema; OLE, open-label extension; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation

In terms of safety associated with ADA status, Subject (b) (6) was reported to have experienced an anaphylactic reaction during Trial CS7 after the NDA data cutoff. This subject presented with high ADA titers (i.e., >1000) beginning at Week 21 in Trial CS5. ADAs remained elevated during Trial CS7 (based on the adis.xpt datasets for Trial CS5 and Trial CS7). This subject was randomized in Trial CS5 on (b) (6), and the anaphylaxis event was reported on (b) (6), following approximately 3 years of treatment with donidalorsen. Of note, this subject also experienced other hypersensitivity reactions (rash, urticaria etc.) prior to the anaphylaxis event. For additional details, refer to Section 7.6.

To evaluate the potential association between ADAs and hypersensitivity reactions, the onset of hypersensitivity reactions from all subjects with high ADA titers (>1000) at any time during Trial CS5 or Trial CS7 were compared with the timing of dosing, the most adjacent ADA titer, and donidalorsen plasma C_{trough}. The Applicant identified 35 subjects who were treated with donidalorsen and had an ADA titer >1000 during any time in Trial CS5 or Trial CS7, including data collected after the NDA data cutoff. Nine subjects were identified as having hypersensitivity reactions derived from the OCMQ of Hypersensitivity. Of these nine subjects, three ((b) (6) (b) (6)) had hypersensitivity reactions that occurred when ADA titer and donidalorsen plasma C_{trough} were high. The remaining subjects experienced hypersensitivity reactions when they were either ADA-negative or presented with low ADA titers. Thus, there is no clear association between high ADA titers and hypersensitivity.

Switch Subjects

The overall incidence rate of treatment-emergent ADAs in Switch Subjects who received donidalorsen-4 was 42.2% (27/64 subjects), with 8/64 (12.5%) subjects ADA-positive at baseline.

The summary of mean trough PKK concentrations and percent change from baseline at Week 17 by ADA status is shown in [Table 64](#). The magnitude of PKK change from baseline was similar irrespective of ADA status.

Table 64. Mean (SD) PKK Concentrations and Percent Change From Baseline by ADA Status in Switch Subjects

Metric and Timepoint	ADA Negative	Treatment-Emergent ADA
Trough plasma PKK concentration (µg/mL)		
Baseline	144.6 (29.2) (N=32)	138.5 (28.2) (N=27)
Week 17	34.0 (22.7) (N=24)	33.7 (17.0) (N=24)
Percent change from baseline (%)		
Week 17	-75.6 (16.5) (N=24)	-74.2 (13.8) (N=24)

Source: Adapted from Table 14.2.3.4.1. CSR CS7

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; N, number of subjects; PKK, prekallikrein; SD, standard deviation

The HAE rate by ADA status in Switch Subjects is summarized in [Table 65](#). The change in HAE attack rate was similar irrespective of ADA status.

Table 65. Mean (SD) HAE Attack Rate Per 4 Weeks and Percent Change From Baseline by ADA Status in Switch Subjects

Time Period	ADA Negative	Treatment-Emergent ADA
Baseline	0.9 (1.2) (N=32)	0.9 (1.5) (N=27)
Week 1 to 53 ^a	0.2 (0.3) (N=32)	0.4 (0.6) (N=27)
Change from baseline to Week 1 to 53	-0.64 (1.1) (N=32)	-0.5 (1.2) (N=27)

Source: Adapted from Table 14.2.3.6.2. CSR CS7

^a The Week 1 to Week 53 HAE attack rate is calculated as the number of HAE attacks occurring from Week 1 to Week 53 divided by the number of days the subject contributed to the period multiplied by 28 days.

Abbreviations: ADA, antidrug antibody; CSR, clinical study report; HAE, hereditary angioedema; SD, standard deviation

14.3. Bioanalytical Method Validation and Performance

The bioanalytical methods that were utilized to analyze donidalorsen concentrations in plasma and urine, as well as PKK concentrations in plasma are summarized in [Table 66](#).

Table 66. Bioanalytical Methods Summary for Donidalorsen and PKK in Human

Method ID	Sample Matrix	Testing Sites	Bioanalytical Method	Assay Description	Validation Status	Assay Validation Report	Clinical Studies
Donidalorsen							
ICD 672 – V1.00	Human plasma	(b) (4)	Hybridization ECL	Quantitation of donidalorsen and total full-length ASO	Validated - (b) (4)	721744-MV04 Addendum 1 Addendum 2	ISIS 721744-CS1 (Phase 1) ISIS 721744-CS2 (Phase 2a) ISIS 721744-CS3 (Phase 2 ^b) ISIS 721744-CS5 (Phase 3) ISIS 721744-CS7 (Phase 3 ^b) ISIS 721744-CS9 (Phase 3 Bioequivalence)
ICD 672.1 (b) (4)	Human urine	(b) (4)	Hybridization ELISA	Quantitation of donidalorsen and total full-length ASO	Partially Validated - (b) (4)	721744-MV05	ISIS 721744-CS1 (Phase 1)
Prekallikrein (PKK)							
US-EL-80-S5	Human serum/ plasma	(b) (4)	Chromogenic ELISA	Quantification of PKK	Validated	PKK-BRV02	ISIS 721744-CS1 (Phase 1) ISIS 721744-CS2 (Phase 2a)
ICD 934 (b) (4) Version 1.00 ICD 934 (b) (4) Version 2.00 ICD 934 (b) (4) Version 2.01 ICD 934 (b) (4) Version 2.02	Human plasma	(b) (4)	Fluorescence ELISA	Quantification of PKK	Validated - (b) (4)	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 ^b) ISIS 721744-CS5 (Phase 3) ISIS 721744-CS9 (Phase 3 Bioequivalence) ISIS 721744-CS7 (Phase 3 ^b)

Source: Table 3, Module 2.7.1. Summary of Biopharmaceutical Studies and Associated Analytical Methods
Abbreviations: ASO, antisense oligonucleotides; ELISA, enzyme-linked immunosorbent assay; PKK, prekallikrein

14.3.1. OSIS Inspection Results

The Office of Study Integrity and Surveillance (OSIS) inspection was requested for both analytical ((b) (4)) and clinical sites (Medpace Inc. Cincinnati, OH) for clinical Trial CS9, which provides pivotal evidence to support the use of the proposed AI presentation. The OSIS conducted a Remote Regulatory Assessment for the analytical site in (b) (4), for other NDA (b) (4) and a clinical inspection for the clinical site in September 2024 for another NDA (218614). OSIS concluded that data from these sites were reliable (DARRTS reference ID: 5471991 and 5450302).

14.3.2. Bioanalytical Methods For Donidalorsen in Plasma and Urine

14.3.2.1. Donidalorsen Concentration in Plasma

The bioanalytical method for analyzing donidalorsen concentrations in plasma are acceptable.

Donidalorsen concentrations in human plasma were determined using a hybridization (ECL) method developed by (b) (4) (Method Validation Report: 721744-MV04). The same bioanalytical method was used in support of Trials CS1, CS2, CS3, CS5, CS7, and CS9. This method assessed the total concentration of full-length ASO including fully conjugated, partially conjugated (i.e., donidalorsen with 1, 2, or 3 GalNAc sugar deletions),

and unconjugated donidalorsen. The reported assay range is 0.02nM to 10nM (i.e., 0.1735 ng/mL to 86.73 ng/mL). The dilution factors of 400- to 10,000-fold were evaluated during method validation and met acceptance criteria. No apparent hook effect was observed up to 5000nM. The method is deemed adequate to detect the full range of donidalorsen concentrations in clinical trials. The assay in-study performance was acceptable for all trials.

The bioanalytical method is summarized in [Table 67](#).

Table 67. Summary of Bioanalytical Method Validation for Quantitation of Donidalorsen in Human Plasma

Validation Report	Method Validation 721744-MV04 Final Report With Addendum 1 and Addendum 2
Method type	Hybridized ECL Assay
Matrix	Human Plasma
Analyte	Total full-length ASO (including fully conjugated, partially conjugated, and unconjugated donidalorsen).
Calibration range	0.02nM (0.1735 ng/mL) to 10nM (86.73 ng/mL)
LLOQ	0.02000nM (0.1735 ng/mL)
Dilution linearity	2000nM diluted 400-, 2000-, and 10,000-fold. No apparent hook effect was observed at concentrations up to 5000nM
QC range	0.02, 0.05, 0.06, 1.5, 7.5, 9, and 10nM
Interassay precision range	≤11.6%
Interassay accuracy range	2.11% to 16.7%
Stability duration at room temperature	24 hours at room temperature
Long-term stability and temperature	176 days at -25°C ±5°C 2480 days at -80°C ±10°C

Source: Reviewer-generated table based on Table 16, Module 2.7.1. Summary of Biopharmaceutical Studies and Associated Analytical Methods

Abbreviations: ASO, antisense oligonucleotides; ECL, electrochemiluminescence; LLOQ, lower limit of quantification; QC, quality control

The cross-reactivity of the assay was assessed with three putative metabolites ISIS 834440, ISIS 834441, and ISIS 834443, which are 19-mer, 10-mer, and 5-mer metabolites of unconjugated donidalorsen. There was a 105% cross-reactivity with ISIS 834440 (19-mer metabolite of unconjugated donidalorsen), while there was only <0.267% cross-reactivity with both ISIS 834441 (10-mer metabolite) and ISIS 834443 (5-mer metabolite). The cross-reactivity potential with 19-mer metabolites may affect the assessment of systemic exposure of full length donidalorsen. However, 19-mer metabolite was not present either in the plasma collected at 2 hours postdose based on Trial CS01-BA04 or in pooled human urine collected over 24 hours after fourth monthly dose of donidalorsen based on Trial 721744-IS10. Although cross-reactivity with other chain-shortened metabolites from 18-mer to 11-mer were not assessed, 11-mer to 18-mer metabolites were not found in the plasma collected at 2 hours postdose based on Trial CS01-BA04. The data suggest that interference due to the presence of these chain shortened metabolites is not likely.

This method was designed to evaluate the total concentrations of full-length ASO, which includes fully conjugated, partially conjugated (i.e., donidalorsen with 1, 2, or 3 GalNAc sugar deletions), and unconjugated donidalorsen in plasma. However, the calibration standard was prepared with fully conjugated donidalorsen (ISIS 721744). The ability of the method to quantify unconjugated donidalorsen (ISIS 780124) was assessed by quantitating low (0.06nM) and high

(7.5nM) QC levels of ISIS 780124 using a fully conjugated donidalorsen (ISIS 721744) standard curve. In addition, to evaluate the ability of the assay to quantitate both full-length ASOs (ISIS 780124 and ISIS 721744) when both are present, low- and high-level QCs of ISIS 780124 and ISIS 721744 were prepared and combined 1:1 to reach both low and high QC levels (0.06nM and 7.5nM, respectively). QC results met the acceptance criteria and demonstrated that the assay is capable of quantifying both total-full length conjugated donidalorsen (ISIS 721744) and unconjugated donidalorsen (ISIS 780124). Based on the principle of the assay (sample is hybridized to a complement capture probe) and results with ISIS 780124 QC samples, the assay is capable of quantifying total full-length ASO regardless of GalNAc conjugation status.

14.3.2.2. Donidalorsen Concentration In Urine

There is insufficient information to determine whether the bioanalytical method is acceptable to measure donidalorsen concentrations in urine.

The bioanalytical method for analyzing donidalorsen concentrations in urine was partially validated at (b) (4) (Method Validation Report: 721744-MV05). The method was used to analyze urine samples from Trial CS1. The detection range was 0.15nM (1.301 ng/mL) to 20nM (173.5 ng/mL). The dilution linearity was assessed between 200-fold and 4000-fold and met acceptance criteria. No apparent hook effect was observed at concentrations up to 53040nM. The bioanalytical method is sufficient to detect the range of donidalorsen urine concentrations in Trial CS1. The summary of bioanalytical method is summarized in [Table 68](#).

Table 68. Summary of Bioanalytical Method Validation of Quantitation of Donidalorsen in Human Urine

Validation Report	721744-MV05
Method type	Hybridization ELISA assay
Matrix	Human urine
Analyte	Total full-length ASO (including fully conjugated, partially conjugated, and unconjugated donidalorsen).
Calibration range	0.15nM (1.301 ng/mL) to 20nM (173.5 ng/mL)
LLOQ	0.15nM (1.301 ng/mL)
Dilution linearity	2000nM diluted 200-, 1000-, and 4000-fold. No apparent hook effect was observed at concentrations up to 53040nM.
QC range	0.15, 0.3, 0.45, 3, 15, 18, and 20nM
Interassay precision range	≤8.82%
Interassay accuracy range	-6.19% to 3.41%
Stability duration at room temperature	Not reported
Long-term stability and temperature	23 days at -80°C ±10°C

Source: Reviewer-generated table based on Table 17, Module 2.7.1. Summary of Biopharmaceutical Studies and Associated Analytical Methods

Abbreviations: ASO, antisense oligonucleotides; ELISA, enzyme-linked immunosorbent assay; LLOQ, lower limit of quantification; QC, quality control

Notably, the long-term sample stability was only assessed for up to 23 days, while samples collected during Trial CS1 were likely stored for a longer duration than the established stability range at the time of analysis, as urine samples were analyzed between November 19, 2018, and January 8, 2019 (60 days). Therefore, there is insufficient information to determine whether the bioanalytical method is acceptable to quantify donidalorsen concentrations in human urine due to the concern of sample stability. However, the observed concentrations of donidalorsen in urine

samples from healthy subjects over 24-hours following both single and multiple doses of donidalorsen in Trial CS1 was consistent with the urine PK data for eplontersen (NDA 217388), another GalNAc-conjugated ASO product with the same nucleotide chain length (20-mer) and identical THA linker to the one found on donidalorsen.¹¹

14.3.3. Bioanalytical Method for PKK In Plasma

The bioanalytical method for analyzing PKK concentrations in human plasma is acceptable.

Two bioanalytical methods were used in the donidalorsen clinical program. The first method, developed by (b) (4), was used to support Trials CS1 and CS2. The other method, developed by (b) (4), was used to support Trials CS3, CS5, CS7, and CS9. The method is deemed adequate to detect the full range of donidalorsen concentrations in clinical trials. The assay in-study performance was acceptable for all trials.

The method developed by (b) (4) (Method Validation Report: PKK-BRV02) utilized commercial Prekallikrein and Kallikrein Human ELISA Kit (Abcam, No. ab171015), which was validated and optimized by (b) (4). The detection range is 7.52 to 2560 µg/mL. The minimum required dilution (MRD) of 1:4000 and a maximum allowed dilution (MAD) of 1:32000 were evaluated.

The method developed by (b) (4) (Method Validation Report: PKK-BRV01) also utilized Human Prekallikrein (Abcam) and was validated and optimized by (b) (4). The detection range was 0.47 ng/mL to 60 ng/mL. The 1:8000 minimum required dilution (MRD) was chosen to ensure all samples were quantified in range. After implementing the MRD, the detection range was 3.76 µg/mL to 480 µg/mL. The detection range after implementing the MRD is sufficient to assess plasma PKK concentrations in clinical samples.

The summary of the two method validations is shown in [Table 69](#).

Table 69. Summary of Bioanalytical Method Validation for Quantitation of PKK in Human Plasma

Validation Report	PKK-BRV02 Report	PKK-BRV01 Report, Addendum 1 and Addendum 2
Method type	Chromogenic ELISA	Fluorescence ELISA
Site	(b) (4)	(b) (4)
Matrix	Human plasma	Human plasma
Analyte	PKK	PKK
Calibration range	7.52- 2560 µg/mL	0.470 to 60.0 ng/mL, After 1:8000 dilution correction: 3.760 to 480 µg/mL
LLOQ	7.52 µg/mL	0.47 ng/mL (after implementing dilution correction: 3.76 µg/mL)
QC range	0.00188 to 0.080 µg/mL (dilutability: x4000 to x32000)	0.839, 4.29, 11.5, 45 ng/mL
Interassay precision range	≤13.5%	≤14.3%

¹¹ See FDA Summary Review for Waniua (eplontersen NDA 217388), dated December 20, 2023, available at <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm>.

Validation Report	PKK-BRV02 Report	PKK-BRV01 Report, Addendum 1 and Addendum 2
Interassay accuracy range	-12.3% to -1.4%	-1.4 to 2.2%
Stability duration at room temperature	7 days at room temperature	24 hours at room temperature
Long-term stability and temperature	9 months at -80°C and 12 months at -20°C	371 days at -80°C

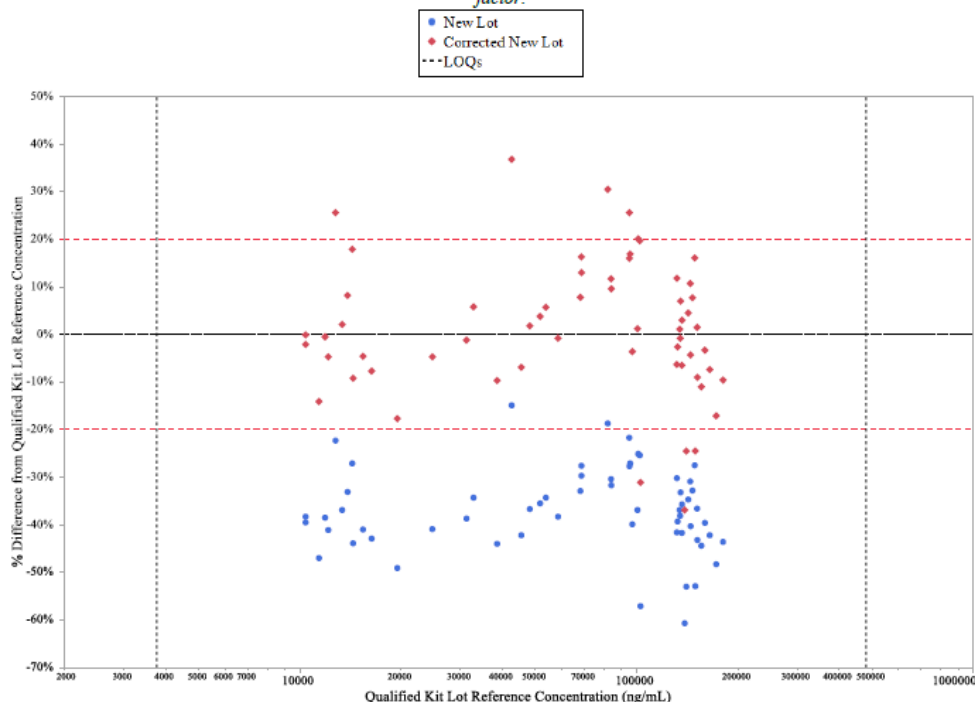
Source: Reviewer-generated table based on Table 19, Module 2.7.1. Summary of Biopharmaceutical Studies and Associated Analytical Method; Final Report PKK-BRV02; Final Report PKK-BRV01 and Addendum 2
Abbreviations: ELISA, enzyme-linked immunosorbent assay; LLOQ, lower limit of quantification; PKK, prekallikrein; QC, quality control

No cross validation was performed between the two bioanalytical methods and two analytical sites. However, based on PD results obtained from Trial CS2 (assessed by (b) (4)) and Trial CS5 (assessed by (b) (4)) in adult subjects with HAE who received the same dosing regimen (donidalorsen-4), a similar nadir in the percent reduction of plasma PKK trough concentration from baseline was demonstrated (about 61% at Week 17 for Trial CS2 and about 74% at Week 25 for Trial CS5), suggesting that these two methods are comparable.

The plasma PKK sample long-term stability was evaluated for up to 613 days (18 months) at -80°C. During the assessment of long-term stability at around 1 year (Day 317), results showed a negative bias of 26% to 56% at both low (1.13 ng/mL and 1.55 ng/mL) and middle level (6.72 ng/mL and 8.66 ng/mL) concentrations until a correction factor was applied to the theoretical values. The correction factor was used to justify the difference in antibody concentrations among different commercial kit lots. The difference among different commercial lots was first discovered during the analysis of samples from Trial CS5, when endogenous quality control concentrations obtained from new kit lots demonstrated consistently lower PKK concentrations compared to historical data. A correction factor was therefore used to correct the new sample concentrations using the new kits lot and resulted in consistent values within ±20% of reference results obtained using the qualified kit lots, as shown in [Figure 64](#).

Figure 64. Percent Difference Between New Kit Lot Concentration and Qualified Kit Lot Reference Concentration

Percent Difference Between the New Kit Lot Concentrations and the Qualified Kit Lots Reference Concentration
Note: Blue circles represent new kit lot original concentrations prior to, and red diamonds represent concentration after application of the correction factor.



Source: Figure 2, 721744-CS05BRA01 Appendix E
Abbreviations: LOQ, limit of quantification

As the new assay kits consistently result in lower PKK concentrations compared to the previously qualified kit for the same samples, the stability assessment based on a comparison to corrected theoretical values is acceptable. After applying the correction factor, 3 out of 4 QC levels were within the acceptance range in the long-term stability assessment at Day 371 (1.13 ng/mL, 1.55 ng/mL and 8.66 ng/mL), supporting the long-term stability for around 1 year. The long-term stability was further assessed at 18 months. Three out of four QC levels were within the acceptable range (0.67 ng/mL, 0.92 ng/mL and 5.14 ng/mL). Overall, sample stability was demonstrated for up to 18 months. The established long-term stability is sufficient to support the stability of trial samples in Trials CS5 and CS9.

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

Immunogenicity of donidalorsen was evaluated in a phase 1 trial (CS1) in healthy subjects as well as in four trials in subjects with HAE (CS2, CS3, CS5, and CS7). Refer to the immunogenicity assay review by the Office of Pharmaceutical Quality Research for details regarding the immunogenicity assay validation (DARRTS reference ID: 5643653). The ADA assay is generally acceptable, and data is interpretable.

Treatment-emergent ADAs were assessed. The definition of treatment-emergent ADAs included both treatment-induced ADAs, defined as ADAs developed de novo following drug administration in a subject without pre-existing ADAs; and treatment-boosted ADAs, defined as pre-existing ADAs that were boosted to a higher-level following drug administration (i.e., any time after the initial drug administration the ADA titer is greater than baseline titer by a factor of 8-fold or more).

The incidence rate of treatment-emergent ADAs was 16.7% following administration of donidalorsen 20 to 80 mg once every 4 weeks for 12 weeks in healthy participants (Trial CS1, dose-dependence not observed), 35.3% following donidalorsen 80 mg once every 4 weeks for 12 weeks in subjects with HAE (Trial CS2), and 20% to 22% following donidalorsen 80 mg once every 4 weeks or every 8 weeks for 24 weeks in subjects with HAE (Trial CS5), respectively. The incidence rate of treatment-emergent ADAs was generally consistent across populations and similar between the donidalorsen-4 and donidalorsen-8 regimens. Treatment-emergent ADAs were characterized with a median onset ranging from 15 days to 175 days postdose and median peak titer ranged from 25 to 800. A summary of immunogenicity incidence rates and peak titers across trials is shown in [Table 70](#).

Table 70. Immunogenicity Incidence Rates From Clinical Trials for Donidalorsen

Study Number (Population)	Treatment and Dosing Schedule	ADA Detected at Baseline ^a n/N (%)	Incidence of Treatment-Emergent ADA n/N (%)	Onset of Treatment-Emergent ADA ^b Median (Range)	Peak Titer Median (Range)
ISIS 721744-CS1 (Healthy participants)	donidalorsen 20 mg Q4W for 12 weeks	0/6 (0%)	0/6 (0%)	NA	NA
	donidalorsen 40 mg Q4W for 12 weeks	1/6 (16.7%)	2/6 (33.3%)	88.5 (1.00, 176)	388 (25.0, 750)
	donidalorsen 60 mg Q4W for 12 weeks	0/6 (0%)	2/6 (33.3%)	175 (174, 176)	25.0 (25.0, 25.0)
	donidalorsen 80 mg Q4W for 12 weeks	0/6 (0%)	0/6 (0%)	NA	NA
	Overall (donidalorsen)	1/24 (4.2%)	4/24 (16.7%)	175 (87.5, 176)	25 (25 to 388)
	Pooled Placebo single/Q4W for 12 weeks	1/8 (12.5%)	0/8 (0%)	1.00 (1.00, 1.00)	750 (750, 750)
ISIS 721744-CS2 (Patients with HAE)	donidalorsen 80 mg Q4W for 16 weeks	3/17 (17.6%)	6/17 (35.3%)	15 (1, 111)	100 (100 to 200)
	Pooled Placebo, Q4W for 16 weeks	1/6 (16.7%)	0/6 (0%)	1.00 (1.00, 100)	25.0 (25, 25)
ISIS 721744-CS3 (Patients with HAE)	Index Placebo (HAE-1/HAE-2)	0/5 (0%)	4/5 (80%)	393.0 (183.0, 774.0)	412.5 (25.0, 1200)
	Index Donidalorsen (HAE-1/HAE-2)	3/12 (25%)	7/12 (58.3%)	56.0 (1.0, 335.0)	800 (400, 1600)
	Overall HAE-1/HAE-2 (donidalorsen)	3/17 (17.6%)	11/17 (64.7%)	84.5 (8.0, 393.0)	800 (300, 1600)
	nC1-INH-HAE	0/3 (0%)	3/3 (100%)	111.0 (15.0, 416.0)	800 (25, 6400)
	Overall HAE-1/HAE-2/nC1-INH-HAE	3/20 (15%)	14/20 (70%)	113.0 (15.0, 449.0)	800 (200, 1600)

Study Number (Population)	Treatment and Dosing Schedule	ADA Detected at Baseline ^a n/N (%)	Incidence of Treatment-Emergent ADA n/N (%)	Onset of Treatment-Emergent ADA ^b Median (Range)	Peak Titer Median (Range)
ISIS 721744-CS5 (Patients with HAE)	donidalorsen 80 mg Q4W for 25 weeks	4/45 (8.9%)	9/45 (20%)	147.0 (87.0, 169.0)	200 (50, 1600)
	donidalorsen 80 mg Q8W for 25 weeks	2/23 (8.7%)	5/23 (21.7%)	88.0 (29.0, 141.0)	100 (25, 800)
	Overall Donidalorsen	6/68 (8.8%)	14/68 (20.6%)	140.5 (29.0, 169.0)	150 (50, 800)
	Pooled Placebo, Q4W and Q8W for 25 weeks	2/22 (9.1%)	1/22 (4.5%)	170.0 (170.0, 170.0)	25.0 (25, 25)
ISIS 721744-CS7 (Patients with HAE)	Index/OLE donidalorsen-4 ^c	4/44 (9.1%)	16/44 (36.4%)	169.0 (113.5, 253.5)	300 (100.0, 2000.0)
	Index donidalorsen-8/OLE donidalorsen-4 ^d	1/6 (16.7%)	3/6 (50.0%)	29.0 (29.0, 232.0)	800 (800, 800)
	Index placebo/OLE donidalorsen-4 ^e	2/19 (10.5%)	6/19 (31.6%)	168.5 (57.0, 169.0)	75.0 (25.0, 100.0)
	OLE donidalorsen-4 ^f	7/69 (10.1%)	25/69 (36.2%)	169.0 (57.0, 171.0)	200 (100, 800)
	Index/OLE donidalorsen-8 ^g	1/14 (7.1%)	3/14 (21.4%)	141.0 (88.0, 169.0)	50.0 (25.0, 100.0)
	Switch Patients (donidalorsen-4) ^h	8/64 (12.5%)	27/64 (42.2%)	109.0 (29.0, 174.0)	400.0 (100.0, 800.0)
	Overall (OLE donidalorsen-4, including Switch Patients) ⁱ	15/133 (11.3%)	52/133 (39.1%)	143.5 (43.0, 172.5)	400.0 (100.0, 800.0)

Source: Table 18, Module 2.7.2 Summary of Clinical Pharmacology

^a ADA Baseline is defined as the ADA status of the last sample prior to the first donidalorsen dose.

^b For Trial ISIS 721744-CS1 and Trial ISIS 721744-CS2, data represents onset of ADA in subjects who are overall positive for ADA, including the subjects who are positive at Baseline (irrespective of whether subjects were treatment-emergent or not).

^c Subjects treated with donidalorsen 80 mg every 4 weeks during both the index and OLE trials.

^d Subjects treated with donidalorsen 80 mg every 8 weeks during the Index Trial and every 4 weeks during the OLE trial.

^e Subjects treated with placebo during the Index Trial and donidalorsen 80 mg every 4 weeks during the OLE trial.

^f All subjects treated with donidalorsen 80 mg every 4 weeks during the OLE part of Trial ISIS 721744-CS7 (regardless of treatment received in the Index Trial).

^g Subjects treated with donidalorsen 80 mg every 8 weeks during both the index and OLE trials.

^h Subjects previously treated with lanadelumab, berotralstat, or C-1 esterase inhibitor who switched to the donidalorsen-4 treatment.

ⁱ All subjects treated with OLE donidalorsen-4 regardless of index treatment (including Switch Subjects).

Abbreviations: ADA, antidrug antibodies; HAE-1, hereditary angioedema type I; HAE-2, hereditary angioedema type II; n, number of subjects per category; N, number of subjects in trial; NA, not applicable; nC1-INH-HAE, hereditary angioedema formerly type III; OLE, open-label extension; Q4W, every 4 weeks; Q8W, every 8 weeks

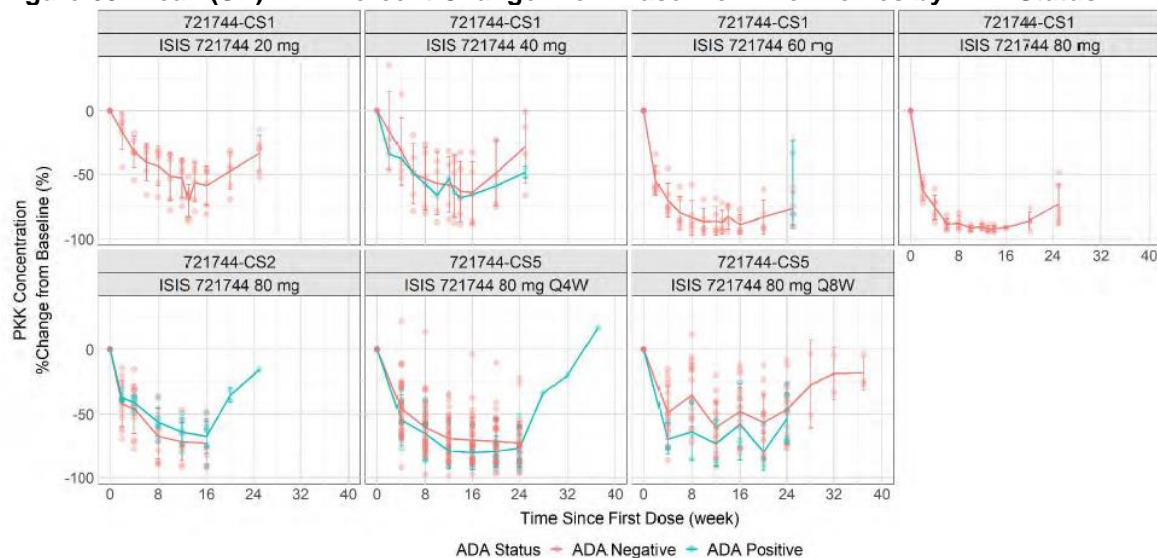
Impact of Immunogenicity on Pharmacokinetics

Based on results from Trial CS5 and Trial CS7, an increase in C_{trough} was observed in some ADA-positive subjects who also had high ADA titers. The increase in C_{trough} was noted in some Rollover subjects from Trial CS5 who continued receiving the same dosing regimen of donidalorsen-4 in Trial CS7. These subjects also experienced an increase in ADA titers during Trial CS7. There is likely an association between presence of ADA and increased $C_{trough,ss}$, possibly due to the binding of ADAs to donidalorsen which extended its plasma half-life. See Section [14.2.6](#) for more details.

Impact of Immunogenicity on Pharmacodynamics

The impact of ADAs on pharmacodynamics was assessed by comparing trough PKK concentration-time profiles by ADA status. ADAs did not appear to impact the mean PD response, as shown in [Figure 65](#).

Figure 65. Mean (SD) PKK Percent Change From Baseline-Time Profiles by ADA Status



ines, mean; error bar, standard deviation; circles, individual observations. Nominal time since first dose was used. Note: values below the lower limit of quantification were excluded from the plot.

Source: Figure 19, Report 721744-PPK01

Abbreviations: ADA, antidrug antibody; PKK, prekallikrein; SD, standard deviation

Impact of Immunogenicity on Efficacy

There was no apparent effect of ADAs on efficacy based on the HAE attack rate observed in Trial CS5 and Trial CS7. However, the small sample size of ADA-positive subjects precludes making a definitive conclusion. See Sections [14.2.4](#) and [14.2.6](#) for more details.

Impact of Immunogenicity on Safety

In Trial CS5, a higher proportion of ADA-positive subjects who received donidalorsen-4 reported injection site reactions, while no ADA-positive subjects from the donidalorsen-8 group reported injection site reactions. Although a higher peak ADA titer was observed among subjects in the donidalorsen-4 group compared to that in the donidalorsen-8 group, the peak titer was reported by Week 13 to Week 21, which was later than the time of the first injection site reaction reported in most subjects. There is thus no clear temporal association between high ADA titers and the onset of injection site reactions. Given the small sample size, a definitive conclusion on the impact of ADAs on safety cannot be drawn. See Section [14.2.4](#) for more details.

In Trial CS7, a trend of increase in C_{trough} was observed in subjects with high ADA titers (>1000). One subject ((b) (6)) who had very high ADA titers (102400) during Trial CS7 reported an anaphylaxis reaction after the date of NDA data cutoff. The association between high

ADA titers and hypersensitivity reactions was explored among subjects who experienced high ADA titers (>1000) at any time during Trial CS5 or Trial CS7. As hypersensitivity reactions were also observed in most subjects who were ADA-negative or had low ADA titers at the time hypersensitivity occurred, there is no clear association between ADA titers and hypersensitivity. See Section [14.2.6](#) for more details.

14.5. Pharmacometrics Assessment

14.5.1. Population PK Analysis

14.5.1.1. Review Summary

In general, the Applicant's population PK analysis is considered acceptable for the purpose of describing the pharmacokinetics/pharmacodynamics of donidalorsen and providing exposure metrics for ER analyses. The Applicant's analyses were verified by the Agency, with no significant discordance identified.

More specifically, the developed model was used to support the current submission as outlined in [Table 71](#).

Table 71. Specific Comments on Applicant’s Final Population PK Model

Utility of the Final Model in Product Labeling		Agency’s Comments	
Support Applicant’s proposed labeling statements about intrinsic and extrinsic factors	Intrinsic factor	Population PK and PD analyses showed no clinically meaningful differences in the pharmacokinetics or pharmacodynamics of donidalorsen based on age, body weight, sex, race, ethnicity, (b) (4) disease status, mild renal impairment (eGFR $\geq$ 60 to $<$ 90 mL/min/1.73 m ²), or mild hepatic impairment (total bilirubin $\leq$ 1 $\times$ ULN and AST $>$ 1 $\times$ ULN, or total bilirubin $>$ 1 to 1.5 $\times$ ULN and any AST. Donidalorsen has not been studied in patients with moderate or severe renal impairment, end-stage renal disease, or moderate or severe hepatic impairment.	The statement is acceptable (Figure 68 and Figure 71). The number of subjects with mild hepatic impairment (n=2) is limited to draw a conclusion on the impact of mild hepatic impairment on PK/PD.
	Pharmacokinetics	Population estimates (geometric mean) of C _{max,ss} , C _{trough,ss} , and AUC _{τ,ss} are presented in the proposed USPI. No accumulation of donidalorsen C _{max} and AUC was observed in plasma after repeated dosing every 4 weeks. The population estimate of the terminal elimination half-life of donidalorsen in a typical patient with HAE is approximately 1 month.	The statement is acceptable (Table 76).
		The population estimate of apparent volume of distribution for the central (V _c /F) and peripheral (V _p /F) compartment were 69.8 L and 1840 L, respectively.	The statement is acceptable (Table 74)
Derive exposure metrics for exposure-response analyses	C _{max,ss} , C _{trough,ss} , AUC _{1344h,ss}		

Source: Module 5.3.3.5, Population Pharmacokinetic/Pharmacodynamic Analysis Report

Abbreviations: AST, aspartate aminotransferase; AUC, area under the concentration-time curve; AUC_{1344h,ss} area under the concentration-time curve at 1344 hours at steady state; AUC _{τ ,ss}, area under the plasma concentration-time curve during a dosage interval (τ) at steady state; C_{max}, maximum plasma concentration; C_{max,ss}, maximum plasma concentration at steady state; C_{trough,ss}, trough concentration at steady state; eGFR, estimated glomerular filtration rate; HAE, hereditary adverse event; n, number of subjects; PD, pharmacodynamic; PK, pharmacokinetic; ULN, upper limit of normal; USPI, U.S. Prescribing Information

14.5.1.2. Introduction

The primary objectives of Applicant's analysis were to:

- Develop a population PK model of donidalorsen using data from healthy volunteers and patients with HAE.
- Develop a population PK/PD model using data from healthy volunteers and patients with HAE.
- Perform simulations using the final models to support dose selection.

14.5.1.3. Model Development

14.5.1.3.1. Data

PK Data

The PK analyses for donidalorsen were based on PK data from one phase 1 trial in healthy subjects, one bioequivalence trial, one phase 2 trial, and one phase 3 trial in patients with HAE. Brief descriptions of the trials included are presented in [Table 72](#).

The below limit of quantification (BLQ) samples were excluded from the population PK modeling. It seems to be reasonable to exclude BLQ samples due to the low percent of BLQ samples (<5%). The final NONMEM data file for donidalorsen PK model development contained 4242 donidalorsen observations from 177 subjects, including 101 healthy volunteers and 76 HAE patients.

[Table 73](#) provides summary statistics of the baseline demographic covariates in the PK analysis dataset.

PD Data

The PK/PD analyses for donidalorsen were based on PK/PD data from one phase 1 trial in healthy subjects, one bioequivalence trial, one phase 2 trial, and one phase 3 trial in patients with HAE. Brief descriptions of the trials included are presented in [Table 72](#).

The BLQ samples were excluded from the population PK/PD modeling. It seems to be reasonable to exclude BLQ samples due to the low percent of BLQ samples (<5%). The final NONMEM data file for donidalorsen PK/PD model development contained 1159 donidalorsen observations from 177 subjects, including 101 healthy volunteers and 76 HAE patients.

[Table 73](#) provides summary statistics of the baseline demographic covariates in the PK/PD analysis dataset.

Table 72. Summary of Trials With PK/PD Sampling Included in Population PK and PK/PD Analysis

Study Number	Study Design	Study Population	Dosing Regimen, Route and Duration of Administration ^a	Total Number of Subjects	PK blood sampling schedule	PD (PKK) sampling schedule
ISIS 721744-CS1	Phase 1, single site, randomized, double-blind, placebo-controlled, multiple-dose, and dose-escalation study	Healthy volunteers	20, 40, 60, and 80 mg Q4W for 13 weeks (total of 4 doses). D1 & D57 SC [arm]; D29 & D85 SC [abdomen or thigh]	N = 32 (8 subjects per cohort; ISIS 721744: placebo = 6:2)	D1 & D85: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 h post-dose. D8, D15, D43, D71, D92, D99, D113, D127, D141, D176: anytime. D29, D57: pre-dose.	D1, D29, D57, D85: pre-dose. D15, D43, D71, D92, D99, D113, D141, D176: anytime.
ISIS 721744-CS9	Phase 1, single dose, single site, randomized, open-label, 2-period, 2-sequence, 2-treatment, crossover, bioequivalence study	Healthy volunteers	Single dose for each period: 80 mg, vial or autoinjector, SC [abdomen] with a 4-week (28 days) washout between study periods	N = 78	D1: pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 168, 336 h post-dose for each study period. Follow-up period at D28, D56, and D91 after last dose: anytime.	Period 1 D1 & Period 2 D1: pre-dose. Period 1 D4, D8, D15: anytime.
ISIS 721744-CS2	Phase 2a global study. Part A: randomized, double-blind, placebo-controlled; Part B: open-label.	Part A: patients with HAE-1/HAE-2; Part B: patients with HAE-nC1-INH	80 mg Q4W SC [arm, abdomen, or thigh] for 13 weeks (total of 4 doses)	Part A: N = 20 (ISIS 721744: placebo = 14:6); Part B: N = 3 PK subgroup: N = 4	D1 & D85: pre-dose, 1, 2, 4, and 6 h post-dose for PK subgroup or pre-dose and 2 h post-dose for all other patients. D29, D57: pre-dose. D15, D113, D141, D176: anytime.	D1, D29, D57, D85: pre-dose. D15, D113, D141, D176: anytime.
ISIS 721744-CS5	Phase 3, global, randomized, double-blind, placebo-controlled study	Patients with HAE-1 and HAE-2	Cohort A: 80 mg Q4W SC [arm, abdomen, or thigh] for 21 weeks (total of 6 doses). Cohort B: 80 mg Q8W SC [arm, abdomen, or thigh] for 21 weeks (total of 3 doses).	N=90 (Cohort A: Cohort B = 2:1; ISIS 721744: placebo = 3:1 in each cohort)	Cohort A: D1 & D141: pre-dose and 2 h post-dose. D29, D57, D85, D113: pre-dose. D169: anytime. Follow-up period at D28, D56, and D91 from end of treatment ^b : anytime. Cohort B: D1 & D113: pre-dose and 2 h post-dose. D57: pre-dose. D29, D85, D141, D169: anytime. Follow-up period at D28, D56, and D91 from end of treatment ^b : anytime.	Cohort A: D1, D29, D57, D85, D113, D141: pre-dose. D169: anytime. Follow-up period at D28, D56, and D91 from end of treatment ^b : anytime. Cohort B: D1, D57, D113: pre-dose. D29, D85, D141, D169: anytime. Follow-up period at D28, D56, and D91 from end of treatment ^b : anytime.

Source: Applicant's population PK report, Table 1

^a End of treatment is defined as 1 dosing interval (i.e., 4 weeks/28 days or 8 weeks/56 days) post-last dose
Abbreviations: D, days; ET, early termination; HAE-1/HAE-2, hereditary angioedema type 1 or 2; HAE-nC1-INH, hereditary angioedema with normal C1-inhibitor; N, number of subjects; OLE, open-label extension; PD, pharmacodynamic; PK, pharmacokinetic; PKK, prekallikrein; Q4W, every 4 weeks; Q8W, every 8 weeks; SC, subcutaneous

Table 73. Summary of Baseline Covariates for PK and PK/PD Analysis

A) Continuous Covariates

	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)

B) Categorical Covariates

	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)

Source: Applicant's population PK report, Tables 5 and 6

^a Hepatic dysfunction category defined as per National Cancer Institute Organ Dysfunction Working Group.

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

Results presented as N (%) for categorical covariates.

Results presented as median (min – max).

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BLPKK, baseline prekallikrein concentration; BSA, body surface area; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate using CKD-EPI formula; HAE, hereditary angioedema; max, maximum; min, minimum; N, number of subjects; PD, pharmacodynamic, PK, pharmacokinetic

14.5.1.3.2. Base Model

PK Base Model

The population PK model was developed using a nonlinear mixed-effects modeling approach in Phoenix NLME (Version 8.4). The structural base model was initially developed using data from Trials ISIS 721744-CS1, ISIS 721744-CS2, and ISIS 721744-CS9 followed by a graphical assessment of covariates including weight, disease status, site of administration, and drug presentation on relevant PK parameters. The PK data from subjects in Trial ISIS 721744-CS5 was then included in the base model development.

Interindividual variability was modelled assuming a log-normal distribution for individual level random effects. Residual variability was described using additive error model on log-transformed data. The quality of the model fit was assessed by diagnostic plots.

PK/PD Base Model

Considering the mechanism of action, an indirect response model was fitted initially to describe the relationship between donidalorsen concentrations and PKK. The population PK/PD model was developed in a similar manner to the population PK model (i.e., sequentially based on available data) and covariates were considered for inclusion into the base model before including data from Trial ISIS 721744-CS5. The PD parameters were estimated using the sequential model building method.

14.5.1.3.3. Covariate Analysis

For both PK and PK/PD models, covariate analysis was performed before and after including data from Trial ISIS 721744-CS5 as described under base model development. Covariates of interest were tested using a forward addition process followed by backward elimination. For forward addition and backward elimination, significance levels of 0.01 and 0.001 were utilized, respectively.

Continuous covariates were evaluated using a power function with the parameter of interest centered at the median value of the covariate in the population. Categorical covariates were parameterized using a proportional structure with the most common level of the covariate being the reference. In addition to covariates in [Table 73](#), covariates evaluated in the development of the PK model included drug presentation and site of administration. The impact of ADA on donidalorsen pharmacokinetics and pharmacodynamics was explored graphically. No apparent impact of ADA on C_{max} was observed, though increased C_{trough} was observed in some ADA-positive subjects. No apparent impact of ADA on PKK concentrations was observed. PK samples at and beyond the onset of ADA were excluded from the population PK analysis dataset due to lack of correlation between antisense oligonucleotide exposure in liver and plasma.

14.5.1.4. Final Model

14.5.1.4.1. PK Final Model

A 2-compartment model with first-order absorption and elimination adequately described the PK data of donidalorsen ([Figure 66](#) and [Figure 67](#)). Statistically significant covariate effects on PK model parameters were the following: body weight on CL/F, Vc/F, Q/F, and Vp/F; site of administration and drug presentation on ka; and disease status on Vc/F and Q/F. Age, sex, race, ethnicity, geographical region, mild hepatic impairment, and mild renal impairment are not significant covariates. The parameter estimates for the final PK model are listed in [Table 74](#). The RSE for all parameters except covariate of site of administration on ka, covariate of drug presentation on ka, correlation between CL/F and ka, correlation between Q/F and ka, and correlation between Vp/F and ka was <30%. All parameters had RSE <41%. Moderate eta-shrinkage (<25%) was observed for all parameters.

The effects of body weight, disease status, drug presentation, and site of administration on the $AUC_{\tau,ss}$, $C_{max,ss}$, and $C_{trough,ss}$ of donidalorsen are explored in [Figure 68](#). Body weight was the most influential covariate on PK exposure. The effect of body weight on donidalorsen pharmacokinetics is unlikely to be clinically meaningful due to lack of exposure-response relationship for efficacy and safety. The effects of other covariates are generally small, and unlikely to be clinically meaningful.

Table 74. 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) ^e	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 _{logadd}	0.296	2.49

Source: Applicant's population PK report, Table 8

^a CL/F = 12.8 × (WTKG / 70) ^{1.52}

^b V_c/F = 69.8 × (WTKG / 70) ^{2.34} × 1.426 [for HAE subject]

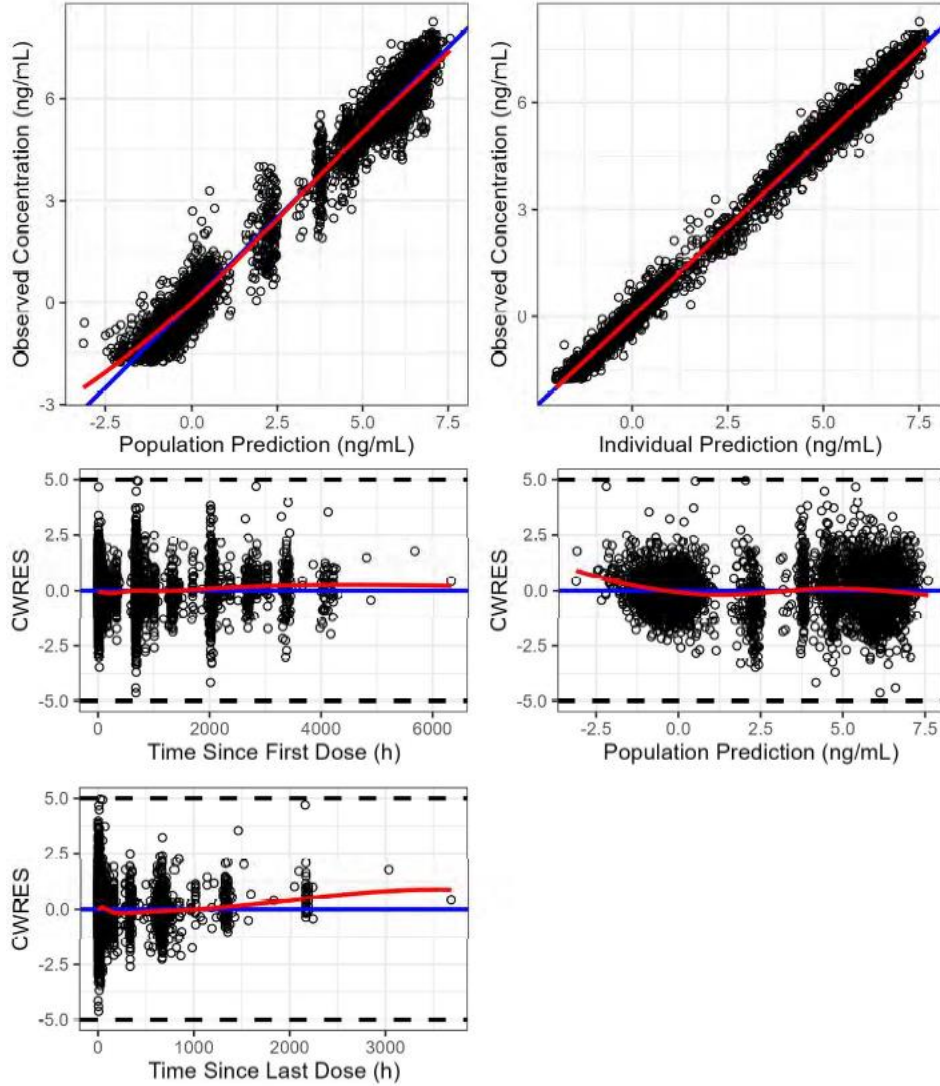
^c Q/F = 2.58 × (WTKG / 70) ^{1.79} × 0.739 [for HAE subject]

^d V_p/F = 1840 × (WTKG / 70) ^{1.60}

^e k_a = 0.952 × 0.662 [for arm injection] × 1.262 [for autoinjector]

Abbreviations: BSV%, between-subject variability expressed as CV%; CL/F, apparent clearance; k_a, first-order absorption rate constant; CV, coefficient of variation; logadd, additive component of the residual error model on log-transformed data; PK, pharmacokinetic; Q/F, apparent intercompartmental 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

Figure 66. Goodness-of-Fit Plots for Final Population Pharmacokinetic Model for Donidalorsen Observations



Source: Applicant's population PK report, Figures 30 and 31

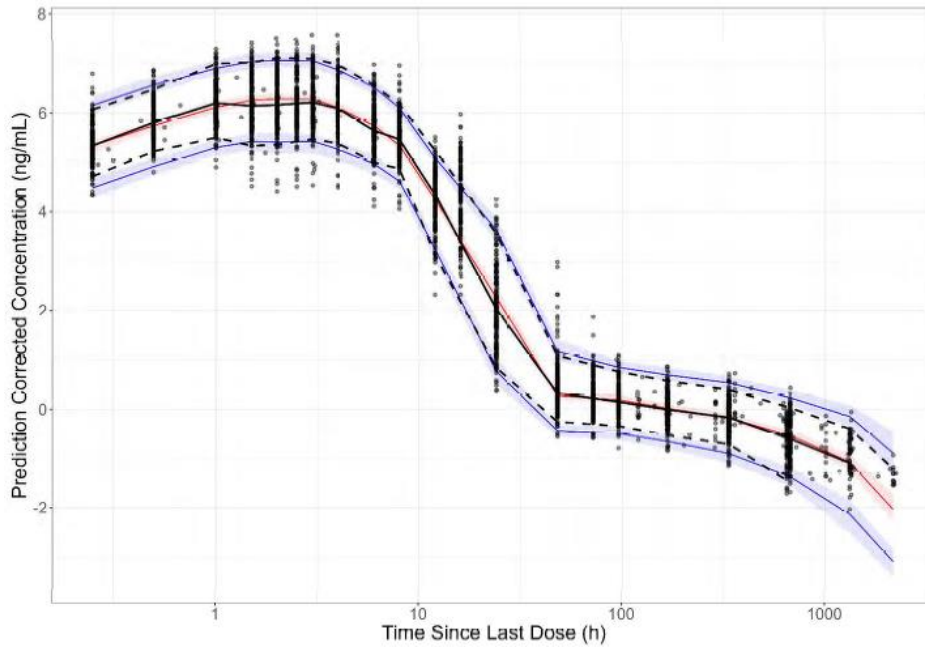
Blue line: line of unity or zero reference line.

Red line: Loess smooth trend line.

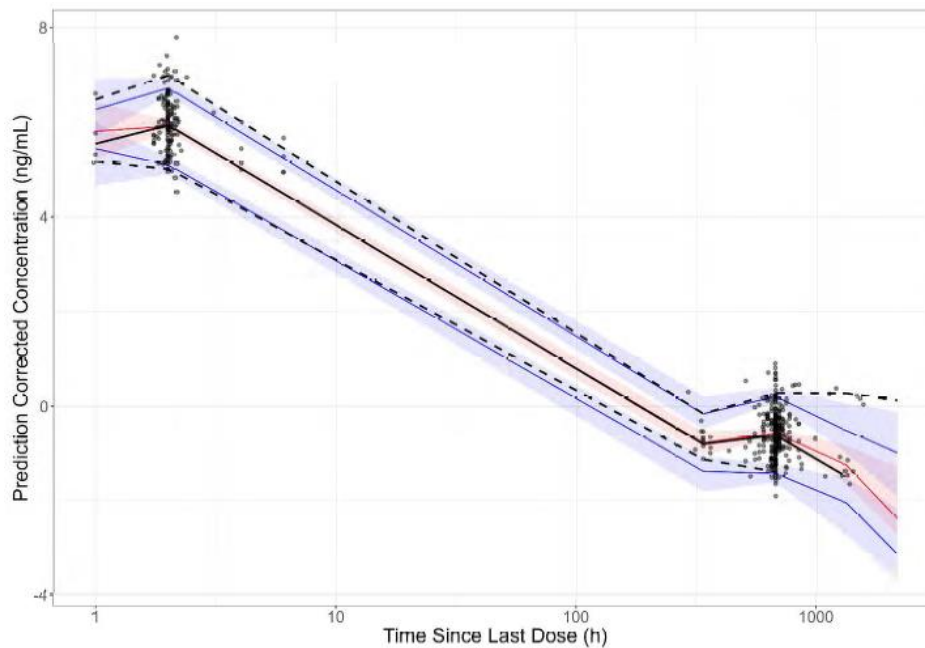
Abbreviations: CWRES, conditional weighted residuals; h, hour; PK, pharmacokinetic

Figure 67. Visual Predictive Check for Donidalorsen Observations From Final Population Pharmacokinetic Model

A) Healthy Subjects

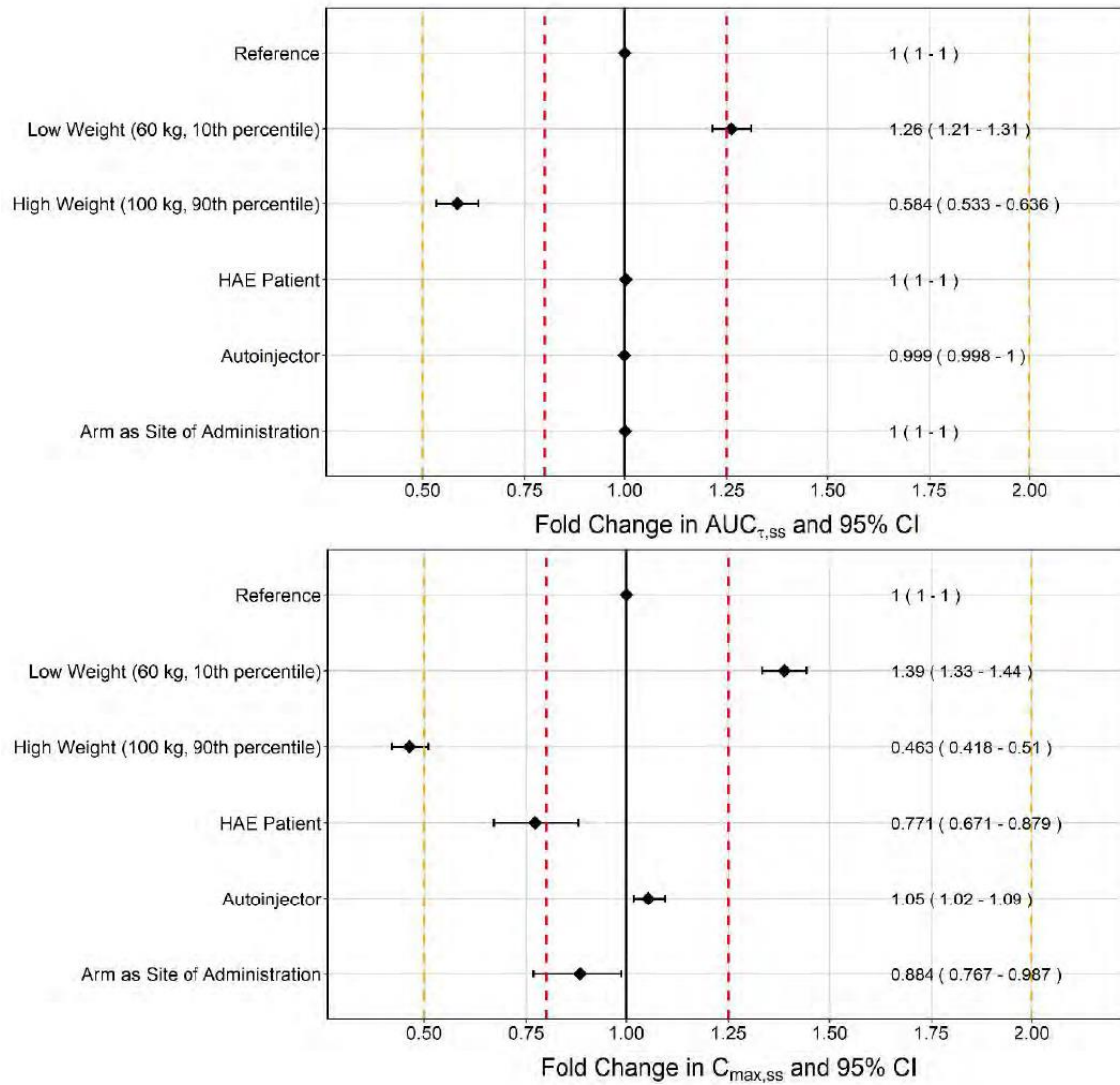


B) HAE Subjects



Source: Applicant's population PK report, Figures 34 and 35
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, and 95th percentiles, and 95% confidence interval around the model-predicted 5th, 50th, and 95th percentiles.
Log transformed concentration data are presented on y-axis. Note: BLQ observations and predictions are censored.
Abbreviations: BLQ, below limit of quantification; h, hour; HAE, hereditary angioedema; PK, pharmacokinetic

Figure 68. Model-Predicted Covariate Effect on Fold Change in $AUC_{\tau,ss}$ and $C_{max,ss}$



Source: Applicant's population PK report, Figures 37 and 39

The solid black line represents the median of the simulated reference subject, defined as a healthy volunteer with a body weight of 70 kg receiving 80 mg donidalorsen Q4W SC into abdomen or thigh using vial as the drug presentation. Dashed red lines represent the 80 to 125% range of the reference subject, and dashed orange lines represent the 50 to 200% range of the reference subject. The symbols and error bars represent the median and 95% confidence interval based on 1000 simulated subjects within each group including uncertainty on the fixed effect.

Abbreviations: $AUC_{\tau,ss}$, area under the plasma concentration-time curve during a dosage interval (τ) at steady state; CI, confidence interval; $C_{max,ss}$, maximum plasma concentration at steady state; HAE, hereditary adverse event; PK, pharmacokinetic; Q4W, every 4 weeks; SC, subcutaneous

14.5.1.4.2. PK/PD Final Model

The final PK/PD model was an indirect effect $E_{\max}$ model with inhibition on the rate of PKK production by donidalorsen. Statistically significant covariate effects on PD model parameters were disease status on baseline PKK and IC_{50} . The pooled PD data were adequately described by the final model ([Figure 69](#) and [Figure 70](#)). The parameter estimates for the final model are listed in [Table 75](#). The RSE for all parameters were <30%. The precision of parameter estimates is reasonable. Eta-shrinkage was low to moderate for baseline (10.0%), IC_{50} (11.7%), and k_{out} (28.6%). The covariate 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 $C_{trough,ss}$ were within the 80% to 125% range ([Figure 71](#)).

Table 75. Final Population PD 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

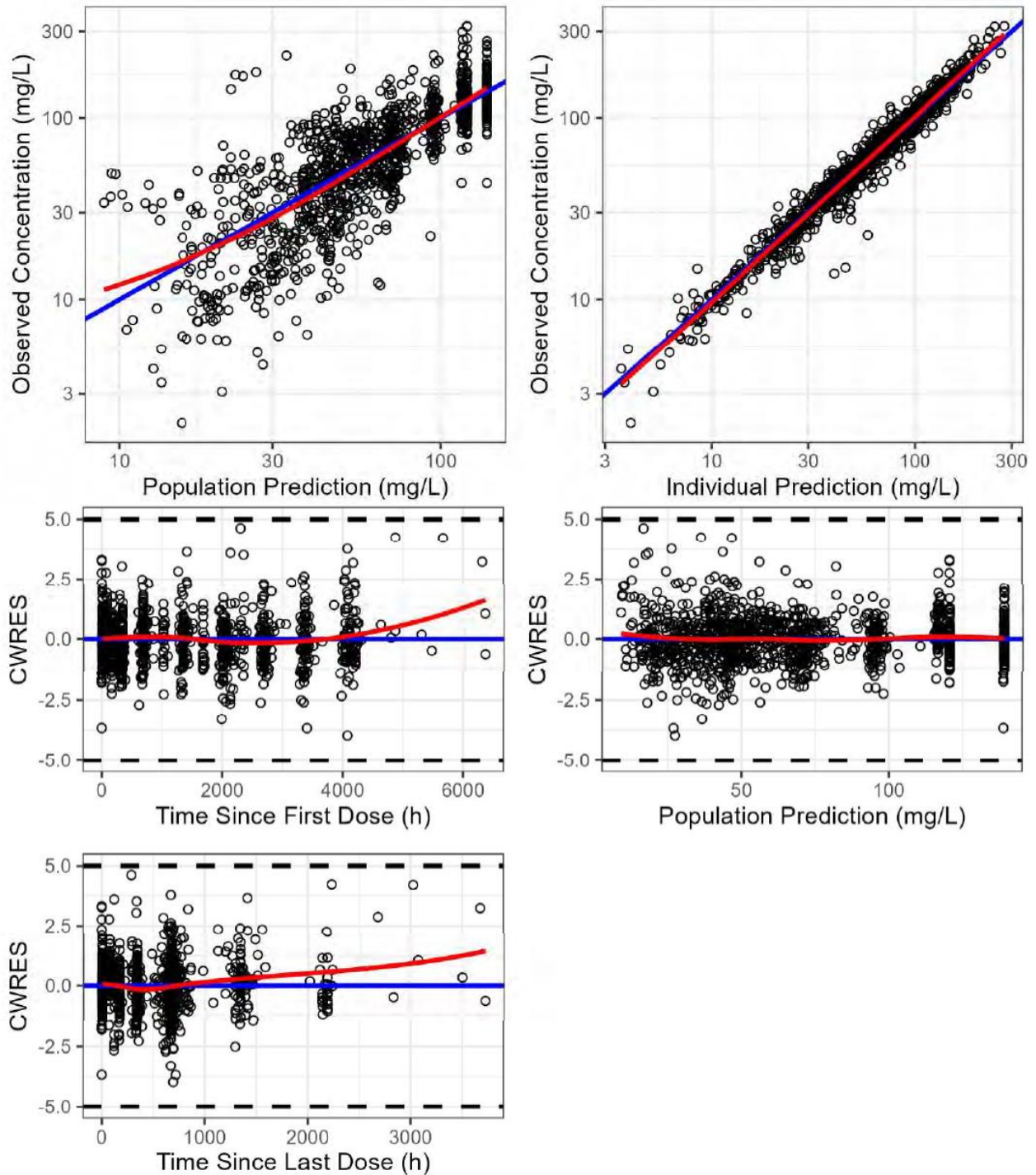
Source: Applicant's population PK report, Table 9

^a BL = 139×0.868 [for HAE subject]

^b IC_{50} = 0.158×1.770 [for HAE subject]

Abbreviations: BL, baseline; BSV%, between-subject variability expressed as CV%; CV, coefficient of variation; HAE, hereditary angioedema; IC_{50} , half-maximal inhibitory concentration; $I_{\max}$, maximal fractional inhibitory capacity; k_{out} , first-order loss rate constant; PD, pharmacodynamic; PK, pharmacokinetic; RSE, relative standard error; Sh, shrinkage for BSV; prop, proportional component of the residual error model

Figure 69. Goodness-of-Fit Plots for Final Population PD Model for Donidalorsen Observations



Source: Applicant's population PK report, Figures 43 and 44

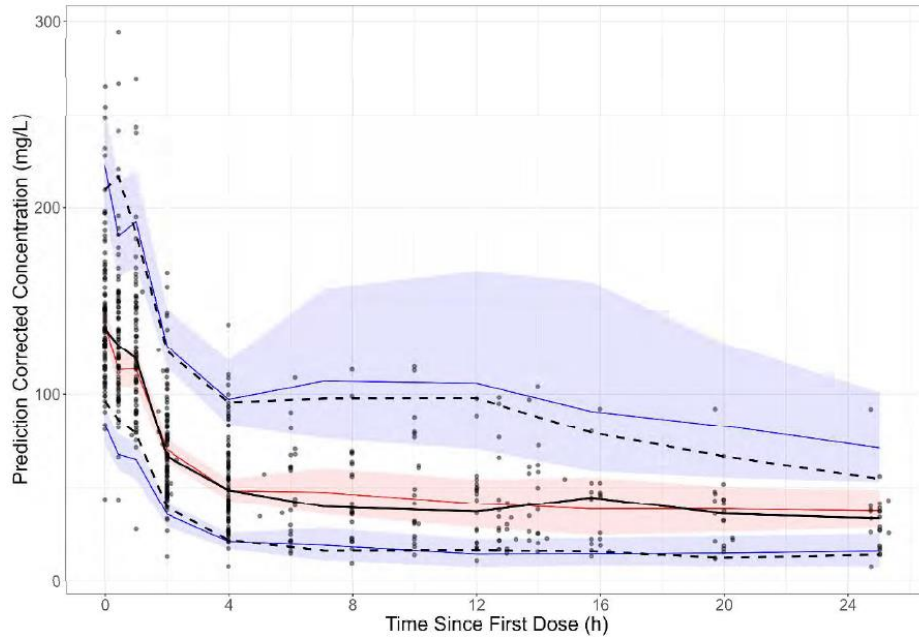
Blue line: Line of unity or zero reference line.

Red line: Loess smooth trend line.

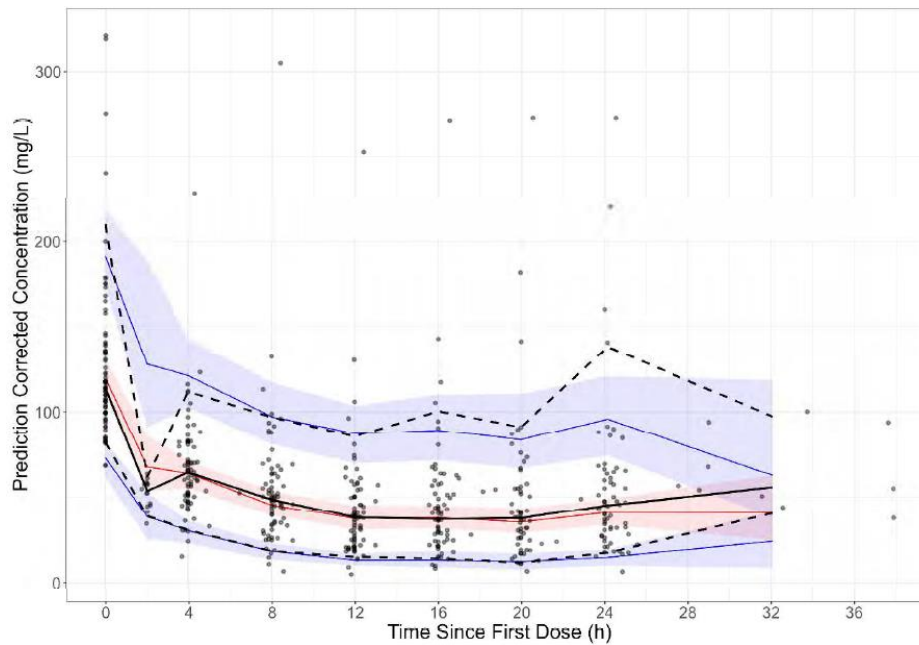
Abbreviations: CWRES, conditional weighted residuals; h, hour; PD, pharmacodynamic; PK, pharmacokinetic

Figure 70. Visual Predictive Check for PKK Observations From Final Population Pharmacodynamic Model

A) Healthy Subjects



B) HAE Subjects



Source: Applicant's population PK report, Figures 47 and 48

Circles: Individual observed data.

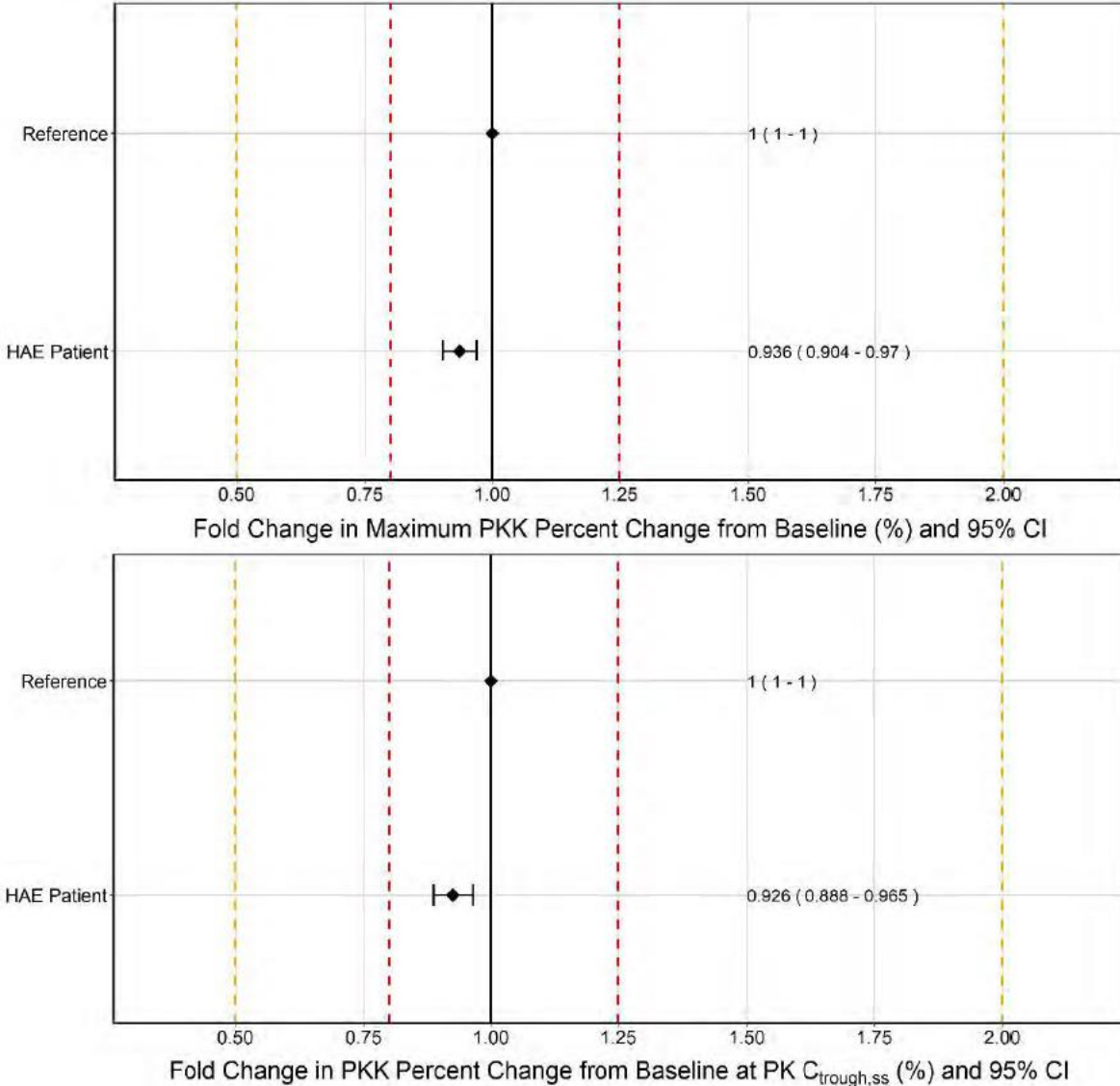
Dashed lines: Observed 5th and 95th percentiles of the observed data.

Solid black line: Observed median concentration.

Colored lines and shaded areas: Model-predicted 5th, 50th, and 95th percentiles, and 95% confidence interval around the model-predicted 5th, 50th, and 95th percentiles.

Abbreviations: h, hour; HAE, hereditary angioedema; PD, pharmacodynamic; PK, pharmacokinetic; PKK, prekallikrein

Figure 71. Model-Predicted Covariate Effect on Fold Change in Maximum PKK Percent Change From Baseline (Nadir) and PKK Percent Change From Baseline at PK C_{trough,ss}



Source: Applicant's population PK report, Figures 50 and 52

The solid black line represents the median of the simulated reference subject, defined as a healthy volunteer with a body weight of 70 kg receiving 80 mg donidalorsen Q4W SC into abdomen or thigh using vial as the drug presentation.

Dashed red lines represent the 80 to 125% range of the reference subject, and dashed orange lines represent the 50 to 200% range of the reference subject.

The symbols and error bars represent the median and 95% confidence interval based on 1000 simulated subjects within each group including uncertainty on the fixed effect.

Abbreviations: CI, confidence interval; C_{trough,ss}, trough concentration at steady state; HAE, hereditary angioedema; PK, pharmacokinetic; PKK, prekallikrein; Q4W, every 4 weeks; SC, subcutaneous

14.5.1.5. Simulations

The exposure metrics ($AUC_{\tau,ss}$, $C_{max,ss}$, and $C_{trough,ss}$) of donidalorsen and PD response following 80 mg Q4W, every month (Q1M), Q8W, or every 2 months (Q2M) were simulated based on the final population PK/PD model and distribution of demographic data in subjects from Trials ISIS 721744-CS2 and ISIS 721744-CS5. The differences in median $AUC_{\tau,ss}$, $C_{max,ss}$, and $C_{trough,ss}$ of donidalorsen between the 80 mg Q4W and Q1M regimens and between the 80 mg Q8W and Q2M regimens was <10% (Table 76 and Figure 72). The median PKK percent change from baseline at the PK $C_{trough,ss}$ 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%) (Figure 73).

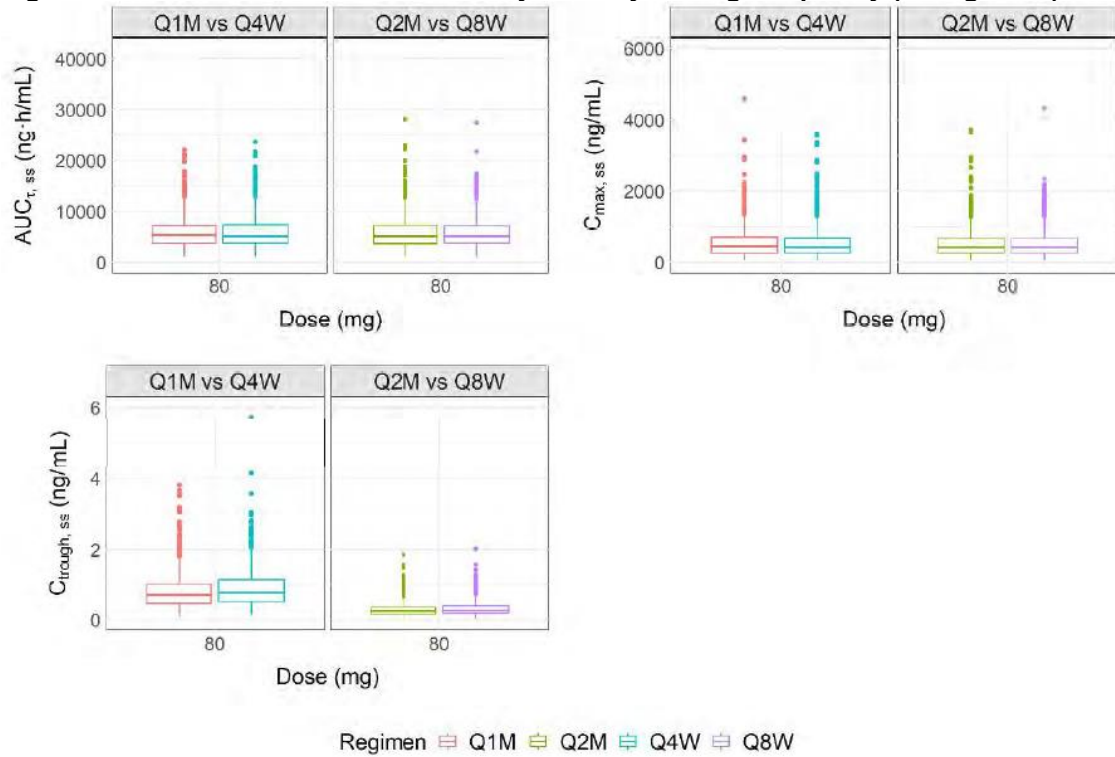
Table 76. Summary of Simulated PK Metrics at Steady State

Regimen	N	Statistic	$AUC_{\tau,ss}$ (ng h/mL)	$C_{max,ss}$ (ng/mL)	$C_{trough,ss}$ (ng/mL)	Accumulation Ratio AUC	Accumulation Ratio C_{max}	Accumulation Ratio C_{trough}	$T_{1/2}$ (h)
80 mg Q1M	1000	Mean	5920	543	0.804	1.08	1.00	2.13	785
		SD	3120	419	0.505	0.0368	0.00113	0.371	195
		CV%	52.7	77.3	62.8	3.40	0.113	17.4	24.9
		GeoMean	5240	424	0.680	1.08	1.00	2.10	762
		GeoMean CV%	52.4	81.4	63.1	3.34	0.113	16.9	24.3
		Min	1180	41.4	0.0997	1.02	1.00	1.33	359
		Median	5280	438	0.688	1.08	1.00	2.09	766
80 mg Q4W	1000	Max	22100	4580	3.81	1.33	1.01	4.42	2070
		Mean	5920	537	0.891	1.09	1.00	2.25	793
		SD	3150	434	0.550	0.0390	0.00130	0.414	202
		CV%	53.2	80.8	61.7	3.59	0.129	18.4	25.5
		GeoMean	5240	417	0.755	1.09	1.00	2.22	769
		GeoMean CV%	52.2	81.2	63.4	3.53	0.129	17.9	25.1
		Min	1180	43.0	0.128	1.02	1.00	1.43	389
80 mg Q2M	1000	Median	5090	417	0.760	1.08	1.00	2.21	771
		Max	23600	3560	5.72	1.28	1.02	4.87	2180
		Mean	5900	530	0.282	1.04	1.00	1.39	778
		SD	3150	408	0.206	0.0244	0.000470	0.163	189
		CV%	53.4	77	72.8	2.34	0.0470	11.7	24.2
		GeoMean	5210	418	0.227	1.04	1.00	1.38	757
		GeoMean CV%	52.6	78.8	75.7	2.31	0.0469	11.3	24.0
80 mg Q8W	1000	Min	1100	40.8	0.0105	1.00	1.00	1.06	345
		Median	5160	417	0.232	1.04	1.00	1.37	757
		Max	28100	3720	1.86	1.17	1.00	2.28	1820
		Mean	5850	523	0.314	1.05	1.00	1.43	774
		SD	2980	384	0.219	0.0246	0.000454	0.174	185
		CV%	50.9	73.3	69.7	2.35	0.0454	12.2	23.9
		GeoMean	5210	416	0.255	1.05	1.00	1.42	753
80 mg Q8W	1000	GeoMean CV%	51.7	78.4	72.9	2.32	0.0454	11.9	24.0
		Min	1070	41.4	0.0307	1.01	1.00	1.07	347
		Median	5140	422	0.255	1.04	1.00	1.41	756
		Max	27500	4310	2.03	1.16	1.00	2.19	1560

Source: Applicant's population PK report, Table 13

Abbreviations: AUC, area under the concentration-time curve; $AUC_{\tau,ss}$, area under the plasma concentration-time curve during a dosage interval (τ) at steady state; C_{max} , maximum plasma concentration; $C_{max,ss}$, maximum plasma concentration at steady state; C_{trough} , trough concentration at steady state; $C_{trough,ss}$, trough concentration at steady state; CV, coefficient of variation; GeoMean, geometric mean; h, hour; max, maximum; min, minimum; N, number of subjects; PK, pharmacokinetic; Q1M, every month; Q2M, every 2 months; Q4W, every 4 weeks; Q8W, every 8 weeks; SD, standard deviation; $t_{1/2}$, terminal half-life

Figure 72. Simulated PK Metrics at Steady State by Dosing Frequency (80-mg Dose)



Source: Applicant's population PK report, Figure 58

The thick horizontal lines (middle of the boxes) represent the median of the data.

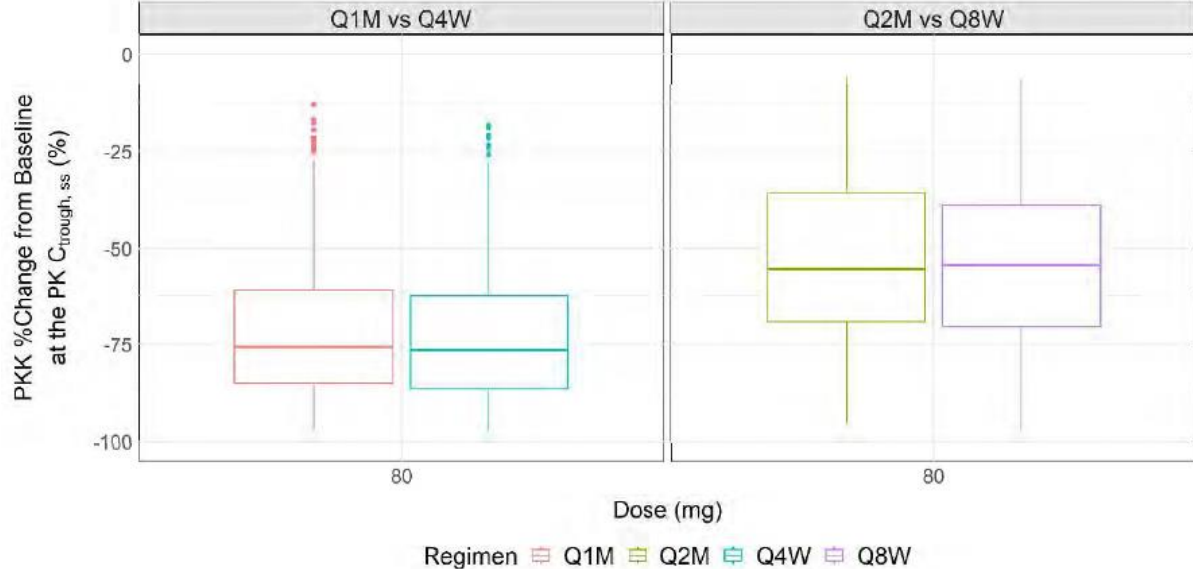
The hinges (top and bottom of the boxes) represent the 25th and 75th percentiles (i.e., IQR).

The top and bottom whiskers extend to the largest and smallest values that are within 1.5 * IQR of the hinges, respectively.

Circles are the simulated data beyond the end of the whiskers.

Abbreviations: $AUC_{\tau,ss}$, area under the plasma concentration-time curve during a dosage interval (τ) at steady state;

$C_{max,ss}$, maximum plasma concentration at steady state; $C_{trough,ss}$, trough concentration at steady state; IQR, interquartile range; PK, pharmacokinetic; Q1M, every month; Q2M, every 2 months; Q4W, every 4 weeks; Q8W, every 8 weeks

Figure 73. Simulated PKK Percent Change From Baseline at the PK $C_{trough,ss}$ by Dosing Frequency (80-mg Dose)

Source: Applicant's population PK report, Figure 60

The thick 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 that are within 1.5 * IQR of the hinges, respectively.

Circles are the simulated data beyond the end of the whiskers.

Abbreviations: $C_{trough,ss}$, trough concentration at steady state; IQR, interquartile range; PK, pharmacokinetic; PKK, prekallikrein; Q1M, every month; Q2M, every 2 months; Q4W, every 4 weeks; Q8W, every 8 weeks

14.6. Pharmacogenetics

Not applicable.

15. Study/Trial Design

15.1. Trial CS5

Title

A phase 3 Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of ISIS 721744 in Patients with Hereditary Angioedema.

Dates

January 25, 2022, to November 9, 2023; CSR completed July 18, 2024.

15.1.1. Definition and Collection of HAE Attacks, Trial CS5

During the trial, subjects were instructed to report details of any HAE attack to the trial 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 subjects approximately weekly, as well as during trial visits, to inquire about attacks.

For each HAE attack, the following data were collected:

- Date/time of symptom onset.
- Location and description of symptoms:
 - Peripheral angioedema: cutaneous swelling involving an extremity, face, neck, torso, and/or genitourinary region.
 - Abdominal angioedema: abdominal pain, with or without abdominal distension, nausea, vomiting, or diarrhea.
 - Laryngeal angioedema: stridor, dyspnea, difficulty speaking, difficulty swallowing, throat tightening, or swelling of the tongue, palate, uvula, or larynx.
- HAE attack severity:
 - Mild: transient or mild discomfort.
 - Moderate: mild to moderate limitation in activity, some assistance needed.
 - Severe: marked limitation in activity, assistance required.
- Need for assistance, medical intervention, emergency room (ER) visit, or hospitalization.
- Medications to treat the attack.
- HAE attack course, including if the HAE attack(s) in question was a typical attack for the subject, or if there was an alternative diagnosis.
- Date/time symptoms resolved.

The presence of these symptoms, including symptoms in one or more of the above locations, was not necessarily considered an investigator-confirmed HAE attack (to be used in efficacy endpoint analyses) unless the diagnosis was confirmed by the investigator. The investigator was permitted to use clinical judgment regarding whether the symptoms represent a HAE attack or not. For example, if the reported event was accompanied by symptoms not consistent with an HAE attack (e.g., urticaria); if the reported event persisted well beyond the typical course of time for an HAE attack (e.g., greater than 7 days); or if there was a likely alternative etiology for the event (e.g., viral gastroenteritis).

An HAE attack was defined as an event with signs or symptoms consistent with an attack in at least one of the locations noted above. A discrete attack was counted if there was at least a 24-hour symptom-free period between attacks. If there was less than 24 hours between the stop

date/time of the first event and the start date/time of the next event, the events were combined and counted as one unique attack.

15.1.2. Data and Safety Monitoring Committee, Trial CS5

A Data and Safety Monitoring Committee was assembled to review safety and tolerability data collected during this trial.

15.1.3. Sample Size Considerations, Trial CS5

The power and sample size estimations were calculated by the Applicant 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 weeks) 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 subjects (2:1 ratio) would provide at least 90% power for the primary endpoint, with a 0.05 significance level. A total of approximately 84 subjects (42 in the donidalorsen-4 group, 21 in the placebo group, and 21 in the donidalorsen-8 group) were planned to be enrolled in this trial to account for potential early dropouts and to facilitate some general safety evaluations.

15.1.4. Protocol Amendments, Trial CS5

The original global protocol, dated June 16, 2021, was amended twice. Key changes to the protocol in the first amendment are summarized in [Table 77](#) below. There were no substantial changes made for the second amendment (dated July 12, 2022). The substantial changes in the first amendment were reviewed by the Agency and deemed acceptable prior to trial subject enrollment, which began in 2022.

Table 77. Protocol Amendments, Trial CS5

Amendment Number and Date	Key Change and Rationale, if Applicable
1; October 1, 2021	- Added the inclusion and rationale for a second dosing cohort with a dose frequency of per 8 weeks (donidalorsen 80 mg once every 8 weeks). - Additional subjects were added to the trial with a new dosing cohort (donidalorsen 80 mg every 8 weeks). - Updated exclusion criteria regarding total bilirubin: Total bilirubin $>1.5 \times$ ULN OR, unless if due to Gilbert's syndrome, (>5 mg/dL). - A specific threshold ($<130,000/\text{mm}^3$) for platelet exclusion was added. - Safety stopping rules for platelet count and actions in subjects with confirmed low platelet count were updated to increase monitoring frequency for platelets for counts between $100,000/\text{mm}^3$ and $125,000/\text{mm}^3$ and added requirement for Applicant approval for

Amendment Number and Date	Key Change and Rationale, if Applicable
	continued dosing when platelets were $\geq 75,000/\text{mm}^3$ to $\leq 100,000/\text{mm}^3$. • Added a safety monitoring plan. • The primary efficacy analysis was updated to reflect that the primary analysis was to compare the 80 mg donidalorsen-4 group versus placebo group. • ECG-related assessment changes were added to the Schedule of Procedures including predose and 2 hours postdose. • Updated Schedule of Procedures by adding a physical exam at every visit to examine for reactions due to trial drug administration. • Added the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2007, to indicate how AEs were graded.

Source: Clinical reviewer

Abbreviations: AE, adverse event; ECG, electrocardiogram; ULN, upper limit of normal

15.1.5. Safety Assessments, Trial CS5

The safety and tolerability assessments included AEs, clinical laboratory parameters (chemistry, coagulation, hematology, and urinalysis), vital signs, physical examinations, and ECG testing according to the Schedule of Assessments (see [Table 78](#)). During the Treatment Period, these assessments were generally collected every 4 weeks. A centralized laboratory was used to enhance data quality.

For this trial, the adverse events of special interest were defined as platelet count $< 50,000/\text{mm}^3$ accompanied by a major bleeding event or clinically relevant non-major bleeding event, or platelet count $< 25,000/\text{mm}^3$, independent of a major bleeding or clinically relevant non-major bleeding event.

Overall, the safety assessments in Trial CS5 were adequate to inform the safety profile of donidalorsen for the proposed use.

Table 78. Schedule of Assessments, Trial CS5

Study Week	Screen	Treatment Period							Post-Treatment Period		
	-8 to -1	1	5 ¹	9 ¹	13 ¹	17 ¹	21 ¹	25	4 ^{1,2}	8 ^{1,2}	13 ^{1,2}
Study Day	-56 to -1	1	29	57	85	113	141	169/ET-L ³ or Tx-ET ⁴	28	56	91/ Post-Tx-ET ⁵
Visit Windows	0	± 3 days									
Informed Consent/Assent	X										
Inclusion/Exclusion	X										
Medical History	X										
HAE Attack History	X										
FSH ⁶	X										
Pregnancy Test ⁷	X	X	X	X	X	X	X	X	X	X	X
Hepatitis B, C, HIV	X										
C1-INH, C1q and C4 Testing ⁸	X										
Height	X							X ²⁰			
Body weight	X	X	X	X	X	X	X	X	X	X	X
Vital Signs ⁹	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/Body Assessments ¹⁰	X	X	X ^{18,19}	X ¹⁸	X	X ¹⁸	X ^{18,19}	X			X
12-lead ECG	X	X			X	X ¹⁷	X ¹⁶	X	X		X
Urinalysis	X	X	X	X	X	X	X	X	X	X	X
Clinical Lab Parameters ^{11,12}	X	X	X	X	X	X	X	X	X	X	X
C5a		X						X			X
Inflammatory Panel		X			X			X			X
Anti-drug Antibody		X	X		X		X	X	X		X
PK Blood Sampling		See Appendix C									
Plasma PKK		X	X	X	X	X	X	X	X	X	X
Archived serum samples	X	X	X	X	X			X	X		X
AE-QoL		X			X			X			X
GAD-7		X			X			X			X
EQ-5D-5L		X			X			X			X
WPAI		WEEKLY THROUGHOUT ENTIRE STUDY									
PGIS		X			X			X			X
PGIC								X			X
AECT		X	X	X	X	X	X	X	X	X	X

Study Week	Screen	Treatment Period							Post-Treatment Period		
	-8 to -1	1	5 ¹	9 ¹	13 ¹	17 ¹	21 ¹	25	4 ^{1,2}	8 ^{1,2}	13 ^{1,2}
Study Day	-56 to -1	1	29	57	85	113	141	169/ET-L ³ or Tx-ET ⁴	28	56	91/ Post-Tx-ET ⁵
Visit Windows	0	± 3 days									
AAS		DAILY THROUGHOUT ENTIRE STUDY									
HAE Attack Assessment ^{13,14}	X	X	X	X	X	X	X	X	X	X	X
ER visits, hospitalizations, total inpatient days		X	X	X	X	X	X	X	X	X	X
Study Drug (ISIS 721744 or placebo) Administration ¹⁵		X	X ¹⁹	X	X ¹⁹	X	X ¹⁹				
Adverse Events	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X

All procedures and study samples collections are to be done pre-dose at respective visits, unless specified otherwise

- ¹ Assessments and procedures may be conducted by a Home Healthcare professional (if available).
- ² Weeks/days from end of treatment. End of treatment is defined as 1 dosing interval (i.e., 4 weeks/28 days for Cohort A and 8 weeks/56 days for Cohort B) post-last dose.
- ³ Landmark visit for patients who discontinue early from the Treatment Period.
- ⁴ For patients who discontinue early from the Treatment Period.
- ⁵ For patients who discontinue early from the Post-Treatment Period.
- ⁶ For confirmation of menopause at Screening per Inclusion Criteria.
- ⁷ For WOCBP only. Serum pregnancy will be performed at Screening, but urine pregnancy will be performed at all other time points. A positive result from a urine pregnancy test will be confirmed with a serum pregnancy test.
- ⁸ C1-INH, C4, and C1q will be obtained at Screening. Historical values can be used for the HAE Inclusion Criteria, if already collected in the last 5 years.
- ⁹ Vital signs may include blood pressure, heart rate, respiratory rate, and body temperature.
- ¹⁰ Physical Exam will be done as a Body Assessment if conducted by a nurse or Home Healthcare professional.
- ¹¹ Including chemistry, hematology, and coagulation.
- ¹² If the platelet value, serum creatinine, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen should be re-drawn as soon as possible (ideally within 7 days); for a complete list of laboratory tests refer to Appendix B.
- ¹³ Site personnel will contact the patient approximately weekly to inquire about HAE attacks experienced by the patient. HAE attacks will also be assessed at each study visit.
- ¹⁴ Patients will be instructed to report details of any HAE attack to the study site within 72 hours of the onset of the attack. The following details relating to each HAE attack will be collected: localization, severity, course of attack, required on-demand treatment(s), and the frequency and dose of on-demand treatment.

- ¹⁵ 7676 Self-administration of Study Drug, or administration by other qualified individuals (e.g., family member), may be allowed after completion of training provided by qualified site personnel. A Home Healthcare professional must be available to complete all pre-dosing procedures prior to Study Drug administration.
- ¹⁶ Cohort A ONLY: Measure ECG at pre-dose and at 2-hours post-dose.
- ¹⁷ Cohort B ONLY: Measure ECG at pre-dose and at 2-hours post-dose.
- ¹⁸ Targeted physical exam/body assessment which must include examination of the skin (including injection site), chest and mouth following Study Drug administration.
- ¹⁹ Cohort A ONLY.
- ²⁰ For pediatric patients only.

Source: CS5 Clinical Study Protocol, Appendix A

Abbreviations: AAS, Angioedema Activity Score; AECT, Angioedema Control Test; AE-QoL, Angioedema Quality of Life Questionnaire; C1-INH, C1 esterase inhibitor; C1q, complement component 1q; C4, complement component 4; C5a, complement component 5a; ECG, electrocardiogram; EQ-5D-5L, EuroQol 5 Dimensions - 5 Levels Questionnaire; ER, emergency room; FSH, follicle-stimulating hormone; GAD-7, general anxiety disorder 7-item scale; HAE, hereditary angioedema; HIV, human immunodeficiency virus; PGIC, patient global impression of change; PGIS, patient global impression of severity; PK, pharmacokinetic; PKK, prekallikrein; WOCBP, women of childbearing potential; WPAI, Work Productivity And Activity Impairment Questionnaire

15.1.6. Grading of Adverse Events, Trial CS5

AEs not included in the below tables for laboratory abnormalities and ISR ([Table 79](#) and [Table 80](#), respectively) were graded by the following criteria:

- Mild: The event is easily tolerated by the subject and does not affect the subject's usual daily activities.
- Moderate: The event causes the subject more discomfort and interrupts the subject's usual daily activities.
- Severe: The event is incapacitating and causes considerable interference with the subject's usual daily activities.

The severity grading of laboratory abnormalities followed [Table 79](#), based largely on the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2007.

Table 79. Laboratory Abnormality Severity Grading Table for Adverse Events, Trial CS5

Adverse Event	Mild	Moderate	Severe
Hematology			
aPTT prolonged	1.0 – 1.2 x ULN	>1.2 – 1.4 x ULN	> 1.4 x ULN
Eosinophils increased	650 – 1,500 cell/mm ³	1,501 - 5,000 cell/mm ³	>5,000 cell/mm ³
Fibrinogen decreased	150 – 200 mg/dL	125 – 149 mg/dL	< 125 mg/dL
Fibrinogen increased	400 – 500 mg/dL	501 – 600 mg/dL	> 600 mg/dL
Hemoglobin decreased			
Male	12.5 – 13.5 g/dL	10.5 – 12.4 g/dL	< 10.5 g/dL
Female	11.0 – 12.0 g/dL	9.5 – 10.9 g/dL	< 9.5 g/dL
INR increased [†]	>1 - 1.5 x ULN; >1 - 1.5 times above baseline if on anticoagulation	>1.5 - 2.5 x ULN; >1.5 - 2.5 times above baseline if on anticoagulation	>2.5 x ULN; >2.5 times above baseline if on anticoagulation
Lymphocyte count decreased	750 – 1,000 cell/mm ³	500 – 749 cell/mm ³	< 500 cell/mm ³
Neutrophil count decreased	1,500 – 2,000 cell/mm ³	1,000 – 1,499 cell/mm ³	< 1,000 cell/mm ³
Platelet count decreased	125,000 – 140,000 cell/mm ³	100,000 – 124,000 cell/mm ³	< 100,000 cell/mm ³
Prothrombin time (PT)	1.0 – 1.1 x ULN	>1.1 – 1.2 x ULN	> 1.2 x ULN
White blood cell decreased	2,500 – 3,500 cell/mm ³	1,500 – 2,499 cell/mm ³	< 1,500 cell/mm ³
White blood cell increased	10,800 – 15,000 cell/mm ³	15,001 – 20,000 cell/mm ³	>20,000 cell/mm ³
Chemistry			
Alanine aminotransferase increased [†]	>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 x ULN
Alkaline phosphatase increased	1.1 – 2.0 x ULN	>2.0 – 3.0 x ULN	> 3 x ULN

NDA 219407
Dawnzera (donidalorsen)

Adverse Event	Mild	Moderate	Severe
Aspartate aminotransferase increased [†]	>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 x ULN
Blood bilirubin increased When accompanied by any increase in liver function test When liver function test is normal	1.1 – 1.25 x ULN 1.1 – 1.5 x ULN	>1.25 – 1.5 x ULN >1.5 – 2.0 x ULN	> 1.5 x ULN > 2 x ULN
Blood urea nitrogen	23 – 26 mg/dL	27 – 31 mg/dL	>31 mg/dL
CPK increased*	>ULN - <6 ULN	6 - 10 x ULN	>10 x ULN
Creatinine increased	1.5 – 1.7 mg/dL	1.8 – 2.0 mg/dL	≥ 2.1 mg/dL
GGT increased [†]	>ULN - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 x ULN
Hypercalcemia	10.5 – 11.0 mg/dL	11.1 – 11.5 mg/dL	≥ 11.6 mg/dL
Hyperglycemia [†]	Fasting glucose value >ULN - 160 mg/dL; Fasting glucose value >ULN - 8.9 mmol/L	Fasting glucose value >160 - 250 mg/dL; Fasting glucose value >8.9 - 13.9 mmol/L	>250 mg/dL; >13.9 mmol/L; hospitalization indicated
Hyperkalemia	5.1 – 5.2 mmol/L	5.3 – 5.4 mmol/L	≥5.5 mmol/L
Hypernatremia	144 – 145 mmol/L	146 – 147 mmol/L	≥148 mmol/L
Hypoalbuminemia	2.8 – 3.1 g/dL	2.5 – 2.7 g/dL	< 2.5 g/dL
Hypocalcemia	8.0 – 8.4 mg/dL	7.5 – 7.9 mg/dL	< 7.5 mg/dL
Hypoglycemia	65 – 69 mg/dL	< 64 mg/dL	< 55 mg/dL AND requires assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions [†]
Hypokalemia	3.5 – 3.6 mmol/L	3.3 – 3.4 mmol/L	< 3.3 mg/dL
Hypomagnesemia	1.3 – 1.5 mg/dL	1.1 – 1.2 mg/dL	< 1.1 mg/dL
Hyponatremia	132 – 134 mmol/L	130 – 131 mmol/L	<130 mg/dL
Hypophosphatemia	2.3 – 2.5 mg/dL	2.0 – 2.2 mg/dL	< 2.0 mg/dL
Hypoproteinemia	5.5 – 6.0 g/dL	5.0 – 5.4 g/dL	< 5.0 g/dL
Lipase increased	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	> 2 x ULN
Serum amylase increased	1.1 – 1.5 x ULN	>1.5 – 2.0 x ULN	> 2 x ULN

Adverse Event	Mild	Moderate	Severe
Urine			
Proteinuria	Trace	1+	≥ 2+
Hematuria	1 - 10 cells per high power field	11 – 50 cells per high power field	> 50 cells per high power field

*Grading for this parameter is derived from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2.0, Nov 2014

†Grading for this parameter is derived from the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, June 2010

‡Modified for consistency with the ADA and Endocrine Society Guidelines (Seaquist ER, Anderson J, Childs B, et al. Hypoglycemia and Diabetes: A Report of a Workgroup of the American Diabetes Association and The Endocrine Society. *Diabetes Care* 2013;36:1384-95)

Source: CS5 Clinical Study Protocol, Appendix D

Abbreviations: ADA, American Diabetes Association; aPTT, activated partial thromboplastin time; CPK, creatine phosphokinase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; ULN, upper limit of normal

The severity grading of injection site reactions followed [Table 80](#), based largely on the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, September 2007.

Table 80. Laboratory Abnormality Severity Grading Table for Injection Site Reactions, Trial CS5

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Source: CS5 Clinical Study Protocol, Appendix D

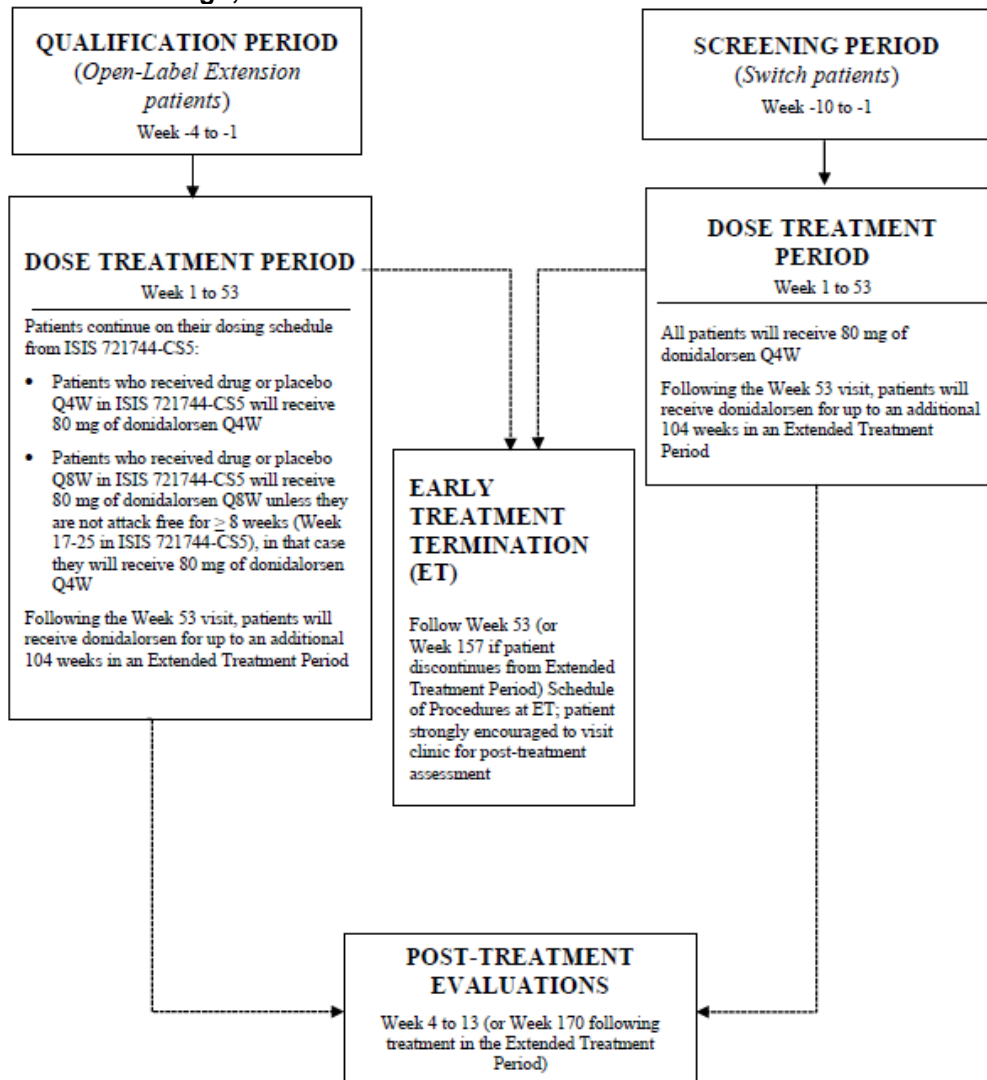
15.2. Trial CS7

15.2.1. Trial Design, Trial CS7

Trial CS7 is an ongoing phase 3b, open-label trial evaluating the long-term safety and efficacy of donidalorsen in subjects with Types I and II HAE. The trial enrolled rollover (referred to in this

review as Rollover, and by the Applicant as OLE) subjects, who completed Trial CS5 (Index Trial), as well as donidalorsen-naïve (referred to in this review as Naïve, and by the Applicant as Switch) subjects. For additional details regarding the Qualification/Screening Period of Trial CS7, see below. The first subject received a dose of trial treatment on August 11, 2022, and the data cutoff date was February 28, 2024, providing approximately 18 months of data as of the cutoff. The trial schematic is shown in [Figure 74](#).

Figure 74. Trial Design, Trial CS7



Source: ISIS 721744-CS7 Clinical Protocol (Appendix 16.1.1)

Terminology: Open-label Extension patients are also referred to as Rollover patients. Switch patients are also referred to as Naïve patients.

Abbreviations: ET, early termination; Q4W, every 4 weeks; Q8W, every 8 weeks

The trial was comprised of four periods, including a Qualification Period for Rollover subjects (Weeks -4 to -1) or a Screening Period for Naïve subjects (Weeks -10 to -1), a Treatment Period (Weeks 1 to 53) for all subjects (Rollover and Naïve), an Extended Treatment Period

(Weeks 54 to 157), and a Post-Treatment Period (end of treatment +13 weeks). Treatment was administered from a vial or AI. Self-administration or caregiver administration was allowed after dosing instructions and training.

In the Treatment Period (Weeks 1 to 53), Rollover subjects who rolled over from Trial CS5 were treated as follows:

- Subjects treated with donidalorsen 80 mg or placebo once every 4 weeks in the Index Trial (Index Trial Cohort A) received the donidalorsen-4 regimen beginning 28 ± 3 days after the last dose in the Index Trial.
- Subjects treated with donidalorsen 80 mg or placebo once every 8 weeks in the Index Trial (Index Trial Cohort B) were to receive the donidalorsen-8 regimen beginning 56 ± 3 days after the last dose in the Index Trial unless they were not attack-free for ≥ 8 weeks (Weeks 17 to 25 during the Index Trial), in which case they were to receive the donidalorsen-4 regimen.

Naïve subjects were to be treated with donidalorsen-4 for the Treatment Period (Weeks 1 to 53).

Following the Week 53 visit, subjects were to receive donidalorsen for up to an additional 104 weeks in the Extended Treatment Period. In this Extended Treatment Period, subjects (whether Rollover or Naïve) 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-4 to donidalorsen-8 or donidalorsen-8 to donidalorsen-4. Change in dose frequency could occur at any time during the Extended Treatment Period. In analyses, the On-Treatment Period includes both the 52-week Treatment Period and the 104-week Extended Treatment Period (i.e., the entire duration of drug administration during Trial CS7). The Integrated Treatment Period (for Rollover subjects only) is the period during which donidalorsen was administered in the Index Trial (CS5).

Rollover subjects who met the Trial CS7 eligibility criteria and expressed interest in enrollment in Trial CS7 were considered to be in the Qualification Period of Trial CS7 during their final 4 weeks of treatment in Trial CS5. Thus, Rollover subjects who were being treated with donidalorsen during Trial CS5 could continue their treatment (in Trial CS7) without interruption.

Naïve subjects began with a Screening Period to confirm eligibility up to 70 days prior to administration of donidalorsen. Naïve subjects 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 Screening and were to continue to take their HAE prophylactic therapy during the Screening Period, and the timing of the switch to donidalorsen was based on the half-life of the individual therapy. No drug-drug interaction with donidalorsen and the prior therapies were anticipated. The schedule for Naïve subjects to take their final dose of the prior HAE prophylactic therapy is summarized in [Table 81](#).

Table 81. Schedule for Naïve Subjects to Switch Prophylactic Therapy to Donidalorsen Treatment, Trial CS7

Prophylactic Therapy	Schedule
Lanadelumab	Subject was to take the last dose of lanadelumab 14 days $\pm$ 3 days prior to Day 1
Berotrastat	Subject was to continue taking a stable dose of berotrastat for 14 days $\pm$ 3 days after Day 1
CI-esterase inhibitor	Subject was to continue taking a stable dose of CI-esterase inhibitor for 14 days $\pm$ 3 days after Day 1

Source: CS7 clinical study report

15.2.1.1. Sample Size Assumptions, Trial CS7

This was an uncontrolled, open-label trial not designed for statistical testing of a hypothesis, but rather for long-term observation of safety and efficacy. The sample size for Trial CS7 was based on the number of subjects enrolled in Trial CS5 and their eligibility to enroll in the open-label extension trial. Eighty-three out of the 90 subjects in Trial CS5 enrolled as Rollover subjects in Trial CS7. For Naïve subjects, sample size was selected based on clinical assessment. A total of 65 Naïve subjects enrolled in Trial CS7.

15.2.2. Schedule of Assessments, Trial CS7

In the Treatment Period through Week 53, trial visits, physical examinations, laboratory monitoring, and AE monitoring occurred per the schedules shown in [Table 82](#) and [Table 83](#). Routine clinical testing, including vital signs and coagulation studies, was generally performed every 3 months, while certain tests were performed monthly: hematology, serum chemistry with hepatic function tests, and pregnancy testing. In the Extended Treatment Period, only pregnancy testing occurred monthly, with the other laboratory tests and vital signs evaluated every 3 months ([Table 84](#)). The schedules of assessments are acceptable for a phase 3b, open-label extension trial.

Table 82. Schedule of Assessments for Rollover Subjects From Index Trial CS5, Trial CS7

	Qual	Treatment Period														Post-Treatment Period		
Study Week	-4 to -1	1	5	9	13	17	21	25	29	33	37	41	45	49	53	4 ¹	8 ¹	13 ¹
Study Day	-28 to -1 ²	1 ³	29 ²	57 ²	85 ²	113 ²	141 ²	169 ²	197 ²	225 ²	253 ²	281 ²	308 ²	337 ²	365/ ET-L ⁴ / Tx-ET	28 ²	56 ²	91 ² /Post -Tx ET
Visit Windows (days)	± 3	0	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4
Informed Consent/Assent	X																	
Inclusion/Exclusion	X	X																
Concomitant Meds and Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight and Vital Signs ⁵	X	X			X			X			X			X	X			X
Height															X ¹⁶			
Pregnancy Test ⁶	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Physical Examination/Body Assessment ⁷	X	X			X ¹⁵			X			X ¹⁵				X			X
12-Lead ECG ¹⁷		X						X							X			X
Urinalysis ⁸	X	X	X	X	X			X			X				X			X
hsCRP		X						X							X			X
Complement ⁸		X						X							X			X
Coagulation ⁸		X			X			X			X				X			X
Chemistry, Hematology ^{8, 9}	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Study Drug Administration (donidalorsen) ¹⁰		Patients continued on their dosing schedule from ISIS 721744-CS5: - Patients treated with drug or placebo Q4W in ISIS 721744-CS5 received donidalorsen 80 mg or placebo once Q4W - Patients treated with drug or placebo Q8W in ISIS 721744-CS5 received donidalorsen 80 mg or placebo once Q8W unless they were not attack free for ≥ 8 weeks (Week 17-25 in ISIS 721744-CS5), in that case they received donidalorsen 80 mg Q4W																
AE-QoL		X			X			X			X				X			X
HAE-QoL		X						X							X			
AECT		X			X			X			X				X			
AAS ¹¹	Completed on days when there were HAE symptoms																	
HAE Attack Assessment ¹²	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
TSQM-II		X						X							X			
ER visits, hospitalizations, total inpatient days ¹³	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADA Blood Sampling		See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)																

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	Qual	Treatment Period														Post-Treatment Period		
Study Week	-4 to -1	1	5	9	13	17	21	25	29	33	37	41	45	49	53	4 ¹	8 ¹	13 ¹
Study Day	-28 to -1 ²	1 ³	29 ²	57 ²	85 ²	113 ²	141 ²	169 ²	197 ²	225 ²	253 ²	281 ²	308 ²	337 ²	365/ ET-L ⁴ / Tx-ET	28 ²	56 ²	91 ² /Post -Tx ET
Visit Windows (days)	± 3	0	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4
PK Blood Sampling		See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)																
PD (PKK) Blood Sampling		See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)																
Archive Serum Samples ¹⁴		X			X			X			X				X			X

Source: Study Protocol, version 3.0, dated 14 Mar 2023, Protocol [Appendix A1](#) (Appendix 16.1.1).

All procedures and study samples collections were done *predose* at respective visits, unless specified otherwise

- ¹ Weeks/days from end of treatment. End of treatment was defined as 1 dosing interval (i.e., 4 weeks/28 days or 8 weeks/56 days) post-last dose
 - ² Assessments and procedures were conducted by either a HHC nurse (if available), or the Study Center as arranged by the Study Center personnel
 - ³ Study Day 1 could be conducted on the same day as the last period visit in the ISIS 721744-CS5 study (all procedures were completed prior to donidalorsen administration). As was operationally feasible, the first dose in this study (ISIS 721744-CS7) was administered 28 ± 3 days (for ISIS 721744-CS5 Cohort A patients) or 56 ± 3 days (for ISIS 721744-CS5 Cohort B patients) after the last dose in ISIS 721744-CS5
 - ⁴ Landmark visit for patients who discontinued early from the Treatment Period
 - ⁵ Vital signs included blood pressure, heart rate, respiratory rate, and body temperature
 - ⁶ Females of childbearing potential only. Serum pregnancy was performed at Qualification, but urine pregnancy was performed at all other time points. A positive result from a urine pregnancy test was confirmed with a serum pregnancy test
 - ⁷ Physical Examination was done as a Body Assessment if conducted by a nurse or Home Healthcare professional
 - ⁸ For a complete list of laboratory tests refer to Protocol [Appendix B](#) (Appendix 16.1.1)
 - ⁹ If the PLT value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen was re-drawn as soon as possible (ideally within 7 days)
 - ¹⁰ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member), was allowed after dosing instructions and training were provided by qualified site personnel. A Home Healthcare professional was available to complete all predosing procedures prior to donidalorsen administration. Autoinjector malfunction was reported into an eCRF
 - ¹¹ The AAS paper questionnaire was completed by patients at the time of having any potential HAE symptoms
 - ¹² During the study, patients were instructed to report details of any HAE attack, including on-demand treatment, to the study site within 72 hours of the onset of the attack
 - ¹³ This was to be recorded in the eCRF monthly
 - ¹⁴ For follow-up exploration of laboratory findings and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc)
 - ¹⁵ Targeted physical examination/body assessment included examination of the skin (including injection site), chest and mouth following donidalorsen administration
 - ¹⁶ For adolescent patients only
 - ¹⁷ On dosing days, the ECG was conducted prior to dose administration
- Abbreviations: AAS = angioedema activity score; ADA = anti-drug antibody; AE = adverse event; AECT = angioedema control test; AE-QoL = angioedema quality of life; eCRF = electronic case report form; ECG = electrocardiogram; ER = emergency room; HAE = hereditary angioedema; HAE-QoL hereditary angioedema quality of life; HHC = home healthcare; hsCRP = c-reactive protein measured by high sensitivity assay; PD = pharmacodynamic(s); PKK = prekallikrein; PLT = platelet; PK = pharmacokinetic(s); Q4W = every 4 weeks; Q8W = every 8 weeks; TSQM-II = treatment satisfaction questionnaire for medication, version II; Tx-ET = treatment early termination

Source: CS7 clinical study report

Abbreviations: ET-L, extended treatment; Qual, qualification

Table 83. Schedule of Assessments for Naïve Subjects, Trial CS7

Study Week	Screening	Treatment Period														Post-Treatment Period			
	-10 to -1	1	5	9	13	17	21	25	29	33	37	41	45	49	53	4 ¹	8 ¹	13 ¹	
Study Day	-70 to -1	1	29 ²	57 ²	85 ²	113 ²	141 ²	169 ²	197 ²	225 ²	253 ²	281 ²	308 ²	337 ²	365/ ET-L ³ / Tx-ET	28 ²	56 ²	91 ² /Post- Tx ET	
Visit Windows (days)	± 3	0	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	
Informed Consent/Assent	X																		
Inclusion/Exclusion	X	X																	
Medical History	X																		
HAE Attack History	X																		
FSH ⁴	X																		
HBV, (HCV), HIV	X																		
Height	X														X ¹⁶				
Prior and Concomitant Meds and Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Body Weight and Vital Signs ⁵	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Pregnancy Test ⁶	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Physical Examination/Body Assessment ⁷	X	X	X ¹⁵	X ¹⁵	X ¹⁵	X		X ¹⁵			X ¹⁵				X			X	
12-Lead ECG ¹⁷	X	X						X							X			X	
Urinalysis ⁸	X	X	X	X	X	X		X			X		X		X			X	
hsCRP		X	X	X	X	X		X			X		X		X			X	
Complement ⁸		X	X	X	X	X		X			X		X		X			X	
C1-INH, C4, C1q ⁸	X																		
Coagulation ⁸	X	X	X	X	X	X		X			X		X		X			X	
Chemistry, Hematology ^{8, 9}	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration (donidalorsen) ¹⁰		X	X	X	X	X	X	X	X	X	X	X	X	X	X				
AE-QoL		X				X		X			X				X			X	
HAE-QoL		X				X		X							X			X	
AECT		X		X		X		X			X				X			X	
ER visits, hospitalizations, total inpatient days	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
AAS ¹¹		Completed on days when there were HAE symptoms																	
HAE Attack Assessment ¹²	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
TSQM-II	X					X									X				
Treatment Preference Questionnaire						X													
ISP Assessment ¹³	X	X	X	X	X	X													
ADA Blood Sampling			See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)																

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Study Week	Screening	Treatment Period														Post-Treatment Period		
	-10 to -1	1	5	9	13	17	21	25	29	33	37	41	45	49	53	4 ¹	8 ¹	13 ¹
Study Day	-70 to -1	1	29 ²	57 ²	85 ²	113 ²	141 ²	169 ²	197 ²	225 ²	253 ²	281 ²	308 ²	337 ²	365/ ET-L ³ / Tx-ET	28 ²	56 ²	91 ² /Post- Tx ET
Visit Windows (days)	± 3	0	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4
PK Blood Sampling		See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)																
PD (PKK) Blood Sampling		See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)																
Archive Serum Samples ¹⁴		X	X	X	X	X		X			X				X			X

Source: Study Protocol, version 3.0, dated 14 Mar 2023, Protocol [Appendix A2](#) (Appendix 16.1.1).

All procedures and study samples collections were done *predose* at respective visits, unless specified otherwise

- ¹ Weeks/days from end of treatment. End of treatment was defined as 1 dosing interval (i.e., 4 weeks/28 days) post-last dose
 - ² Assessments and procedures were conducted by either a HHC nurse (if available), or the Study Center as arranged by the Study Center personnel
 - ³ Landmark visit for patients who discontinued early from the Treatment Period
 - ⁴ For confirmation of menopause at Screening per Inclusion Criteria, as applicable
 - ⁵ Vital signs included blood pressure, heart rate, respiratory rate, and body temperature
 - ⁶ Females of childbearing potential only. Serum pregnancy was performed at Screening, but urine pregnancy was performed at all other time points. A positive result from a urine pregnancy test was confirmed with a serum pregnancy test.
 - ⁷ Physical Examination was done as a Body Assessment if conducted by a nurse or HHC professional
 - ⁸ For a complete list of laboratory tests refer to Protocol [Appendix B](#) (Appendix 16.1.1). Samples for C4, C1q and C1-INH assays were obtained at Screening. Historical values could be used for the HAE Inclusion Criteria
 - ⁹ If the PLT value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen was re-drawn as soon as possible (ideally within 7 days)
 - ¹⁰ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member), may have been allowed after dosing instructions and training were provided by qualified site personnel. A HHC professional completed all pre-dosing procedures prior to donidalorsen administration. Autoinjector malfunction was reported into the eCRF.
 - ¹¹ The AAS paper questionnaire was completed by patients at the time of having any potential HAE symptoms
 - ¹² During the study patients were instructed to report details of any HAE attack, including on-demand treatment, to the study site within 72 hours of the onset of the attack
 - ¹³ Injection Site Pain (ISP) assessment was recorded at the time of every injection and 5 minutes after each injection during Screening and through Week 17. During Screening the patients recorded the ISP of the prophylactic treatment they received before switching to donidalorsen. Patients were provided at Screening with paper ISP questionnaire for use during the Screening and Treatment Periods
 - ¹⁴ For Follow-Up exploration of laboratory findings and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc)
 - ¹⁵ Targeted physical examination/body assessment included examination of the skin (including injection site), chest and mouth following donidalorsen administration
 - ¹⁶ For adolescent patients only
 - ¹⁷ On dosing days, an ECG was conducted prior to dose administration
- Abbreviations: AAS = angioedema activity score; ADA = anti-drug antibody; AEs = adverse events; AECT = angioedema control test; AE-QoL = angioedema quality of life; C1-INH = C1 inhibitor; C1q = complement 1q; C4 = complement factor 4; eCRF = electronic case report form; ECG = electrocardiogram; ER = emergency room; ET-L = extended treatment; FSH = follicle stimulation hormone; HAE = hereditary angioedema; HAE-QoL hereditary angioedema quality of life; HBV = hepatitis B virus; HCV = hepatitis C virus; ; HHC =home health care; hsCRP = c-reactive protein measured by high sensitivity assay; HIV = human immunodeficiency virus; ISP = injection site pain; PD = pharmacodynamics; PLT = platelet; PK =pharmacokinetic(s); PKK = prekallikrein; TSQM-II = treatment satisfaction questionnaire for medication, version II; Tx-ET = treatment early termination.

Source: CS7 clinical study report

Table 84. Schedule of Assessments for Extended Treatment Period for All Subjects, Trial CS7

	Extended Treatment Period												
Study Week	57	61	65	69	73	77	81	85	89	93	97	101	105
Study Day	393 ¹¹	421 ¹¹	449 ¹¹	477 ¹¹	505 ¹¹	533 ¹¹	561 ¹¹	589 ¹¹	617 ¹¹	645 ¹¹	673 ¹¹	701 ¹¹	729 ¹¹
Visit Windows (Days)	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4
Study Visit ¹			X			X			X				X
Concomitant Medications and Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight and Vital Signs ²			X			X			X				X
Urine Pregnancy Test ^{3,4}	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/ Body Assessment													X
Height													X ¹²
12-Lead ECG ¹³						X							X
Complement ⁵						X							X
Plasma PKK	See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)												
Chemistry, Hematology, Coagulation ^{5,6}			X			X			X				X
Study Drug Administration (donidalorsen) ^{7,8}	• Patients continued to receive donidalorsen 80 mg as per their prior dosing schedule (Q4W or Q8W). • Patients treated with donidalorsen 80 mg Q8W can change dosing at any time to donidalorsen 80 mg once Q4W as determined by clinical symptoms. • Patients treated with donidalorsen 80 mg Q4W can change dosing at any time to donidalorsen 80 mg once Q8W.												
Immunogenicity and PK	See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)												
AE-QoL and HAE-QoL						X							X
AAS ⁹ and HAE Attack Assessment ¹⁰	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis ⁵			X			X			X				X

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	Extended Treatment Period													Post-Treatment Period
Study Week	109	113	117	121	125	129	133	137	141	145	149	153	157/ET	170 (13 weeks after Week 157 or ET)
Study Day	757 ¹¹	785 ¹¹	813 ¹¹	841 ¹¹	869 ¹¹	897 ¹¹	925 ¹¹	953 ¹¹	981 ¹¹	1009 ¹¹	1037 ¹¹	1065 ¹¹	1093	1184 ¹¹
Visit Windows (Days)	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4	± 4
Study Visit ¹			X			X			X				X	X
Concomitant Medications and Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight and Vital Signs ²			X			X			X				X	X
Urine Pregnancy Test ^{3,4}	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/ Body Assessment ¹²													X	
Height													X ¹²	
12-Lead ECG ¹³						X							X	
Complement ⁵						X							X	
Plasma PKK	See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)													
Chemistry, Hematology, Coagulation ^{5,6}			X			X			X				X	X
Study Drug Administration (donidalorsen) ^{7,8}	- Patients continued to receive donidalorsen 80 mg as per their prior dosing schedule (once Q4W or Q8W). - Patients treated with donidalorsen 80 mg Q8W can change dosing at any time to donidalorsen 80 mg once Q4W as determined by clinical symptoms. - Patients treated with donidalorsen 80 mg Q4W can change dosing at any time to donidalorsen 80 mg once Q8W.													
Immunogenicity and PK	See Study Protocol, version 3.0, dated 14 Mar 2023, Protocol Appendix C (Appendix 16.1.1)													
AE-QoL and HAE-QoL						X							X	
AAS ⁹ and HAE Attack Assessment ¹⁰	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis ⁵			X			X			X				X	X

Source: Study Protocol, version 3.0, dated 14 Mar 2023, Protocol [Appendix A3](#) (Appendix 16.1.1).
All procedures and study samples collections were done *predose* at respective visits, unless specified otherwise

- ¹ Procedures, assessments, and confirmation of Study Drug administration were conducted via telephone contact by qualified site personnel on non-study visit/HHC days
- ² Vital signs included blood pressure, heart rate, respiratory rate, and body temperature
- ³ Females of childbearing potential only
- ⁴ Patient took a home pregnancy test (Ionis to supply) and reported the results to the site. Except on site/HHC visit days
- ⁵ For a complete list of laboratory tests refer to Protocol [Appendix B](#) (Appendix 16.1.1)

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- ⁶ If the platelet value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen was re-drawn as soon as possible (ideally within 7 days)
- ⁷ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member), may have been allowed after dosing instructions and training were provided by qualified site personnel. A HHC professional completed all predosing procedures prior to Study Drug administration. Autoinjector malfunction was reported into the eCRF.
- ⁸ If the patient discontinued the Study Drug, they then entered the Post-Treatment Period
- ⁹ The AAS questionnaire was only completed by patients if an HAE attack occurred
- ¹⁰ During the study, patients were instructed to report details of any HAE attack, including on-demand treatment to the study site within 72 hours of the onset of the attack
- ¹¹ Assessments and procedures were conducted by either a HHC nurse (if available), or the Study Center as arranged by the Study
- ¹² For adolescent patients only
- ¹³ On dosing day, an ECG was conducted prior to dose administration

Abbreviations: AAS = angioedema activity score; AE = adverse events; AE-QoL = angioedema quality of life; ECG = electrocardiogram; ET = end of treatment; HAE = hereditary angioedema; HAE-QoL = hereditary angioedema quality of life; HCC = home healthcare; PKK = prekallikrein; PK = pharmacokinetic(s); Q4W = every 4 weeks; Q8W = every 8 weeks

Source: CS7 clinical study report

15.2.3. Primary, Secondary, and Exploratory Objectives and Endpoints, Trial CS7

The primary objective of this trial was to evaluate the long-term safety of donidalorsen as prophylaxis in patients with HAE. The primary endpoint was the incidence and severity of TEAEs.

The secondary objectives of this trial were to evaluate the long-term efficacy and the effects of donidalorsen on the number of HAE attacks and their impact on the QoL of patients with HAE. The secondary endpoints were:

- 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 subjects 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 HAE therapy from Week 5 to Week 53.
- The Angioedema Quality of Life questionnaire total score over 52 weeks.

The exploratory objectives of this trial were to characterize the effects of donidalorsen on HAE attacks, additional PROs, and biomarkers. The exploratory endpoints were:

- All subjects:
 - The time-normalized number of investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 157.
 - The Angioedema Quality of Life questionnaire total score over 157 weeks.
 - Percentage of total days during the Treatment Period that subjects were attack free.
 - Percentage of total months during the Treatment Period that subjects were attack free.
 - Duration in days of the longest attack-free interval.
 - Percent of subjects who were attack free for 6 or 12 consecutive months.
 - Mean/median longest attack-free interval.
 - Rate of HAE attacks that involve the larynx.
 - Plasma PKK levels over 52 weeks.
 - AECT over 52 weeks.
 - Hereditary Angioedema Quality of Life questionnaire total score over 52 weeks.

- Treatment Satisfaction Questionnaire for Medication, version 11 (TSQM-11) score over 52 weeks.
- Incidence of ER visits, all-cause hospitalization, and total inpatient days over 52 weeks.
- Pharmacokinetic exposure over time and potential exposure-response analysis using relevant exposure parameters and biomarkers.
- Rollover subjects only
 - The time-normalized number of investigator-confirmed HAE attack rate (per 4 weeks) compared to Trial ISIS 721744-CS5 (Index Trial) from Week 1 to Week 53.
- Naïve subjects only
 - The time-normalized number of investigator-confirmed HAE attacks (per 4 weeks) from Week 1 to Week 17.

15.2.4. Analysis Populations, Trial CS7

The following analysis sets were defined by the Applicant in the SAP, version 1.0, dated April 3, 2024:

- The Safety Set was to include all enrolled subjects who received at least one dose of donidalorsen.
- The Full Analysis Set was to include all enrolled subjects who received at least one dose of donidalorsen.
- The Per Protocol Set was to include all subjects 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 subjects who enrolled and received at least one dose of donidalorsen and had at least one evaluable PK sample.

15.2.5. Statistical Analysis Plan, Trial CS7

For all endpoints, HAE attacks refers to investigator-confirmed HAE attacks.

For both Rollover and Naïve subjects, the time-normalized number of HAE attacks, as well as the changes and the percent changes from baseline from Week 1 to Week 53, was summarized using descriptive statistics. Trial CS5 Run-In baseline was used for the Rollover subjects, and Trial CS7 baseline was used for the Naïve subjects. For Rollover subjects only, this endpoint was also compared to the Trial CS5 on-treatment baseline. A summary using integrated data from Trial CS5 and Trial CS7 was planned. The time-normalized number of HAE attacks from Trial CS5 Week 1 to Trial CS7 Week 157, and from Index Trial Week 5 to OLE Week 157 was compared to the Trial CS5 Run-In baseline. In addition, a summary of every 4 weeks was provided by grouping HAE attacks into 28-day intervals.

The time-normalized number of HAE attacks from Week 5 to Week 53 was summarized similarly for both Rollover and Naïve subjects.

For Naïve subjects only, the time-normalized number of investigator-confirmed attack rate from Week 1 to Week 17 and from Week 5 to Week 17 were summarized using the similar methods. To explore whether attack rates remained stable after transitioning for Naïve subjects, p-values using paired t-tests were provided for Week 1 to Week 17 attack rates compared to baseline attack rates.

The time-normalized number of moderate or severe HAE attacks from Week 5 to Week 53 was summarized similarly for both Rollover and Naïve subjects.

The time-normalized number of HAE attacks requiring acute therapy from Week 5 to Week 53 was summarized similarly for both Rollover and Naïve subjects.

15.2.6. Protocol Amendments, Trial CS7

The original protocol (dated February 28, 2022) was amended twice. Key changes in Amendment #1 were the addition of the Extended Treatment Period and an increase in the planned enrollment number of Naïve subjects from 30 to 60 to obtain additional safety information. Amendment #2 allowed use of an AI to administer donidalorsen.

15.2.7. Interim Analyses, Trial CS7

The interim analyses presented in the Applicant's CSR were performed after the data cutoff date of February 28, 2024 (final database lock on April 10, 2024). The results of the interim analyses were intended for regulatory submission, not to be used for stopping or adapting the trial or adjusting the sample size.

15.2.8. Data and Safety Monitoring Committee, Trial CS7

The safety of donidalorsen was continually monitored throughout the trial by the investigator or designee and the Applicant or designee. No Data and Safety Monitoring Committee was utilized for this OLE trial. Safety was evaluated by the collection and measurement of AEs, clinical laboratory parameters (including hematology, serum chemistry, and urinalysis), concomitant medication/procedure information, vital signs, and ECGs.

15.3. Trial CS2

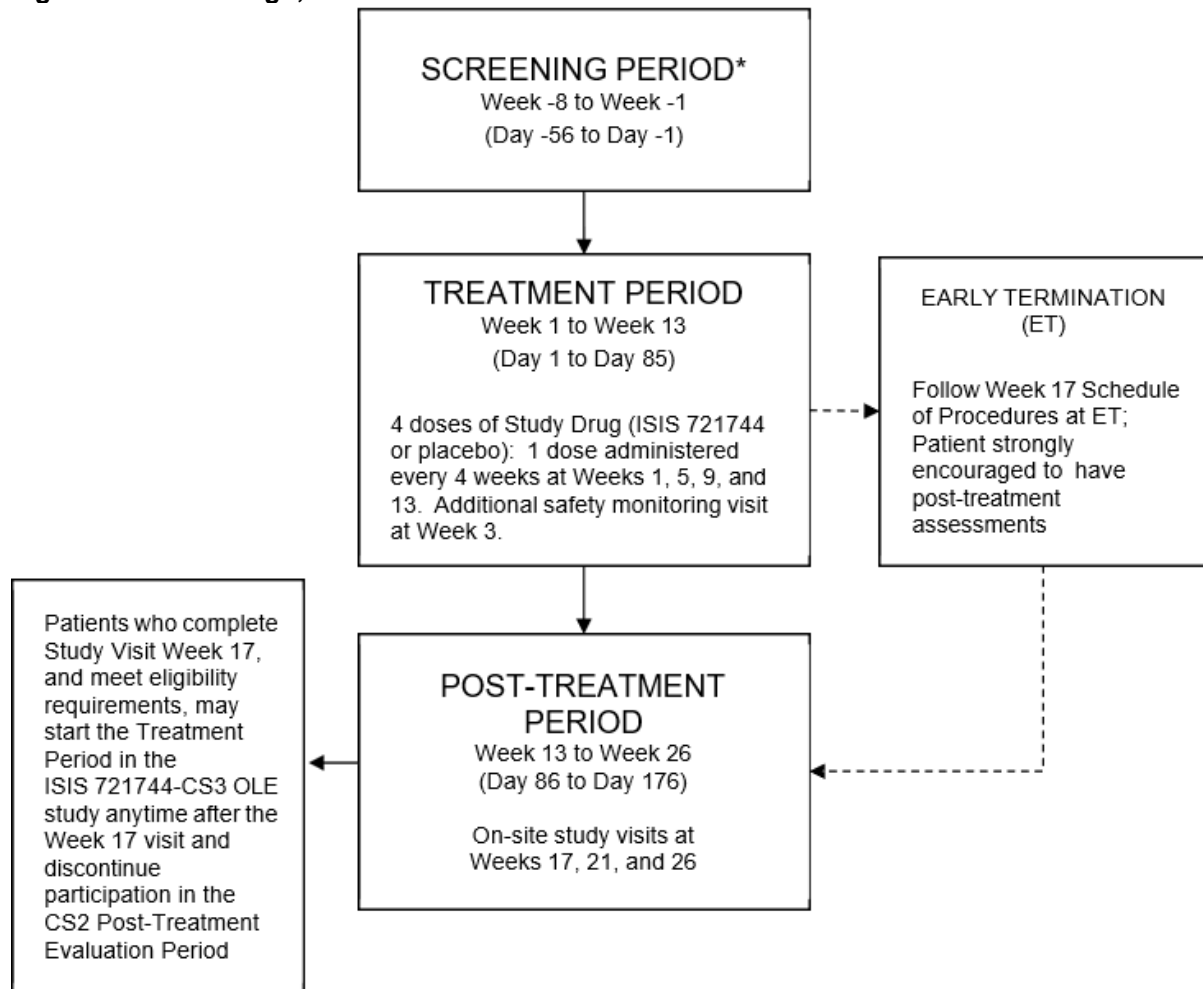
15.3.1. Trial Design, Trial CS2

Results from Trial CS2 support the efficacy and safety of donidalorsen for HAE attack prevention. This was a two-part, phase 2, multicenter trial. Subjects were allocated into Part A or Part B according to type of HAE, with either type I or II in Part A and type III in Part B. Parts A and B were conducted concurrently. The trial visit schedule and procedures were nearly identical in Parts A and B. Part B enrolled three adults with HAE type III for open-label treatment with donidalorsen-4. Because Part B was uncontrolled and had a small sample size, it is not considered adequate and well-controlled, and does not support efficacy or safety; the Agency's review focuses on Part A of Trial CS2 only.

Part A was a randomized, placebo-controlled, parallel-group trial in 20 adults with HAE type I or II in a 2:1 ratio to donidalorsen 80 mg (n=14) or placebo (n=6) SC every 4 weeks. The trial schedule included a Screening Period of up to 8 weeks, a 12-week Treatment Period when subjects received treatment every 4 weeks during 4 on-site trial visits, and a 4- to 13-week Post-Treatment Period, with the duration determined by whether a subject enrolled in Trial CS3, the follow-on OLE trial. Subjects completed post-treatment follow-up visits at Weeks 17, 21, and 26/Early Termination. Alternatively, subjects who completed the Week 17 trial visit and met eligibility requirements started the Treatment Period in Trial CS3 any time after the Week 17 visit and discontinued participation in the Trial CS2 Post-Treatment Period. For subjects not enrolling in the OLE trial, the final trial visit was the Week 26/Early Termination Visit. The trial design is shown in [Figure 75](#).

The length of trial participation for each subject was up to 8 months. Written and signed informed consent was obtained prior to performance of any trial-related procedures, including Screening. Each enrolled subject was randomized to begin trial treatment as soon as the subject experienced ≥ 2 HAE attacks during the Screening Period.

Figure 75. Trial Design, Trial CS2



Source: CS2 clinical study report
Abbreviations: OLE, open-label extension

15.3.2. Schedule of Assessments, Trial CS2

In Trial CS2, trial visits, physical examinations, laboratory monitoring, and AE monitoring occurred per the schedule shown in [Table 85](#). Routine clinical testing, including vital signs and coagulation studies, was generally performed every 2 or 4 weeks. The schedule of assessments is acceptable for a phase 2 trial.

Table 85. Schedule of Assessments, Trial CS2

Study Week/Day	Screening ¹	Treatment Period (12 Weeks)					Post-Treatment Period ¹⁶ (13 Weeks)		
	Weeks -8 to -1 Days -56 to -1	Week 1 Day 1	Week 3 Day 15 ¹⁷	Week 5 Day 29 ¹⁷	Week 9 Day 57 ¹⁷	Week 13 Day 85 ¹⁷	Week 17 Day 113 ¹⁷ or ET	Week 21 Day 141 ¹⁷	Week 26 Day 176 ¹⁷
Visit Window (Days)	± 0	± 0	± 2	± 2	± 2	± 2	± 2	± 2	± 3
Informed Consent	X								
Inclusion/Exclusion	X								
Demographics and Medical History (including detailed HAE history)	X								
Prior/Concomitant Medications (including HAE treatment)	X	X	X	X	X	X	X	X	X
Body Weight and Height ²	X	X	X	X	X	X	X	X	X
Physical Examination	X	X		X	X	X	X	X	X
Vital Signs ³	X	X	X	X	X	X	X	X	X
HIV, Hepatitis B, and Hepatitis C	X								
FSH ⁴	X								
Pregnancy Test ⁵	X	X		X	X	X	X	X	X
Clinical Laboratory Parameters (chemistry, hematology, complement, and coagulation) ^{6,7}	X	X	X	X	X	X	X	X	X

Study Week/Day	Screening ¹	Treatment Period (12 Weeks)					Post-Treatment Period ¹⁶ (13 Weeks)		
	Weeks -8 to -1 Days -56 to -1	Week 1 Day 1	Week 3 Day 15 ¹⁷	Week 5 Day 29 ¹⁷	Week 9 Day 57 ¹⁷	Week 13 Day 85 ¹⁷	Week 17 Day 113 ¹⁷ or ET	Week 21 Day 141 ¹⁷	Week 26 Day 176 ¹⁷
Visit Window (Days)	± 0	± 0	± 2	± 2	± 2	± 2	± 2	± 2	± 3
Urinalysis	X	X	X	X	X	X	X	X	X
Threshold-Stimulated Kallikrein Activity (if applicable) ⁸	X								
Genetic Diagnostic Testing (if applicable) ⁹	X								
AE-QoL	X	X			X		X		X
Angioedema Activity Score ¹⁰	X	X	X	X	X	X	X	X	X
HAE Attack Assessment (including on-demand treatment) ^{11, 12}	X	X	X	X	X	X	X	X	X
12-Lead ECG	X	X			X		X		X
Randomization ¹³		X							
Study Drug Administration (ISIS 721744 or placebo)		X		X	X	X			
Adverse Events	X	X	X	X	X	X	X	X	X
Immunogenicity Testing		X ^a	X	X ^a	X ^a		X		X

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Study Week/Day	Screening ¹	Treatment Period (12 Weeks)					Post-Treatment Period ¹⁶ (13 Weeks)		
	Weeks -8 to -1 Days -56 to -1	Week 1 Day 1	Week 3 Day 15 ¹⁷	Week 5 Day 29 ¹⁷	Week 9 Day 57 ¹⁷	Week 13 Day 85 ¹⁷	Week 17 Day 113 ¹⁷ or ET	Week 21 Day 141 ¹⁷	Week 26 Day 176 ¹⁷
Visit Window (Days)	± 0	± 0	± 2	± 2	± 2	± 2	± 2	± 2	± 3
PK Blood Sampling ¹⁴		X ^b	X	X ^a	X ^a	X ^b	X	X	X
PD Blood Sampling ¹⁵	X	X ^a	X	X ^a	X ^a	X ^a	X	X	X
Inflammatory Panel	X	X ^a	X	X ^a	X ^a	X ^a	X	X	X

Note: When multiple procedures were scheduled at the same visit, clinical assessments and procedures were to be performed prior to Study Drug administration, unless otherwise indicated, and were to proceed in the following order: vital signs, physical examinations, patient-reported outcomes (such as the AAS and the AE-QoL questionnaire), ECGs, and collection of blood and/or urine for laboratory assessments.

- ¹ The Screening Period was up to 8 weeks in duration. A patient may have been randomized after fewer than 8 weeks of Screening if the patient experienced ≥ 2 HAE attacks in less than 8 weeks and had completed all other screening activities and met all other eligibility requirements.
- ² Height was measured at Screening only.
- ³ Vital signs included blood pressure, heart rate, respiratory rate, and body temperature and were to be taken after sitting for at least 5 min.
- ⁴ For confirmation of menopause at Screening per inclusion criteria.
- ⁵ Only for women who were not surgically sterile or postmenopausal. A serum pregnancy test was performed during Screening and urine pregnancy tests were performed at all other study visits.
- ⁶ If the platelet value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis, or quantity not sufficient) or missing, a repeat blood specimen was to be redrawn as soon as possible (ideally within 7 days).
- ⁷ For a complete list of laboratory analytes, refer to Table 5.
- ⁸ Assessed only in patients who had HAE-nC1-INH without a factor XII mutation or the plasminogen or angiotensin-1 mutation.
- ⁹ Performed only for patients who did not have HAE genetic diagnostic testing results prior to Screening.
- ¹⁰ The AAS questionnaire was completed by patients on a daily basis (minimum of 4 daily assessments per week) for the duration of the study. Patients were instructed on the use of the AAS during Screening and recorded their HAE attacks using the AAS throughout the Screening Period (daily during up to 8 consecutive weeks) to confirm study eligibility; patients were required to experience at least 2 HAE attacks (assessed by the AAS and confirmed by the Investigator) during the Screening Period to be eligible for enrollment. If a patient experienced a third attack, the patient was to be randomized as soon as possible, assuming all other screening activities had been completed and the patient met all other eligibility requirements; in such cases, the Screening Period may have been shorter than 8 weeks. Enrolled patients were to continue to record HAE attacks using the AAS throughout the Treatment and Post-Treatment Periods.
- ¹¹ Historical HAE attack information was collected at Screening. 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, Treatment, and Post-Treatment Periods, site personnel contacted the patient once a week (at approximately 7 days after the last contact with the patient) to inquire about any attack that may have occurred. In addition, during study visits, site personnel inquired about any new HAE attack information that was not provided through patient contact with the site.
- ¹² The following details relating to the localization, severity, and course of all HAE attacks that occurred since the previous visit were collected: date/time of symptom onset; description of symptoms; location of symptoms (peripheral angioedema, abdominal angioedema, or laryngeal angioedema); impact of HAE attack on activity level; HAE attack severity (mild, moderate, or severe); need for assistance, medical intervention, emergency room visit, or hospitalization; medications to treat the attack; HAE attack course, including if the HAE attack(s) in question was a typical attack for the patient, or if there was an alternative diagnosis; and date/time symptoms resolved. The number of HAE attacks that required on-demand treatment, and the frequency and dose of on-demand treatment, were also b recorded.
- ¹³ After all screening assessments had been completed and the Investigator had verified that the patient was eligible.
- ¹⁴ Refer to Table 3 for the PK Sampling Schedule.
- ¹⁵ To be collected at each visit to assess plasma PKK, plasma proenzyme activation, and cHK levels.
- ¹⁶ Patients who completed Study Visit Week 17, and met eligibility requirements, may have started the Treatment Period in the ISIS 721744-CS3 OLE study anytime after the Week 17 visit and discontinued participation in the CS2 Post-Treatment Evaluation Period.
- ¹⁷ Assessments and procedures may have been conducted by either a Home Healthcare professional (if available) or the Study Center as arranged by the Study Center personnel. Physical Exam was labeled Body Assessment if conducted by a Home Healthcare professional. Study Drug administration by a Home Healthcare professional may have been done only after Study Drug for home use was available.

Time (in reference to Study Drug administration):

^a Pre-dose.

^b Pre-dose, 1, 2, 4, and 6 hours post-dose (PK Subgroup) or pre-dose and 2-hours post-dose.

Source: CS2 clinical study report

Abbreviations: AAS, Angioedema Activity Score; AE-QoL, Angioedema Quality Of Life Questionnaire; cHK, cleaved high molecular weight kininogen; ECG, electrocardiogram; ET, end of treatment; FSH, follicle-stimulating hormone; HAE, hereditary angioedema; HAE-nC1-INH, hereditary angioedema with normal C1-inhibitor; HIV, human immunodeficiency virus; OLE, open-label extension; PD, pharmacodynamic; PK, pharmacokinetic; PKK, prekallikrein

15.3.3. Primary, Secondary, and Exploratory Objectives and Endpoints, Trial CS2

The primary objective of Trial CS2 Part A was to evaluate the clinical efficacy of donidalorsen in patients with HAE types I and II. The primary endpoint was the time-normalized number of HAE attacks (per month) from Week 1 to Week 17. HAE attack information was collected as described in Section [15.1.1](#). An HAE attack was defined as an event with signs or symptoms consistent with an attack in at least one of the following locations: peripheral, abdominal, or laryngeal. A discrete attack was counted if there was at least a 24-hour symptom-free period between attacks. This definition of HAE attacks is subtly different than the investigator-confirmed definition used in the phase 3 trials, but not expected to impact the Agency's overall conclusions, as this trial was intended to provide proof-of-concept and not SEE.

The secondary objectives of Trial CS2 Part A were to evaluate the safety and tolerability of donidalorsen in patients with HAE types I and II and to evaluate the effects of donidalorsen on plasma PKK and other biomarkers. The secondary endpoints, which were not tested with Type I error control, included:

- 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 subjects with a clinical response (defined as a $\geq 50\%$, $\geq 70\%$, or $\geq 90\%$ 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.
- Prekallikrein activity at Weeks 9 and 17.
- Consumption of on-demand medication at Weeks 9 and 17.
- Angioedema Quality of Life questionnaire score at Weeks 9 and 17.

The exploratory objectives were to evaluate PK over time and assess potential PK/PD correlations on biomarkers and clinical outcomes. The exploratory endpoints included:

- The number of HAE attack-free subjects by Week 17.
- Pharmacokinetic parameters, including, but not limited to: $C_{\max}$, $T_{\max}$, AUC, and half-life ($t_{1/2}$).
- Potential exposure response analysis using relevant exposure parameters and biomarkers.

15.3.4. Eligibility Criteria, Trial CS2

Key eligibility criteria for Part A are summarized below. The trial enrolled subjects ≥ 18 years of age with clinically confirmed HAE and an attack frequency that would likely warrant prophylactic HAE therapy. The inclusion and exclusion criteria are acceptable and consistent with those used in previous trials of approved products for HAE prophylaxis.

Key Inclusion Criteria

- Male or female ≥ 18 years of age at the time of informed consent.
- Diagnosed with clinically confirmed C1-INH (Type I or Type II) HAE based on all of the following:
 - Documented clinical history consistent with HAE (SC or mucosal, nonpruritic swelling episodes without accompanying urticaria).
 - 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 LLN or when a known pathogenic mutation in the *SERPING1* gene had been demonstrated.
 - At least one of the following: age at HAE onset ≤ 30 years; family history consistent with Type I or II HAE; or complement component 1q within the normal range.
- Experienced ≥ 2 HAE attacks during the Screening Period.
- Female subjects: nonpregnant, nonlactating, and either surgically sterile, postmenopausal, abstinent, or using acceptable contraceptive methods.
- Male subjects: either surgically sterile or using an acceptable contraceptive method.

Key Exclusion Criteria

- Concomitant diagnosis of another form of angioedema, such as idiopathic or acquired angioedema, recurrent angioedema associated with urticaria, or Type III HAE.
- Exposure to any of the following medications:
 - ACE inhibitors or any estrogen containing medications with systemic absorption (such as oral contraceptive or hormonal replacement therapy) within 4 weeks prior to Screening.
 - 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/Beriner 2000/3000® subcutaneously/Cinryze/Ruconest within 2 weeks prior to Screening, Orladeyo within 3 weeks prior to Screening).
 - Oligonucleotides within 4 months of Screening (if a single administration) or 12 months of Screening (if multiple doses received); does not apply to vaccines.
- History of acquired coagulopathies or bleeding diathesis (e.g., thrombocytopenia) and inherited bleeding disorders (e.g., hemophilia).
- Any clinically significant renal or hepatic diseases.

Consistent with the eligibility criteria, continued use of a stable dose of androgens was allowed while on trial. The use of on-demand medication to treat acute HAE attacks was also allowed.

15.3.5. Statistical Analysis Plan, Trial CS2

The final SAP, version 1.0, is dated February 17, 2021.

For Trial CS2, the ITT population included all enrolled or randomized subjects. The per-protocol (PP) population included all subjects in the ITT population who were treated according to the protocol without any major deviations.

The primary efficacy endpoint, the time-normalized number of 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 donidalorsen-4 and placebo in Part A using a Poisson regression model and Pearson chi-square scaling of standard errors to account for potential overdispersion. The model included a 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 subject was observed during the period as an offset variable. The primary analysis of the primary endpoint was conducted in the ITT population.

All secondary endpoints that concerned time-normalized varieties of HAE attacks were analyzed using the same method as the primary endpoint.

The number of subjects with a clinical response (defined as $\geq 50\%$, $\geq 70\%$, or $\geq 90\%$ reduction from Baseline in HAE attack rate) by Week 17 was analyzed as a risk difference with a corresponding exact 95% confidence interval and p-value from Fisher's Exact test using the ITT and per-protocol populations. For each subject, a Treatment Period HAE attack rate from Week 5 to Week 17 and Run-In Period HAE attack rate was calculated. The percentage reduction will be 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. Subjects who discontinued treatment early due to lack of efficacy or AEs were considered as nonresponders and included in the denominator for the calculation of the proportion in the ITT population. Subjects who discontinued due to other reason were excluded from analysis.

The number and percentage of subjects who achieved HAE attack free (no HAE attacks) by Week 17 (end of the On-Treatment Period) were tabulated by treatment group and by Part. Subjects who discontinued treatment early due to lack of efficacy or AEs were considered as nonresponders and included in the denominator for the calculation of the proportion in the ITT population. Subjects who discontinued due to other reason were excluded from analysis. The risk difference between donidalorsen-4 group to the placebo group in Part A, and the corresponding exact 95% confidence interval were provided along with the Fisher's Exact test p-value. The analysis was conducted in both ITT and per-protocol populations.

15.3.6. Sample Size Considerations, Trial CS2

The primary endpoint was the time-normalized number of HAE attacks (per month) from Week 1 to Week 17. From historical data, the placebo group was estimated to have 6.8 HAE attacks per 4-month period. Assuming the donidalorsen-4 group has 2.8 HAE attacks per 4-month period, then with a 0.05 significance level and using Poisson model, the sample size of 18 subjects (12 subjects administered donidalorsen-4, and 6 subjects administered placebo) provided at least 90% power for the primary endpoint, considering a 10% missing data or dropout rate for both active treatment and placebo.

15.3.7. Data and Safety Monitoring Committee, Trial CS2

A Data and Safety Monitoring Committee reviewed safety, tolerability, and efficacy (as needed) data collected during the trial.

15.3.8. Protocol Amendments, Trial CS2

The original protocol (dated May 3, 2019) was amended three times. Key changes in Amendment #1 were the addition of several exclusion criteria (related to elevated PTT, history of coagulopathy or bleeding diathesis, and renal and hepatic diseases); these were added prior to the first subject's enrollment. Amendment #2 allowed subjects who completed the Week 17 trial visit to pursue enrollment in the OLE trial (CS3) at any time after that. Amendment #3 allowed flexibility to conduct some trial procedures via home healthcare, in response to the COVID-2019 pandemic. These protocol amendments are not expected to have substantially impacted the trial results.

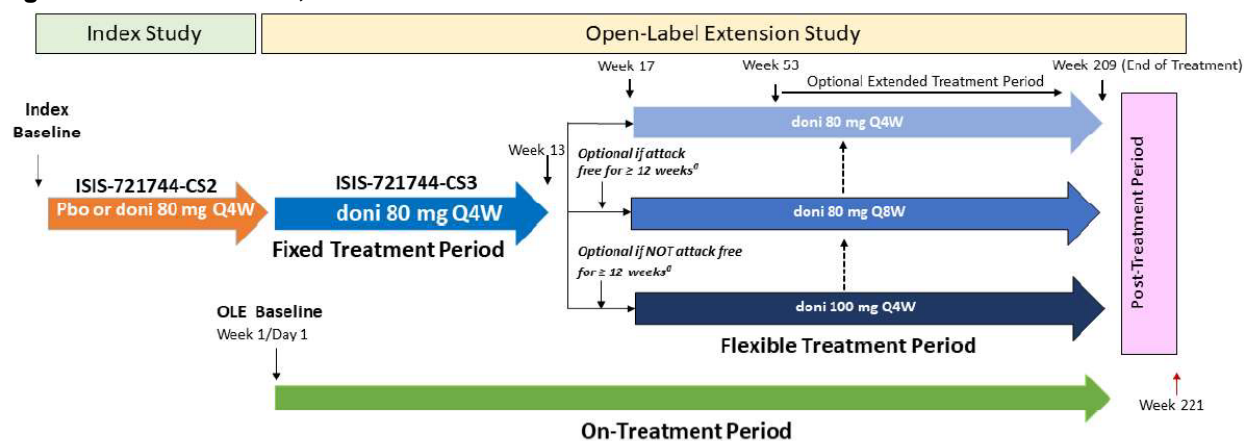
15.4. Trial CS3

15.4.1. Trial Design, Trial CS3

Trial CS3 is an ongoing phase 2, randomized, open-label extension trial for adults with HAE Type I or II who completed Trial CS2. The Trial CS3 CSR submitted with the NDA has a data cutoff date of February 26, 2024. In Trial CS3, 20 subjects were treated with donidalorsen in two dosing periods: the Fixed Dosing Period followed by the Flexible Dosing Period. In the Fixed Dosing Period, every subject received donidalorsen-4 for 12 weeks. In the Flexible Dosing Period, based on Investigator and Sponsor Medical Monitor recommendation, a subject was treated with either: (1) continued donidalorsen 80 mg every 4 weeks; (2) donidalorsen 80 mg once every 8 weeks (if attack free for the prior 12 weeks); or (3) donidalorsen 100 mg once every 4 weeks (if not attack free for the prior 12 weeks). At the end of the 52-week Treatment Period (Fixed Dosing and Flexible Dosing), subjects had the option of: (1) 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 (2) enter the 12-week Post-Treatment Period. The trial design is shown in [Figure 76](#).

Figure 76. Trial Schema, Trial CS3



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.

Source: CS3 clinical study report

15.4.1.1. Sample Size Assumptions, Trial CS3

The sample size for Trial CS3 was dictated by the number of subjects enrolled in Trial CS2.

15.4.2. Schedule of Assessments, Trial CS3

In Trial CS3, trial visits, physical examinations, laboratory monitoring, and AE monitoring occurred per the schedules shown in [Table 86](#), [Table 87](#), [Table 88](#), and [Table 89](#). Routine clinical testing, including vital signs, hematology, serum chemistry with hepatic function tests, and pregnancy testing was performed monthly, with coagulation studies performed every 3 months. For participation beyond Week 53, only pregnancy testing occurred monthly, with the other laboratory tests and vital signs evaluated every 3 months. The schedules of assessments are acceptable for a phase 3b, open-label extension trial.

Table 86. Schedule of Assessments, Year 1, Trial CS3, Protocol Amendment 3, Version 4.0

Assessment	Qual ¹	Treatment Period 1 Fixed Dosing Period				Treatment Period 2 Flexible Dosing Period										Post-Treatment Period
		1	5	9	13	17	21	25	29	33	37	41	45	49	53/ET	
Study Week	-4 to 1	1	5	9	13	17	21	25	29	33	37	41	45	49	53/ET	65
Study Day	-30 to -1	1 ²	29	57	85	113	141	169	197	225	253	281	308	337	365	449
Visit Windows (days)	0	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3
Informed Consent	X															
Inclusion/Exclusion	X															
Study Visit	X	X	X ¹¹	X ¹¹	X ¹¹	X ¹¹	X ¹¹	X ¹¹	X ¹¹	X ¹¹	X	X ¹¹	X ¹¹	X ¹¹	X	X ¹¹
Concomitant Medications & Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight and Vital Signs ³	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test ⁴	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/ Body Assessment	X	X	X		X			X			X				X	X
12-lead ECG		X			X			X			X				X	X
Inflammatory Panel ⁵		X			X			X			X				X	X
Complement ⁵		X			X			X			X				X	X
Coagulation ⁵	X	X	X		X			X			X				X	X
Chemistry, Hematology ^{5,6}	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug Administration (donidalorsen) ⁷		X	X	X	X	Option 1: Patients continue 80 mg donidalorsen every 4 weeks. Option 2: For patients who were attack-free for ≥ 12 weeks, after entering this OLE Study, the Investigator could initiate a switch to 80 mg donidalorsen every 8 weeks. The switch could begin at any Study Center visit starting at Week 17. If patients were not adequately controlled on 80 mg every 8 weeks then dosing could return to 80 mg donidalorsen every 4 weeks. Option 3: For patients who were not attack-free for ≥ 12 weeks, after entering this OLE Study, the Investigator could initiate a switch to 100 mg donidalorsen every 4 weeks. The switch could begin at any Study Center 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.										
AE-QoL		X			X			X			X				X	X
Angioedema Activity Score ⁸	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HAE Attack Assessment ⁹	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis ⁵	X	X	X	X	X		X		X		X		X		X	X
Immunogenicity Testing		See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)														
PK Blood Sampling		See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)														
PD Blood Sampling		See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)														
Archived Serum Samples ¹⁰		X	X	X	X		X		X		X		X		X	X

Source: Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix A (Appendix 16.1.1)

¹ Patient eligibility for the study was to be determined within 30 days prior to study entry. A Qualification Period longer than 30 days may be considered after discussion with the Sponsor Medical Monitor, but the intention was to minimize the treatment pause between the 2 studies (see Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Section 6.1.1 [Appendix 16.1.1]). Safety clinical laboratory parameters and assessments from the Index Study could have been used for determination of eligibility (as Qualification for this OLE Study) if they were obtained within the Qualification Period (i.e., 30 days prior to enrollment in the study or longer if approved by the Sponsor Medical Monitor).

² Study Day 1 could have been conducted on the same day as ISIS 721744-CS2 Study Visit Week 17 (All procedures were to be completed prior to study drug administration). As is operationally feasible, the first dose in this study (ISIS 721744-CS3) should have been administered 28 ± 2 days after the last dose in ISIS 721744-CS2.

³ Vital signs included blood pressure, heart rate, respiratory rate, and body temperature.

⁴ Females of childbearing potential only.

⁵ For a complete list of laboratory tests refer to Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix B (Appendix 16.1.1).

⁶ If the platelet value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen should have been re-drawn as soon as possible (ideally within 7 days).

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⁷ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member, HHC nurse), may have been allowed after dosing instructions and training were provided by qualified site personnel and HHC nurse was available to complete all predosing procedures.

⁸ The AAS questionnaire was to be completed by patients daily (minimum of 4 daily assessments per week) during Treatment Period 1 and Treatment Period 2. During the Post-Treatment Period, the AAS was only completed by patients if an HAE attack occurred.

⁹ During the study patients were instructed to report details of any HAE attack, including on-demand treatment, to the study site within 72 hours of the onset of the attack.

¹⁰ For follow-up exploration of laboratory findings and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc.).

¹¹ Assessments and procedures to be conducted by either an HHC nurse (if available), or the Study Center as arranged by the Study Center personnel.

Abbreviations: AAS = angioedema activity score; AE = adverse event(s); AE-QoL = angioedema quality of life; ECG = electrocardiogram; ET = early termination; HAE = hereditary angioedema; HHC = home healthcare; OLE = open-label extension; PD = pharmacodynamic(s); PK = pharmacokinetic(s); PKK = prekallikrein.

Source: CS3 clinical study report

Abbreviations: Qual, qualification

Table 87. Schedule of Assessments, Year 2, Trial CS3, Protocol Amendment 3, Version 4.0

Assessment	Extended Treatment Period (Flexible Dosing)													Post-Treatment Period
	Study Week	57	61	65	69	73	77	81	85	89	93	97	101	105/ET
Study Day	393 ¹¹	421 ¹¹	449 ¹¹	477 ¹¹	505 ¹¹	533 ¹¹	561 ¹¹	589 ¹¹	617 ¹¹	645 ¹¹	673 ¹¹	701 ¹¹	729	785 ¹¹
Visit Windows (Days)	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3
Study Visit ¹			X			X			X			X		X
Concomitant Medications & Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight & Vital Signs ²			X			X			X				X	X
Urine Pregnancy Test ^{3,4}	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/Body Assessment													X	
12-lead ECG													X	
Complement ⁵						X							X	
Plasma PKK	See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)													
Chemistry, Hematology, Coagulation ^{5,6}			X			X			X				X	X
Study Drug Administration (donidalorsen) ^{7,8}	Option 1: Patients continue 80 mg donidalorsen every 4 weeks. Option 2: Patients continue 80 mg donidalorsen every 8 weeks. If patients were not adequately controlled on 80 mg every 8 weeks, then dosing could return to 80 mg donidalorsen every 4 weeks. Option 3: Patients continue 100 mg donidalorsen every 4 weeks. If patients developed any tolerability or safety issue the dose could be reduced back to 80 mg every 4 weeks.													
Immunogenicity & PK	See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)													
AE-QoL						X							X	
AAS ⁹ and HAE Attack Assessment ¹⁰	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis ⁵			X			X			X				X	X

Source: Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix A (Appendix 16.1.1)

¹ Procedures, assessments, and confirmation of study drug administration were to be conducted via telephone contact by qualified site personnel on non-study visit/HHC days.

² Vital signs included blood pressure, heart rate, respiratory rate, and body temperature.

³ Females of childbearing potential only.

⁴ Patient were to take a home pregnancy test (Ionis to supply) and provide a picture of the stick to the site. Except on site/HHC visit days.

⁵ For a complete list of laboratory tests refer to Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix B (Appendix 16.1.1).

⁶ If the platelet value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen was to be re-drawn as soon as possible (ideally within 7 days).

⁷ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member), may have been allowed after dosing instructions and training were provided by qualified site personnel. An HHC professional must have been available to complete all predosing procedures prior to study drug administration.

⁸ If the patient was not continuing for another year, then no dose was to be given at Week 105.

⁹ The AAS questionnaire was only completed by patients if an HAE attack occurred.

¹⁰ During the study patients were instructed to report details of any HAE attack, including on-demand treatment, to the study site within 72 hours of the onset of the attack.

¹¹ Assessments and procedures were to be conducted by either an HHC nurse (if available), or the Study Center as arranged by the Study Center personnel.

Abbreviations: AAS = angioedema activity score; AE-QoL = angioedema quality of life; ECG = electrocardiogram; ET = early termination; HAE = hereditary angioedema; HHC = home healthcare; PK = pharmacokinetic(s); PKK = prekallikrein.

Source: CS3 clinical study report

Table 88. Schedule of Assessments, Year 3, Trial CS3, Protocol Amendment 3, Version 4.0

Assessment	Extended Treatment Period (Flexible Dosing)													Post-Treatment Period
	109	113	117	121	125	129	133	137	141	145	149	153	157/ET	
Study Week	757 ¹¹	785 ¹¹	813 ¹¹	841 ¹¹	869 ¹¹	897 ¹¹	925 ¹¹	953 ¹¹	981 ¹¹	1009 ¹¹	1037 ¹¹	1065 ¹¹	1093	1177 ¹¹
Study Day														
Visit Windows (Days)	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3
Study Visit ¹			X			X			X				X	X
Concomitant Medications & Concurrent Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body Weight & Vital Signs ²			X			X			X				X	X
Urine Pregnancy Test ^{3,4}	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/ Body Assessment													X	
12-lead ECG													X	
Complement ⁵						X							X	
Plasma PKK	See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)													
Chemistry, Hematology, Coagulation ^{5,6}			X			X			X				X	X
Study Drug Administration (donidalorsen) ^{7,8}	Option 1: Patients continue 80 mg donidalorsen every 4 weeks. Option 2: Patients continue 80 mg donidalorsen every 8 weeks. If patients were not adequately controlled on 80 mg every 8 weeks then dosing could return to 80 mg donidalorsen every 4 weeks. Option 3: Patients continue 100 mg donidalorsen every 4 weeks. If patients developed any tolerability or safety issue the dose could be reduced back to 80 mg every 4 weeks.													
Immunogenicity & PK	See Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix C (Appendix 16.1.1)													
AE-QoL						X								X
AAS ⁹ and HAE Attack Assessment ¹⁰	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis ⁵			X			X			X				X	X

Source: Study Protocol, version 4.0, dated 17 Feb 2023, Protocol [Appendix A](#) (Appendix 16.1.1)

¹ Procedures, assessments, and confirmation of study drug administration were to be conducted via telephone contact by qualified site personnel on non-study visit/HHC days.

² Vital signs included blood pressure, heart rate, respiratory rate, and body temperature.

³ Females of childbearing potential only.

⁴ Patient were to take a home pregnancy test (Ionis to supply) and provide a picture of the stick to the site. Except on site/HHC visit days.

⁵ For a complete list of laboratory tests refer to Study Protocol, version 4.0, dated 17 Feb 2023, Protocol [Appendix B](#) (Appendix 16.1.1).

⁶ If the platelet value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen was to be re-drawn as soon as possible (ideally within 7 days).

⁷ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member), may have been allowed after dosing instructions and training were provided by qualified site personnel. A Home Healthcare professional must have been available to complete all predosing procedures prior to study drug administration.

⁸ If the patient was not continuing for another year, no dose was given at Week 157.

⁹ The AAS questionnaire was only to be completed by patients if an HAE attack occurred.

¹⁰ During the study patients were instructed to report details of any HAE attack, including on-demand treatment, to the study site within 72 hours of the onset of the attack.

¹¹ Assessments and procedures were to be conducted by either an HHC nurse (if available), or the Study Center as arranged by the Study Center personnel.

Abbreviations: AAS = angioedema activity score; AE-QoL = angioedema quality of life; ECG = electrocardiogram; ET = early termination; HAE = hereditary angioedema; HHC = home healthcare; PK = pharmacokinetic(s); PKK = prekalikrein.

Source: CS3 clinical study report

Table 89. Schedule of Assessments, Year 4, Trial CS3, Protocol Amendment 3, Version 4.0

Assessment	Extended Treatment Period (Flexible Dosing)													Post-Treatment Period
	161	165	169	173	177	181	185	189	193	197	201	205	209/ET	221
Study Week	161	165	169	173	177	181	185	189	193	197	201	205	209/ET	221
Study Day	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1541
Dose Windows (Days)	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3
Study Visit ¹			X			X			X				X	X
Concomitant Medications & Concurrent Procedures	Throughout													
Adverse Events	Throughout													
Body Weight & Vital Signs ²			X			X			X				X	X
Urine Pregnancy Test ^{3,4}	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination/ Body Assessment													X	
12-lead ECG													X	
Complement ⁵													X	
Chemistry, Hematology, Coagulation ^{5,6}			X			X			X				X	X
Study Drug Administration (donidalorsen) ⁷	Option 1: Patients continue 80 mg donidalorsen every 4 weeks. Option 2: Patients continue 80 mg donidalorsen every 8 weeks. If patients were adequately controlled on 80 mg every 8 weeks, then dosing could return to 80 mg donidalorsen every 4 weeks. Option 3: Patients continue 100 mg donidalorsen every 4 weeks. If patients developed any tolerability or safety issue the dose could be reduced back to 80 mg every 4 weeks.													
AAS ⁸ and HAE Attack Assessment ⁹	Throughout													
Urinalysis ⁵			X			X			X				X	X

Source: Study Protocol, version 4.0, dated 17 Feb 2023, Protocol Appendix A (Appendix 16.1.1)

¹ Procedures, assessments, and confirmation of study drug administration were to be conducted via telephone contact by qualified site personnel on non-study visit.

² Vital signs included blood pressure, heart rate, respiratory rate, and body temperature.

³ Females of childbearing potential only.

⁴ Patient were to take a home pregnancy test (Ionis to supply) and provide a picture of the stick to the site. Except on site visit days.

⁵ For a complete list of laboratory tests refer to Protocol Appendix B (Appendix 16.1.1).

⁶ If the platelet value, serum creatinine, or liver enzyme tests were uninterpretable (e.g., due to clumping, hemolysis or quantity not sufficient) or missing, a repeat blood specimen should have been re-drawn as soon as possible (ideally within 7 days).

⁷ Self-administration of donidalorsen, or administration by other qualified individuals (e.g., family member), may have been allowed after dosing instructions and training were provided by qualified site personnel.

⁸ The AAS questionnaire was only to be completed by patients if an HAE attack occurs.

⁹ During the study patients were instructed to report details of any HAE attack, including on-demand treatment, to the study site within 72 hours of the onset of the attack.

Abbreviations: AAS = angioedema activity score; ECG = electrocardiogram; ET = early termination; HAE = hereditary angioedema.

Source: CS3 clinical study report

15.4.3. Primary, Secondary, and Exploratory Objectives and Endpoints, Trial CS3

The primary objective of Trial CS3 was to evaluate the safety of extended dosing and alternative dosing and/or dose frequency of donidalorsen in patients with HAE. The primary endpoint was the incidence and severity of TEAEs.

The secondary objectives of this trial were to evaluate the efficacy of extended dosing and alternative dosing and/or dose frequency in patients with HAE. The secondary endpoints were:

- The time-normalized HAE attacks (per 4 weeks) by treatment.
- Consumption of on-demand medications.

The exploratory objectives of this trial were to characterize the effects of donidalorsen on PPK levels, plasma proenzyme activation, and cHK levels; to evaluate the effects of donidalorsen on the clinical and AE-QoL endpoints; and to evaluate PK exposure over time. The exploratory endpoints were:

- Prekallikrein levels, plasma proenzyme activation (only during Year 1), and cHK levels.
- Angioedema Quality of Life questionnaire score.

15.4.4. Analysis Populations, Trial CS3

The following analysis populations were defined by the Applicant in the final SAP, version 3.0, dated March 14, 2024:

- The safety population was to include all enrolled subjects who received at least one dose of donidalorsen.
- The ITT population was to include all enrolled subjects.
- The per protocol population was to include all subjects 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 compromise the interpretation of efficacy were to be determined prior to the database lock.
- The PK population was to include all subjects who enrolled and received at least one dose of donidalorsen and had at least one evaluable PK sample.

15.4.5. Statistical Analysis Plan, Trial CS3

Per the final SAP, dated March 14, 2024, the primary efficacy endpoint was the time-normalized number of HAE attacks. The HAE attack rates, as well as the changes and the percent changes from baseline during the On-Treatment Period and Fixed Dosing Period, were summarized using descriptive statistics by the Index Trial treatment group and overall for HAE Type I, II, and III. The investigator-confirmed HAE attack rate was calculated for each subject as the number of HAE attacks occurring during the On-Treatment Period divided by number of days the subject contributed to the period multiplied by 28 days.

For the On-Treatment Period and Fixed Dosing Period, three sets of analysis were performed. For the first set of analysis, the Trial CS2-on-treatment baseline was used to derive change and percent change from baseline, regardless of which trial drug the subjects took in the Index Trial. For the second set of analysis, subjects who took the active drug in the Index Trial used Trial CS2-Run-In baseline and subjects who took placebo in the Index Trial used OLE baseline to derive change and percent change from baseline. For the third set of analysis, all subjects used Trial CS2-Run-In baseline.

Summaries for all analysis periods during the Flexible Dosing Period were only summarized by dose options, using Trial CS2-Run-In baseline for subjects who took the active drug in the Index Trial and OLE baseline for subjects who took placebo in the Index Trial. The analysis was repeated using Trial CS2-Run-In baseline for all subjects. This analysis was also repeated using the Fixed Dosing Period HAE attack rate as the baseline.

For subjects who received donidalorsen-4 in the Index Trial, an integrated analysis of HAE attack rates was also done by including data in the Index Trial. Trial CS2-Run-In baseline was used for derivation of change and percent change from baseline. The time-normalized HAE attack rate for each subject was calculated as the number of HAE attacks occurring from first dose of the Index Trial to the end of Treatment Period in the OLE trial divided by number of days in the period multiplied by 28 days. The data were presented by HAE type.

Similar analyses were performed for the secondary efficacy endpoints of time-normalized number of moderate or severe HAE attacks and time-normalized number of HAE attacks requiring acute therapy. The secondary efficacy endpoints of response rate (subjects who achieve 50%, 70%, 90% reduction from baseline in HAE attack rate) and HAE attack free response rate were summarized by number and percentage of subjects.

15.4.6. Protocol Amendments, Trial CS3

The original protocol (dated January 17, 2020) was amended three times. Key changes in each respective amendment were to provide the option for each subject to participate in an additional year of trial participation (with relevant adjustments to the trial schedule of assessments), lengthening the trial duration to 4 years of treatment with Amendment #3. Additionally, Amendment #1 provided the option to reduce dosing to 80 mg every 8 weeks in the event of tolerability or safety issues. Amendment #2 allowed continued flexible dosing of donidalorsen after Week 41.

15.4.7. Interim Analyses, Trial CS3

The interim analyses presented in the Applicant's CSR were performed after the data cutoff date of February 26, 2024 (final database lock on March 26, 2024). The results of the interim analyses were intended for regulatory submission, not to be used for stopping or adapting the trial or adjusting the sample size.

15.4.8. Data and Safety Monitoring Committee, Trial CS3

The safety of donidalorsen was continually monitored throughout the trial by the investigator or designee and the Applicant or designee. No Data and Safety Monitoring Committee was utilized for this OLE trial (CS3). Safety was evaluated by the collection and measurement of AEs, clinical laboratory parameters (including hematology, serum chemistry, and urinalysis), concomitant medication/procedure information, vital signs, physical examinations, and ECGs.

16. Efficacy

16.1. Results of Statistical Analyses, Trial CS2

16.1.1. Subject Disposition and Exposure, Trial CS2

Disposition information is presented in [Table 90](#). In Part A, a total of 23 subjects were screened, 20 subjects were randomized and received trial drug, and 19 subjects completed trial treatment. One subject voluntarily withdrew due to travel time to the site and work schedule. There were 17 subjects who rolled over to the open-label extension trial (CS3).

Table 90. Disposition, Intent-to-Treat Population, Trial CS2 Part A

Disposition	Donidalorsen 80 mg Every 4 Weeks		Total N=20
	N=14	Placebo N=6	
Treatment discontinuation reason			
Voluntary withdrawal	1 (7.1)	0	1 (5.0)
Completed	13 (92.9)	6 (100.0)	19 (95.0)
Follow-up discontinuation reason			
Roll over to CS3	12 (85.7)	5 (83.3)	17 (85.0)
Withdrawal of consent	1 (7.1)	0	1 (5.0)

Source: Clinical study report Table 14.1.2 (Pages 116-122); findings reproduced by statistical reviewer using adsl.xpt
Abbreviations: N, number of subjects

16.1.2. Subject Demographics and Baseline Characteristics, Trial CS2

The demographics and baseline clinical characteristics of the ITT population are summarized in [Table 91](#). Imbalances are noted, including in age categories and family history of HAE. However, imbalances are expected in a small trial.

Table 91. Baseline Demographics and Clinical Characteristics, Intent-to-Treat Population, Trial CS2 Part A

Characteristic	Donidalorsen 80 mg Every 4 Weeks	
	N=14	Placebo N=6
Sex, n (%)		
Female	9 (64.3)	4 (66.7)
Male	5 (35.7)	2 (33.3)
Age, years		
Mean (SD)	37.8 (14.4)	40.0 (13.8)
Median	32.5	44.5
IQR	26.0, 47.0	24.0, 49.0
Min, max	21.0, 66.0	22.0, 56.0
Age categories, n (%)		
18-39	9 (64.3)	2 (33.3)
40-64	4 (28.6)	4 (66.7)
≥65	1 (7.1)	0
Race, n (%)		
Black or African American	0	1 (16.7)
White	14 (100)	5 (83.3)
Ethnicity, n (%)		
Hispanic or Latino	1 (7.1)	0
Not Hispanic or Latino	13 (92.9)	6 (100)
Baseline BMI (kg/m ²)		
Mean (SD)	29.6 (7.5)	26.2 (3.4)
Median	28.2	24.7
IQR	24.8, 34.6	24.2, 27.0
Min, max	19.6, 48.1	23.6, 32.8
HAE subtype, n (%)		
Type I	13 (92.9)	5 (83.3)
Type II	1 (7.1)	1 (16.7)

Characteristic	Donidalorsen 80 mg	Placebo
	Every 4 Weeks N=14	N=6
Number of attacks in the 12 months prior to Screening		
Mean (SD)	23.1 (16.2)	25.3 (22.9)
Median	24.0	20.0
IQR	10.0, 26.0	15.0, 23.0
Min, max	4.0, 60.0	4.0, 70.0
Family history of HAE, n (%)	13 (92.9)	4 (66.7)

Source: Clinical study report Table 7 and Table 8 (Pages 63, 64-65); findings reproduced by statistical reviewer using adsl.xpt and adef.xpt Statistical Reviewer Analysis; adsl.xpt
Abbreviations: BMI, body mass index; HAE, hereditary angioedema; IQR, interquartile range; max, maximum; min, minimum; N, number of subjects; SD, standard deviation

16.1.3. Primary Efficacy Results, Trial CS2

[Table 92](#) summarizes the efficacy results for the time-normalized number of HAE attacks per 4 weeks from Week 1 to Week 17 for the primary efficacy endpoint comparison between donidalorsen-4 and placebo. After adjusting for baseline attack rate and treatment group, the estimated least square mean time-normalized number of HAE attacks per 4 weeks was lower in the donidalorsen-4 (LS mean =0.22, 95% CI =0.10, 0.50) group compared to placebo (LS mean =2.29, 95% CI =1.54, 3.41). The estimated attack rate ratio for the donidalorsen-4 group relative to placebo was 0.10 (95% CI =0.04, 0.24). Additionally, the percentage reduction in the LS means for the donidalorsen-4 group was 90% (95% CI =76%, 96%) relative to placebo.

Table 92. Primary Analysis: Time-Normalized HAE Attack Rate From Week 1 to Week 17 in Intent-to-Treat Population, Trial CS2 Part A

Parameter	Donidalorsen 80 mg	Placebo
	Every 4 Weeks N=14	N=6
Run-In Period HAE attack rate, mean ^a (SD)	2.82 (1.53)	2.44 (1.03)
Time-normalized HAE attack rate from Week 1 to Week 17, mean ^b (SD)	0.23 (0.27)	2.21 (1.56)
Least square mean rate ^c (95% CI)	0.22 (0.10, 0.50)	2.29 (1.54, 3.41)
Model adjusted mean rate ratio ^c (95% CI)	0.10 (0.04, 0.24)	-
Percentage difference relative to placebo ^d (95% CI)	-90 (-96, -76)	-
p-value	<0.0001	-

Source: Clinical study report Table 9 (Page 67); adef.xpt

^a The Run-In Period (Screening) attack rate (per 4 weeks) for each subject was calculated as the number of HAE attacks that occurred during the Run-In Period divided by the number of days the subject contributed to the Run-In Period, and then multiplied by 28.

^b The time-normalized HAE attack rate (per 4 weeks) was calculated as number of HAE attacks that occurred from Week 1 to Week 17, divided by the number of days the subject contributed to that period, and then multiplied by 28.

^c The Poisson regression model included treatment group, baseline (the Run-In Period HAE attack rate), and the logarithm of time in every 4 weeks that each subject was observed from Week 1 to Week 17 used as an offset. Pearson chi-square scaling of standard errors was used to account for potential overdispersion.

^d The percentage difference in mean 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 were transformed by subtracting 1 and multiplying by 100% to calculate 95% confidence intervals for the percentage change.

Abbreviations: CI, confidence interval; HAE, hereditary angioedema; N, number of subjects; SD, standard deviation; SE, standard error

16.1.4. Type III HAE, Trial CS2

In Trial CS2 Part B, six subjects with HAE-nC1-INH (Type III HAE) were screened, and three subjects were dosed and completed trial treatment. All three subjects were female, the mean age was 34 years (range 25 to 40), all were White, and the mean body mass index was 38.4 kg/m² (range 36 to 42). All three subjects had experienced prior peripheral and abdominal HAE attacks, and one subject 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) and the mean HAE attack rate in the Run-In Period was 4.23 (values: 2.3, 3, 7.4). The Treatment Period (Week 1 to Week 17) mean HAE attack rate was 1.52 (values: 0, 0.5, 4.1). The mean reduction in monthly HAE attack rate was 75% (values: 45, 83, 100).

Due to the open-label conduct and sample size of Trial CS2 Part B, no conclusive evidence about efficacy can be drawn from this trial.

17. Clinical Safety

17.1. Safety Results, Trial CS7

17.1.1. Subject Disposition and Demographics, Trial CS7

Table 93. Subject Disposition and Demographics, Trial CS7

Subject Disposition	Rollover Subjects Donidalorsen Q4W (N=69) n (%)	Rollover Subjects Donidalorsen Q8W (N=14) n (%)	Naïve Subjects (N=65) n (%)	All Subjects (N=148) n (%)
Subjects dosed	69 (100)	14 (100)	64 (98.5)	147 (99.3)
Analysis set safety set ^a	69 (100)	14 (100)	64 (98.5)	147 (99.3)
Full analysis set ^b	69 (100)	14 (100)	64 (98.5)	147 (99.3)
Per-protocol set ^c	66 (95.7)	12 (85.7)	59 (90.8)	137 (92.6)
PK set ^d	69 (100)	9 (64.3)	63 (96.9)	141 (95.3)
Subjects who completed one-year trial treatment	5 (7.2)	2 (14.3)	12 (18.5)	19 (12.8)
Subjects who completed three-year trial treatment	0	0	0	0

Subject Disposition	Rollover Subjects Donidalorsen Q4W (N=69) n (%)	Rollover Subjects Donidalorsen Q8W (N=14) n (%)	Naïve Subjects (N=65) n (%)	All Subjects (N=148) n (%)
Subjects who early terminated trial treatment	2 (2.9)	0	8 (12.3)	10 (6.8)
Primary reason for discontinuation: AE	0	0	1 (1.5)	1 (0.7)
Lack of efficacy	0	0	4 (6.2)	4 (2.7)
Lost to follow-up	0	0	1 (1.5)	1 (0.7)
Other	0	0	1 (1.5)	1 (0.7)
Withdrawal by subject	2 (2.9)	0	1 (1.5)	3 (2.0)
Discontinuation due to COVID-19 related impact	0	0	0	0

Source: Applicant's Information Request response received February 24, 2025, edited by clinical reviewer

^a The safety set includes all enrolled subjects who receive at least one dose of trial drug.

^b The full analysis set includes all enrolled subjects who receive at least one dose of trial drug.

^c The per-protocol set includes all subjects in the full analysis set who are treated according to the protocol without any major deviations.

^d The PK set includes all subjects who are enrolled and receive at least one dose of trial drug and have at least one evaluable PK sample.

% = 100 × n/N.

Rollover subjects who changed dosing could be counted under both treatment groups (donidalorsen 80 mg Q4W and Q8W).

Abbreviations: AE, adverse event; NA, not applicable; PK, pharmacokinetic; Q4W, every 4 weeks; Q8W, every 8 weeks

The median duration of exposure for Rollover subjects was similar between the donidalorsen treatment groups: 227 days (P25, P75: 163 to 272) in the Rollover donidalorsen-4 group and 226 days (P25, P75: 148 to 288) in the Rollover donidalorsen-8 group. The median duration of exposure for Naïve subjects was 253 days (P25, P75: 159 to 340).

[Table 94](#) provides the demographics and baseline characteristics of the subjects enrolled in Trial CS7. The demographics and baseline characteristics are similar to those of Trial CS5 and generally representative of the broader HAE population.

Table 94. Baseline Demographic and Clinical Characteristics, Safety Population, Trial CS7

Characteristic	Donidalorsen-Naïve N=64	Rollover Donidalorsen-4^a N=69	Rollover Donidalorsen-8^b N=14	Total N=147
Sex, n (%)				
Female	38 (59.4)	40 (58.0)	5 (35.7)	83 (56.5)
Male	26 (40.6)	29 (42.0)	9 (64.3)	64 (43.5)
Age, years				
Mean (SD)	41.6 (14.5)	38.9 (14.2)	30.8 (9)	39.3 (14.2)
Median (min, max)	42.5 (12, 69)	37 (12, 69)	31 (15, 47)	38 (12, 69)
Age group, years, n (%)				
12 to 17	4 (6.2)	5 (7.2)	2 (14.3)	11 (7.5)
18 to 39	24 (37.5)	32 (46.4)	10 (71.4)	66 (44.9)
40 to 64	34 (53.1)	31 (44.9)	2 (14.3)	67 (45.6)
≥65	2 (3.1)	1 (1.4)	0	3 (2.0)

Characteristic	Donidalorsen-Naïve N=64	Rollover Donidalorsen-4 ^a N=69	Rollover Donidalorsen-8 ^b N=14	Total N=147
Race, n (%)				
American Indian or Alaska Native	0	3 (4.3)	0	3 (2.0)
Asian	1 (1.6)	1 (1.4)	0	2 (1.4)
Black or African American	2 (3.1)	1 (1.4)	0	3 (2.0)
Multiple	1 (1.6)	1 (1.4)	0	2 (1.4)
Other	3 (4.7)	1 (1.4)	0	4 (2.7)
White	57 (89.1)	62 (89.9)	14 (100)	133 (90.5)
Ethnicity, n (%)				
Hispanic or Latino	3 (4.7)	4 (5.8)	1 (7.1)	8 (5.4)
Not Hispanic or Latino	61 (95.3)	65 (94.2)	13 (92.9)	139 (94.6)
Country of participation, n (%)				
Great Britain	4 (6.2)	3 (4.3)	2 (14.3)	9 (6.1)
Italy	4 (6.2)	7 (10.1)	2 (14.3)	13 (8.8)
Netherlands	4 (6.2)	6 (8.7)	2 (14.3)	12 (8.2)
Turkey	0	15 (21.7)	3 (21.4)	18 (12.2)
United States	39 (60.9)	13 (18.8)	1 (7.1)	53 (36.1)
Others	13 (20.3)	25 (36.2)	4 (28.6)	42 (28.6)
Is in United States, n (%)				
United States	39 (60.9)	13 (18.8)	1 (7.1)	53 (36.1)
Non-United States	25 (39.1)	56 (81.2)	13 (92.9)	94 (63.9)
Treatment prior to switch, n (%)				
Lanadelumab	31 (48.4)	NA	NA	31 (21.1)
C1-esterase inhibitor	22 (34.4)	NA	NA	22 (15.0)
Berotrastat	11 (17.2)	NA	NA	11 (7.5)
HAE type, n (%)				
Type I	57 (89.1)	65 (94.2)	13 (92.9)	135 (91.8)
Type II	7 (10.9)	4 (5.8)	1 (7.1)	12 (8.2)
Number of HAE attacks in past 12 months				
Mean (SD)	11 (13.6)	41.3 (37.5)	32.6 (19.2)	27.3 (31.3)
Median (min, max)	5 (0, 52)	36 (0, 208)	36.5 (1, 70)	19 (0, 208)
Family history of HAE, n (%)				
Yes	55 (85.9)	60 (87.0)	11 (78.6)	126 (85.7)
No	9 (14.1)	9 (13.0)	3 (21.4)	21 (14.3)

Source: Clinical data scientist using adsl.xpt; Software: R

^a All rollover subjects treated with donidalorsen 80 mg every 4 weeks (regardless of treatment received in the Index Trial)

^b All rollover subjects treated with donidalorsen 80 mg every 8 weeks (regardless of treatment received in the Index Trial).

Abbreviations: HAE, hereditary angioedema; N, number of subjects in treatment group; NA, not applicable; n, number of subjects with given characteristic; SD, standard deviation

17.1.2. Overview of Treatment-Emergent Adverse Events Summary, Trial CS7

As summarized in [Table 95](#), most subjects experienced at least one TEAE, the majority of which were mild or moderate in severity. No deaths were reported during Trial CS7. Five (3%) subjects experienced an SAE, none of which were considered related to donidalorsen. One Naïve subject and no Rollover subjects experienced a TEAE that led to permanent treatment discontinuation.

The TEAE summary data do not reveal a new safety signal in Trial CS7.

Table 95. Overview of Treatment-Emergent Adverse Events, Rollover and Naïve Subjects, Trial CS7

Parameter	Rollover Subjects Donidalorsen Q4W (N=69) n (%)	Rollover Subjects Donidalorsen Q8W (N=14) n (%)	Naïve Subjects (N=65) n (%)	All Subjects (N=148) n (%)
Subjects with any TEAEs	56 (81.2)	10 (71.4)	50 (78.1)	116 (78.9)
Subjects with any TEAEs by maximum severity				
Severe and worse	2 (2.9)	1 (7.1)	5 (7.8)	8 (5.4)
Moderate	26 (37.7)	4 (28.6)	26 (40.6)	56 (38.1)
Mild	28 (40.6)	5 (35.7)	19 (29.7)	52 (35.4)
Subjects with any TEAEs leading to dose reduction or interruption	1 (1.4)	0	0	1 (0.7)
TEAE leading to dose reduction	0	0	0	0
TEAE leading to dose interruption	1 (1.4)	0	0	1 (0.7)
Subjects with any TEAEs leading to permanent treatment discontinuation	0	0	1 (1.6)	1 (0.7)
Subjects with any serious TEAEs	4 (5.8)	0	1 (1.6)	5 (3.4)
Subjects with any TEAEs leading to death	0	0	0	0
Subjects with any life-threatening serious TEAE	1 (1.4)	0	0	1 (0.7)

Source: Applicant's Information Request response received February 24, 2025, edited by clinical reviewer

% = 100 × n/N.

Adverse events were coded using MedDRA Version 26.1.

TEAEs are defined as AEs that either start or worsen in severity on or after the date/time of first dose of trial drug in Trial ISIS 721744-CS7.

For rollover subjects, each adverse event is counted under the dose regimen that the subject was assigned to at the onset of the event.

Subjects who changed dosing could be counted under both treatment groups (donidalorsen 80 mg Q4W and Q8W).

At each level of summarization, subjects reporting at least one relevant event are counted only once. At each level of subject summarization of severity, a subject is classified according to the highest severity if the subject reported one or more events.

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; Q4W, every 4 weeks; Q8W, every 8 weeks; TEAE, treatment-emergent adverse event

17.1.3. Deaths, Trial CS7

There were no deaths in Trial CS7.

17.1.4. Serious Treatment-Emergent Adverse Events, Trial CS7

Four (6%) subjects in the Rollover donidalorsen-4 group, and one (2%) in the Naïve group, experienced serious TEAEs; all events were considered unrelated, and two led to treatment discontinuation. No subject in the Rollover donidalorsen-8 group experienced serious TEAEs.

Table 96. Listing of Subjects With Serious Treatment-Emergent Adverse Events^a by System Organ Class and Preferred Term, Safety Population, Trial CS7

Trial Group, Unique Subject Identifier	Age/Sex HAE Type	SAE PT	SAE Onset Trial Day No.	AE Duration (Days)	Treatment DC?
Rollover doni-4, (b) (6)	61/M Type I	Meningioma, pulmonary oedema	210	33	Y
Rollover doni-4, (b) (6)	50/F Type I	Cardiac valve disease	50	Ongoing at DBL	N
Rollover doni-4, (b) (6)	23/F Type I	Mastoiditis, otitis media chronic	150	4	N
Rollover doni-4, (b) (6)	46/F Type I	Uterine leiomyoma	50	39	N
Naïve, (b) (6)	58/F Type I	Cardiac failure acute, renal infarct	61 and 62	Ongoing at DBL	Y

Source: Clinical reviewer-generated from the CS7 CSR

^a Serious adverse events defined as any untoward medical occurrence that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Abbreviations: AE, adverse event; CSR, clinical study report; DBL, database lock; DC, discontinuation; doni, donidalorsen; F, female; HAE, hereditary angioedema; M, male; No., number; PT, preferred term; SAE, serious adverse event

Subject (b) (6) was a 61-year-old White male who was treated with donidalorsen-4 in Trial CS5 and continued this treatment in Trial CS7. On (b) (6), he received his Week-29 dose of donidalorsen-4. On (b) (6), he was hospitalized due to a life-threatening SAE of atypical meningioma of severe intensity. He presented with drowsiness, fever, fatigue, difficulty in articulation, cough, and difficulty breathing. A head CT revealed a tumor in the frontal lobe with mass effect and midline shift, leading to diagnosis of a brain tumor. He was also diagnosed with severe pneumonia. On (b) (6), he was intubated due to his Glasgow Coma Scale score and underwent a craniotomy and excision of the brain tumor. He was diagnosed with influenza A on (b) (6), and developed life-threatening pulmonary edema on (b) (6). On an unknown date, he was extubated, but was reintubated on (b) (6), due to irritability, wheezing, and inspiratory stridor. He was extubated on (b) (6), and discharged from the hospital on (b) (6). During the hospitalization, administration of the trial drug was paused, and has not been resumed. Because meningiomas are slow growing tumors that may progress over years before detection, it is unlikely that this SAE was related to donidalorsen-4.

Subject (b) (6) was a 50-year-old White female who was treated with donidalorsen-4 in Trial CS5 and continued this treatment in Trial CS7. On (b) (6), she received the Week-9 dose of donidalorsen-4 and, on the same day, experienced an SAE of cardiac valve disease (moderate in intensity). She was clinically asymptomatic, but echocardiography revealed moderate mitral regurgitation, moderate mitral stenosis, and moderate aortic stenosis. Her valvular disease is likely related to acute rheumatic fever in childhood. The recommended clinical management was follow-up with a repeat echocardiogram in 6 months. This SAE is not related to donidalorsen-4.

Subject (b) (6) was a 23-year-old White female who was treated with donidalorsen-8 in Trial CS5 and transitioned to donidalorsen-4 in Trial CS7. On (b) (6), she received the Week 21 dose of donidalorsen-4. On (b) (6), she was hospitalized for the SAE of

chronic suppurative otitis media and mastoiditis, both severe in intensity. Her presenting symptom was right ear pain. On (b) (6), she underwent tympanoplasty and was treated with antibiotics. No action was taken with the trial drug. On (b) (6), the SAEs resolved, and she was discharged from the hospital. It is unlikely that donidalorsen was related to the SAEs. On (b) (6), the subject tested positive for pregnancy with a last menstrual period approximately 34 days prior, with an estimated due date in (b) (6). She had been on desogestrel oral contraceptive, which was stopped, and the trial drug was discontinued. The pregnancy was ongoing at the time of data cutoff.

Subject (b) (6) was a 46-year-old American Indian or Alaska native female who was treated with placebo in Trial CS5 (enrolled in (b) (6)) and rolled over to Trial CS7 (first dose of donidalorsen-4 on (b) (6)). She had a past medical history of uterine leiomyoma since (b) (6). On (b) (6), she was noted to have multiple new growths of uterine leiomyoma, and on (b) (6), she was hospitalized and underwent a hysterectomy (SAE). She continued treatment with donidalorsen through the data cutoff on (b) (6). This SAE does not appear to be related to donidalorsen.

Subject (b) (6) was a 58-year-old White female, previously treated with lanadelumab, whose first dose of trial treatment was administered on (b) (6). Relevant ongoing medical conditions at enrollment included asthma (since (b) (6)), hypertension (since (b) (6)), drug abuse (since (b) (6)), cardiac failure congestive (since (b) (6)), asthma-chronic obstructive pulmonary disease overlap syndrome and pulmonary hypertension (since (b) (6)), and wound infection (since July (b) (6)). On Trial Day 57, the subject received the Week 9 dose of donidalorsen-4. On Trial Day 61 ((b) (6)), the subject presented to the emergency room with worsening congestive heart failure indicated by shortness of breath and distress, lower extremity swelling, enlarged kidneys, and left flank pain. On the same day (Trial Day 61), the subject was hospitalized for an SAE of exacerbation of acute cardiac failure of severe intensity. A venous ultrasound of the left lower extremity was negative for deep vein thrombosis. A chest x-ray showed cardiomegaly with pulmonary vascular congestion and interstitial thickening, correlated for congestive heart failure, and no large airspace opacity. On Trial Day 62, a renal Doppler revealed a mass, measuring 3 cm by 3 cm, in the right interpolar region that is T2 hypointense with heterogeneous delayed enhancement, and the SAE of T2 hypointense lesion within the right kidney of moderate intensity was reported. Laboratory test results showed troponin I of 0.05 ng/mL (reference range: ≤ 0.02 ng/mL), chloride of 97 mmol/L (reference range: 98 to 108 mmol/L), and normal values of sodium, potassium, blood urea nitrogen, and creatinine. A transthoracic echocardiogram showed left ventricular function was moderately reduced with a left ventricular ejection fraction of 30% to 35%. Moderate concentric left ventricular hypertrophy was noted along with regional wall motion abnormalities. A medium-sized thrombus was noted in the left ventricle. The right ventricular systolic function was reduced, accompanied by the enlargement of the right ventricle and severe dilation of the right atrium. There was moderate to severe tricuspid regurgitation, and right ventricular systolic pressure was elevated. On the same day (Trial Day 62), the AEs of emphysema, pulmonary artery aneurysm, and chronic hypoxic respiratory failure of moderate intensity were reported. The subject was treated with oral apixaban for acute cardiac failure (exacerbation); intravenous furosemide, subcutaneous enoxaparin sodium, oral carvedilol, oral spironolactone, and oral empagliflozin for acute cardiac failure (exacerbation) and congestive heart failure (combined systolic/diastolic) with cor pulmonale. On Trial Day 67, the nonserious adverse events of lower extremity edema

resolved, and the AE of acute cardiac failure (exacerbation) resolved. The event of T2 hypointense lesion within the right kidney persisted as a nonserious AE of renal infarct, right side. On the same day (Trial Day 67), she was discharged from the hospital. On Trial Day 79, the trial drug was discontinued, and the subject started lanadelumab. Overall, these SAEs are most likely related to progression of chronic pre-existing medical conditions, though contribution from treatment with donidalorsen cannot be excluded.

The observed SAEs do not change the risk/benefit assessment of donidalorsen for HAE.

17.1.5. Adverse Events and Office of New Drugs Custom Medical Queries Leading to Treatment Discontinuation, Trial CS7

One (2%) subject in the Naïve group (Subject (b) (6)), as described in [Table 96](#) and no subjects in the Rollover donidalorsen-4 or Rollover donidalorsen-8 group permanently discontinued the trial drug due to TEAEs. Overall, the incidence of TEAEs leading to discontinuation was low.

17.1.6. Treatment-Emergent Adverse Events, Trial CS7

Common TEAEs, defined as those occurring in $\geq 5\%$ of the total subjects, are shown in [Table 97](#). Noting the limitations of these data without a control group for comparison, no new safety signal is identified based on these results.

Table 97. Common TEAEs by PT Occurring With Incidence $\geq 5\%$ of the Total Subjects, Safety Population, Trial CS7

Preferred Term	Rollover Subjects Donidalorsen 80 mg Q4W (N=69) n (%)	Rollover Subjects Donidalorsen 80 mg Q8W (N=14) n (%)	Switch Subjects (N=64) n (%)	All Subjects (N=147) n (%)
Nasopharyngitis	9 (13.0)	4 (28.6)	12 (18.8)	25 (17.0)
Upper respiratory tract infection	9 (13.0)	0	14 (21.9)	23 (15.6)
Headache	8 (11.6)	1 (7.1)	7 (10.9)	16 (10.9)
Influenza	12 (17.4)	2 (14.3)	1 (1.6)	15 (10.2)
Injection site erythema	3 (4.3)	0	9 (14.1)	12 (8.2)
Back pain	7 (10.1)	2 (14.3)	2 (3.1)	11 (7.5)
COVID-19	7 (10.1)	1 (7.1)	1 (1.6)	9 (6.1)
Fatigue	1 (1.4)	2 (14.3)	6 (9.4)	9 (6.1)
Nausea	3 (4.3)	2 (14.3)	4 (6.3)	9 (6.1)
Injection site discolouration	4 (5.8)	1 (7.1)	3 (4.7)	8 (5.4)

Preferred Term	Rollover Subjects Donidalorsen 80 mg Q4W (N=69) n (%)	Rollover Subjects Donidalorsen 80 mg Q8W (N=14) n (%)	Switch Subjects (N=64) n (%)	All Subjects (N=147) n (%)
Injection site pruritus	1 (1.4)	0	7 (10.9)	8 (5.4)
Sinusitis	3 (4.3)	0	5 (7.8)	8 (5.4)

Source: Applicant's Information Request response received February 24, 2025, edited by clinical reviewer
% =100x n/N.

Adverse events were coded using MedDRA Version 26.1.

TEAEs are defined as AEs that either start or worsen in severity on or after the date/time of first dose of trial drug in Trial ISIS 721744-CS7.

For rollover subjects, each adverse event is counted under the dose regimen that the subject was assigned to at the onset of the event.

Subjects who changed dosing could be counted under both treatment groups (donidalorsen 80 mg Q4W and Q8W).

At each level of summarization, subjects reporting at least one relevant event are counted only once. At each level of subject summarization of severity, a subject is classified according to the highest severity if the subject reported one or more events.

Abbreviations: AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities; PT, preferred term; Q4W, every 4 weeks; Q8W, every 8 weeks; TEAE, treatment-emergent adverse event

17.1.7. Laboratory Findings and Vital Signs, Trial CS7

In the Treatment Period through Week 53, routine clinical testing, including vital signs and coagulation studies, was performed every 3 months, while certain tests were performed on a monthly basis: (1) hematology; (2) serum chemistry with hepatic function tests; and (3) pregnancy testing. In the Extended Treatment Period, only pregnancy testing occurred monthly, with the other laboratory tests and vital signs evaluated every 3 months. See Section [15.2.2](#) for full details.

Noting the limitations of these data without a control group for comparison, no new safety signal is identified based on the vital sign findings. A discussion of the platelet, coagulation, and hepatic function laboratory values can be found in Section [7.7.2](#) and Section [7.7.3](#).

17.2. Safety Results, Trials CS2 and CS3

Overall, the safety results from Part A of Trial CS2 and for Trial CS3 were consistent with those of Trial CS5; no new risks were identified. See Section [15.3](#) and Section [15.4](#) for details of these trial designs. The key safety review issues included analysis of Trials C2 and CS3. See Section [7.7](#) for details.

17.3. Subgroup Analyses, Trial CS5

The Agency conducted additional safety analyses by sex, age, race and geography using system organ class and preferred terms to assess for potential safety imbalances. These post hoc subgroup analyses conducted on a small population have limited interpretability. But overall, no imbalance was identified.

17.4. 120-Day Safety Update

The Applicant submitted a 120-day safety update on December 19, 2024. The report included data from ongoing open-label trials (CS3 and CS7) as of the cutoff dates of June 25, 2024, and June 27, 2024, respectively. The report included updated pooled safety data from Trial CS2, Trial CS3, Trial CS5, and Trial CS7; the 176 subjects exposed to donidalorsen in these trials had a median donidalorsen exposure duration of 433 days, versus 168 days for placebo recipients.

Updated safety data from Trial CS3 and Trial CS7 included one fatal event of completed suicide, recorded as a death, SAE, and permanent treatment discontinuation. This subject was a 48-year-old male with a past medical history of headache, on-trial TEAEs of headache and basilar migraine, and no known history of depression or anxiety. One subject experienced an SAE of cellulitis. One subject permanently discontinued trial treatment due to TEAEs of ALT increased and AST increased (subject met the protocol-defined stopping rules for ALT $>5\times$ ULN). Commonly reported TEAEs were generally consistent with the safety profile observed to date. The safety update did not identify any new safety signals for donidalorsen.

17.5. Additional Safety Information

17.5.1. ASKCOV Trial

ASKCOV was an investigator-initiated, placebo-controlled clinical trial in Brazil, to evaluate whether a single dose of 120 mg SC donidalorsen would lead to improved outcomes for patients with COVID-19 prior to the availability of vaccines. The trial was funded by Ionis. The investigators hypothesized that targeting and inhibiting prekallikrein would prevent abnormal activation of the kallikrein-kinin system that leads to angioedema, lung inflammation, and ultimately vessel blockage, which are complications of severe COVID-19 infections.

A total of 111 hospitalized patients with moderate to severe COVID-19 on supplemental oxygen were enrolled. There was no statistically significant difference for the primary outcome (number of days subjects were alive and free of oxygen support up to 15 days after randomization). Incidences of mortality at Day 30 and the use of mechanical ventilation within 15 days were approximately doubled in the donidalorsen group versus the placebo group.

The incidence and pattern of TEAEs in the trial did not reveal a concerning imbalance. No TEAEs led to trial drug discontinuation (as it was a single dose). There were 78 SAEs reported in 32 subjects. The PT of respiratory failure occurred most frequently (26/78 [33%] events), followed by septic shock (9/78 [12%] events), severe acute respiratory syndrome (5/78 [6%] events), cardio-respiratory arrest and chronic kidney disease (4/78 [5%] events). None of these SAEs were reported as related to the trial drug by the investigator. Because all these SAEs are confounded by the background moderate-to-severe COVID-19 infection, applicability of these results to the safety profile of donidalorsen in HAE is very limited.

18. Clinical Virology

Not applicable.

19. Clinical Microbiology

The clinical microbiology assessment recommendation is that the submission is adequate. The drug product is [REDACTED] (b) (4). The PFS are then assembled into a nonsterile AI device. [REDACTED] (b) (4). [REDACTED] (b) (4). The Applicant has provided up to 12 months of long-term stability data that support the maintenance of microbiological quality for the subject drug at 2 to 8°C.

20. Mechanism of Action/Drug Resistance

Not applicable

21. Other Drug Development Considerations

Not applicable.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

A clinical inspection was conducted as donidalorsen is a new molecular entity. Dr. Andrew Smith was inspected in support of NDA 219407, covering one trial, ISIS 721744-CS5. A total of 3 subjects were screened and 2 subjects were enrolled and randomized at this site. Based on this inspection, the trial appears to have been conducted adequately, and the data generated by the clinical investigator site appears acceptable in support of the respective indication. See the Clinical Inspection Summary dated April 21, 2025, for further details.

23. Labeling: Key Changes

This PI review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the Applicant's draft PI ([Table 98](#)). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

Table 98. Key Labeling Changes and Considerations

Full PI Sections¹	Rationale for Major Changes to Finalized PI² Compared to Applicant's Draft PI
BOXED WARNING	Not applicable.
1 INDICATIONS AND USAGE	Dawnzera™ is indicated for prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older.
2 DOSAGE AND ADMINISTRATION	The recommended dosage is 80 mg administered subcutaneously every 4 weeks. A dosage of 80 mg every 8 weeks may also be considered. The dosage in final labeling does not indicate switching from a more frequent dosing regimen (every 4 weeks) to a less frequent dosing regimen (every 8 weeks) for all patients based on the disease control for at least 3 months (b) (4) However, as a positive dose response relationship was demonstrated for every 4 weeks compared to every 8 weeks in OASIS-HAE (Trial CS5), every 4 weeks is the preferred dosing regimen. Every 8 weeks may also be considered for some subjects based on the efficacy demonstrated in Trial CS5 and the sustained efficacy demonstrated in CS7. (b) (4)
4 CONTRAINDICATIONS	Dawnzera is contraindicated in patients with a history of serious hypersensitivity reactions, including anaphylaxis, to donidalorsen or any of the excipients in Dawnzera.
5 WARNINGS AND PRECAUTIONS	Includes a Risk of Hypersensitivity Reactions, including Anaphylaxis because of a case reported during clinical trials.
6 ADVERSE REACTIONS	The safety of Dawnzera reflects the exposure among patients in three clinical trials (CS5, CS3, and CS7), but one clinical trial, OASIS-HAE (Trial CS5), informed the safety and common adverse reactions observed from treatment with Dawnzera. Trial CS2 was not included as it provided limited safety data due to the small sample size and short trial duration. Decrease in platelet counts and increase in liver function tests are included due to safety signal from clinical trials with donidalorsen and the known class effect of ASOs. The incidence of anaphylaxis is not included because there was one case in a rare disease population, which could be misleading.
7 DRUG INTERACTIONS	Omitted because there were no clinically relevant drug interactions.
8 USE IN SPECIFIC POPULATIONS (e.g., Pregnancy, Lactation, Females and Males of Reproductive Potential, Pediatric Use, Geriatric Use, Renal Impairment, Hepatic Impairment)	8.1 Pregnancy and 8.2 Lactation subsections were developed consistent with the PLLR Review Tool and available animal data. 8.4 Pediatric Use subsection includes the pediatric use statement for pediatric patients aged 12 years and older, which is supported

Full PI Sections ¹	Rationale for Major Changes to Finalized PI ² Compared to Applicant's Draft PI
	by extrapolation from adults in the OASIS-HAE (Trial CS5) trial with additional PK analysis and PD response. 8.5 Geriatric Use: Because only two subjects ≥ 65 years of age were enrolled in Trial CS5, the Geriatric Use subsection includes a statement that clinical studies of Dawnzera did not include sufficient numbers of patients 65 years of age and older to determine whether they respond differently from younger adult patients. 8.7 Hepatic Impairment includes language in which Dawnzera is not recommended in patients with moderate or severe hepatic impairment. This statement is supported by the following: donidalorsen is distributed to the liver and its accumulation in the liver and long-term safety following repeat dosing in patients with moderate or severe hepatic impairment is not known as donidalorsen has not been studied in patients with moderate or severe renal impairment.
9 DRUG ABUSE AND DEPENDENCE	Not applicable.
10 OVERDOSAGE	Omitted
12 CLINICAL PHARMACOLOGY	12.3 PK subsection was revised to provide general PK information following administration of donidalorsen 80 mg every 4 weeks or every 8 weeks.
13 NONCLINICAL TOXICOLOGY	13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility subsection was revised to provide the results from a long-term animal study that evaluated carcinogenic potential of donidalorsen.
14 CLINICAL STUDIES	OASIS-HAE (Trial CS5) was a 24-week, multicenter, randomized, double-blind placebo-controlled trial that included patients 12 years of age and older with HAE. The demographics and baseline characteristics of patients in OASIS-HAE are provided in a table. The efficacy results of HAE attacks per 4 weeks are provided in a table along with a Figure that shows the mean HAE attack rate for both dosages over time. Several secondary endpoints are provided, including the proportion of patients with a percent reduction from baseline in the HAE attack rate, although only 70% and 100% was multiplicity controlled. Of note, the numbering of the Week in the label is different than in the CSRs and the review. Week 0 in the label = Week 1 elsewhere (e.g. Week 0-24 label = Week 1-25 review/CSRs)
17 PATIENT COUNSELING INFORMATION	Included administration instructions and risk of hypersensitivity reactions, including anaphylaxis information for healthcare providers to convey to patients during a counseling discussion.
Product Quality Sections (i.e., DOSAGE FORMS AND STRENGTHS, DESCRIPTION, HOW SUPPLIED/STORAGE AND HANDLING)	Sections 3, 11, and 16 were developed consistent with the considerations for product quality information in PI. Section 16: Formatted <i>How Supplied</i> information in a table to improve readability.

Source: Labeling Information Requests dated March 28, June 13, July 29, and August 5, 2025

¹ Product quality sections (Sections 3, 11, and 16) are pooled under the last row in this table; Section 15 (REFERENCES) is not included in this table.

² For the purposes of this document, the finalized PI is the PI that will be approved or is close to being approved.

Abbreviation(s): CSR, clinical study report; LFT, liver function test; PI, Prescribing Information; ASO, antisense oligonucleotides

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved: The following labeling documents were reviewed:

- Prescribing Information
- Patient Package Insert
- Instructions for Use
- Carton/container

The substantially complete PI, Patient Package Insert, and Instructions for Use were reviewed by the labeling consultants (DMEPA, OPDP, and DMPP/Patient Labeling).

24. Postmarketing Requirements and Commitments

PMR 4862-1

Complete the ongoing four-year open-label extension (OLE) trial (ISIS 721744-CS3) and the three-year OLE (ISIS 721744-CS7) to evaluate the potential for drug induced liver injury with Dawnzera.

ISIS 721744-CS3

Trial Completion:	January 2025
Final Report Submission:	September 2025

ISIS 721744-CS7

Trial Completion:*	November 2026
Final Report Submission:*	September 2027

* Both trials should be completed by the trial completion milestone and the final report submission should include data from both trials.

NDA 219407
Dawnzera (donidalorsen)

PMR 4862-2

Complete the ongoing 2-year carcinogenicity study in rats administered donidalorsen.

Study Completion: The 2-year carcinogenicity study in rats was ongoing (> Week 74 treatment) at the time of NDA submission.

End of Life: January 2025
Interim/Other - Final Pathology Report: September 2025
Final Report Submission: February 2026

25. Financial Disclosure

Table 99. Covered Clinical Studies: Trial CS5

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 211		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 1		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 1 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 2		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Abbreviation: FDA, Food and Drug Administration

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NDA 219407
Dawnzera (donidalorsen)

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27. Review Team

Table 100. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory project manager	Cindy Chee
Nonclinical reviewer	Eleni Salicru
Nonclinical team leader	Carmen Booker
OCP reviewer(s)	Qianni Wu
OCP team leader(s)	Amer Al-Khouja/Yunzhao Ren
Clinical reviewer	Rachel Bean
Clinical team leader	Miya Paterniti
Biometrics reviewer	Emily Shives
Biometrics team leader	Yongman Kim/Weiya Zhang
Cross-discipline team leader	Miya Paterniti
Associate Director for Therapeutic Review (clinical)	Kelly Stone
Office director (or designated signatory authority)	Kathleen Donohue

Abbreviations: OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology

Table 101. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Ghaled Hamad/Craig Bertha (ATL) Brian Cawrse/Larry Perez
OPMA Facility	Chaoying Ma/Shujun Chen
Microbiology	Catherine Gilbert/Yan Zheng
OPDP	Quynh-Nhu Capasso/Adewale Adeleye
OMP/DMPP	Mary Carroll/Marcia Williams
OSI	Suyoung Tina Chang/Phillip Kronstein
OSE/DEPI	Margie Goulding/Mingfeng Zhang
OSE/DMEPA	Sherly Abraham/Damon Birkemeier
OSE/DRISK	Donella Fitzgerald/Jackie Sheppard
CDRH	Tiffani Chance
CDS	Elizabeth Booth/Ling Cao

Abbreviations: CDRH, Center for Devices and Radiological Health; CDS, clinical data scientist; DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations

27.1. Reviewer Signatures

Table 27-102 Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Secondary Reviewer	Miya Paterniti OII DPACC	Sections: 1, 2, 3, 4, 6, 7, 8.3, 9, 10, 11, 12, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Miya Paterniti		Digitally signed by Miya Paterniti		
		Date: 8/20/2025 7:25 AM EDT GUID: 202582011251		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Secondary Reviewer	Craig Bertha OPQAII DPQAVIII	Sections: 9	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Craig Bertha		Digitally signed by Craig Bertha		
		Date: 8/20/2025 7:25 AM EDT GUID: 2025820112521		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Tertiary Reviewer	Yunzhao Ren OCP DIIP	Sections: 5, 6, 7, 8, 14	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Yunzhao Ren		Digitally signed by Yunzhao Ren Date: 8/20/2025 7:32 AM EDT GUID: 2025820113232		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Secondary Reviewer	Yongman Kim OB DBIII	Sections: 6.2, 6.3, 15.3, 16.1	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Yongman Kim		Digitally signed by Yongman Kim Date: 8/20/2025 7:42 AM EDT GUID: 202582011426		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Secondary Reviewer	Amer Al- Khouja OCP DIIP	Sections: 5.2, 6.1, 6.3, 8.1, 8.2, 14	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Amer Al-Khouja		Digitally signed by Amer Al-Khouja Date: 8/20/2025 7:43 AM EDT GUID: 202582011438		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non- clinical Discipline Primary Reviewer	Eleni Salicru OII DPTII	Sections: 5.1 7.1 7.7.1 7.7.2 7.7.3 8.3 8.4 13 24	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Eleni Salicru		Digitally signed by Eleni Salicru Date: 8/20/2025 7:57 AM EDT GUID: 202582011573		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Tertiary Reviewer	Weiya Zhang OB DBIII	Sections: Sections 6, 15, and 16	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Weiya Zhang		Digitally signed by Weiya Zhang Date: 8/20/2025 7:57 AM EDT GUID: 2025820115719		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Data Analyst Discipline Secondary Reviewer	Ling Cao	Sections: 7.6, 17	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Ling Cao		Digitally signed by Ling Cao Date: 8/20/2025 8:46 AM EDT GUID: 2025820124630		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Pharmacology Discipline Primary Reviewer	Qianni Wu OCP DIIP	Sections: 5.2, 6.1, 6.3, 8.1, 8.2, 14	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Qianni Wu		Digitally signed by Qianni Wu		
		Date: 8/20/2025 8:47 AM EDT GUID: 2025820124723		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline Primary Reviewer	Cindy Chee ORO DROII	Sections: 12	Based on my assessment of the application: ☐ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☒ Not applicable.	
Signature: Cindy Chee		Digitally signed by Cindy Chee		
		Date: 8/20/2025 8:49 AM EDT GUID: 2025820124949		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Biostatistics Discipline Primary Reviewer	Emily Shives OB DBIII	Sections: 6.2, 6.3, 15, 16	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Emily Shives		Digitally signed by Emily Shives Date: 8/20/2025 8:53 AM EDT GUID: 2025820125330		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharmacometrics Reviewer Discipline Primary Reviewer	Huali Wu OCP DPM	Sections: 14.5	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Huali Wu		Digitally signed by Huali Wu Date: 8/20/2025 9:16 AM EDT GUID: 202582013169		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director for Labeling Discipline Primary Reviewer	Miya Paterniti OII DPACC	Sections: 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Miya Paterniti		Digitally signed by Miya Paterniti Sign on behalf of Jessica Lee Date: 8/20/2025 9:20 AM EDT GUID: 2025820132022		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Associate Director for Labeling Discipline Secondary Reviewer	Miya Paterniti OII DPACC	Sections: 23	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Miya Paterniti		Digitally signed by Miya Paterniti Date: 8/20/2025 9:21 AM EDT GUID: 2025820132122		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharm-tox/Non-clinical Discipline Secondary Reviewer	Carmen Booker OII DPTII	Sections: 5, 7, 13	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Carmen Booker		Digitally signed by Carmen Booker		
		Date: 8/20/2025 9:41 AM EDT GUID: 2025820134120		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Regulatory Project Manager Discipline CPMS	Ji Hyun Larose ORO DROII	Sections: 121	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Ji Hyun Larose		Digitally signed by Ji Hyun Larose		
		Date: 8/20/2025 10:27 AM EDT GUID: 202582014273		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Product Quality Microbiology Reviewer Discipline Primary Reviewer	Catherine Gilbert OPMA DPMIII	Sections: Microbiology pg. 119	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Catherine Gilbert Digitally signed by Catherine Gilbert Date: 8/20/2025 10:50 AM EDT GUID: 202582014507				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Product Quality Microbiology Reviewer Discipline Secondary Reviewer	David Anderson OPMA DPMIV	Sections: Product quality - Microbiology p. 119	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: David Anderson Digitally signed by David Anderson Date: 8/20/2025 10:51 AM EDT GUID: 2025820145123				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Clinical Discipline Tertiary Reviewer	Kelly Stone OII DPACC	Sections: All	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Kelly Stone		Digitally signed by Kelly Stone		
		Date: 8/20/2025 11:32 AM EDT		
		GUID: 2025820153210		

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
CMC (OPQ/ONDP) Discipline Primary Reviewer	Ghaled Hamad OPQAII DPQAVII	Sections: Section 9. Product Quality (Including 9.1 Product).	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Ghaled Hamad		Digitally signed by Ghaled Hamad		
		Date: 8/20/2025 11:50 AM EDT		
		GUID: 2025820155045		

NDA 219407
 Dawnzera (donidalorsen)

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory
Pharmacometrics Reviewer Discipline Secondary Reviewer	Jingyu Yu OCP DPM	Sections: 14.5	Based on my assessment of the application: ☒ <u>No</u> deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	
Signature: Jingyu Yu		Digitally signed by Jingyu Yu Date: 8/20/2025 12:40 PM EDT GUID: 2025820164038		

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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08/20/2025 12:47:09 PM

KELLY D STONE
08/20/2025 01:42:47 PM

KATHLEEN M DONOHUE
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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 219407
Supporting document/s: 1 and 7
Applicant's letter date: 08/21/2024 and 10/11/2024
CDER stamp date: 08/21/2024 and 10/11/2024
Product: Donidalorsen (ISIS 721744)
Pharmacologic Class Prekallikrein (PKK)-targeted *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO)
Indication: Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older
Applicant: Ionis Pharmaceuticals Inc.
Clinical Division: Division of Pulmonology, Allergy, and Critical Care (DPACC)
PharmTox Division: Division of Pharmacology Toxicology for Immunology and Inflammation (DPT-II)
Reviewer: Eleni Salicru, PhD
Supervisor/Team Leader: Carmen Booker, PhD
Acting Clinical Division: Banu Karimi Shah, MD
Director:
Project Manager: Cindy Chee, PharmD

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 219407 are owned by Ionis Pharmaceuticals Inc. or are data for which Ionic Pharmaceuticals Inc. has obtained a written right of reference.

Any information or data necessary for approval of NDA 219407 that Ionis Pharmaceuticals Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 219407.

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1 Executive Summary

1.1 Introduction

Ionis Pharmaceuticals Inc. (Applicant) submitted a 505(b)(1) New Drug Application (NDA) for subcutaneous (SC) administration of donidalorsen (ISIS 721744) as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older. ISIS 721744 is an *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO), which binds to human prekallikrein (PKK) messenger RNA (mRNA) to reduce PKK protein production. The GalNAc molecule preferentially delivers ISIS 721744 to the liver by binding to the asialoglycoprotein receptor (ASGPR) on liver cells.

This review focuses on the nonclinical safety assessment of extractables and leachables for the donidalorsen drug product container closure system (CCS), which constitutes a staked needle glass prefilled syringe (PFS) closed with a (b) (4) rubber plunger stopper that is assembled into an autoinjector to deliver a single dose of 80 mg donidalorsen in 0.8 mL (100 mg/mL). The extractables and leachables information and study reports were submitted on 08/21/2024 (SDN 1) and 10/11/2024 (SDN 7).

Refer to **Section 3.3** (Previous Reviews Referenced) for a list of the other nonclinical reviews related to the nonclinical evaluation for NDA 219407.

1.2 Brief Discussion of Nonclinical Findings

The extractables and leachables studies for the donidalorsen drug product CCS included a controlled extraction study, accelerated leachable study, and leachable study as part of the stability assessment. As summarized below, there are no nonclinical safety concerns based on results from these extractables and leachables studies.

In the controlled extraction study, the CCS was filled with aqueous buffer solution (pH 5 or pH 9.5) or water-isopropyl alcohol (50:50 v/v) and sealed with plunger stoppers sterilized by (b) (4) (in order to determine the potential impact of sterilization on the extractables profile). The detected extractables (b) (4)

(b) (4) are considered common extractables/leachables with the materials of the CCS. These compounds were not detected in the leachable studies (accelerated or stability). Further, it did not appear that the sterilization process ((b) (4)) affected the extractable profile.

In the accelerated leachable study (Study #1 equivalent to approximately 1 year at 5°C and Study #2 equivalent to slightly less than 3 years at 5°C), two representative ASO drug products were filled into 1 mL and 2.25 mL PFS CCS comprising a staked needle (b) (4) syringe with a (b) (4) tip cap and (b) (4) gray (b) (4) rubber plunger/stopper ((b) (4)). No volatile, semi-volatile, or non-

volatile organic leachables were detected (b) (4) analytical evaluation thresholds (AETs). (b) (4) was detected (b) (4) AET in the filled primary container sample in Study #2 at (b) (4) µg/mL but was also detected in the control (drug product that has not been in contact with the CCS) at (b) (4) µg/mL and is likely not a leachable.

As part of the stability assessment, three registration stability batches of the 100 mg/mL donidalorsen autoinjector have been evaluated for leachables at time point 0 (T0); 6 months at 25°C/65% relative humidity (6 months), and 12 months at 5°C/ambient relative humidity transitioned to 30°C/75% relative humidity 6 weeks prior to the pull date to simulate the patient in-use period (12 months). (b) (4) was detected as a volatile leachable (b) (4) AET in three lots at T0 ((b) (4) µg/mL), 6 months, ((b) (4) µg/mL), and 12 months ((b) (4) µg/mL). (b) (4) is listed as a Class 3 solvent (regarded as less toxic and of lower risk to human health) in ICH Q3C with an amount of ≤50 mg/day considered acceptable without justification. The exposure of patients to potential (b) (4) from the CCS of the donidalorsen drug product for SC administration based on leachable stability data to date was below 50 mg/day and therefore there is no toxicological concern. (b) (4) was detected in all lots at T0 ((b) (4) µg/mL). (b) (4) is listed as a Class 3 solvent in ICH Q3C and with a permitted daily exposure (PDE) of (b) (4) mg/day. The exposure of patients to potential (b) (4) from the CCS of the donidalorsen drug product for SC administration based on leachable stability data to date was below (b) (4) mg/day and therefore there is no toxicological concern.

Refer to **Sections 2.5** (Comments on Extractables and Leachables Studies) and **11** (Integrated Summary and Safety Evaluation).

Conclusion: Overall, there were no nonclinical safety concerns with potential leachables from the CCS for the donidalorsen drug product for SC administration.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, NDA 219407 is recommended for approval. There are no outstanding nonclinical issues.

1.3.2 Additional Nonclinical Recommendations

As described in the NDA 219407 Nonclinical Primary Review dated 12/19/2024, the Applicant should submit the final study report for the 2-year carcinogenicity study in rats as a post marketing requirement (PMR) (Refer to IND 142564 pre-NDA Meeting Preliminary Comments dated 04/10/2024).

1.3.3 Labeling

Nonclinical sections of the product label are evaluated separately.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

2304692-48-4 (free acid)

2304701-45-7 (sodium salt)

Trade Name

Dawnzera

Generic Name

Donidalorsen

Code Name

ISIS 721744

Chemical Name (IUPAC)

all-P-ambo-5'-O-[[[(6-{5-[(tris{3-[6-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)hexylamino]-3-oxopropoxymethyl})methyl]amino-5-oxopentanamido}hexyl)]phospho}-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioguanilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methylcytidilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenilyl-(3'-O→5'-O)-2'-deoxy-P-thioguanilyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-P-thioguanilyl-(3'-O→5'-O)-2'-deoxy-P-thioguanilyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenosine, icosasodium salt

Molecular Formula/Molecular Weight

C₂₉₆H₄₃₅N₈₃O₁₅₁P₂₀S₁₅ (free acid)

C₂₉₆H₄₁₅N₈₃O₁₅₁P₂₀S₁₅Na₂₀ (sodium salt)

8672.66 Da (free acid)

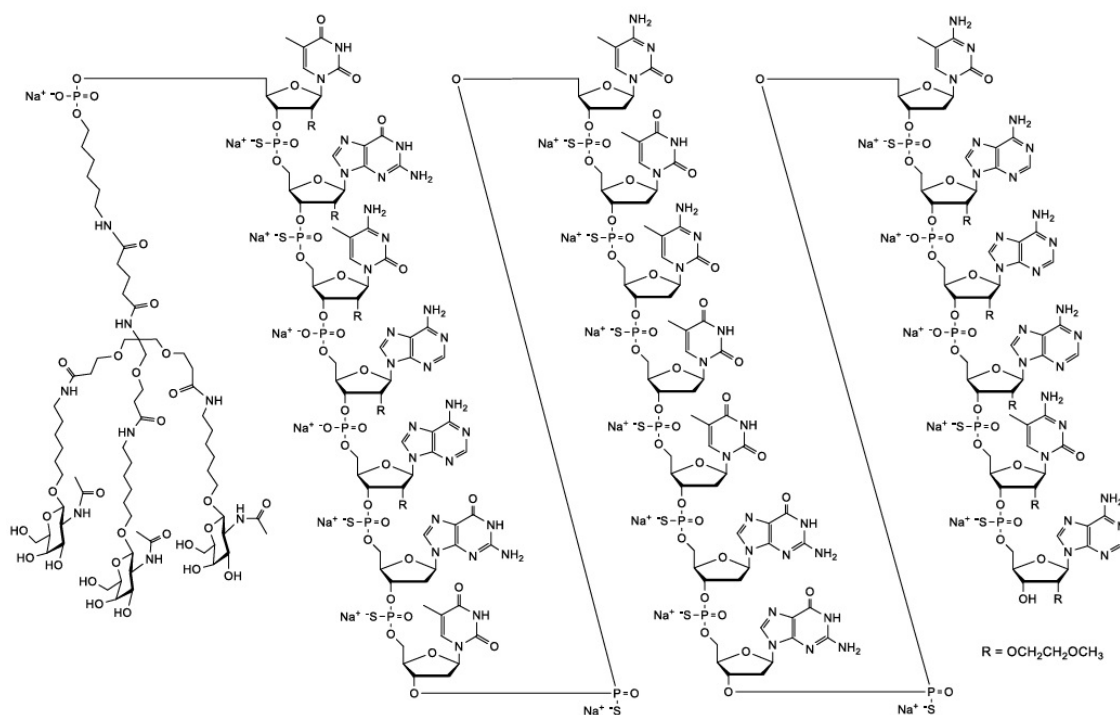
9112.27 Da (sodium salt)

Structure or Biochemical Description

The structure of donidalorsen sodium is shown in **Figure 1** (Applicant's Figure).

ISIS 721744 is a 20-mer chimeric 2'-O-methoxymethyl (2'-MOE)-modified ASO with a mixed backbone made up of phosphorothioate (PS) and phosphodiester (PO) internucleotide linkages, which targets the mRNA of human PKK. The ASO is conjugated to triantennary GalNAc via a trishexylamino (THA)-C6 linker, which preferentially delivers ISIS 721744 to the liver by binding to the ASGPR on liver cells.

Figure 1 Structure of Donidalorsen Sodium (Applicant's Figure)



Pharmacologic Class
 PKK-targeted GalNAc-conjugated ASO
 PKK-directed ASO

2.2 Relevant INDs, NDAs, BLAs and DMFs

Relevant IND

IND 142564 (active): Donidalorsen (ISIS 721744) by Ionis Pharmaceuticals Inc. for prophylaxis to prevent attacks of HAE in Center for Drug Evaluation and Research/Office of New Drugs/Office of Immunology and Inflammation/Division of Pulmonology, Allergy, and Critical Care (CDER/OND/OII/DPACC)

Relevant DMFs (Letters of Authorization provided in NDA 219407 Module 1.4.2)

DMF [redacted] (b) (4)

DMF [redacted] (b) (4)

[redacted] (b) (4)

DMF [redacted] (b) (4)

[redacted] (b) (4)

DMF [redacted] (b) (4)

[redacted] (b) (4)

DMF [redacted] (b) (4)
 [redacted] (b) (4)

DMF [redacted] (b) (4)
 [redacted] (b) (4)

2.3 Drug Formulation

The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The drug product formulation is shown in **Table 1** (Applicant's Table). The drug product is packaged in a staked needle glass PFS closed with a [redacted] (b) (4) rubber plunger stopper and assembled into an autoinjector to deliver 0.8 mL of solution containing 80 mg of donidalorsen (80 mg/0.8 mL = 100 mg/mL).

Refer to **Section 2.5** (Comments on Extractables and Leachables Studies) for additional information on the CCS.

Refer to NDA 219407 Office of Pharmaceutical Quality Integrated Quality Assessment dated 05/05/2025 for more details on the drug formulation from the quality perspective.

Table 1 Donidalorsen Drug Product Formulation (Applicant's Table)

Ingredients	Quality Standards ^j	Quantity per Autoinjector	Quantity per Unit Dose (0.8mL)	Pharmaceutical Function
Donidalorsen ^a	Professed ^b	(b) (4)mg (equivalent to (b) (4)mg as sodium salt)	80 mg (equivalent to 84 mg as sodium salt)	Active
Sodium dihydrogen phosphate (b) (4)	USP-NF	[redacted] (b) (4)		
Disodium hydrogen phosphate (b) (4)	USP-NF			
Sodium chloride	USP-NF			
Hydrochloric acid ^f	USP-NF	QS ^{g, h}	QS ^h	pH adjustment
Sodium hydroxide ^f	USP-NF	QS ^h	QS ^h	pH adjustment
Water for Injection (WFI)	USP-NF	QS to (b) (4) mL ⁱ	QS to 0.8 mL	Vehicle

^a Donidalorsen drug substance is supplied as the sodium salt. The label claim is expressed as the free acid concentration

^b Per Section 3.2.S.4.1

[redacted] (b) (4)

^f Added [redacted] (b) (4) for pH adjustment

^g QS: quantity sufficient

^h Ingredient may be added to adjust pH; therefore, the quantity is variable

ⁱ To ensure that no less than 0.8 mL is dosed upon injection, a (b) (4) mL fill is targeted

^j USP - United States Pharmacopoeia; NF- National Formulary

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product formulation. All the excipients are within levels found in other FDA-approved SC drug products.

2.5 Comments on Extractables and Leachables Studies

The Applicant provided data from a controlled extraction study and an accelerated leachable study with the donidalorsen drug product CCS. The Applicant also indicated that a leachable study is ongoing as part of the registration stability protocol and available data were provided to the NDA. The Applicant indicated that the extractables and leachables assessment for the CCS was in line with the recommendations for evaluation of primary packaging in USP <1661>, extractables/leachables testing per USP <1663>, USP <1664>, and the Product Quality Research Institute (PQRI) Leachables and Extractables Working Group Initiatives for Parenteral and Ophthalmic Products.^{1,2,3}

This section describes the donidalorsen drug product CCS as well as the study design and results for the extractables and leachables studies with the CCS. The nonclinical safety evaluation for potential leachables is included in **Section 11** (Integrated Summary and Safety Evaluation).

Refer to NDA 219407 Office of Pharmaceutical Quality Integrated Quality Assessment dated 05/05/2025 for more details on the extractables and leachables assessment from the quality perspective.

CCS for Donidalorsen Drug Product

As described by the Applicant (Module 3.2.P.7. Container Closure System): Donidalorsen Solution for Injection, 100 mg/mL, is packaged in a 1 mL Long (1 mL L) staked needle syringe with a plunger stopper (which together constitute the primary CCS). The (b) (4) syringe is the (b) (4) 1 mL L glass syringe with a rigid needle shield (RNS) closure and a 0.5 inch (12.7 mm), 27 gauge, (b) (4) staked needle. The plunger stopper is the (b) (4) rubber plunger stopper. The drug product is filled into the syringe and closed with the plunger stopper to create the filled primary CCS. Of note, none of the components of the staked needle syringe are made with natural rubber latex or any derivatives from natural rubber latex and the formulation used in the plunger stopper is not made with latex or dry rubber. The filled primary CCS is assembled into an integral, single-use, single-dose (b) (4) autoinjector (secondary CCS). The (b) (4) autoinjector is used in other FDA approved combination products.

¹Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, Product Quality Research Institute, Arlington VA, September 8, 2006

²D Jenke PQRI PODP Workshop Feb.22-23, 2011

³D Jenke, PDA J. Pharm. Sci. and Tech. 2013, 67, 448-551

According to the Applicant (Module 3.2.P.2.4 Container Closure System), the components of the filled primary CCS comply with the relevant pharmacopeia standards as shown in **Table 2** (Applicant's Table).

Table 2 Regulatory Compliance of Donidalorsen Primary Container Closure System (Applicant's Table)

Component and Manufacturer	Regulatory Compliance
Glass barrel tubing (b) (4)	- USP <660> "Containers: glass" - Ph. Eur. 3.2.1. "Glass containers for pharmaceutical use", (b) (4) - JP 7.01 "Tests for glass containers for injection"
Barrel (b) (4) and Plunger stopper (b) (4)	- USP (b) (4) - Ph. Eur. (b) (4)
Needle lubricant (b) (4)	- USP (b) (4) - Ph. Eur. 3.1.8 "Silicone oil used as a lubricant"
Plunger stopper (b) (4) and Elastomeric needle shield (b) (4)	- USP <381> "Elastomeric Closures for Injection", chemical tests - Ph. Eur. 3.2.9 "Rubber closures for containers for aqueous parenteral preparations for powders and for freeze-dried powders," chemical tests

Controlled Extraction Study

Study Design

The Applicant described the study design for the controlled extraction study as follows: The commercial CCS plunger stoppers were sterilized by (b) (4) in order to determine the potential impact of sterilization on the extractables profile. The CCSs were filled with aqueous buffer solution (pH 5 or pH 9.5) or water-isopropyl alcohol (50:50 v/v) and sealed with the plunger stopper. The samples were stored horizontally at 55°C for 72 hours.

Extracts from the aqueous samples (pH 5.0 and pH 9.5) and individual components of the PFS were analyzed for volatile organic extractables by headspace gas chromatography-mass spectrometry (GC-MS). The individual components of the PFS (glass barrel, plunger stopper, and tip cap) were analyzed directly in place of the organic extract. The Sample IDs of the individual components were identified as follows:

- Glass Barrel with Needle ((b) (4) 1.0 mL 27G STW 0.5 inch staked PFS with a silicone oil lubricant) Component wt. 3.07682 g
- Stopper/Plunger ((b) (4) 7 mm (b) (4) rubber Grey (b) (4) Component wt. 0.24077 g (b) (4); 0.2422 g (b) (4)
- Black Rubber (b) (4) Component wt. 0.47409 g

Extracts from water-isopropyl alcohol (50:50 v/v) samples were not analyzed for volatile organic extractables (b) (4).

Extracts from aqueous samples (pH 5.0 and pH 9.5) and water-isopropyl alcohol (50:50 v/v) samples were analyzed for semi-volatile organic extractables by direct inject GC-MS, non-volatile organic extractables by ultra-high pressure liquid chromatography with photodiode array and quadrupole-time-of-flight mass spectrometry (HPLC-PDA-QTOF-MS), and inorganic extractables by inductively coupled plasma mass spectrometry (ICP-MS).

The Applicant calculated the AET for the drug product based on a safety concern threshold (SCT) of 1.5 µg/day (ICH M7 limit for mutagenic impurities), as follows:

$$\text{AET} = \text{(b) (4)} \mu\text{g/mL}$$

The Applicant applied an uncertainty factor of (b) (4) which resulted in a final AET value of (b) (4) µg/mL.

The Applicant expressed the AET for the individual components as µg/component to represent the entire surface area of each component, whereas only a fraction is in contact with the drug product.

$$\text{AET} = \text{(b) (4)} \text{ g/component}$$

The Applicant applied an uncertainty factor of (b) (4) which resulted in a final AET value of (b) (4) µg/component.

The Applicant calculated the limit of detection (LOD) expressed in µg/component as follows:

$$\text{LOD } \mu\text{g/g} \times \text{g/component} = \mu\text{g/component.}$$

Results

Volatile Organic Results

No volatile organic extractables were detected (b) (4) AET in the aqueous buffer samples (pH 5.0 or pH 9.5) of CCS with plunger stoppers sterilized by (b) (4).

No volatile organic extractables were detected (b) (4) AET from the individual components of the PFS (i.e., glass barrel, plunger stoppers sterilized by (b) (4) and tip cap).

Semi-Volatile Organic Results

No semi-volatile organic extractables were detected (b) (4) AET in the aqueous buffer samples (pH 5.0 or pH 9.5) of CCS with plunger stoppers sterilized by (b) (4).

Two semi-volatile organic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) ((b) (4) (b) (4) at (b) (4) µg/mL and (b) (4) at (b) (4) µg/mL).

Two semi-volatile organic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) at (b) (4) µg/mL and (b) (4) at (b) (4) µg/mL).

Non-Volatile Organic Results

No non-volatile organic extractables were detected (b) (4) AET in the aqueous buffer samples (pH 5.0 or pH 9.5) of CCS with plunger stoppers sterilized by (b) (4)

Three non-volatile organic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, and (b) (4) at (b) (4) µg/mL).

Five non-volatile organic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, and (b) (4) at (b) (4) µg/mL).

Inorganic Results

No inorganic extractables were detected (b) (4) AET in the aqueous buffer samples (pH 5.0 or pH 9.5) of CCS with plunger stoppers sterilized by (b) (4)

No inorganic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4).

Refer to **Section 11** (Integrated Summary and Safety Evaluation) for the nonclinical safety evaluation of the controlled extraction study data.

(b) (4)
The Applicant indicated that (b) (4) were evaluated as extractables in the (b) (4) gray (b) (4) rubber plunger/stopper.

With regard to (b) (4), the Applicant indicated that: The (b) (4) Grey rubber formulation was tested for (b) (4) after reflux extraction with water-isopropyl alcohol (50:50 v/v) by GC-MS. The LOD for each (b) (4) was calculated on a µg/mL basis based on the detection limit determined for the GC-MS method (reported in µg/g) using the average weight in grams of the plunger stopper (0.244 g) and a dose of 0.8 mL. The result was related to patient safety based on the SCT of 1.5 µg/day and the established AET of (b) (4) µg/mL for the product (the AET was used directly for (b) (4) since the response factor is known and therefore no uncertainty factor was needed).

As shown in **Table 3** (Applicant's Table), no (b) (4) was detected (b) (4) LOD in the extraction study of the (b) (4)/Grey rubber formulation. Refer to **Section 11**

(Integrated Summary and Safety Evaluation) for the nonclinical safety evaluation of (b) (4) based on request from the Quality Review Team.

With regard to (b) (4) the Applicant indicated that (b) (4) confirmed that the (b) (4) Grey rubber formulation does not contain (b) (4) (Quality Certificates from (b) (4) were provided).

Table 3 Limit of Detection (LOD) for (b) (4) Analysis for (b) (4) Extraction Study (Applicant's Table)

Analyte	Result (µg/mL)	LOD (µg/mL)
(b) (4)		

ND: Not Detected

Accelerated Leachable Study

Study Design

The Applicant described the study design for the accelerated leachable study as follows:

Based on the extractable study results for the (b) (4) sterilized stopper (see above), the stoppers sterilized by (b) (4) were considered

equivalent. As such, the accelerated leachable study used (b) (4) sterilized stoppers on two representative ASO drug products filled into 1 mL and 2.25 mL PFS CCS comprising a staked needle (b) (4) syringe with a (b) (4) tip cap and (b) (4) gray (b) (4) rubber plunger/stopper. The Applicant indicated that the 1 mL and 2.25 mL CCS have identical materials of construction, and the extraction power of ASO drug products are similar as they are aqueous based formulations containing only ASO and inorganic excipients. The Applicant further indicated that the 1 mL CCS is identical to the system used for the 80 mg donidalorsen solution for injection.

The Applicant described the two accelerated leachable studies as follows:

- Study #1: 200 mg/mL ASO in water formulated at pH 8.0, filled into a 2.25 mL PFS primary container sealed with (b) (4) stoppers and stored horizontally for 1 month at the accelerated condition of 40°C, which is equivalent to approximately 1 year at 5°C.
- Study #2: 75 mg/mL ASO in isotonic (b) (4) formulated at pH 7.4, filled into a 1 mL PFS primary container sealed with (b) (4) stoppers and stored horizontally for 3 months at the accelerated condition of 40°C, which is equivalent to slightly less than 3 years at 5°C.

The samples were then analyzed for volatile organic leachables by headspace GC-MS, semi-volatile organic leachables by direct injection GC-MS, non-volatile organic leachables by HPLC-PDA-MS and inorganic leachables by ICP-MS.

The Applicant set an AET of (b) (4) µg/mL for Study #1 and (b) (4) µg/mL (includes (b) (4) uncertainty factor) for Study #2. The Applicant indicated that the AET's were based on PQRI's SCT of 1.5 µg/day¹ and either a (b) (4) mL (Study #1) or (b) (4) mL (Study #2) delivered dose volume.

Results

Volatile, Semi-Volatile, and Non-Volatile Organic Results

No volatile, semi-volatile, or non-volatile organic leachables were detected (b) (4) AETs in either Study #1 or Study #2, respectively.

Inorganic Results

No inorganic leachables were detected (b) (4) AET in Study #1.

(b) (4) was detected (b) (4) AET in the filled primary container sample in Study #2 at (b) (4) µg/mL but was also detected in the control (drug product that has not been in contact with the CCS) at (b) (4) µg/mL.

Refer to **Section 11** (Integrated Summary and Safety Evaluation) for the nonclinical safety evaluation of the accelerated leachable study data.

Leachable Study on Stability

Study Design

The Applicant described the study design for the leachable study on stability as follows:

Registration stability batches of the 100 mg/mL autoinjector are being monitored on stability. At the time of the NDA submission, three registration stability batches were evaluated at T0, 6 months at 25°C/65% relative humidity, and 12 months at 5°C/ambient relative humidity transitioned to 30°C/75% relative humidity 6 weeks prior to the pull date to simulate the patient in-use period.

As stated in the study report, the leachable studies on stability were performed on three lots of the donidalorsen drug product at T0, 6 months, and 12 months (lots (b) (4) 01A, (b) (4) 02B, and (b) (4) 03A at T0 and 6 months and lots (b) (4) 01R12, (b) (4) 02R12, and (b) (4) 03R12 at 12 months) and a bulk control (i.e., drug product formulation, stored upright at 2 to 8°C in an inert container and cap; lots Q1618-2-2 and Q1596-72-2).

Samples were analyzed for volatile organic compounds by headspace GC-MS, semi-volatile organic compounds by direct injection GC-MS, nonvolatile organic compounds by HPLC-PDA-MS, and inorganic elemental impurities by ICP-MS.

The Applicant indicated that the AET values for the drug products were based on a SCT of 1.5 µg/day (the ICH M7 limit for mutagenic impurities).

The Applicant calculated the AET values as follows: $\text{AET } \mu\text{g/mL} = \frac{\text{(b) (4)}}{\text{mL/day}} = \text{(b) (4)} \mu\text{g/mL}$

The Applicant applied an uncertainty factor of (b) (4) for a final AET value of (b) (4) µg/mL ((b) (4) AET).

The Applicant indicated that the methods aimed to target classes of potential leachables based upon the materials of construction of the primary container components and extractables detected in the controlled extraction studies (see above). The validated target compounds for the leachable study are shown in **Table 4** (Applicant's Table). As stated in **Table 4** (Applicant's Table), the PDE for (b) (4) was derived, based on ICH principles and guidance to support an increased limit of quantitation (LOQ) (b) (4) AET. The PDE of (b) (4) µg/kg/day was proposed which is significantly higher than the SCT of 1.5 µg/day used to calculate the AET. Refer to **Section 11** (Integrated Summary and Safety Evaluation) for the nonclinical safety evaluation of (b) (4) based on request from the Quality Review Team.

Table 4 Validated Target Compounds for Leachable Study (Applicant’s Table)

Method	Analyte	Justification	Validated Range (µg/mL)	LOQ (µg/mL)
Volatile Organics (Headspace GC-MS)	(b) (4)			
Semi-Volatile Organics (Direct Injection GC-MS)				
Method	Analyte	Justification	Validated Range (µg/mL)	LOQ (µg/mL)
Non-Volatile Organics (HPLC-PDA-MS)	(b) (4)			
Elemental Impurities (ICP-MS)				

^a Permitted daily exposures (PDEs) were derived, based on ICH principles and guidance to support an increased LOQ (b) (4) AET. The PDE of (b) (4) µg/kg bw/day was proposed which is significantly higher than the SCT of (b) (4) µg/day used to calculate the AET.

^b (b) (4) not an elemental impurity of concern and as such no PDE is included in ICH Q3D. (b) (4) a leading indicator of (b) (4) and was included with a relevant limit to monitor for early indications of (b) (4) attack.

Results

The leachable data from stability at T0, 6 months, and 12 months are summarized in **Table 5** (Applicant’s Table). Of note, the Applicant provided a commitment to continue

the long-term leachable study through the proposed shelf-life of the product (up to 60 months).

(b) (4) was detected (b) (4) AET in three lots analyzed for volatiles by headspace GC-MS at T0 ((b) (4) µg/mL), 6 months, ((b) (4) µg/mL), and 12 months ((b) (4) µg/mL).

No other targeted or untargeted organic leachables were detected (b) (4) respective AETs at any time point.

No inorganic leachables (metals) were detected (b) (4) AET at any time point.

(b) (4) was detected in all lots analyzed for non-volatiles by HPLC-PDA-MS at T0 ((b) (4) µg/mL).

Refer to **Section 11** (Integrated Summary and Safety Evaluation) for the nonclinical safety evaluation of the leachable study on stability data.

Table 5 Summary of Data from Leachable Study on Stability (Applicant's Table)

Method	Time-Point and Storage Condition	Result
Volatiles by headspace GC-MS	T0	(b) (4) detected in the 3 lots at (b) (4) µg/mL No other targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Semi-volatiles by direct injection GC-MS		No targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Non-volatiles by HPLC-PDA-MS		No targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL (b) (4) detected in all lots at (b) (4) µg/mL ^b
Metals by ICP-MS		No inorganic leachables ≥ AET of (b) (4) µg/mL ^a
Volatiles by headspace GC-MS	6 months at 25 °C ± 2 °C / 60% relative humidity	(b) (4) detected in the 3 lots at (b) (4) µg/mL. No other targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Semi-volatiles by direct injection GC-MS		No targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Non-volatiles by HPLC-PDA-MS		No targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Metals by ICP-MS		No inorganic leachables ≥ AET of (b) (4) µg/mL ^a
Volatiles by headspace GC-MS	12 months at 5 °C ± 3 °C / ambient relative humidity transitioned to 30 °C ± 2 °C/75% ± 5% RH 6 weeks prior to the scheduled pull date to simulate the patient in-use period	(b) (4) detected in the 3 lots at (b) (4) µg/mL. No other targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Semi-volatiles by direct injection GC-MS		No targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Non-volatiles by HPLC-PDA-MS		No targeted leachables ≥ AET of (b) (4) µg/mL ^a No untargeted leachables detected ≥ (b) (4) AET of (b) (4) µg/mL
Metals by ICP-MS		No inorganic leachables ≥ AET of (b) (4) µg/mL ^a

^a Target leachable compounds and element are reported at the AET since the response factor is known and therefore no uncertainty factor is needed

^b (b) (4) is part of the drug substance process and is not considered a CCS leachable

2.6 Proposed Clinical Population and Dosing Regimen

Donidalorsen is indicated for prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. The recommended dosage is 80 mg administered SC (b) (4). A dosing interval of (b) (4) may be considered in some patients.

2.7 Regulatory Background

Provided below is regulatory background for the donidalorsen development program as it relates to extractables and leachables.

The following comment was provided to the Applicant in IND 142564 Type C written responses dated 12/19/2023:

“The clinical drug product is packaged with 0.8 mL deliverable volume in an ISO 2R Type (b) (4) clear glass vial that is stoppered with a (b) (4) rubber closure and sealed with an aluminum overseal. During the phase 3 Open Label Extension study, the intended commercial configuration will be introduced, which comprises a glass prefilled syringe (PFS) fitted into an autoinjector (AI). A safety assessment of extractables and leachables should be provided in the NDA for all primary container closure components of the device that are in contact with the drug product during storage (i.e., AI and potentially the vial if it is also used as a commercial configuration), as well as secondary container closure components where there is a potential for migration of leachables into the drug product. See USP Chapters for 1663 and 1664 for the design of extractables and leachables studies, respectively.”

During the filing review of the original submission for this NDA 219407 (SDN 1, 08/21/2024), it appeared that the Applicant had not provided the study reports associated with the extractables and leachables studies. The following information request was sent to the Applicant on 09/27/2024:

“We are reviewing your August 21, 2024, NDA submission and have the following requests for information:

The results of extractable and leachable studies conducted with the drug product primary container closure system are summarized in Pharmaceutical Development (Module 3.2.P.2) for the Container Closure System (Module 3.2.P.2.4). To facilitate the review of your application, provide the finalized study reports associated with these studies or provide their specific location in the submission.”

The Applicant provided the following response to the information request on 10/11/2024 (SDN 7):

“The product-specific study reports for donidalorsen drug product are provided with this response.

Module 3.2.P.2.4 Section 7.2 provides a detailed extractables and leachables assessment for the container closure system (CCS). The assessment is in line with the recommendations for evaluation of primary packaging in USP <1661>, extractables/leachables testing per USP <1663>, USP <1664>, and the Product Quality Research Institute (PQRI) Leachables and Extractables Working Group Initiatives for Parenteral and Ophthalmic Products.

To confirm the low risk of leachables from the CCS assessment, a product-specific leachable study is ongoing as part of the registration stability protocol (Module 3.2.P.8.1). Three (3) registration stability batches of the donidalorsen drug product are being monitored on stability. A tabular summary of leachable results obtained through 12 months storage at the long-term storage condition of 2–8 °C (with 6 weeks in use at 30 °C) and at T=0 and 6 months of storage at 25 °C/60% RH are summarized in Module 3.2.P.2.4 Table 20 and the reports associated with these studies are provided as

Appendix 1 of this response. A commitment to continue the longterm leachable study through the proposed shelf-life of the product (up to 60 months) is provided in Module 3.2.P.8.2.”

3 Studies Submitted

3.1 Studies Reviewed

Information in NDA 219407 Module 3 Quality (SDN 1, 08/21/2024)

- Module 3.2.P.2.4 Container Closure System (Donidalorsen Solution for Injection)
- Module 3.2.P.7. Container Closure System
- Module 3.2.P.8.1 Stability Summary and Conclusions
- Module 3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment (Donidalorsen Solution for Injection)

Leachable study reports submitted by the Applicant (SDN 7, 10/11/2024):

- Routine Leachable Study PKK-L (Donidalorsen, ISIS 721774) Drug Product (Time Zero); Element Job No. 270307
- Routine Leachable Study PKK-L (Donidalorsen, ISIS 721774) Oligonucleotide Drug Product (6-Month Time Point); Element Report No. 23-011-97257-1
- Routine Leachable Study PKK-L (Donidalorsen, ISIS 721774) Oligonucleotide Drug Product (12-Month Time Point); Element Report No. 24-011-98754 Rev 1

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 142564	Eleni Salicru, PhD	08/13/2019	Nonclinical primary review of primary pharmacology, safety pharmacology (in vivo cardiovascular, respiratory, and central nervous systems in monkey), PK/ADME, general toxicology (39-week in monkeys), and genetic toxicology (in vitro Ames assay and in vitro chromosome aberration assay)
NDA 219407	Eleni Salicru, PhD	12/19/2024	Nonclinical primary review of genetic toxicology

Application	Reviewer	Date in DARRTS	Notes
			(mammalian micronucleus assay in mice) and carcinogenicity (6-months in Tg.rasH2 mice)
NDA 219407	Eleni Salicru, PhD	03/05/2025	Nonclinical primary review of reproductive and developmental toxicology in mice
NDA 219407	Eleni Salicru, PhD	03/25/2025	Nonclinical primary review of pharmacology, safety pharmacology (in vitro hERG assay), PK/ADME, and general toxicology (26-week in rats)
NDA 219407	Eleni Salicru, PhD	05/09/2025	Nonclinical primary review of impurities including a 13-week repeat-dose SC toxicity and impurity qualification study of ISIS 721744 and associated test article mixtures (TAMs) in ICR mice

11 Integrated Summary and Safety Evaluation

The Applicant is seeking approval for donidalorsen for prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. The recommended dosage is 80 mg administered SC (b) (4). A dosing interval of (b) (4) (b) (4) may be considered in some patients. The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The drug product is packaged in a staked needle glass PFS closed with a (b) (4) rubber plunger stopper and assembled into an autoinjector to deliver a single dose of 0.8 mL of 80 mg donidalorsen (80 mg/80 mL = 100 mg/mL).

This review provides a nonclinical safety assessment of potential extractables and leachables from the primary CCS for the donidalorsen drug product for SC administration based on data submitted by the Applicant and other available information. Of note, although the drug product is delivered (b) (4) (b) (4) any comparisons made in this evaluation are based on acceptable levels per day. Adjustments for dosing intervals were not made to provide a conservative safety assessment. In general, for the leachable evaluation, any organic (nonmetal) compound with expected patient exposure below the PQRI thresholds of 5 µg/day (for compounds lacking genotoxic potential and irritant potential) and 1.5 µg/day (for compounds with genotoxic potential) was considered qualified for safety.¹ For compounds not qualified for safety based on PQRI thresholds, a nonclinical safety assessment to establish PDE

levels for each compound based on experimental data from the literature, where available, and considering modifying factors (following the ICH Q3C Guideline 2021) was used. Of note, (b) (4) leachables or other special case leachables (such as (b) (4)) would necessitate additional evaluation to determine a qualified threshold.

Comment on Controlled Extraction Study Results with Donidalorsen Drug Product CCS for SC Administration

The Applicant conducted a controlled extraction study in which the CCS was filled with aqueous buffer solution (pH 5 or pH 9.5) or water-isopropyl alcohol (50:50 v/v) and sealed with plunger stoppers sterilized by (b) (4) (in order to determine the potential impact of sterilization on the extractables profile). Refer to **Section 2.5** (Comments on Extractables and Leachables Studies).

Based on the Applicant's data:

Two semi-volatile organic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) (b) (4) at (b) (4) µg/mL and (b) (4) at (b) (4) µg/mL) or by (b) (4) ((b) (4) at (b) (4) µg/mL and (b) (4) at (b) (4) µg/mL). Five total non-volatile organic extractables were detected (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) ((b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, and (b) (4) at (b) (4) µg/mL) or by (b) (4) (five = (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, (b) (4) at (b) (4) µg/mL, and (b) (4) at (b) (4) µg/mL).

In general, the detected extractables ((b) (4) and (b) (4)) are considered common extractables/leachables with the materials of the CCS. Of note, it did not appear that these compounds were detected in the leachable studies (accelerated or stability). Further, it did not appear that the sterilization process (b) (4) affected the extractable profile.

Comment on (b) (4) and (b) (4) Extractables

The Applicant indicated that the (b) (4) Grey rubber formulation was tested for (b) (4) after reflux extraction with water-isopropyl alcohol (50:50 v/v) by GC-MS. Based on the Applicant's data, no (b) (4) was detected (b) (4) LOD. Refer to **Section 2.5** (Comments on Extractables and Leachables Studies).

In an email dated 11/21/2024, the Quality Reviewer Dr. Ghaled Hamad, requested input from the Nonclinical Review Team on whether the Applicant's use of (b) (4) µg/day as the SCT for (b) (4) in the extractables and leachables testing of donidalorsen drug product for SC administration was acceptable.

As described by the National Cancer Institute:⁴

(b) (4)

(b) (4)

(b) (4). Some members of the (b) (4) class were shown to have carcinogenic, genotoxic, and immunotoxic properties, developmental toxicity and affect reproductive health.

From a toxicological perspective, (b) (4) encompass compounds with a cohort of concern due to their carcinogenic potential.^{5,6} While in general a SCT of 1.5 µg/day would be considered acceptable for qualifying compounds with genotoxic potential¹, based on internal nonclinical discussion, it was considered that the SCT for each (b) (4) would need to be lower than 1.5 µg/day. After feedback from the PTCC Extractables and Leachables Subcommittee, it was considered that based on the known information for (b) (4) as potential leachables, using the LOD for each (b) (4) (see **Table 3**, Applicant's Table) would be acceptable. From the nonclinical safety perspective, the lack of detectable (b) (4) (b) (4) LOD is reassuring.

With regard to (b) (4) the Applicant indicated that (b) (4) confirmed that the (b) (4) Grey rubber formulation does not contain (b) (4) (Quality Certificates from (b) (4) were provided). For internal descriptive purposes only, it is noted that a nonclinical safety assessment of (b) (4) has been conducted by the PTCC Extractables and Leachables Subcommittee, which established an acceptable daily intake level (ADI) of (b) (4) mg/day, following the recommendation from the ICH Q3C Guideline 2021 (e.g., PDE = [no observed adverse effect level [NOAEL] from valid and well-conducted toxicity study x 50 kg adult body weight]/F1 x F2 x F3 x F4 x F5 x F6).

Safety Evaluation of Accelerated Leachable Study Results with Donidalorsen Drug Product CCS for SC Administration

The Applicant conducted an accelerated leachable study (Study #1 equivalent to approximately 1 year at 5°C and Study #2 equivalent to slightly less than 3 years at 5°C) using (b) (4) sterilized stoppers on two representative ASO drug products filled into 1 mL and 2.25 mL PFS CCS comprising a staked needle (b) (4) syringe with a (b) (4) tip cap and (b) (4) gray (b) (4) rubber plunger/stopper. Refer to **Section 2.5** (Comments on Extractables and Leachables Studies).

No volatile, semi-volatile, or non-volatile organic leachables were detected (b) (4) AETs. (b) (4) was detected (b) (4) AET in the filled primary container sample in Study #2 at (b) (4) µg/mL but was also detected in the control (drug product that has not been in contact with the CCS) at (b) (4) µg/mL and is likely not a leachable.

(b) (4)

Safety Evaluation of Leachable Study on Stability Results with Donidalorsen Drug Product CCS for SC Administration

The Applicant conducted a leachable study on three registration stability batches of the 100 mg/mL donidalorsen autoinjector at T0, 6 months, and 12 months. Of note, the Applicant provided a commitment to continue the long-term leachable study through the proposed shelf-life of the product (up to 60 months). Refer to **Section 2.5** (Comments on Extractables and Leachables Studies).

(b) (4) was detected as a volatile leachable (b) (4) AET in three lots at T0 ((b) (4) µg/mL), 6 months, ((b) (4) µg/mL), and 12 months ((b) (4) µg/mL). (b) (4) is listed as a Class 3 solvent (regarded as less toxic and of lower risk to human health) in ICH Q3C with an amount of ≤50 mg/day considered acceptable without justification. As shown in **Table 6**, the exposure of patients to potential (b) (4) (b) (4) from the CCS of the donidalorsen drug product for SC administration based on leachable stability data to date was below 50 mg/day and therefore there is no toxicological concern. Of note, the exposure to patients was calculated considering the maximum daily dose of the finished SC product (i.e., 0.8 mL/day = 80 mg/day ÷100 mg/mL).

(b) (4) was detected in all lots at T0 ((b) (4) µg/mL). The Applicant indicated that (b) (4) is part of the drug substance process and is not considered a leachable. Further, (b) (4) is listed as a Class 3 solvent in ICH Q3C and a PDE of (b) (4) mg/day was calculated based upon the no observed effect level (NOEL) from a subchronic inhalation study in rats (considered to be the most relevant published animal study for deriving a PDE). As shown in **Table 6**, the exposure of patients to potential (b) (4) from the CCS of the donidalorsen drug product for SC administration based on leachable stability data to date was below (b) (4) mg/day and therefore there is no toxicological concern. Of note, the exposure to patients was calculated considering the maximum daily dose of the finished SC product (i.e., 0.8 mL/day = 80 mg/day ÷100 mg/mL).

Table 6 Leachable Study Stability Risk Assessment of Donidalorsen Drug Product CCS for SC Administration

Leachable Compound	Highest Concentration Measured in Leachable Study (µg/mL)	Human Maximum Daily Exposure (µg/day) ¹
(b) (4)	(b) (4)	(b) (4)

Abbreviations: (b) (4) = (b) (4)

¹Human exposure was calculated considering the maximum daily dose of 0.8 mL/day for the SC finished product

Safety Evaluation of (b) (4) as a Potential Leachable with Donidalorsen Drug Product CCS for SC Administration

Although (b) (4) was detected in the controlled extraction study as a non-volatile organic extractables (b) (4) AET in water-isopropyl alcohol samples of CCS with plunger stoppers sterilized by (b) (4) ((b) (4) µg/mL) or by (b) (4) ((b) (4)

µg/mL), it was not detected in the leachable studies (accelerated or stability). The Applicant considered (b) (4) a targeted leachable and proposed a derived PDE of (b) (4) µg/kg/day. Refer to **Section 2.5** (Comments on Extractables and Leachables Studies).

In an email dated 03/19/2025, the Quality Reviewer Dr. Ghaled Hamad, requested input from the Nonclinical Review Team on the Applicant's PDE for (b) (4) of (b) (4) µg/kg/day, which significantly exceeds the AET calculation basis of 1.5 µg/day SCT.

Below is the nonclinical assessment of (b) (4) As summarized from PubChem⁷: (b) (4) (b) (4) (b) (4). From a toxicological perspective, the concern for (b) (4) is low given its abundance in tissues and the circulation in various forms. (b) (4) (b) (4)

As described by the United States Department of Agriculture (USDA), based on data from the Continuing Survey of Food Intakes by Individuals (CSFII) survey, the average intake of (b) (4) is (b) (4) g/d for women and (b) (4) g/d for men, representing (b) (4)% and (b) (4)% (b) (4) for women and men, respectively.⁸

(b) (4) is listed in the FDA's Substances Added to Food database for use as an (b) (4) (b) (4) Food additive and GRAS regulations (21 CFR Parts (b) (4)) include: (b) (4) (b) (4)

(b) (4) is found as an inactive ingredient (excipient) in FDA-approved (b) (4) (b) (4) drug products. For internal descriptive purposes only, it is noted that (b) (4) is found as an excipient in (b) (4) (b) (4) (b) (4)

(b) (4) and comparable (b) (4), do not exert genotoxic or skin sensitizing potential.^{10, 11, 12}

(b) (4)

Based on limited toxicity data with (b) (4) by the SC route, toxicity data with comparable (b) (4) were used for the derivation of a potential PDE. As described by the European Chemicals Agency (ECHA)¹⁰: (b) (4)



The calculation of a PDE for (b) (4) uses the NOAEL of (b) (4) mg/kg/day from the 6-week oral toxicity study in rats with (b) (4) and the following uncertainty factors:

- F1 = (b) (4)
- F2 = (b) (4)
- F3 = (b) (4)
- F4 = (b) (4)
- F5 = (b) (4)
- F6 = (b) (4)

PDE Calculation:

NO(A)EL = (b) (4) mg/kg

PDE = (b) (4)

As calculated, the PDE for (b) (4) is (b) (4)

In comparison, the Applicant's proposed PDE for (b) (4) would be (b) (4)



(b) (4) which are (b) (4) the calculated PDE based on toxicity data with (b) (4).

The following supportive information is included for internal descriptive purposes only: An ICH Q3E Guideline for Extractables and Leachables is currently being drafted by an Expert Working Group (EWG), which has been discussed extensively within the PTCC Extractables and Leachables Subcommittee. Based on these discussions, (b) (4) and other (b) (4) are considered as Class 3 leachables, which the draft Guidance describes as compounds considered to have relatively low potency for systemic toxicity with derived chronic parenteral PDE in excess of the levels at which leachables are typically observed (i.e., PDE $\geq$ 1 mg/day using the methodology which will be described in the Guideline). Class 3 leachables would not require further safety qualification if observed at daily exposure levels $<$ 1 mg/day. The draft Guideline states that a toxicological evaluation should be conducted for Class 3 leachables present at levels above 1 mg/day (1000 μ g/day). The following (b) (4) are potentially being considered to be included in the guidance as Class 3 leachables: (b) (4)

(b) (4)

There are various draft PDE worksheets for (b) (4) (based on (b) (4) PDE) that have been discussed within the PTCC Extractables and Leachables Subcommittee, as they are being considered for potential inclusion in the ICH Q3E Guideline for Extractables and Leachables. In all cases, the various PDEs for (b) (4) for the parenteral route are (b) (4) than the proposed Class 3 PDE of 1 mg/day.

Taken together, the Applicant's proposed PDE of (b) (4) would be acceptable. (b) (4)

Conclusion: The Applicant conducted extractables and leachables studies of the container closure systems for the donidalorsen drug product for SC administration. As described above, there were no nonclinical safety concerns based on potential leachables. Further toxicological risk assessment of the extractables and leachables was not considered necessary.

Refer to NDA 219407 Office of Pharmaceutical Quality Integrated Quality Assessment dated 05/05/2025 for more details on the extractables and leachables assessment from the quality perspective.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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07/24/2025 12:59:07 PM

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 219407
Supporting document/s: 1
Applicant's letter date: 08/21/2024
CDER stamp date: 08/21/2024
Product: Donidalorsen (ISIS 721744)
Pharmacologic Class: Prekallikrein (PKK)-targeted *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO)
Indication: Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older
Applicant: Ionis Pharmaceuticals Inc.
Clinical Division: Division of Pulmonology, Allergy, and Critical Care (DPACC)
PharmTox Division: Division of Pharmacology Toxicology for Immunology and Inflammation (DPT-II)
Reviewer: Eleni Salicru, PhD
Supervisor/Team Leader: Carmen Booker, PhD
Acting Clinical Division: Banu Karimi Shah, MD
Director:
Project Manager: Cindy Chee, PharmD

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Ionis Pharmaceuticals Inc. (Applicant) submitted a 505(b)(1) New Drug Application (NDA) for subcutaneous (SC) administration of donidalorsen (ISIS 721744) as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older. ISIS 721744 is an *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO), which binds to human prekallikrein (PKK) messenger RNA (mRNA) to reduce PKK protein production. The GalNAc molecule preferentially delivers ISIS 721744 to the liver by binding to the asialoglycoprotein receptor (ASGPR) on liver cells.

This review focuses on the nonclinical safety assessment of ISIS 721744 impurities through review of a 13-week repeat-dose SC toxicity and impurity qualification study of ISIS 721744 and associated test article related mixtures (TAMs) in ICR mice and also comparison of impurity levels in the ISIS 721744 clinical batch to the toxicology batch used in the 39-week repeat-dose toxicity study in monkeys.

Refer to the NDA 219407 Nonclinical Primary Review dated 03/25/2025 and Investigational New Drug (IND) 142564 Nonclinical Primary review dated 08/13/2019 for a review of the nonclinical pharmacology, safety pharmacology, pharmacokinetics/absorption, distribution, metabolism, and excretion (PK/ADME), and general toxicology studies for the ISIS 721744 nonclinical program.

Refer to the NDA 219407 Nonclinical Primary Review dated 03/05/2025 for a review of the reproductive and developmental toxicology studies with ISIS 721744 (i.e., combined fertility and developmental toxicity study in mice and pre- and postnatal development [PPND] study in mice).

Refer to the NDA 219407 Nonclinical Primary Review dated 12/19/2024 for a review of the 6-month carcinogenicity study of ISIS 721744, as well as the mouse surrogate, ISIS 722059, by SC injection in CByB6F1- Tg(HRAS)2Jic Hemizygous Transgenic mice (Tg.rasH2 mice).

1.2 Brief Discussion of Nonclinical Findings

The Applicant submitted a 13-week repeat-dose SC toxicity and impurity qualification study in which ICR mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection/solution [saline]) or (b) (4) mg/kg total oligonucleotide dose level of ISIS 721744, TAM 1, and TAM 2 once every two weeks (Q2W) for 13-weeks. The two TAMs that were evaluated in the study were made up of ISIS 721744 and associated impurities (i.e., (b) (4) impurities for TAM 1 and (b) (4) impurity for TAM 2). The findings in TAM 1- and TAM 2-treated groups were similar to those in ISIS 721744-treated groups, which included increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), increases in

liver weights, and histopathology findings of mononuclear cell infiltration and karyocytomegaly in the liver and vacuolated granular macrophages at the injection site. Overall, there were no meaningful differences between the findings in either of the TAM groups (ISIS 721744 + associated impurities) compared to the ISIS 721744 group alone and it did not appear that the added impurities affected the toxicity profile of ISIS 721744. Refer to **Sections 2.5** and **6.2**.

The Applicant conducted a pivotal Good Laboratory Practice (GLP)-compliant 39-week repeat-dose toxicity study of ISIS 721744 (0, 2, 6, 12, and 30 mg/kg/week) in Cynomolgus monkeys with a 13-week interim sacrifice and 26-week treatment-free recovery period (Refer to IND 142564 Nonclinical Primary review dated 08/13/2019). The impurity levels seen in the toxicology batch at the no observed adverse effect level (NOAEL) of 6 mg/kg/week provide support for the proposed impurity limits for the clinical batch. Refer to **Section 2.5**.

Conclusion: Taken together, the 13-week repeat-dose SC toxicity and impurity qualification study in ICR mice and comparisons of impurity levels between the 39-week toxicology study in monkeys and clinical batches of the drug support the proposed impurity limits. There are no outstanding nonclinical concerns.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, NDA 219407 is recommended for approval. There are no outstanding nonclinical issues.

1.3.2 Additional Non Clinical Recommendations

As described in the NDA 219407 Nonclinical Primary Review dated 12/19/2024, the Applicant should submit the final study report for the 2-year carcinogenicity study in rats as a post marketing requirement (PMR) (Refer to IND 142564 pre-NDA Meeting Preliminary Comments dated 04/10/2024).

1.3.3 Labeling

Nonclinical sections of the product label are evaluated separately.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
2304692-48-4 (free acid)
2304701-45-7 (sodium salt)

Trade Name
Dawnzera

Generic Name
Donidalorsen

Code Name
ISIS 721744

Chemical Name (IUPAC)

all-P-ambo-5'-O-[[[(6-{5-[(tris{3-[6-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)hexylamino]-3-oxopropoxymethyl})methyl]amino-5-oxopentanamido}hexyl)]phospho}-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioguanilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methylcytidilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenilyl-(3'-O→5'-O)-2'-deoxy-P-thioguanilyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-P-thioguanilyl-(3'-O→5'-O)-2'-deoxy-P-thioguanilyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenosine, icosasodium salt

Molecular Formula/Molecular Weight

C₂₉₆H₄₃₅N₈₃O₁₅₁P₂₀S₁₅ (free acid)

C₂₉₆H₄₁₅N₈₃O₁₅₁P₂₀S₁₅Na₂₀ (sodium salt)

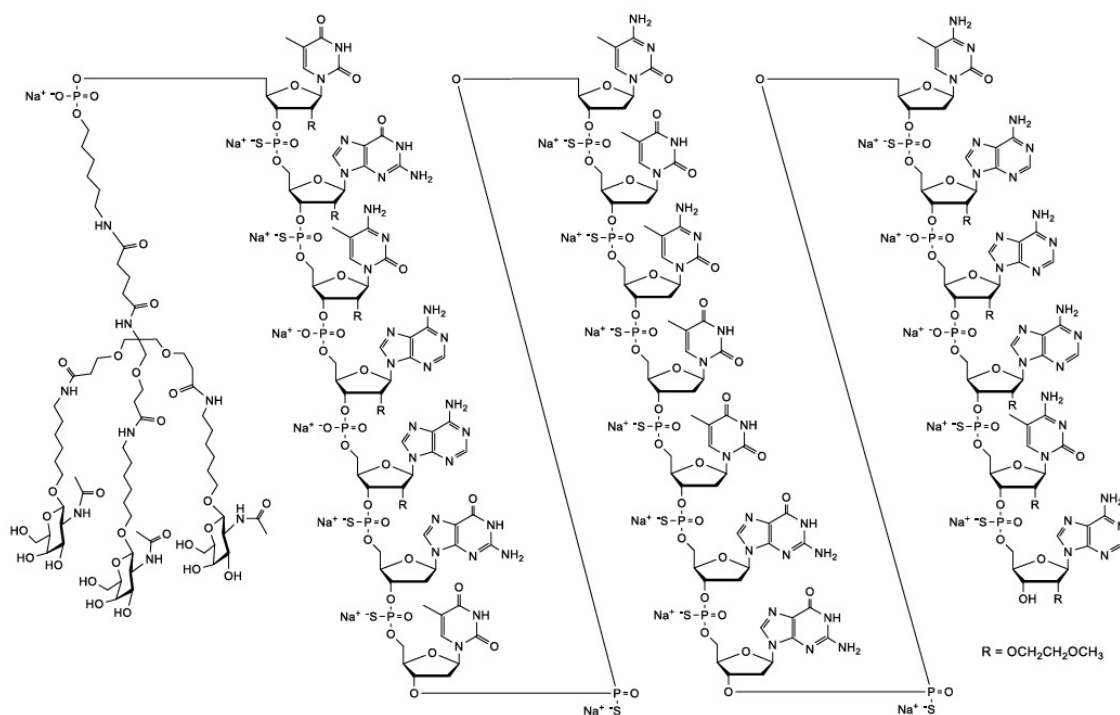
8672.66 Da (free acid)

9112.27 Da (sodium salt)

Structure or Biochemical Description

The structure of donidalorsen sodium is shown in **Figure 1** (Applicant's Figure).

ISIS 721744 is a 20-mer chimeric 2'-MOE-modified ASO with a mixed backbone made up of phosphorothioate (PS) and phosphodiester (PO) internucleotide linkages, which targets the mRNA of human PKK. The ASO is conjugated to triantennary GalNAc via a trishexylamino (THA)-C6 linker, which preferentially delivers ISIS 721744 to the liver by binding to the ASGPR on liver cells.

Figure 1 Structure of Donidalorsen Sodium (Applicant's Figure)

Pharmacologic Class
 PKK-targeted GalNAc-conjugated ASO
 PKK-directed ASO

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 142564 (active): Donidalorsen (ISIS 721744) by Ionis Pharmaceuticals Inc. for prophylaxis to prevent attacks of HAE in Center for Drug Evaluation and Research/Office of New Drugs/Office of Immunology and Inflammation/Division of Pulmonology, Allergy, and Critical Care (CDER/OND/OII/DPACC)

2.3 Drug Formulation

The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The qualitative and quantitative drug product formulation is shown in **Table 1** (Applicant's Table). The drug product is packaged in a staked needle glass prefilled syringe (PFS) closed with a (b) (4) rubber plunger stopper. The PFS is assembled into an autoinjector for the final drug product presentation. The autoinjector delivers a single dose of 80 mg donidalorsen in 0.8 mL (100 mg/mL).

A nonclinical safety evaluation of extractables and leachables of the container closure system for the drug product is provided in a separate review.

Refer to NDA 219407 Office of Pharmaceutical Quality Integrated Quality Assessment dated 05/05/2025 for more details on the drug formulation.

Table 1 Donidalorsen Drug Product Formulation (Applicant's Table)

Ingredients	Quality Standards ^j	Quantity per Autoinjector	Quantity per Unit Dose (0.8mL)	Pharmaceutical Function
Donidalorsen ^a	Professed ^b	(b) (4) mg (equivalent to (b) (4) mg as sodium salt)	80 mg (equivalent to 84 mg as sodium salt)	Active
Sodium dihydrogen phosphate (b) (4)	USP-NF	(b) (4)		
Disodium hydrogen phosphate (b) (4)	USP-NF			
Sodium chloride	USP-NF			
Hydrochloric acid ^f	USP-NF	QS ^{g, h}	QS ^h	pH adjustment
Sodium hydroxide ^f	USP-NF	QS ^h	QS ^h	pH adjustment
Water for Injection (WFI)	USP-NF	QS to (b) (4) mL ⁱ	QS to 0.8 mL	Vehicle

^a Donidalorsen drug substance is supplied as the sodium salt. The label claim is expressed as the free acid concentration

^b Per Section 3.2.S.4.1

(b) (4)

ⁱ Added (b) (4) for pH adjustment

^g QS: quantity sufficient

^h Ingredient may be added to adjust pH; therefore, the quantity is variable

ⁱ To ensure that no less than 0.8 mL is dosed upon injection, a (b) (4) mL fill is targeted

^j USP - United States Pharmacopoeia; NF - National Formulary

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product formulation. All the excipients are within levels found in other FDA-approved SC drug products.

2.5 Comments on Impurities/Degradants of Concern

In an internal email dated 03/12/2025, the Quality Reviewer for the drug substance (Dr. Brian Cawrse) solicited input from the Nonclinical Review Team on the impurity levels in the donidalorsen drug substance.

The Quality Reviewer summarized the following information:

The Applicant provided the structures, origins, and fates for the oligonucleotide-related impurities, including those derived from the starting materials and process-related impurities. For the purposes of reporting, identifying, and qualifying impurities, the Applicant grouped the impurities on the basis of structure and formation mechanism, which is typical for oligonucleotide products. The release specification limits total grouped impurities to between (b) (4)%, based on previous manufacturing experience and batch analysis. The Quality Reviewer considered the Applicant's approach consistent with other oligonucleotide products, but asked if there were any nonclinical concerns regarding the impurity levels. For internal descriptive purposes only, the Quality Reviewer referenced NDA 217779 (Rytelo [imetelstat] by Geron Corp.)

and NDA 217388 (Wainua [eplontersen] by AstraZeneca) for comparison to other approved ASOs.

Of note, the Quality Review did not identify any concerns with any of the impurities or their proposed limits. Further, there were no ICH Q3C Class 1 solvents used in the manufacture and there were no genotoxic or (b) (4) impurities in the drug substance. Refer to NDA 219407 Office of Pharmaceutical Quality Integrated Quality Assessment dated 05/05/2025 for more details.

The Applicant's proposed specification acceptance criteria for the donidalorsen sodium drug substance are shown in **Table 2** (Applicant's Table).

Table 2 Specification for Donidalorsen Sodium Drug Substance (Applicant's Table)

Test	Method	Acceptance Criterion
Appearance	Visual inspection	White to yellow solid free from visual evidence of contamination
Identification by Mass	IP-HPLC-UV-MS	(b) (4)
Identification, Sequence Confirmation	Duplex Melting Temperature (T_m)	
Identity and Quantity of Counterion	ICP-OES	
Assay	IP-HPLC-UV-MS	
Purity	IP-HPLC-UV-MS	
Specified Oligonucleotide Impurities	IP-HPLC-UV-MS	
(b) (4)		
Unspecified Oligonucleotide Impurities	IP-HPLC-UV-MS	
Total Oligonucleotide Degradation Products ^a	IP-HPLC-UV-MS	
Total Oligonucleotide Impurities ^b	IP-HPLC-UV-MS	

Test	Method	Acceptance Criterion
Residual Solvents (b) (4)	Gas Chromatography	(b) (4)
Bacterial Endotoxins	USP <85> Ph. Eur. 2.6.14 JP 4.01	
Microbial Examination of Nonsterile Products Total Aerobic Microbial Count Total Yeast and Mold Count	USP <61> Ph. Eur. 2.6.12 JP 4.05 I	
Water Content	Karl-Fischer	

Abbreviations:

ICP-OES = Inductively coupled plasma optical emission spectroscopy; IP-HPLC-UV-MS = Ion-pair high performance liquid chromatography with ultraviolet and mass spectrometry detection; NLT = Not less than; NMT = Not more than

^a Defined (b) (4)

When observed, unspecified degradation products are also added to the total.

^b Defined as the sum of Specified and Unspecified Oligonucleotide Impurities.

Nonclinical Integrated Summary and Safety Evaluation on Impurities

As communicated to the Applicant in the IND 142564 Study May Proceed letter dated 09/30/2019 (**Section 2.7**): One approach for the determination of appropriate impurity specifications could involve comparisons of impurity levels in toxicology and clinical batches of the drug. If impurity levels at the no observed adverse effect level (NOAEL) of the 39-week monkey study (or 13-week interim sacrifice) provide adequate safety margins over impurity levels at the maximum proposed clinical dose, this could assist in determination of appropriate specifications.

The safety margins for the donidalorsen specified oligonucleotide impurities based on comparing the proposed acceptance criterion at the proposed clinical dose to the impurity levels in the toxicology lot in the 39-week monkey study are shown in **Table 3** or **Table 4** based on body weight and body surface area, respectively.

Table 3 Safety Margins for Donidalorsen Specified Oligonucleotide Impurities Based on 39-Week Monkey Study using Body Weight (mg/kg)

	Drug Substance Acceptance Criterion - Clinical	Maximum Accepted Impurity in 80 mg Q4W (2.7 mg/kg Q4W) DAWNZERA ¹	Impurity Levels in Toxicology Lot # CA721744-001 ²	Amount of Toxicology Lot Impurity at 6 mg/kg/week NOAEL from 39-Week Monkey Study	Safety Margin ³
(b) (4)					1.2x
					4.8x
					1.3x
					5.1x
					<0.1x
					<0.6x
					0.7x
					2.7x
					0.5x
					2.1x
					0.4x
					1.8x
					1.1x
					4.3x
1.6x					
6.5x					
0.6x					
2.3x					
0.9x					
3.6x					
0.6x					
2.5x					

Abbreviations: (b) (4) ND = not determined; NMT = no more than; NOAEL = no observed adverse effect level; (b) (4) donidalorsen sodium; (b) (4) donidalorsen sodium; Q4W = once every four weeks; Q8W = once every eight weeks

Notes:

¹The recommended dosage of DAWNZERA (donidalorsen) is SC administration of 80 mg Q4W or Q8W in adult and pediatric patients 12 years of age and older. For calculation purposes, the maximum clinical dose of 2.7 mg/kg Q4W is determined by dividing 80 mg by the average body weight of 29.5 kg for the adolescent patient population.

²ISIS 721744 Toxicology Lot # CA721744-001 was used in the 39-week monkey study.

³In the 39-week monkey study, the monkeys were dosed more frequently (i.e., loading doses on Days 1, 5, 9, and 14 and once weekly thereafter) than is proposed in the clinical population (i.e., Q4W or Q8W). The top safety margin does not account for the difference in dosing frequency between the toxicity study and the proposed clinical population while the second safety margin accounts for the difference (about 4x).

⁴Per the Applicant, the limit for Total(n+1) is at the proposed qualification threshold of 1.5% so no toxicology assessment is required.

Table 4 Safety Margins for Donidalorsen Specified Oligonucleotide Impurities Based on 39-Week Monkey Study using Body Surface Area (mg/m²)

	Drug Substance Acceptance Criterion - Clinical	Maximum Accepted Impurity in 80 mg Q4W (2.7 mg/kg Q4W) DAWNZERA ¹	Impurity Levels in Toxicology Lot # CA721744-001 ²	Amount of Toxicology Lot Impurity at 1.94 mg/kg/week HED NOAEL from 39-Week Monkey Study ³	Safety Margin ⁴
(b) (4)					0.4x
					1.6x
					0.4x
					1.6x
					<0.05x
					<0.2x
					0.2x
					0.9x
					0.2x
					0.7x
					0.1x
					0.6x
					0.3x
					1.4x
0.5x					
2.1x					
0.2x					
0.7x					
0.3x					
1.1x					
0.2x					
0.8x					

Abbreviations: (b) (4); HED = human equivalent dose; ND = not determined; NMT = no more than; NOAEL = no observed adverse effect level; (b) (4) (b) (4) donidalorsen sodium; (b) (4) donidalorsen sodium; Q4W = once every four weeks; Q8W = once every eight weeks

Notes:

¹The recommended dosage of DAWNZERA (donidalorsen) is SC administration of 80 mg Q4W or Q8W in adult and pediatric patients 12 years of age and older. For calculation purposes, the maximum clinical dose of 2.7 mg/kg Q4W is determined by dividing 80 mg by the average body weight of 29.5 kg for the adolescent patient population.

²ISIS 721744 Toxicology Lot # CA721744-001 was used in the 39-week monkey study.

³The NOAEL of 6 mg/kg/week from the 39-week monkey study was divided by a scaling factor of 3.1 to convert based on body surface area (mg/m²) to a HED of 1.94 mg/kg/week.

⁴In the 39-week monkey study, the monkeys were dosed more frequently (i.e., loading doses on Days 1, 5, 9, and 14 and once weekly thereafter) than is proposed in the clinical population (i.e., Q4W or Q8W). The top safety margin does not account for the difference in dosing frequency between the toxicity study and the proposed clinical population while the second safety margin accounts for the difference (about 4x).

⁵Per the Applicant, the limit for Total(n+1) is at the proposed qualification threshold of 1.5% so no toxicology assessment is required.

Based on Capaldi et al 2017,¹ one approach for evaluating toxicology coverage for impurities is to multiply the amount of impurity present in the drug substance by the NOAEL and divide the product by the clinical dose (i.e., 2.7 mg/kg Q4W [80 mg Q4W considering an average patient weight of 29.5 kg for the adolescent patient population]). Refer to **Table 5** (Applicant’s Table) for toxicology coverage based on this approach.

Per communication with the Quality Reviewer, the Capaldi et al 2017 reference is well known for oligonucleotide impurities and commonly referenced. The approach for calculating toxicology coverage is standard and does not raise any concerns from the quality perspective.

Table 5 Impurity Qualification based on 39-Week Repeat-Dose Toxicity Study of ISIS 721744 with a 13-Week Interim Necropsy in Monkeys (Applicant’s Table)

Oligonucleotide Impurity	721744-AS02	
	Amount in CA721744-001 (%)	Coverage (% of clinical dose) ^a
		(b) (4)

^a Coverage = Impurity level × NOAEL determined in tox study (24 mg/kg/month) / clinical dose (80 mg/month = 2.7 mg/kg/month); calculated values are rounded to the nearest 0.1%

Taken together, the proposed impurity specification levels for donidalorsen at the proposed clinical dose are considered acceptable based on the impurity levels in the toxicology lot at the NOAEL from the 39-week monkey study.

In addition to relying on the 39-week monkey study to qualify the oligonucleotide impurities, the Applicant conducted a 13-week repeat-dose SC toxicity and impurity qualification study in which ICR mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection/solution [saline]) or (b) (4) mg/kg total oligonucleotide dose level of ISIS 721744, TAM 1, and TAM 2 Q2W for 13-weeks (Refer

¹Capaldi D, Teasdale A, Henry S, Akhtar N, den Besten C, Gao-Sheridan S, Kretschmer M, Sharpe N, Andrews B, Burm B, Foy J. Impurities in Oligonucleotide Drug Substances and Drug Products. Nucleic Acid Ther. 2017 Dec;27(6):309-322.

to **Section 6.2**). The two TAMs that were evaluated in the study were made up of ISIS 721744 and associated impurities (i.e., (b) (4) impurities for TAM 1 and (b) (4) impurity for TAM 2). The findings in TAM 1- and TAM 2-treated groups were similar to those in ISIS 721744-treated groups. Overall, there were no meaningful differences between the findings in either of the TAM groups (ISIS 721744 + associated impurities) compared to the ISIS 721744 group alone and it did not appear that the added impurities affected the toxicity profile of ISIS 721744. In this case, based on the concepts presented in Capaldi et al 2017, the toxicology findings observed are likely due to ISIS 721744 rather than the added impurities and, therefore, the amounts of impurities are qualified.

Table 6 (Applicant’s Table) shows the impurity qualification based on the 13-week repeat-dose toxicity study of ISIS 721744 and associated TAMs in mice.

Table 6 Impurity Qualification based on 13-Week Repeat-Dose Toxicity Study of ISIS 721744 and Associated TAMs in Mice (Applicant’s Table)

Impurity	Composition (%) ^a	
	Test Article 1	Test Article 2
(b) (4)		

Considering the impurity qualification based on the 39-week toxicity study in monkeys and the 13-week repeat-dose toxicity study and associated TAMs in mice, the proposed impurity specifications are acceptable and there are no outstanding nonclinical safety concerns.

Refer to Office of Pharmaceutical Quality NDA 219407 Integrated Quality Assessment dated 05/05/2025 for more details on impurities/degradants.

2.6 Proposed Clinical Population and Dosing Regimen

Donidalorsen is indicated for prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. The recommended dosage is 80 mg administered SC (b) (4). A dosing interval of (b) (4) may be considered in some patients.

2.7 Regulatory Background

Provided below is regulatory background for the donidalorsen development program as it relates to impurities.

The following comment was provided in the IND 142564 Study May Proceed letter dated 09/30/2019:

“Provide structures of any impurities and degradants of the drug substance and drug product in your IND submission. Monitor impurities and degradation products of all active ingredients and refer to ICH Guidance Q3A(R) and Q3B(R) for possible qualification requirements. The specific impurity thresholds described in these guidances do not apply to oligonucleotide-related impurities; however, the general principles of these guidances do apply. It is expected that you would identify and measure impurities as well as determine appropriate impurity specifications as IND development proceeds. One approach for the determination of appropriate impurity specifications could involve comparisons of impurity levels in toxicology and clinical batches of the drug. If impurity levels at the no observed adverse effect level (NOAEL) of the 39-week monkey study (or 13-week interim sacrifice) provide adequate safety margins over impurity levels at the maximum proposed clinical dose, this could assist in determination of appropriate specifications.”

3 Studies Submitted

3.1 Studies Reviewed

A 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice (Study No. G122105; Applicant Reference No. 721744-AS11); GLP-compliant

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 142564	Eleni Salicru, PhD	08/13/2019	Nonclinical primary review of primary pharmacology, safety pharmacology (in vivo cardiovascular, respiratory, and central nervous systems in monkey), PK/ADME, general toxicology (39-week in monkeys), and genetic toxicology (in vitro Ames assay and in vitro chromosome aberration assay)
NDA 219407	Eleni Salicru, PhD	12/19/2024	Nonclinical primary review of genetic toxicology (mammalian micronucleus assay in mice) and

Application	Reviewer	Date in DARRTS	Notes
			carcinogenicity (6-months in Tg.rasH2 mice)
NDA 219407	Eleni Salicru, PhD	03/05/2025	Nonclinical primary review of reproductive and developmental toxicology in mice
NDA 219407	Eleni Salicru, PhD	03/25/2025	Nonclinical primary review of pharmacology, safety pharmacology (in vitro hERG assay), PK/ADME, and general toxicology (26-week in rats)

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: A 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice

Study no.: G122105 (721744-AS11)
 Study report location: Electronic Document Room (EDR)
 Supporting Document Number (SDN) 1
 Conducting laboratory and location: ^{(b) (4)}
 Date of study initiation: 01/02/2023
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: ISIS 721744, bulk test article lot # CA721744-003, 92.6% purity

TAM 1 and TAM 2 are made up of ISIS 721744 and associated impurities (i.e., ^{(b) (4)} impurities for TAM 1 and ^{(b) (4)} impurity for TAM 2).

TAM 1 (ISIS 721744 ^{(b) (4)} impurities), bulk test article lot # ARM721744-12, 97.8% UV purity, 94.3% full length n ISIS 721744

The Certificate of Analysis indicates that the following impurities are incorporated in the product oligonucleotide, producing ^{(b) (4)} impurities that are indistinguishable from the parent and that they were synthesized with ^{(b) (4)}  with the following impurities by mole:

^{(b) (4)}

TAM 2 (ISIS 721744 ^{(b) (4)} impurity), bulk test article lot # ARM721744-13,

(b) (4)

Key Study Findings

- In a 13-week repeat-dose toxicity impurity qualification study, ICR mice were treated by SC administration with vehicle control (0.9% sodium chloride for injection/solution [saline]) or (b) (4) mg/kg total oligonucleotide dose level of ISIS 721744, TAM 1 ((b) (4) impurities), and TAM 2 ((b) (4) impurity) Q2W for 13-weeks.
- There were no unscheduled deaths during the study.
- Red blood cell values were minimally decreased only in females at (b) (4) mg/kg/dose TAM 2.
- BUN values were decreased in males and females at (b) (4) mg/kg/dose TAM 2; AST values were increased only in males at (b) (4) mg/kg/dose ISIS 721744, TAM 1, and TAM 2; ALT values were increased only in males at (b) (4) mg/kg/dose ISIS 721744, TAM 1, and TAM 2; and triglycerides were decreased in females at (b) (4) mg/kg/dose ISIS 721744 and males at (b) (4) mg/kg/dose TAM 2.
- Liver weights (absolute and/or relative to body weight) were increased only in males at (b) (4) mg/kg/dose ISIS 721744 and TAM 1, and (b) (4) mg/kg/dose TAM 2.
- Histopathology findings attributed to treatment with ISIS 721744, TAM 1, and/or TAM 2 were noted in the liver and injection site.
 - Liver: Minimal to slight mononuclear cell infiltration in males and/or females at (b) (4) mg/kg/dose ISIS 721744, TAM 1, and/or TAM 2. Minimal karyocytomegaly in males only at (b) (4) mg/kg/dose ISIS 721744, TAM 1, and TAM 2, which correlated with minimally increased ALT and AST and increased liver weight in males. Liver karyocytomegaly was attributed to uptake and accumulation of ASOs into the liver.
 - Injection Site: Minimal to slight vacuolated granular macrophages in males and females at (b) (4) mg/kg/dose ISIS 721744, (b) (4) mg/kg/dose TAM 1, and (b) (4) mg/kg/dose TAM 2. Injection site findings were attributed to uptake and accumulation of ASOs into the macrophages.
- Mean concentrations of unconjugated ISIS 721744 in the mouse liver on Day 87 were comparable for ISIS 721744-, TAM 1-, and TAM 2-treated groups.
- **Conclusion: The effects observed in TAM 1- and TAM 2-treated groups were similar to effects observed in ISIS 721744-treated groups. Overall, it did not appear that the added impurities affected the toxicity profile of ISIS 721744.**

Methods

Doses: ISIS 721744: (b) (4) mg/kg total oligonucleotide dose level

TAM 1: (b) (4) mg/kg total oligonucleotide dose level

TAM 2: (b) (4) mg/kg total oligonucleotide dose level

As stated in the study report: Dose levels represent total oligonucleotide concentration which is inclusive of ISIS 721744 and ASO-related impurities. A (b) (4) mg/kg total oligonucleotide dose yields approximately 4 and 12 mg/kg ISIS 721744, respectively, which has been administered to mice in previous repeat-dose toxicity studies.

Frequency of dosing: Q2W for 13 weeks (Days 1, 15, 29, 43, 57, 71, and 85; total of seven doses)

Route of administration: SC

Dose volume: 10 mL/kg

Formulation/Vehicle: 0.9% sodium chloride for injection/solution (saline)

Species/Strain: Mouse/CD-1

Number/Sex/Group: 10

Age: 7 to 8 weeks (age range on study)

Weight: 32.6 to 40.5 g for males and 25.2 to 32.5 g for females at the start of dosing.

Satellite groups: No

Unique study design: This study was designed as an impurity qualification study to evaluate the potential toxicity of the ISIS 721744-associated TAM 1 ((b) (4) impurities) or TAM 2 ((b) (4) impurity) as compared to ISIS 721744 alone in CD-1 mice after 13 weeks of SC administration.

Deviation from study protocol: None of the protocol deviations listed in the study report were deemed to have impacted the integrity or conclusions of the study.

Observations and Results

Mortality

Checks for mortality and morbidity were conducted twice per day (AM and PM) during the treatment period.

There were no unscheduled deaths during the study.

Clinical Signs

Clinical sign observations were made once per day during the pretreatment period, twice per day during the treatment period, and once prior to necropsy. Detailed clinical sign observations were made at least once per week during the treatment period. Injection site observations were made prior to dosing on each dosing day.

There were no clinical sign or injection site findings during the study that were attributed to treatment with ISIS 721744, TAM 1, or TAM 2.

Body Weights

Body weights were recorded at least once per week during the treatment period and once prior to necropsy.

There were no body weight changes during the study that were attributed to treatment with ISIS 721744, TAM 1, or TAM 2.

Feed Consumption

Food consumption was measured at least once per week during the treatment period.

There were no feed consumption changes during the study that were attributed to treatment with ISIS 721744, TAM 1, or TAM 2.

Ophthalmoscopy

Ophthalmic examinations using binocular indirect ophthalmoscope after treatment with mydriatic agent were conducted once during the pretreatment period and once during Week 13 of the treatment period.

There were no ophthalmic findings during the study that were attributed to treatment with ISIS 721744, TAM 1, or TAM 2.

ECG

Not evaluated

Hematology

Blood samples were collected from the last 5 animals/sex/group for hematology evaluations prior to terminal sacrifice in Week 13 (Day 87). A representative panel of hematology parameters was evaluated.

There were no changes in hematology parameters during the study that were attributed to treatment with ISIS 721744 or TAM 1. Red blood cell values were minimally decreased only in females at (b) (4) mg/kg/dose TAM 2 (-7% difference from vehicle control) (**Table 7**).

Table 7 Notable Hematology Findings for 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice

	Males (Day 87)							Females (Day 87)						
	Veh	ISIS 721744 (mg/kg/dose)		TAM 1 (mg/kg/dose)		TAM 2 (mg/kg/dose)		Veh	ISIS 721744 (mg/kg/dose)		TAM 1 (mg/kg/dose)		TAM 2 (mg/kg/dose)	
	0	(b) (4)												
RBC x10 ⁶ /mL	9.71	9.99	9.95	9.89	9.83	9.66	9.66	9.59	9.17	9.40	9.62	9.41	9.33	8.92*
% Diff	-	2.9	2.5	1.9	1.2	-0.5	-0.5	-	-4.4	-2.0	0.3	-1.9	-2.7	-7.0
tCtrl	-	1.03	1.02	1.02	1.01	0.99	0.99	-	0.96	0.98	1.00	0.98	0.97	0.93

Abbreviations: % Diff = percent difference from vehicle control; RBC = red blood cells; TAM = test article mixture; tCtrl = fold change from vehicle control; Veh = vehicle control

Notes:

*Applicant's statistical analyses Dunnett LSD Test Significant at the 0.05 level

Clinical Chemistry

Blood samples were collected from the first 5 animals/sex/group for clinical chemistry evaluations prior to terminal sacrifice in Week 13 (Day 87). A representative panel of clinical chemistry parameters was evaluated.

Clinical chemistry changes attributed to treatment with ISIS 721744, TAM 1, and/or TAM 2 were seen in values for BUN, AST, ALT, and/or triglycerides (**Table 8**).

BUN values were decreased at (b) (4) mg/kg/dose TAM 2 in males (up to -29% difference from vehicle control) and females (up to -38% difference from vehicle control).

AST values were increased only in males at (b) (4) mg/kg/dose ISIS 721744 (1.47x of vehicle control), (b) (4) mg/kg/dose TAM 1 (1.84x of vehicle control), and (b) (4) mg/kg/dose TAM 2 (1.27x of vehicle control).

ALT values were increased only in males at (b) (4) mg/kg/dose ISIS 721744 (2.48x of vehicle control), (b) (4) mg/kg/dose TAM 1 (2.44x of vehicle control), and (b) (4) mg/kg/dose TAM 2 (2.43x of vehicle control).

Triglyceride values were decreased in females at (b) (4) mg/kg/dose ISIS 721744 (up to -47% difference from vehicle control) and males at (b) (4) mg/kg/dose TAM 2 (-53% difference from vehicle control).

Table 8 Notable Clinical Chemistry Findings for 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice

	Males (Day 87)							Females (Day 87)						
	Veh	ISIS 721744 (mg/kg/dose)	TAM 1 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	Veh	ISIS 721744 (mg/kg/dose)	TAM 1 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)
	0	(b) (4)												
BUN mg/dL	28.7	26.1	27.3	26.1	23.0	20.5 *	20.8 *	28.3	26.2	25.8	23.7	24.3	17.6 **	21.7 **
% Diff	-	-9.1	-4.9	-9.1	-	-	-	-	-7.4	-8.8	-	-	-	-
tCtrl	-	0.91	0.95	0.91	0.80	0.71	0.72	-	0.93	0.91	0.84	0.86	0.62	0.77
AST IU/L	59.7	59.4	87.8	54.7	109.9	49.8	75.9 *	60.7	62.4	66.6	71.4	68.7	57.0	62.8
% Diff	-	-0.5	47.1	-8.4	84.1	-	27.1	-	2.8	9.7	17.6	13.2	-6.1	3.5
tCtrl	-	0.99	1.47	0.92	1.84	0.83	1.27	-	1.03	1.10	1.18	1.13	0.94	1.03
ALT IU/L	41.3	46.2	102.5 *	44.3	100.9 **	43.4	100.2 **	37.8	45.6	37.3	49.2	40.7	33.4	42.7
% Diff	-	11.9	148.2	7.3	144.3	5.1	142.6	-	20.6	-1.3	30.2	7.7	-	13.0
tCtrl	-	1.12	2.48	1.07	2.44	1.05	2.43	-	1.21	0.99	1.30	1.08	0.88	1.13
TRIG mg/dL	121.8	113.2	102.5	88.9	94.9	98.7	57.6 *	112.9	59.7 *	61.2 *	77.9	74.3	65.3	100.6
% Diff	-	-7.1	-	-	-	-	-	-	-	-	-	-	-	-
tCtrl	-	0.93	0.84	0.73	0.78	0.81	0.47	-	0.53	0.54	0.69	0.66	0.58	0.89

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; % Diff = percent difference from vehicle control; TAM = test article mixture; tCtrl = fold change from vehicle control; TRIG = triglycerides; Veh = vehicle control

Notes:

*Applicant's statistical analyses Dunnett LSD Test Significant at the 0.05 level or Dunn Rank Sum Test Significant at the 0.05 level

** Applicant's statistical analyses Dunnett LSD Test Significant at the 0.01 level or Dunn Rank Sum Test Significant at the 0.01 level

Urinalysis

Not evaluated

Gross Pathology

Terminal sacrifice was performed on Day 87 (about 48 hours after the last dose on Day 85). Complete necropsy examinations were performed.

There were no gross pathology findings during the study that were attributed to treatment with ISIS 721744, TAM 1, or TAM 2.

Organ Weights

The following organs were weighed at the time of scheduled necropsy: adrenal glands, brain, heart, kidneys, liver with gallbladder, lung (with bronchi), ovaries (with oviduct), pituitary gland, prostate, seminal vesicle (with coagulating gland), spleen, salivary glands (sublingual and submandibular), testes, thymus, thyroids (with parathyroids), and uterus (with cervix). Paired organs were weighed together.

Liver weights (absolute and/or relative to body weight) were increased only in males at (b) (4) mg/kg/dose ISIS 721744 (up to 1.13x of vehicle control), (b) (4) mg/kg/dose TAM 1 (1.11x of vehicle control), and (b) (4) mg/kg/dose TAM 2 (1.09x of vehicle control) (**Table 9**). The increase in liver weight at (b) (4) mg/kg/dose ISIS 721744 correlated with the histopathology finding of karyocytomegaly in the liver of males at (b) (4) mg/kg/dose.

Table 9 Notable Organ Weight Findings for 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice

	Males (Day 87)							Females (Day 87)						
	Veh	ISIS 721744 (mg/kg/dose)		TAM 1 (mg/kg/dose)		TAM 2 (mg/kg/dose)			ISIS 721744 (mg/kg/dose)		TAM 1 (mg/kg/dose)		TAM 2 (mg/kg/dose)	
	0	(b) (4)												
Liver with Gall bladder Weight (g)	2.30	2.56 *	2.59 *	2.52	2.44	2.39	2.51	1.80	1.93	1.81	1.88	1.84	1.74	1.83
% Diff	-	11.6	12.8	9.8	6.4	4.2	9.3	-	7.2	0.7	4.7	2.0	-3.2	1.8
tCtrl	-	1.12	1.13	1.10	1.06	1.04	1.09	-	1.07	1.01	1.05	1.02	0.97	1.02
Liver with Gall bladder (% BW)	5.14	5.67 *	5.69 *	5.71 **	5.62 **	5.40	5.59 **	5.16	5.20	5.27	5.38	5.33	5.13	5.39
% Diff	-	10.5	10.7	11.3	9.4	5.1	8.8	-	0.8	2.2	4.4	3.3	-0.5	4.6
tCtrl	-	1.10	1.11	1.11	1.09	1.05	1.09	-	1.01	1.02	1.04	1.03	0.99	1.05
Liver with Gall bladder (% BrW)	477	511	501	496	490	485	516	364	378	361	377	359	353	369
% Diff	-	7.2	5.1	4.0	2.9	1.7	8.3	-	3.9	-0.8	3.5	-1.4	-2.9	1.4

	Males (Day 87)							Females (Day 87)						
	Veh	ISIS 721744 (mg/kg/dose)		TAM 1 (mg/kg/dose)		TAM 2 (mg/kg/dose)			ISIS 721744 (mg/kg/dose)		TAM 1 (mg/kg/dose)		TAM 2 (mg/kg/dose)	
	0	(b) (4)												
tCtrl	-	1.07	1.05	1.04	1.03	1.02	1.08	-	1.04	0.99	1.03	0.99	0.97	1.01

Abbreviations: BW = body weight; BrW = brain weight; g = grams; %Diff = percent difference from vehicle control; TAM = test article mixture; tCtrl = fold change from vehicle control; Veh = vehicle control

Notes:

*Applicant's statistical analyses Dunnett LSD Test Significant at the 0.05 level or Dunn Rank Sum Test Significant at the 0.05 level

** Applicant's statistical analyses Dunnett LSD Test Significant at the 0.01 level or Dunn Rank Sum Test Significant at the 0.01 level

Histopathology

Adequate Battery

Yes. Refer to **Table 10** (Applicant's Table).

Table 10 Tissue List for 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice (Applicant's Table)

Tissue Preserved	Organ Weight	Collect & Preserved	Microscopic Examination
Abnormal lesion		√	√
Adrenal glands	√	√	√
Animal ID ^a		√	
Aorta (thoracic)		√	√
Bone, Femur (including the tibiofemoral joint)		√	√
Bone marrow (femur)		√	√
Bone marrow (sternum)		√	√
Bone, Sternum		√	√
Bone marrow smear ^b		√	
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	√	√	√
Cecum		√	√
Colon		√	√
Duodenum		√	√
Epididymides		√	√
Esophagus		√	√
Eyes		√	√
Gall bladder ^c		√	√
GALT (Peyer's patch) ^d		√	√
Harderian gland		√	√

Heart	√	√	√
Ileum		√	√
Inguinal lymph node		√	√
Injection sites		√	√
Jejunum		√	√
Kidneys	√	√	√
Lacrimal gland (exorbital)		√	√
Larynx		√	√
Liver ^c	√	√	√
Lung (with bronchi)	√	√	√
Mammary gland (female only)		√	√
Mandibular lymph node		√	√
Mesenteric lymph node		√	√
Optic nerve (attached to the eye) ^e		√	√
Ovaries (with oviduct)	√	√	√
Pancreas		√	√
Parotid salivary gland		√	√
Pituitary gland	√	√	√
Prostate	√	√	√
Rectum		√	√
Sciatic nerve		√	√
Seminal vesicle (with coagulation gland)	√	√	√
Skeletal muscle (biceps femoris)		√	√
Skin (inguinal)		√	√
Spinal cord (cervical, thoracic, lumbar)		√	√
Spleen	√	√	√
Stomach (glandular, non-glandular)		√	√
Sublingual salivary gland ^f	√	√	√
Submandibular salivary gland ^f	√	√	√
Testes	√	√	√
Thymus	√	√	√
Thyroids (with parathyroids) ^e	√	√	√
Tongue		√	√
Trachea		√	√
Ureters		√	√
Urinary bladder		√	√

Uterus (with cervix)	√	√	√
Vagina		√	√

^s Collected but not prepared for histopathology.

^b Bone marrow smears were prepared from animals' femur only at scheduled necropsy. Evaluation was not performed.

^c Liver and gallbladder were weighed together.

^d Gut Associated Lymphoid Tissue.

^e Measured or examined only if present in the routine section.

^f Sublingual and submandibular salivary glands were weighed together.

Peer Review

Yes. A peer review was conducted by an expert Peer Review Pathologist (facility not in compliance with current regulatory GLP requirements). As stated in the study report, the Peer Review Pathologists followed a robust scientific methodology, ensured that slides were stored properly, and that records related to the chain of custody of the slides.

Histological Findings

Histopathology findings attributed to treatment with ISIS 721744, TAM 1, and/or TAM 2 were noted in the liver and injection site (**Table 11**).

Liver

Minimal to slight mononuclear cell infiltration was seen in males and/or females at (b) (4) mg/kg/dose ISIS 721744, TAM 1, and/or TAM 2.

Minimal karyocytomegaly was seen in males only at (b) (4) mg/kg/dose ISIS 721744, TAM 1, and TAM 2. Hepatocellular karyocytomegaly correlated with minimally increased ALT and AST and increased liver weight in males. This finding was attributed to uptake and accumulation of ASOs into the liver.

Reviewer's Note: Karyocytomegaly reflects hepatocyte polyploidy that occurs when there is duplication of nuclear material in the absence of cytokinesis. Changes can consist of increased numbers of diploid nuclei per hepatocyte or an increase in the ploidy level of a single hepatocyte nucleus. Spontaneous occurrence of karyocytomegaly is commonly seen in aging rodents and is not typically associated with increased liver weight. This aging change, often referred to as polyploidy, is more common in mice than in rats (NTP).²

Injection Site

Minimal to slight vacuolated granular macrophages were seen in males and females at (b) (4) mg/kg/dose ISIS 721744, (b) (4) mg/kg/dose TAM 1, and (b) (4) mg/kg/dose TAM 2. This finding was attributed to uptake and accumulation of ASOs into the macrophages.

²National Toxicology Program. US Department of Health and Human Services. Nonneoplastic Lesion Atlas. <https://ntp.niehs.nih.gov/atlas/nnl/hepatobiliary-system/liver/Hepatocyte-Karyomegaly>

Table 11 Histopathology Findings for 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice

	Males (Day 87)							Females (Day 87)						
	V	ISIS 721744 (mg/kg/dose)	TAM 1 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 1 (mg/kg/dose)	TAM 2 (mg/kg/dose)	V	ISIS 721744 (mg/kg/dose)	TAM 1 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 2 (mg/kg/dose)	TAM 1 (mg/kg/dose)	TAM 2 (mg/kg/dose)
	0	(b) (4)												
Liver n =	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Infiltration, mononuclear cell	2	3	4	4	2	3	2	1	7	5	2	5	3	6
...minimal	2	2	3	4	2	2	2	1	5	4	1	4	3	5
...slight	0	1	1	0	0	1	0	0	2	1	1	1	0	1
Karyocytomegaly	0	0	3	0	3	0	3	0	0	0	0	0	0	0
...minimal	0	0	3	0	3	0	3	0	0	0	0	0	0	0
Injection Site(s) n =	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Vacuolated/granular macrophage	0	0	2	2	6	5	5	0	0	6	4	4	1	1
...minimal	0	0	2	1	5	5	5	0	0	6	4	4	1	1
...slight	0	0	0	1	1	0	0	0	0	0	0	0	0	0

Abbreviations: n = number examined; TAM = test article mixture; V = vehicle control

Special Evaluation

None

Toxicokinetics

Liver and kidney samples were collected from the first 6 animals/sex/group on Day 87 (48 hours after the last dose on Day 85).

Liver samples were analyzed for concentrations of unconjugated ISIS 721744 by UHPLC-MS/MS method.

Mean concentrations of unconjugated ISIS 721744 in the mouse liver on Day 87 were comparable for ISIS 721744-, TAM 1-, and TAM 2-treated groups (**Table 12**, Applicant's Table).

Table 12 Mean Concentration of Unconjugated ISIS 721744 in Liver for 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice (Applicant's Table)

Group	Treatment	Dose Level	Tissue	Necropsy Day	N	Gender	Mean ± SD (µg/g)
G2	ISIS 721744	(b) (4) ng/kg	Liver	87	6	M	21.4 ± 3.4
						F	17.6 ± 8.3
G3	ISIS 721744	(b) (4) mg/kg	Liver	87	6	M	38.2 ± 7.3
						F	40.2 ± 4.8
G4	TAM #1	(b) (4) ng/kg	Liver	87	6	M	19.3 ± 3.5
						F	14.4 ± 3.1
G5	TAM #1	(b) (4) mg/kg	Liver	87	6	M	43.3 ± 8.2
						F	31.2 ± 9.1
G6	TAM #2	(b) (4) ng/kg	Liver	87	6	M	20.8 ± 3.3
						F	18.4 ± 3.0
G7	TAM #2	(b) (4) mg/kg	Liver	87	6	M	46.1 ± 4.6
						F	31.0 ± 5.3

Dosing Solution Analysis

The dose formulations for ISIS 721744, TAM 1, and TAM 2 were provided ready-to-use to the conducting laboratory by the Applicant. The Applicant verified concentrations of ISIS 721744, TAM 1, and TAM 2 using a qualified UV spectroscopy or IP-HPLC-UV-MS procedure. The Applicant verified stability of TAM 1 and TAM 2 using a qualified IP-HPLC-UV-MS procedure.

The measured concentrations of the dosing solutions met the acceptance criterion of (b) (4) of the label claim. There were no significant changes in the impurity profiles of the test articles based on stability testing.

11 Integrated Summary and Safety Evaluation

Refer to **Section 2.5**.

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/s/

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 219407
Supporting document/s: 1
Applicant's letter date: 08/21/2024
CDER stamp date: 08/21/2024
Product: Donidalorsen (ISIS 721744)
Pharmacologic Class: Prekallikrein (PKK)-targeted *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO)
Indication: Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older
Applicant: Ionis Pharmaceuticals Inc.
Clinical Division: Division of Pulmonology, Allergy, and Critical Care (DPACC)
PharmTox Division: Division of Pharmacology Toxicology for Immunology and Inflammation (DPT-II)
Reviewer: Eleni Salicru, PhD
Supervisor/Team Leader: Timothy Robison, PhD, DABT
Acting Clinical Division Director: Banu Karimi Shah, MD
Project Manager: Cindy Chee, PharmD

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

Ionis Pharmaceuticals Inc. (Applicant) submitted a 505(b)(1) New Drug Application (NDA) for subcutaneous (SC) administration of donidalorsen (ISIS 721744) as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older. ISIS 721744 is an *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO), which binds to human prekallikrein (PKK) messenger RNA (mRNA) to reduce PKK protein production. The GalNAc molecule preferentially delivers ISIS 721744 to the liver by binding to the asialoglycoprotein receptor (ASGPR) on liver cells.

This review focuses on the nonclinical pharmacology, safety pharmacology, pharmacokinetics/absorption, distribution, metabolism, and excretion (PK/ADME), and general toxicology studies for the ISIS 721744 nonclinical program that were not previously reviewed. Refer to the IND 142564 Nonclinical Primary Review dated 08/13/2019 for the primary pharmacology, safety pharmacology, PK/ADME, and general toxicology studies that were previously reviewed.

Refer to the NDA 219407 Nonclinical Primary Review dated 12/19/2024 for a review of the 6-month carcinogenicity study of ISIS 721744, as well as the mouse surrogate, ISIS 722059, by SC injection in CByB6F1- Tg(HRAS)2Jic Hemizygous Transgenic mice (Tg.rasH2 mice) and to NDA 219407 Nonclinical Primary Review dated 03/05/2025 for a review of the reproductive and developmental toxicology studies with ISIS 721744 (i.e., combined fertility and developmental toxicity study in mice and pre- and postnatal development [PPND] study in mice).

Together, these studies entail the nonclinical studies to support the safety of donidalorsen for marketing approval.

1.2 Brief Discussion of Nonclinical Findings

Primary Pharmacology

Donidalorsen is a GalNAc-conjugated ASO that binds to PKK mRNA in the liver which causes ribonuclease H1 (RNase H1)-mediated degradation of PKK mRNA to reduce production of PKK protein by the liver. 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.

As described by the Applicant: Over 1300 ASOs were designed to target various sites within the human PKK gene and were evaluated for their ability to inhibit the level of PKK mRNA in vitro in the human hepatocellular carcinoma cell line HepaRG. Of those

ASOs, ISIS 546254, an unconjugated 2'-O-methoxyethyl (2'-MOE) gapmer ASO with a uniform phosphorothioate (PS) backbone, was identified as a lead candidate. Donidalorsen (ISIS 721744) was derived from ISIS 546254 as a 2'-MOE gapmer ASO with the same nucleobase sequence as ISIS 546254 but with a mixed PS and phosphodiester (PO) backbone and conjugated to a trivalent GalNAc₃.

Specificity analysis of ISIS 721744 to PKK mRNA was performed with the unconjugated PKK ASO, ISIS 546254. ISIS 546254 is perfectly matched to only PKK mRNA in human transcriptome (targets site free of meaningful sequence variation). Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In an in vitro study, ISIS 721744 and unconjugated PKK ASO, ISIS 546254, induced a dose-dependent reduction of PKK mRNA in human hepatocytes with supporting stromal with IC₅₀ values of 0.19 mcM vs. 8.73 mcM, respectively. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In an in vitro study, unconjugated PKK ASO, ISIS 546254, reduced human PKK mRNA levels by greater than 90% in a concentration-dependent manner in human hepatocellular cell lines HepaRG and Hep3b with corresponding IC₅₀ of 0.31 mcM and 0.35 mcM, respectively.

In an in vivo study in mice, SC administration of unconjugated PKK ASO mouse surrogate, ISIS 482584, for 3 weeks caused a dose-dependent reduction of PKK liver mRNA and PKK plasma protein. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In an in vivo study in mice, SC administration of unconjugated PKK ASO mouse surrogate, ISIS 482584, for 3 weeks caused inhibition of basal vascular permeability, captopril-induced vascular permeability, and C1 INH ASO-induced vascular permeability in the colon of mice. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In an in vivo study in mice, SC administration of unconjugated PKK ASO mouse surrogate ISIS 482584 for 3 weeks reduced basal activated high molecular weight kininogen cleavage in blood of male mice. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In an in vivo study in human KLKB1/PKK transgenic mice, single SC administration of ISIS 721744 on Day 0 resulted in a dose- and time-dependent reduction of plasma PKK protein that approached 91% at the highest dose tested (10 mg/kg) on Day 14 after treatment. PKK levels were still 20% below baseline levels by Day 56 (last time point evaluated) at 10 mg/kg.

Safety Pharmacology

The safety pharmacology evaluations for the cardiovascular, respiratory, and central nervous system (CNS) were combined into one study in Cynomolgus monkeys. There were no test article-related effects on cardiovascular, respiratory, or CNS parameters in

male Cynomolgus monkeys evaluated for up to 24 hours after SC administration of 12 and 30 mg/kg ISIS 721744. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In the in vitro human ether-a-go-go-related gene (hERG) assay, ISIS 721744 did not inhibit hERG potassium channel current in human embryonic kidney 293 (HEK-293) mammalian cells stably transfected with hERG complementary DNA (cDNA) compared to the vehicle control. In general, oligonucleotides are not thought to interact with the hERG channel.

PK/ADME

In an in vitro evaluation of plasma protein binding of ISIS 721744 (0.5, 5, and 150 mcg/mL) in human, monkey, and mouse plasma, ISIS 721744 was highly bound ($\geq 96.81\%$) to whole plasma proteins in all three species. The extent of plasma protein binding of ISIS 721744 was similar across all species. Refer to IND 142564 Nonclinical Review dated 08/13/2019.

The in vitro mean blood to plasma ratio of ISIS 721744 was between 0.630 and 0.824 in human, 0.468 and 0.566 in monkey, and 0.501 and 0.661 in rat suggesting that ISIS 721744 distributes mostly in the plasma compartment across the concentrations tested in all three species.

Based on metabolic profiling and identification of ISIS 721744 oligonucleotide-related metabolites in urine from mice, monkeys, and humans, the Applicant described that the primary route of elimination of donidalorsen is initial rapid hydrolysis of the GalNAc conjugate in tissues (presumed to be primarily in the liver based on directed distribution to the liver), followed by slow nuclease-mediated metabolism of the unconjugated oligonucleotide, and subsequent excretion of chain-shortened metabolites, as well as full-length oligonucleotides, albeit minor in urine and feces. There were no unique metabolites identified in humans compared to animal species.

Based on metabolic profiling, identification, and quantitation of ISIS 721744 linker-related metabolites in plasma, urine, and/or fecal samples from mice, monkeys, and/or humans, the Applicant described that the GalNAc moiety is hydrolyzed intracellularly following uptake into tissues, and the trishexylamino (THA) linker undergoes extensive oxidative metabolism, and is nearly completely eliminated via biliary excretion, and to a lesser extent, renal excretion, within 24 hours after administration.

Toxicology

In the pivotal Good Laboratory Practice (GLP)-compliant chronic toxicity study in Cynomolgus monkeys (pharmacologically relevant species), monkeys received SC doses of ISIS 721744 at 0, 2, 6, 12, or 30 mg/kg/dose. The monkeys were dosed for 39-weeks (dosing on Days 1, 5, 9, and 14 [loading doses] and once weekly thereafter) with a 13-week interim sacrifice (monkeys at 30 mg/kg/dose were dosed only until the interim sacrifice). A 26-week treatment-free recovery period was included after both the 13-week interim dosing interval (30 mg/kg/dose) and the 39-week dosing interval (0, 2,

6, 12 mg/kg/dose) to assess potential reversibility of any observed effects. Refer to Nonclinical Primary Review dated 08/13/2019 for details. In brief, the main test article-related findings included thrombocytopenia in two animals (12 and 30 mg/kg/dose) and histopathology findings of basophilic granules in various organs/tissues (including kidneys, liver, lymph nodes, and injection site) that in the absence of correlating histopathology were considered consistent with oligonucleotide uptake and not considered adverse. There were dose-dependent decreases in hepatic PKK mRNA expression and dose-dependent and time-dependent decreases in PKK plasma protein after treatment with ISIS 721744 at ≥ 2 mg/kg/dose at the interim and/or terminal sacrifices. Both hepatic PKK mRNA expression and plasma protein increased by the end of the recovery periods. Tissue concentrations of unconjugated ISIS 721744 in liver or kidney increased in a dose-dependent manner. The no observed adverse effect level (NOAEL) was 6 mg/kg/week based on thrombocytopenia in two monkeys at the higher dose levels of 12 and 30 mg/kg/dose. The NOAEL corresponded to a C_{max} of 6.57 mcg/mL and an AUC_{0-48hr} of 37.6 mcg*hr/mL on Day 273.

In the pivotal chronic toxicity study in mice (not a pharmacologically relevant species), mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (1, 4, or 12 mg/kg/dose), or mouse surrogate, ISIS 722059, (4 mg/kg/dose) once every two weeks (Q2W) for 26 weeks with a 13-week treatment-free recovery period (vehicle control and 12 mg/kg/dose ISIS 721744 dose groups only). This study, as designed, could assess potential off-target effects of ISIS 721744 and potential on-target effects using the mouse surrogate, ISIS 722059. Changes in clinical chemistry parameters at the end of the 26-week treatment period included increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and/or alkaline phosphatase (ALP) in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744. Effects on clinical chemistry parameters (including ALT, AST, and ALP) were less clear at 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period, increases in ALT and/or AST were still seen in males and/or females at 12 mg/kg/dose ISIS 721744, while increases in ALP were no longer seen. The toxicological significance of increases in ALT and AST were unclear as histopathology findings in the liver appeared to be partially reversible and levels of unconjugated ISIS 721744 had declined below the lower limit of quantitation. Histopathology findings after the 26-week treatment period with ISIS 721744 were seen in the liver, skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen. Histopathology findings after the 26-week treatment with mouse surrogate ISIS 722059 were seen in the skin/subcutis, SC dose sites, spleen, and testis.

- Liver: Minimal or slight centrilobular hepatocellular hypertrophy was noted in males at 1, 4, and 12 mg/kg/dose ISIS 721744 and in one female (moderate) at 12 mg/kg/dose ISIS 721744. This finding correlated with increased mean liver weights at 4 and 12 mg/kg/dose ISIS 721744 and increased liver enzymes (AST, ALT, and/or ALP). Despite a background incidence of hepatocyte vacuolation in vehicle control males, there was an increase in the severity (slight to marked) of hepatocyte vacuolation in males at 4 and 12 mg/kg/dose ISIS 721744, which correlated with gross pathology findings of liver discoloration at all dose levels. These changes were attributed to uptake of ISIS 721744 into the liver.

- Skin/Subcutis: Increased incidences and/or severity of infiltrates of mononuclear cells, mixed cells, and/or vacuolated macrophages in the skin/subcutis were noted in males and/or females at 4 and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. Increased incidences and/or severity of vacuolated fibrocytes in the skin/subcutis were noted in males and/or females at 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059. Slight to moderate inflammation in the skin was noted in females at 4 and 12 mg/kg/dose ISIS 721744. These findings in the skin/subcutis correlated with increased leukocyte counts.
- SC dose sites: Increased incidences and/or severity of infiltrates of vacuolated macrophages, mononuclear cells, and mixed cells, as well as fibrocyte vacuolation at the SC injection sites were noted in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. These findings at the SC injection sites correlated with increased leukocyte counts.
- Inguinal Lymph Nodes: Increased incidences and severities (minimal to marked) of increased lymphocytes in males at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and one female at 12 mg/kg/dose ISIS 721744 were noted in the inguinal lymph node. This finding in the inguinal lymph node correlated with increased leukocyte counts. Amyloid in the inguinal lymph node was noted in one male at 4 mg/kg/dose ISIS 722059.
- Spleen: Increased incidence and/or severity of extramedullary hematopoiesis in the spleen was noted in males and/or females at 1, 4 and/or 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059 and correlated with increased mean spleen weight in males administered 12 mg/kg/dose ISIS 721744.
- Testis: Moderate interstitial cell hyperplasia, minimal sperm stasis, and minimal vacuolation were noted in the testis of males at 4 mg/kg/dose ISIS 722059.

Histopathology findings after the 13-week recovery period at 12 mg/kg/dose ISIS 721744 were still noted in the liver as hepatocellular vacuolation in males and females, which correlated with increased liver weights. Histopathology findings in the skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen at 12 mg/kg/dose ISIS 721744 were not seen at the end of the recovery period.

Unconjugated ISIS 721744 was measurable in a dose-dependent manner in the kidney (greater than dose proportional) and liver (dose proportional) after 26 weeks of treatment with ISIS 721744. The mean concentrations of unconjugated ISIS 721744 were similar in the kidney and liver except at 12 mg/kg/dose where mean unconjugated ISIS 721744 concentrations were greater in the kidney than in the liver. Mean unconjugated ISIS 721744 concentrations were higher in females than in males in the kidney but similar in the liver. After the 13-week treatment-free recovery period at 12 mg/kg/dose ISIS 721744, mean unconjugated ISIS 721744 concentrations were below or near the lower limit of quantitation of 0.107 mcg/g, suggesting complete clearance of ISIS 721744.

Mouse PKK mRNA in mouse liver was reduced by 77% (statistically significant) compared to vehicle control in mice treated with mouse surrogate ISIS 722059 at 4 mg/kg/dose for 26 weeks.

The NOAEL was 12 mg/kg/dose ISIS 721744, as test article-related findings were considered related to the oligonucleotide uptake.

Conclusions

The nonclinical pharmacology and toxicology studies were adequate to support the safety of donidalorsen for marketing. There are no outstanding concerns from the nonclinical perspective.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, NDA 219407 is recommended for approval. There are no outstanding nonclinical issues.

1.3.2 Additional Nonclinical Recommendations

As described in the NDA 219407 Nonclinical Primary Review dated 12/19/2024, the Applicant should submit the final study report for the 2-year carcinogenicity study in rats as a post marketing requirement (PMR) (Refer to IND 142564 pre-NDA Meeting Preliminary Comments dated 04/10/2024).

1.3.3 Labeling

Nonclinical sections of the product label are evaluated in a separate review.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

2304692-48-4 (free acid)

2304701-45-7 (sodium salt)

Trade Name

Dawnzera

Generic Name

Donidalorsen

Code Name

ISIS 721744

Chemical Name (IUPAC)

all-P-ambo-5'-O-[[[6-{5-[(tris{3-[6-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)hexylamino]-3-oxopropoxymethyl})methyl]amino-5-

oxopentanamido}hexyl)]phospho}-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioguanlyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methylcytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-deoxy-P-thioguanlyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-P-thioguanlyl-(3'-O→5'-O)-2'-deoxy-P-thioguanlyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenosine, icosasodium salt

Molecular Formula/Molecular Weight

$C_{296}H_{435}N_{83}O_{151}P_{20}S_{15}$ (free acid)

$C_{296}H_{415}N_{83}O_{151}P_{20}S_{15}Na_{20}$ (sodium salt)

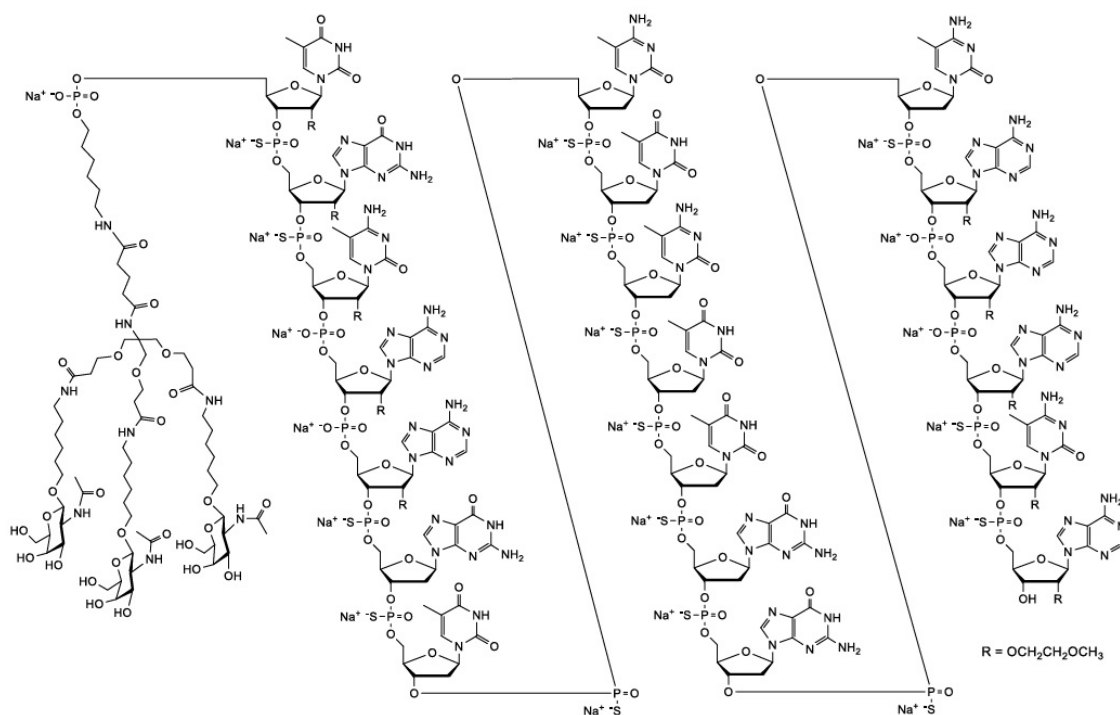
8672.66 Da (free acid)

9112.27 Da (sodium salt)

Structure or Biochemical Description

The structure of donidalorsen sodium is shown in **Figure 1** (Applicant's Figure).

ISIS 721744 is a 20-mer chimeric 2'-MOE-modified ASO with a mixed backbone made up of PS and PO internucleotide linkages, which targets the mRNA of human PKK. The ASO is conjugated to triantennary GalNAc via a THA-C6 linker, which preferentially delivers ISIS 721744 to the liver by binding to the ASGPR on liver cells.

Figure 1 Structure of Donidalorsen Sodium (Applicant's Figure)

Pharmacologic Class
 PKK-targeted GalNAc-conjugated ASO

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 142564 (active): Donidalorsen (ISIS 721744) by Ionis Pharmaceuticals Inc. for prophylaxis to prevent attacks of HAE in Center for Drug Evaluation and Research/Office of New Drugs/Office of Immunology and Inflammation/Division of Pulmonology, Allergy, and Critical Care (CDER/OND/OII/DPACC)

2.3 Drug Formulation

The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The qualitative and quantitative drug product formulation is shown in **Table 1** (Applicant's Table). The drug product is packaged in a staked needle glass prefilled syringe (PFS) closed with a (b) (4) rubber plunger stopper. The PFS is assembled into an autoinjector for the final drug product presentation. The autoinjector delivers a single dose of 80 mg donidalorsen.

Table 1 Donidalorsen Drug Product Formulation (Applicant's Table)

Ingredients	Quality Standards ^j	Quantity per Autoinjector	Quantity per Unit Dose (0.8mL)	Pharmaceutical Function
Donidalorsen ^a	Professed ^b	(b) (4) mg (equivalent to (b) (4) mg as sodium salt)	80 mg (equivalent to 84 mg as sodium salt)	Active
Sodium dihydrogen phosphate (b) (4)	USP-NF	(b) (4)		
Disodium hydrogen phosphate (b) (4)	USP-NF			
Sodium chloride	USP-NF			
Hydrochloric acid ^f	USP-NF	QS ^{g, h}	QS ^h	pH adjustment
Sodium hydroxide ^f	USP-NF	QS ^h	QS ^h	pH adjustment
Water for Injection (WFI)	USP-NF	QS to (b) (4) mL ⁱ	QS to 0.8 mL	Vehicle

^a Donidalorsen drug substance is supplied as the sodium salt. The label claim is expressed as the free acid concentration

^b Per [Section 3.2.S.4.1](#)

(b) (4)

ⁱ Added (b) (4) for pH adjustment

^g QS: quantity sufficient

^h Ingredient may be added to adjust pH; therefore, the quantity is variable

ⁱ To ensure that no less than 0.8 mL is dosed upon injection, a (b) (4) mL fill is targeted

^j USP - United States Pharmacopoeia; NF- National Formulary

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product formulation. All the excipients are within levels found in other FDA-approved SC drug products.

2.5 Comments on Impurities/Degradants of Concern

Nonclinical comments on impurities/degradants of concern are provided in a separate review.

A nonclinical safety evaluation of extractables and leachables of the container closure system for the drug product is provided in a separate review.

2.6 Proposed Clinical Population and Dosing Regimen

Donidalorsen is indicated for prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. The recommended dosage is 80 mg administered SC (b) (4). A dosing interval of (b) (4) may be considered in some patients.

2.7 Regulatory Background

Provided below is a summary of the regulatory background for the donidalorsen development program.

There was no pre-IND under the associated IND 142564. New IND 142564 was received on 07/15/2019 for a phase 2a clinical study (Protocol No. ISIS 721744-CS2) to assess the clinical efficacy of 80 mg ISIS 721744 (once monthly for 12-weeks) in patients with HAE. The clinical study was allowed to proceed based on the 30-day safety review and subsequently nonhold comments were provided in the Study May Proceed letter dated 09/30/2019.

A Type B End of Phase 2 (EOP2) meeting teleconference meeting between the Sponsor and the FDA occurred on 08/30/2021 to discuss the adequacy of the existing and proposed nonclinical and clinical development package to support a marketing application for ISIS 721744 for prophylaxis to prevent attacks of HAE in patients 12 years of age and older. The Nonclinical Team indicated that the nonclinical evaluation of the metabolism of ISIS 721744 and its THA linker was limited. As such, the Sponsor was asked to provide data that characterizes the metabolism of ISIS 721744 and its THA linker through literature (Sponsor's work and/or non-proprietary literature) and actual metabolism studies in monkey and mouse (as needed) prior to the start of phase 3 clinical studies to demonstrate reasonable comparability to human metabolism. In addition, the FDA agreed that an embryofetal development (EFD) study of ISIS 721744 in rabbits was not needed. Refer to IND 142564 EOP2 Meeting Minutes dated 10/18/2021 for added details. Also, refer to NDA 219407 Nonclinical Primary Review dated 03/05/2025 for added details on the regulatory history related to the reproductive and developmental toxicology program.

The initial Pediatric Study Plan (iPSP) for ISIS 721744 for HAE was submitted on 11/18/2021, with subsequent revisions submitted on 05/03/2022, and the agreed iPSP submitted on 06/06/2022. As part of the iPSP, the Sponsor indicated that they planned to conduct a 13-week repeat-dose toxicity study of ISIS 721744 in juvenile CD-1 mice with a 6-week recovery period to support the inclusion of patients with HAE between 2 to <12 years of age. FDA communicated that a draft unaudited report of the juvenile toxicity study in mice should be available prior to the start of the pediatric clinical trial and a final report would be expected within 120 days after providing the draft unaudited report. As such, the Sponsor submitted the draft protocol for the juvenile toxicity study in mice on 07/11/2023 (see IND 142564 Nonclinical Primary Review dated 08/28/2023). The FDA communicated comments on the draft protocol to the Sponsor on 08/31/2023.

A Special Protocol Assessment (SPA) 1 for the 2-year study in rats was submitted on 08/05/2022 and denied on 08/17/2022 due to the lack of SEND datasets for the supporting 16-week dose range finding study in rats. SPA 1 was resubmitted on 09/30/2022 with the SEND datasets. FDA agreed with the proposed 2-year carcinogenicity study in rats on 11/04/2022. SPA 2, which proposed a 6-month carcinogenicity study in Tg.rasH2 mice, was submitted on 10/12/2022, and was agreed

upon on 11/16/2022 for male mice but not for female mice (as no acceptable basis for dose selection could be identified).

In pre-NDA meeting preliminary comments (Refer to IND 142564 pre-NDA Meeting Preliminary Comments dated 04/10/2024), the FDA agreed that it would be acceptable to submit the report of the 6-month carcinogenicity study in Tg.rasH2 mice and a summary of the in-life data for the 2-year carcinogenicity study in rats at the time of the NDA submission and to submit the final report as a PMR.

Refer to the NDA 219407 Nonclinical Primary Review dated 12/19/2024 for added details on the regulatory history related to the genetic toxicology and carcinogenicity programs.

NDA 219407 for donidalorsen was submitted on 08/21/2024, and the application was filed on 10/20/2024, for standard review, with a user fee goal date of 08/21/2025.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Title	Study Number	GLP	Reviewed
Evaluation of the Effect of Prekallikrein ASO ISIS 482584 on the Basal Vascular Permeability in Mice	721744-1887	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019
Evaluation of the Effect of Prekallikrein ASO ISIS 482584 on the Captopril Induced Vascular Permeability in Mice	721744-1888	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019
Evaluation of the Dose-Responsive Effects of Prekallikrein ASO ISIS 482584 on the Captopril-Induced Vascular Permeability in Mice	721744-2064	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019
Evaluation of the Dose-Responsive Effects of Prekallikrein ASO ISIS 482584 on the Basal Vascular Permeability in Mice	721744-3223	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019

Title	Study Number	GLP	Reviewed
In Silico and In Vitro Characterization of Human Prekallikrein (PKK) ASO ISIS 721744 Specificity	ECID129370	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019
In vitro Characterization of Human Prekallikrein Antisense Oligonucleotide ISIS 721744 Activity	ECID129371	No	Current review
In Vivo Evaluation of ISIS 721744 Activity in Human KLKBI/PKK Transgenic Mice – Analysis of Plasma PKK Protein Levels	ELN 23-100d	No	Current review
Evaluation of the Effect of Prekallikrein ASO ISIS 482584 on the C1 Inhibitor ASO-Induced Vascular Permeability in Mice	EXP-19-AE2418	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019
Characterization of ISIS 721744 Pharmacology in Primary Human Hepatocytes Co-Cultured with Mouse Fibroblasts (HepatoPac®)	GEN2.5-RS83 (PART F)	No	Summary in IND 142564 Nonclinical Review dated 08/13/2019

Safety Pharmacology

Title	Study Number	GLP	Reviewed
Evaluation of Cardiovascular, Respiratory, and Central Nervous Systems Following Subcutaneous Administration of ISIS 721744 in Cynomolgus Monkeys	11752; 721744-AS03	Yes	IND 142564 Nonclinical Review dated 08/13/2019
Effect of ISIS 721744 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	210830.JTJ; 721744-IS08	Yes	Current review

PK/ADME

Title	Study Number	GLP	Reviewed
Absorption, Distribution, Metabolism and Excretion of [3H]ISIS 721744 following a Single Subcutaneous Dose Administration to Sprague Dawley Rats	IPI/06; 721744-APK02	Yes	Current review Absorption
Metabolite Profiling and Identification Studies following a Single Subcutaneous Dose Administration of [3H]ISIS 721744 to Male and Female Rats	IPI/07; 721744-APK02	No	Current review Absorption
A 6-Week Subcutaneous Repeat-Dose Pharmacokinetic Study of ISIS 721744 in CD-1 Mice	G122111; 721744-APK03	Yes	Current review Absorption
In Vitro Evaluation of the Extent of Plasma Protein Binding of ISIS 721744 in Human, Monkey, and Mouse Plasma by Ultrafiltration	IIS-M4-298; 721744-IS03	No	IND 142564 Nonclinical Review dated 08/13/2019 Distribution
In Vitro Evaluation of Whole Blood and Plasma Concentration Ratio of ISIS 721744 (Donidalorsen) in Human, Monkey and Rat	IIS-P2-008; 721744-IS11	No	Current review Distribution
Method Development, Qualification and Sample Analysis of a Bioanalytical Method for the Determination of M8 in Liver and Kidney Samples Generated from Rat using LC-MS/MS	IPI/08; 721744-IS17	No	Current review Distribution
Metabolic Profiling and Identification of ISIS 721744 Oligonucleotide-Related Metabolites by HPLC-HRMS in Select Urine Samples from Mice, Monkeys, and Humans	727-085, 727-081, ISIS 721744-CS1; 721744-IS10	No	Current review Metabolism

Title	Study Number	GLP	Reviewed
Profiling, Identification, and Quantitation of ISIS 721744 Linker-Related Metabolites by HPLC-MS/MS in Select Plasma, Urine, and Fecal Samples from Mice, Monkeys, and Humans	721744-IS12	No	Current review Metabolism

Repeat-Dose Toxicity

Title	Study Number	GLP	Reviewed
A 39-Week Repeat Dose Toxicity Study of ISIS 721744 in Cynomolgus Monkeys with a 13-Week Interim Necropsy and 26-Week Recovery	727-081; 721744-AS02	Yes	IND 142564 Nonclinical Review dated 08/13/2019
A 26-Week Repeat Dose Toxicity Study of ISIS 721744 in Mice with a 13-Week Recovery	8405032; 721744-AS04	Yes	Current Review

Genotoxicity

Title	Study Number	GLP	Reviewed
ISIS 721744: Bacterial Reverse Mutation Assay	G115138; 721744-IS01	Yes	IND 142564 Nonclinical Review dated 08/13/2019
ISIS 721744: In Vitro Chromosome Aberration Assay in Chinese Hamster Lung (CHL) Cells	G115139; 721744-IS02	Yes	IND 142564 Nonclinical Review dated 08/13/2019
Mammalian Micronucleus Assay in Bone Marrow Erythrocytes Following Subcutaneous Injection of ISIS 721744 to ICR Mice	G120005; 721744-AS05	Yes	NDA 219407 Nonclinical Review dated 12/19/2024

Carcinogenicity

Title	Study Number	GLP	Reviewed
A 6-Month Study of ISIS 721744 by Subcutaneous Bolus Injection in CByB6F1-Tg(HRAS) ^{2Jic} Hemizygous Transgenic Mouse	727-140; 721744-AS07	Yes	NDA 219407 Nonclinical Review dated 12/19/2024

Title	Study Number	GLP	Reviewed
A 14-Week Repeat Dose Toxicity Study of ISIS 721744 and ISIS 722059 IN CBYB6F1-TG(HRAS)2JIC Hemizygous Transgenic Mice	721744-AS07P	No	IND 142564 Nonclinical Review dated 12/12/2022
A 16-Week Repeat-Dose Toxicity Study of ISIS 721744 in Sprague Dawley Rats	721744-AS08P	No	IND 142564 Nonclinical Review dated 12/08/2022

Reproductive and Developmental Toxicity

Title	Study Number	GLP	Reviewed
A Combined Fertility and Developmental Toxicity Study of ISIS 721744 in Mice via Subcutaneous Administration	727-134; 721744-AS06	Yes	NDA 219407 Nonclinical Review dated 03/05/2025
A Combined Fertility and Developmental Toxicity Study of ISIS 546254 and ISIS 482584 Administered by Subcutaneous Injection in CD-1 Mice	(b) (4) 090.60; 546254-AS04	Yes	Summary in NDA 219407 Nonclinical Review dated 03/05/2025
An Assessment of Effects of ISIS 546254 on Embryo-Fetal Development in New Zealand White Rabbits	(b) (4) 090.61; 546254-AS05	Yes	Summary in NDA 219407 Nonclinical Review dated 03/05/2025
Study for Toxic Effects of ISIS 721744 on Pre- and Postnatal Development, Including Maternal Function in Mice	727-142; 721744-AS09	Yes	NDA 219407 Nonclinical Review dated 03/05/2025

Other Toxicity

Title	Study Number	GLP	Reviewed
A 13-Week Repeat Dose Subcutaneous Toxicity and Impurity Qualification Study of ISIS 721744 and associated TAMs in ICR Mice	G122105; 721744-AS11	Yes	Separate NDA 219407 review

3.2 Studies Not Reviewed

Analytical Methods and Validation Reports

Title	Study Number	GLP
Validation of a UHPLC/MS/MS Method for the Quantitation of Unconjugated ISIS 721744 (ISIS 780124) in Mouse Tissue	(b) (4) -192083; 721744-MV01	Yes
Validation of a Hybridization ECL Method for the Quantitation of ISIS 721744 or Total Full-Length ASO in Cynomolgus Monkey Plasma	(b) (4) -192045; PBA-192045; 721744-MV02	Yes
Partial Validation of a UHPLC/MS/MS Method for the Quantitation of Unconjugated ISIS 721744 (ISIS 780124) in Monkey Tissue	(b) (4) -192085; 721744-MV03	Yes
Validation of an ELISA Method for the Detection of Anti-ISIS 721744 Antibodies in Cynomolgus Monkey Plasma	ICDIM 361.1; RORL2; 721744-MV07	No
Validation of a Hybridization ECL Method for the Quantitation of ISIS 721744 in CD-1 Mouse Plasma Containing Dipotassium EDTA	ICD 672.2; QAAM2; ISIS 721744-MV08	No

Pharmacokinetic Drug Interactions

(Defer to Clinical Pharmacology Review Team)

Title	Study Number	GLP
In Vitro Evaluation of Human Plasma Protein Binding of ISIS 721744 and its Interaction with Warfarin and Ibuprofen by Ultrafiltration	IIS-M4-453; 721744-IS05	No
In Vitro Assessment of Cytochrome P450 Induction Potential of ISIS 721744 in Plated Cryopreserved Human Primary Hepatocytes	2109081-03; 721744-IS06	No
Assessment of ISIS 721744 as an inhibitor or a substrate of human OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, BCRP, P-gp, and BSEP mediated transport	OPT-2021-146; 721744-IS07	No
Cell Uptake and In Vitro Evaluation of ISIS 721744 as an Inhibitor of Cytochrome P450 Expression in Human Hepatocytes	2109082-03; 721744-IS09	No

Title	Study Number	GLP
In Vitro Cytochrome P450 Induction Study on the M8 Linker Metabolite Using Human Liver Hepatocytes	PF23M-0038; 721744-IS13	No
In Vitro Cytochrome P450 Inhibition Study on the M8 Linker Metabolite Using Human Liver Microsome	PF23M-0039; 721744-IS14	No
Assessment of M8 as an inhibitor or a substrate of human OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, BCRP, P-gp, and BSEP mediated transport	OPT-2023-121, OPT-2024-002; 721744-IS15	No
In Vitro Cytochrome P450 Phenotyping Study on M8 Linker Metabolite Using Human Liver Microsomes and Recombinant CYP Enzymes	PF23M-0040; 721744-IS16	No

Repeat-Dose Toxicity

Title	Study Number	GLP
A 13-Week Repeat Dose Toxicity Study of ISIS 721744 in Mice with a 13-Week Recovery Period	727-085; 721744-AS01	Yes

3.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 142564	Eleni Salicru, PhD	08/13/2019	Nonclinical primary review of primary pharmacology, safety pharmacology (in vivo cardiovascular, respiratory, and central nervous systems in monkey), PK/ADME, general toxicology (39-week toxicity study in monkeys), and genetic toxicology (in vitro Ames assay and in vitro chromosome aberration assay)
IND 142564	Eleni Salicru, PhD	12/08/2022	Nonclinical SPA review of 16-week dose range finding study in rats to provide the dose selection for the 2-year carcinogenicity study in rats

Application	Reviewer	Date in DARRTS	Notes
IND 142564	Eleni Salicru, PhD	12/12/2022	Nonclinical SPA review of 14-week dose range finding study in Tg.rasH2 mice to provide the dose selection for the 6-month carcinogenicity study in Tg.rasH2 mice
NDA 219407	Eleni Salicru, PhD	12/19/2024	Nonclinical primary review of mammalian micronucleus assay in mice and 6-month carcinogenicity study in Tg.rasH2 mice
NDA 219407	Eleni Salicru, PhD	03/05/2025	Nonclinical primary review of reproductive and developmental toxicology studies

4 Pharmacology

4.1 Primary Pharmacology

As described in **Section 3.1**, most of the primary pharmacology studies for the nonclinical development of ISIS 721744 were previously reviewed (refer to the IND 142564 Nonclinical Review dated 08/13/2019). Included below is a review of two primary pharmacology studies not previously reviewed.

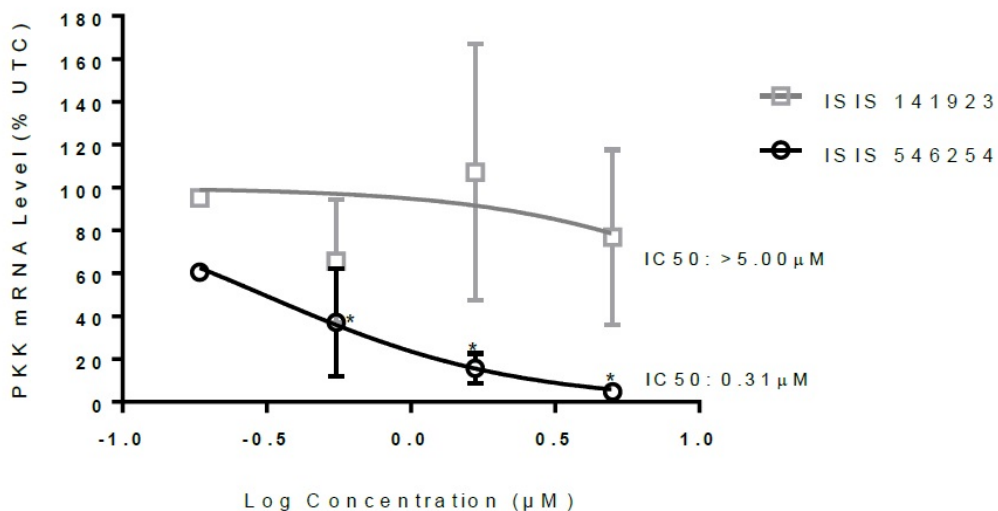
In Vitro Characterization of Human Prekallikrein Antisense Oligonucleotide ISIS 721744 Activity (Study No. ECID129371)

Two human hepatocellular carcinoma cell lines (HepaRG and Hep3b) were electroporated with ISIS 546254 (unconjugated parent compound of ISIS 721744 with the same sequence) or ISIS 141923 (control oligonucleotide with no matches to any sites within the human transcriptome) and incubated for 24 hours under the experimental conditions of the study, lysed for RNA isolation, and analyzed for human PKK mRNA. As described in the study report, the sequence of ISIS 546254 was identified from screens and dose-response confirmations of over 1300 unconjugated ASOs that were designed throughout the human PKK primary transcript and then determined to be a lead compound for its ability to inhibit the level of PKK mRNA at a single concentration in the human hepatocellular carcinoma cell line, HepaRG.

As shown in **Figure 2** (Applicant's Figure), ISIS 546254 reduced human PKK mRNA levels by greater than 90% in a concentration-dependent manner in human hepatocellular cell lines HepaRG and Hep3b with corresponding IC_{50} of 0.31 mcM and 0.35 mcM, respectively. The control oligonucleotide ISIS 141923 did not reduce PKK mRNA in either cell line.

Figure 2 Concentration-Dependent Reduction of Human PKK mRNA in Human Hepatocellular Carcinoma Cells by ISIS 546254 (Applicant's Figure)

A.



B.

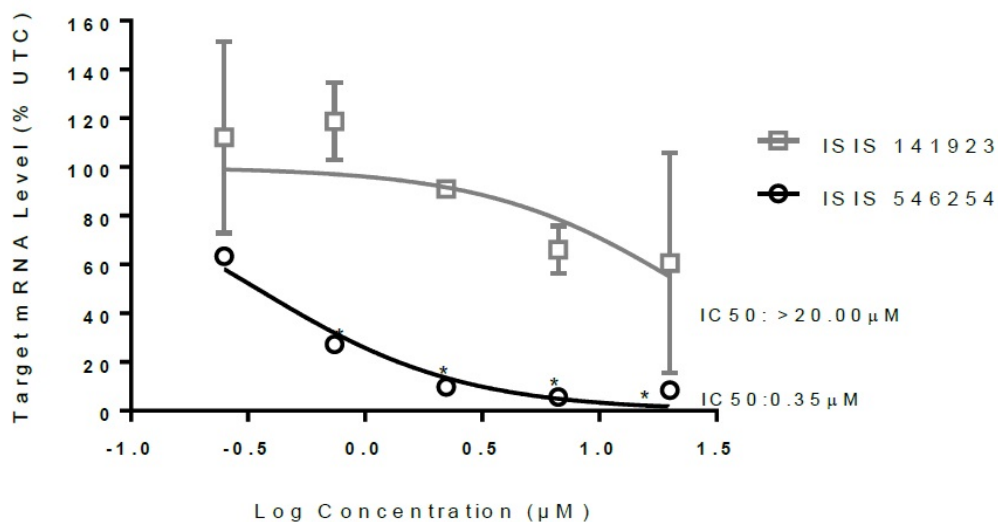


Figure 2: Concentration-Dependent Reduction of Human PKK mRNA in Human Hepatocellular Carcinoma Cells upon ISIS 546254 Treatment

ISIS 546254 was evaluated for its ability to reduce human PKK mRNA in 2 different human hepatocellular carcinoma cell lines (A) HepaRG and (B) Hep3B. Compounds were transfected into the cells with electroporation (t = 24 hours). Results represent the mean $\pm$ standard deviation as percentages relative to UTC (* P < 0.05 vs. UTC).

In Vivo Evaluation of ISIS 721744 Activity in Human KLKB1/PKK Transgenic Mice (Study No. ELN 23-100d)

Transgenic mice expressing the human KLKB1/PKK gene (n = 2 males/group + 2 females/group) were treated with single SC dose levels of ISIS 721744 at 0.1, 0.3, 1, 3, and 10 mg/kg. Blood was collected on Days -1, 3, 7, 14, 28, 42 and 56 and human PKK plasma protein levels were evaluated by ELISA.

As shown in **Figure 3** (Applicant's Figure), ISIS 721744 reduced plasma human PKK protein in human KLKB1/PKK transgenic mice in a dose- and time-dependent manner. Human PKK protein was reduced up to a maximum of about 91% on Day 14 after treatment with 10 mg/kg ISIS 721744. After Day 14, plasma PKK protein levels gradually increased to baseline levels and were restored by Day 42 or Day 56 at 1 mg/kg or 3 mg/kg ISIS 721744, respectively. At Day 56, PKK levels plasma PKK protein levels at 10 mg/kg were still 20% below baseline levels.

As shown in **Figure 4** (Applicant's Figure), based on non-linear curve fit analysis, ED_{50} at Days 7 and 14 after treatment with ISIS 721744 was 0.67 and 0.75 mg/kg, respectively.

Figure 3 Dose- and Time-Dependent Reduction of Plasma PKK Protein in Human KLKB1/PKK Transgenic Mice Treated with ISIS 721744 (Applicant's Figure)

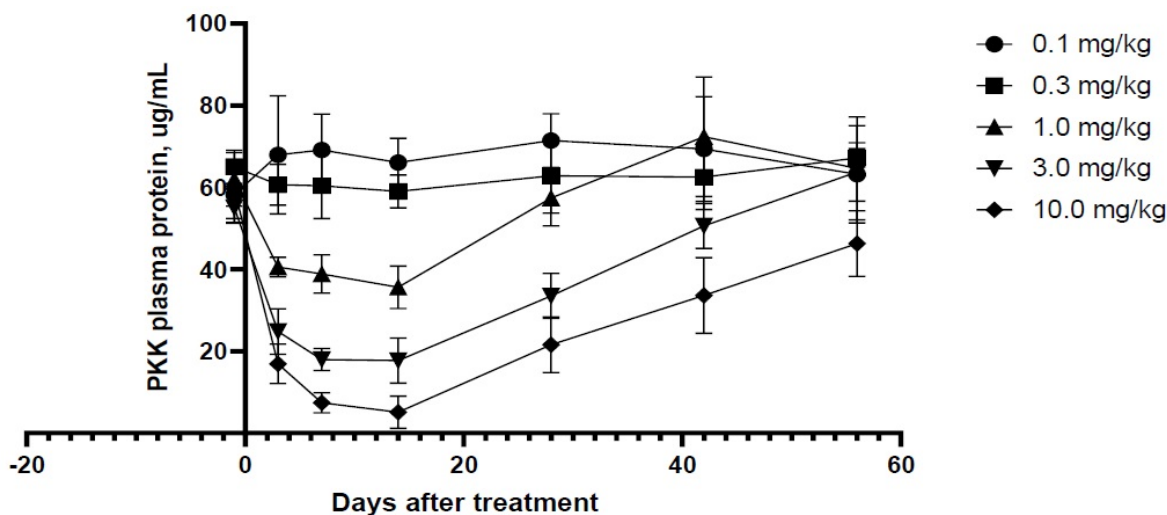


Figure 1: Dose and Time-Dependent Reduction of Plasma PKK Protein in Human KLKB1/PKK Transgenic Mice Treated with ISIS 721744

Human *KLKB1/PKK* transgenic mice (N = 4/group) were dosed with ISIS 721744 at 10, 3, 1, 0.3 or 0.1 mg/kg on Day 0. PKK plasma protein levels were evaluated on Day -1, 3, 7, 14, 28, 42 and 56. Dosing was performed by SC injection. Mean PKK plasma protein levels (ug/mL) $\pm$ SD are plotted.

Figure 4 Dose-Dependent Reduction of Plasma PKK Protein in Human *KLKB1*/*PKK* Transgenic Mice at Days 7 and 14 after Treatment with ISIS 721744 (Applicant's Figure)

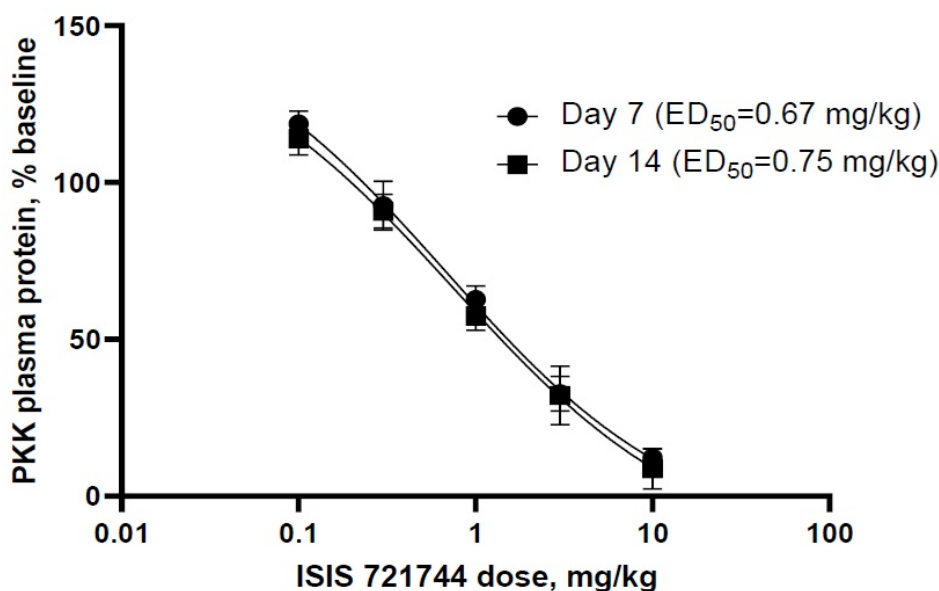


Figure 2: Dose-Dependent Reduction of Plasma PKK Protein in Human *KLKB1*/*PKK* Transgenic Mice at Days 7 and 14 After the Treatment with ISIS 721744

Human *KLKB1*/*PKK* transgenic mice (N = 4/group) were dosed with ISIS 721744 at 10, 3, 1, 0.3 or 0.1 mg/kg on Day 0. PKK plasma protein levels were evaluated on Day 7 and 14. Dosing was performed by SC injection. PKK plasma protein levels are plotted as Mean $\pm$ SD and expressed as a percent relative to the baseline.

4.2 Secondary Pharmacology

There were no secondary pharmacology studies conducted.

4.3 Safety Pharmacology

As described in **Section 3.1**, the GLP-compliant safety pharmacology study for the evaluation of cardiovascular, respiratory, and CNS parameters following SC administration of ISIS 721744 in *Cynomolgus* monkeys was previously reviewed (refer to the IND 142564 Nonclinical Review dated 08/13/2019). Included below is a review of the GLP-compliant hERG assay with ISIS 721744.

Effect of ISIS 721744 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study No. 210830.JTJ; 721744-IS08)

HEK-293 cells were stably transfected with hERG cDNA and treated with ISIS 721744 (30 and 300 mcM) at near-physiological temperature to measure the effect on ionic currents by whole-cell patch-clamp technique.

ISIS 721744 inhibited hERG current by 0.5% and 1.9% at 30 mcM and 300 mcM, respectively (not statistically significant) versus 2.4% for the vehicle control. The IC_{50} for

the inhibitory effect of ISIS 721744 on hERG potassium current was not calculated but was estimated to be greater than 300 mcM. The positive control article (60 nM terfenadine) inhibited hERG potassium current by 85.6%. In general, oligonucleotides are not thought to interact with the hERG channel.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

As described in **Section 3.1**, the in vitro evaluation of the extent of plasma protein binding of ISIS 721744 in human, monkey, and mouse plasma by ultrafiltration was previously reviewed (refer to IND 142564 Nonclinical Review dated 08/13/2019). Included below are the PK/ADME studies not previously reviewed.

Absorption, Distribution, Metabolism and Excretion of [3H]ISIS 721744 following a Single Subcutaneous Dose Administration to Sprague Dawley Rats (Study No. IPI/06; 721744-APK02)

This study determined the ADME properties of [3H]ISIS 721744 (2 or 10 mg/kg) after single SC administration to non-pigmented Sprague Dawley rats.

- PK parameters and tissue distribution of total radioactivity were evaluated in blood, plasma, and tissues after single SC administration of [3H]ISIS 721744 at 2 mg/kg and a radioactive dose level of 200 μ Ci/kg to male non-pigmented Sprague Dawley rats.
- Mass balance and routes and extent of excretion of total radioactivity were evaluated after single SC administration of [3H]ISIS 721744 at 2 mg/kg and a radioactive dose level of 200 μ Ci/kg to male non-pigmented Sprague Dawley rats.
- Tissue distribution of radioactivity was evaluated using Quantitative Whole Body Autoradiography (QWBA) after single SC administration of [3H]ISIS 721744 at 10 mg/kg and a radioactive dose level of 500 μ Ci/kg to male and female non-pigmented Sprague Dawley rats.
- Metabolite identification and profiling was evaluated after single SC administration of [3H]ISIS 721744 at 10 mg/kg and a radioactive dose level of 500 μ Ci/kg to male and female non-pigmented Sprague Dawley rats.
- One male non-pigmented Sprague Dawley rat served as a control (i.e., received a single SC dose of sterile isotonic saline).

Following a single SC dose of [3H]ISIS 721744 at 2 mg/kg to male non-pigmented Sprague Dawley rats, C_{max} of 0.375 and 0.538 μ g equiv./mL were reached in the blood and plasma, respectively, at 1 hour for both, after which radioactivity declined in a biphasic manner. The blood:plasma ratio calculated from $AUC_{0-\infty}$ was 0.76. C_{max} (μ g equiv./g) of 24.40 at 24 hours and 14.89 at 168 hours were reached in the liver and kidney, respectively. Most tissues achieved concentrations similar to or slightly above the blood radioactivity concentrations. Tissue:blood ratios at 24 hours were highest in the liver and kidney (1877 and 850, respectively) consistent with uptake of ISIS 721744. The thyroid gland, pancreas, large and small intestine, spleen, lymph node, and bone marrow had tissue:blood ratios between 24.2 to 69.2 at 24 hours.

Following a single SC dose of [3H]ISIS 721744 at 2 mg/kg to male non-pigmented Sprague Dawley rats, the mean cumulative recovery (0 to Day 56) of radioactivity in excreta was 76.8% (17.14% from urine + 57.46% from feces + 2.16% from cage wash). Residual radioactivity in the carcass on Day 56 accounted for 5.84% of the administered dose resulting in an overall recovery of 82.6%.

Following a single SC dose of [3H]ISIS 721744 at 10 mg/kg to male and female non-pigmented rats, radioactivity was distributed to tissues by the first sampling time at 2 hours and reached maximal concentrations within 2 to 48 hours postdose with highest concentrations of radioactivity in the kidney, liver, intestinal mucosa, salivary gland, bone marrow, lymph nodes and splenic red pulp. Concentrations of radioactivity in most organs/tissues decreased by the end of the sampling period at 336 hours, except for the kidney where radioactivity levels remained at similar levels throughout the sampling period.

Recovery of radioactivity in urine and feces from non-pigmented rats following a single SC dose of [3H]ISIS 721744 at 10 mg/kg was 0.35% and 1.37% of dose, respectively for males and 0.30% and 1.31% of dose, respectively for females. See also below.

Metabolite Profiling and Identification Studies following a Single Subcutaneous Dose Administration of [3H]ISIS 721744 to Male and Female Rats (Study No. IPI/07; 721744-APK02)

This study evaluated the metabolic profile of ISIS 721744 in rat whole blood and plasma, liver and kidney tissues, and in the excreta (urine and feces) by liquid chromatography mass spectrometry (LC-MS) after single SC administration of [3H]ISIS 721744 at 10 mg/kg to male and female non-pigmented rats.

A summary of metabolites is shown in **Table 2** (Applicant's Table).

ISIS 721744 was seen in the liver, kidney, plasma and whole blood. ISIS 721744 was the major component at 2 hours in plasma and blood but was not detected at 24 hours in plasma and blood. Unconjugated ISIS 721744 (i.e. ISIS 780124) was the major component in liver (2 and 24 hours) and kidney (24 hours). Two major components were present in the kidney at 2 hours: unconjugated ISIS 721744 (i.e. ISIS 780124) and ISIS 721744 minus 3 GalNAc sugars (i.e. ISIS 721744 -3 GalNAc). Two major components were detected in feces samples at 0 to 24 hours: ISIS 780124 3'-N-18 and ISIS 780124 3'-N-17. Several ISIS 780124 shortmers were identified as major components in urine at 0 to 24 hours, from ISIS 780124 3'-N-10 to ISIS 780124 3'-N-14.

As described in the study report, all identified metabolites were consistent with nuclease-mediated metabolism to chain shortened oligonucleotides with full, partial, or without GalNAc or linker-conjugation.

Table 2 Metabolites in Urine, Feces, Kidney, Liver, Blood, and Plasma from Rats dosed with [3H]ISIS 721744 at 10 mg/kg (Applicant's Table)

Component Description	Molecular weight ^a (Da)	Molecular formula	Nominal retention time (min)	Region on HPLC Radiometric-Chromatogram					
				Rat urine	Rat faeces	Rat plasma	Rat whole blood	Rat liver	Rat kidney
M341: MOE G	341	C ₁₃ H ₁₉ N ₅ O ₆	9.0 / 11.0 ^b	x	x	x	x	x	x
M736: ISIS 780124 3'N-18	736	C ₂₆ H ₃₈ N ₇ O ₁₄ PS	17.0 ^c	x	[F2]	x	x	x	x
M1129: ISIS 780124 3'N-17	1129	C ₃₉ H ₅₈ N ₁₀ O ₂₁ P ₂ S ₂	21.2 ^c	[U5]	[F4]	x	x	x	x
M2249: ISIS 780124 3'N-14	2249	C ₇₅ H ₁₀₆ N ₂₅ O ₄₀ P ₆ S ₃	23.7 ^c	[U6]	x	x	x	[L1]	x
M2569: ISIS 780124 3'N-13	2569	C ₈₅ H ₁₁₉ N ₂₇ O ₄₆ P ₈ S ₄	24.0 ^c	[U6]	x	x	x	[L1]	[K1]
M2888: ISIS 780124 3'N-12	2888	C ₈₅ H ₁₃₃ N ₃₀ O ₅₁ P ₇ S ₅	24.3 ^c	[U7]	x	x	x	[L2]	[K1]
M1904: ISIS 834443 (also known as ISIS 780124 3'N-15)	1904	C ₆₅ H ₆₄ N ₂₀ O ₃₅ P ₄ S ₂	24.4 ^b	x	x	x	x	x	x
M3209: ISIS 780124 3'N-11	3209	C ₁₀₅ H ₁₄₆ N ₃₂ O ₅₇ P ₈ S ₆	24.6 ^c	[U8]	x	x	x	[L3]	[K2]
M3528: ISIS 834441 (also known as ISIS 780124 3'N-10)	3528	C ₁₁₅ H ₁₈₀ N ₃₅ O ₆₂ P ₉ S ₇	25.0 ^c	[U8]	x	x	x	[L3]	[K2]
M1897: ISIS 834444 (also known as ISIS 780124 5'N-15)	1897	C ₆₅ H ₆₃ N ₂₃ O ₃₂ P ₄ S ₂	25.1 ^c	✓	x	x	x	✓	✓
M3547: ISIS 834442 (also known as ISIS 780124 5'N-10)	3547	C ₁₁₅ H ₁₅₇ N ₄₀ O ₅₉ P ₉ S ₇	25.3 ^c	✓	x	✓	✓	x	x
M3848: ISIS 780124 3'N-9	3848	C ₁₂₅ H ₁₇₃ N ₃₇ O ₆₈ P ₁₀ S ₈	25.4 ^c	x	x	x	x	[L4]	[K3]
M4168: ISIS 780124 3'N-8	4168	C ₁₃₅ H ₁₈₈ N ₃₉ O ₇₄ P ₁₁ S ₉	25.7 ^c	x	x	x	x	[L5]	[K4]
M4514: ISIS 780124 3'N-7	4514	C ₁₄₅ H ₁₉₈ N ₄₄ O ₇₉ P ₁₂ S ₁₀	25.9 ^c	x	x	x	x	[L5]	[K4]
M6750: ISIS 834440 (also known as ISIS 780124 3'N-1)	6750	C ₂₁₇ H ₂₉₈ N ₇₀ O ₁₁₆ P ₁₈ S ₁₄	29.7 ^c	x	x	x	x	[L6]	[K5]
M5048: ISIS 721744 3'N-10	5048	C ₁₈₁ H ₂₇₉ N ₄₃ O ₉₁ P ₁₀ S ₇	30.0 ^c	x	x	[P1]	[B1]	x	x
M4728: ISIS 721744 3'N-11	4728	C ₁₇₁ H ₂₆₅ N ₄₀ O ₈₆ P ₉ S ₆	30.0 ^c	x	x	[P1]	x	x	x
M5368: ISIS 721744 3'N-9	5368	C ₁₉₁ H ₂₉₂ N ₄₅ O ₉₇ P ₁₁ S ₈	30.5 ^c	x	x	[P1]	[B1]	x	x

^a Molecular weight quoted refer to unlabelled components

^b Retention times are nominal and are taken from accurate mass extracted ion chromatograms for corresponding reference standard

^c Retention times are taken from the relevant accurate mass extracted ion chromatograms for the urine, faeces, plasma, whole blood, liver and kidney samples and represent a range across all samples

[xx] Component confirmed as present by LC-MS and in radio-chromatogram

✓ Component confirmed as present by LC-MS only

x Component not detected by LC-MS

Component Description	Molecular weight ^a (Da)	Molecular formula	Nominal retention time (min)	Region on HPLC Radiometric-Chromatogram					
				Rat urine	Rat faeces	Rat plasma	Rat whole blood	Rat liver	Rat kidney
M7153: ISIS 780124 (also known as unconjugated ISIS 721744)	7153	C ₂₃₀ H ₃₁₆ N ₇₅ O ₁₂₂ P ₁₉ S ₁₅	30.7 ^c	[U9]	x	[P1]	x	[L7]	[K6]
M6033: ISIS 721744 3'N-7	6033	C ₂₁₁ H ₃₁₇ N ₅₂ O ₁₀₈ P ₁₃ S ₁₀	30.8 ^c	x	x	[P1]	[B1]	x	x
M5688: ISIS 721744 3'N-8	5688	C ₂₀₁ H ₃₀₅ N ₄₇ O ₁₀₃ P ₁₂ S ₉	30.9 ^c	x	x	[P1]	[B1]	x	x
M5165: ISIS 721744 - 1 GalNac 3'N-9	5165	C ₁₈₃ H ₂₇₆ N ₄₄ O ₉₂ P ₁₁ S ₈	32.2 ^c	x	x	✓	x	x	x
M8269: ISIS 721744 3'N-1	8269	C ₂₈₃ H ₄₁₇ N ₇₈ O ₁₄₅ P ₁₆ S ₁₄	35.2 ^c	x	x	[P2]	[B2]	x	x
M8657: ISIS 721744 S to O	8657	C ₂₉₈ H ₄₃₅ N ₈₃ O ₁₅₂ P ₂₀ S ₁₄	35.5 ^c	x	x	[P2]	[B2]	x	x
M8066: ISIS 721744 -1 GalNac 3'N-1	8066	C ₂₇₅ H ₄₀₄ N ₇₇ O ₁₄₀ P ₁₉ S ₁₄	36.8 ^c	x	x	[P2]	x	x	x
M8673: ISIS 721744	8673	C ₂₉₈ H ₄₃₅ N ₈₃ O ₁₅₁ P ₂₀ S ₁₅	36.9 ^c	x	x	[P2]	[B2]	[L8]	[K7]
M4438: ISIS 721744 -3 GalNac 3'N-10	4438	C ₁₅₇ H ₂₄₀ N ₄₀ O ₇₈ P ₁₀ S ₇	37.5 ^c	x	x	x	x	[L8]	[K7]
M3799: ISIS 721744 -3 GalNac 3'N-12	3799	C ₁₃₇ H ₂₁₃ N ₃₅ O ₆₅ P ₈ S ₅	37.5 ^c	x	x	x	x	[L8]	[K7]
M4758: ISIS 721744 -3 GalNac 3'N-9	4758	C ₁₆₇ H ₂₅₃ N ₄₂ O ₈₂ P ₁₁ S ₈	37.8 ^c	x	x	x	x	[L8]	[K7]
M4119: ISIS 721744 -3 GalNac 3'N-11	4119	C ₁₄₇ H ₂₂₈ N ₃₇ O ₇₁ P ₉ S ₆	37.8 ^c	x	x	x	x	[L8]	[K7]
M3479: ISIS 721744 -3 GalNac 3'N-13	3479	C ₁₂₇ H ₁₉₉ N ₃₂ O ₆₀ P ₇ S ₄	37.9 ^c	x	x	x	x	[L8]	[K7]
M5424: ISIS 721744 -3 GalNac 3'N-7	5424	C ₁₈₇ H ₂₇₈ N ₄₉ O ₉₃ P ₁₃ S ₁₀	38.0 ^c	x	x	x	x	x	[K7]
M5079: ISIS 721744 -3 GalNac 3'N-8	5079	C ₁₇₇ H ₂₆₆ N ₄₄ O ₈₈ P ₁₂ S ₉	38.1 ^c	x	x	x	x	x	[K7]
M8469: ISIS 721744 -1 GalNac	8469	C ₂₈₈ H ₄₂₂ N ₈₂ O ₁₄₈ P ₂₀ S ₁₅	38.4 ^c	x	x	[P3]	[B3]	[L8]	[K7]
M8266: ISIS 721744 -2 GalNac	8266	C ₂₈₀ H ₄₀₉ N ₈₁ O ₁₄₁ P ₂₀ S ₁₅	40.2 ^c	x	x	[P4]	[B4]	[L9]	[K8]
M7660: ISIS 721744 -3 GalNac 3'N-1	7660	C ₂₅₉ H ₃₇₈ N ₇₅ O ₁₃₀ P ₁₉ S ₁₄	41.3 ^c	x	x	x	x	[L10]	[K8]
M8063: ISIS 721744 -3 GalNac	8063	C ₂₇₂ H ₃₉₈ N ₈₀ O ₁₃₈ P ₂₀ S ₁₅	42.0 ^c	x	x	[P5]	[B5]	[L10]	[K8]

^a Molecular weight quoted refer to unlabelled components

^b Retention times are nominal and are taken from accurate mass extracted ion chromatograms for corresponding reference standard

^c Retention times are taken from the relevant accurate mass extracted ion chromatograms for the urine, faeces, plasma, whole blood, liver and kidney samples and represent a range across all samples

[xx] Component confirmed as present by LC-MS and in radio-chromatogram

✓ Component confirmed as present by LC-MS only

x Component not detected by LC-MS

A 6-Week Subcutaneous Repeat-Dose Pharmacokinetic Study of ISIS 721744 in CD-1 Mice (Study No. G122111; 721744-APK03)

In this study, male ICR mice were treated by SC administration with ISIS 721744 (10 or 20 mg/kg/week once weekly) for 6 weeks (Days 1, 8, 15, 22, 29, 36 and 43) to determine the PK profile and distribution of ISIS 721744 to kidney and liver. As described in the study report, concentrations of total full-length ISIS 721744, including fully-conjugated, partially-conjugated (minus 1-, 2-, and 3-GalNAc sugars), and unconjugated ISIS 721744 (ISIS 780124), were determined in mouse plasma by a validated hybridization electrochemiluminescence (ECL) assay, while concentrations of unconjugated ISIS 721744 in kidney and liver samples were analyzed by a validated high-performance liquid chromatography mass spectrometry mass spectrometry (HPLC-MS/MS) bioanalytical method.

As shown in **Table 3** (Applicant's Table), T_{max} values were 0.5 hours on Days 1 and 43 at 10 and 20 mg/kg ISIS 721744. C_{max} and AUC increased in a slightly greater than dose proportional manner on Days 1 and 43. There was lack of accumulation after repeat dosing.

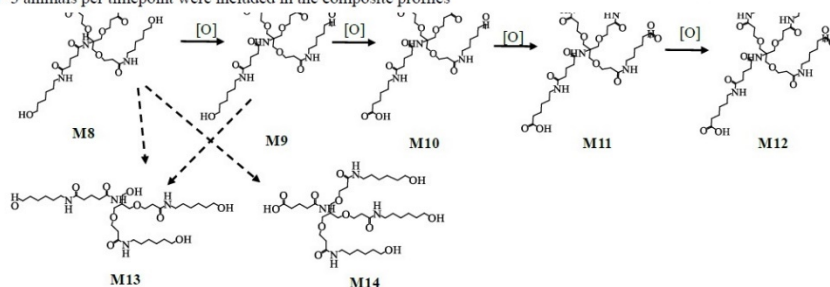
Table 3 Plasma Pharmacokinetics from 6-Week Subcutaneous Repeat-Dose Study of ISIS 721744 in CD-1 Mice (Applicant's Table)

Group	Dose Level (mg/kg)	Number of Doses	Profile Day	T_{max} (h)	C_{max} ($\mu\text{g/mL}$)	AUC_{0-24h} ($\mu\text{g}^*\text{h/mL}$)	AUC_{0-48h} ($\mu\text{g}^*\text{h/mL}$)	$CL_{0-24h/F}$ (mL/h/kg)	$CL_{0-48h/F}$ (mL/h/kg)	MRT_{0-48h} (h)
1	10	1	1	0.50	5.95	7.04	7.31	1420	1370	2.83
1	10	7	43	0.50	8.20	8.99	9.84	1110	1020	5.56
2	20	1	1	0.50	16.0	18.8	19.3	1060	1040	2.30
2	20	7	43	0.50	16.6	29.3	31.4	683	636	4.65

Note: Data presented were calculated based on composite mean profiles obtained from sparse sampling. ISIS 721744 concentration measured as total full-length antisense oligonucleotide, which includes fully conjugated, partially conjugated, and unconjugated ISIS 721744.

CL/F parameters were calculated with the following equation: Dose [mg/kg] x 1000 [$\mu\text{g}/\text{mg}$] / AUC [$\mu\text{g}^*\text{h}/\text{mL}$]

N=3 animals per timepoint were included in the composite profiles



As shown in **Table 4** (Applicant's Table) unconjugated ISIS 721744 was measured in the liver and kidney after repeat-dose treatment with ISIS 721744. Concentrations of unconjugated ISIS 721744 were higher in the kidney than in the liver. Concentrations of unconjugated ISIS 721744 in the liver were similar at 10 and 20 mg/kg/week possibly due to saturation of tissue uptake at these dose levels.

Table 4 Unconjugated ISIS 721744 Concentrations in Kidney and Liver from 6-Week Subcutaneous Repeat-Dose Study of ISIS 721744 in CD-1 Mice (Applicant's Table)

Matrix	Group	Dose Level (mg/kg)	Number of Doses	Study Day	Time from Last Dose (day)	N	Unconjugated ISIS 721744	
							(μ M)	(μ g/g) ^a
Kidney	1	10	1	1	2	3	8.37 $\pm$ 0.241	59.8 $\pm$ 1.72
Kidney	1	10	7	43	2	2 ^b	17.7 $\pm$ 5.44	126 $\pm$ 38.9
Kidney	2	20	1	1	2	3	16.6 $\pm$ 2.41	119 $\pm$ 17.3
Kidney	2	20	7	43	2	3	21.9 $\pm$ 6.14	156 $\pm$ 43.9
Liver	1	10	1	1	2	3	6.23 $\pm$ 0.717	44.6 $\pm$ 5.13
Liver	1	10	7	43	2	3	10.9 $\pm$ 7.71	77.7 $\pm$ 55.1
Liver	2	20	1	1	2	3	8.86 $\pm$ 3.71	63.4 $\pm$ 26.5
Liver	2	20	7	43	2	3	9.31 $\pm$ 0.946	66.6 $\pm$ 6.77

Note: Values reported as mean $\pm$ SD.

^a Unconjugated ISIS 721744 concentration (μ g/g) in tissue was calculated by ([concentration in μ M] x [(Molecular Weight of Unconjugated ISIS 721744 / 1000)]; ISIS 721744 Molecular Weight = 8672.6599 g/Mol

^b The kidney concentration for Animal 1M052 on Day 43 is potentially outlying and was removed from summary statistics. Inclusion of that data results in mean concentrations of 28.8 $\pm$ 19.7 μ M and 206 $\pm$ 141 μ g/g.

SD = standard deviation

In Vitro Evaluation of Whole Blood and Plasma Concentration Ratio of ISIS 721744 (Donidalorsen) in Human, Monkey and Rat (Study No. IIS-P2-008; 721744-IS11)

This study determined the in vitro blood to plasma ratio of ISIS 721744 in human whole blood (neat, 0.01, 0.03, 0.1, 0.3, 1, and 2 mcg/mL) and monkey and rat whole blood (neat, 0.1, 0.3, 1, 3, 10, 30, 100, and 200 mcg/mL) using a hybridization ECL detection method.

As shown in **Table 5** (Applicant's Table), the in vitro mean blood to plasma ratio of ISIS 721744 was between 0.630 and 0.824 in human, 0.468 and 0.566 in monkey, and 0.501 and 0.661 in rat suggesting that ISIS 721744 distributes primarily in the plasma compartment across the concentrations tested in all three species.

Table 5 In Vitro Whole Blood to Plasma Ratio of ISIS 721744 in Human, Monkey, and Rat (Applicant's Table)

ISIS 721744 Nominal Whole Blood Concentration (µg/mL)	Blood to Plasma Ratio		
	Human	Monkey	Rat
0.000	NA	NA	NA
0.0100	0.766 ± 0.0207	NA	NA
0.0300	0.824 ± 0.0196	NA	NA
0.100	0.673 ± 0.0137	0.566 ± 0.0256	0.661 ± 0.0377
0.300	0.630 ± 0.0200	0.543 ± 0.0479	0.623 ± 0.0426
1.00	0.659 ± 0.0287	0.515 ± 0.0370	0.600 ± 0.0103
2.00	0.631 ± 0.0223	NA	NA
3.00	NA	0.480 ± 0.0226	0.571 ± 0.0420
10.0	NA	0.468 ± 0.0263	0.596 ± 0.00545
30.0	NA	0.562 ± 0.0762	0.501 ± 0.0381
100	NA	0.534 ± 0.0272	0.622 ± 0.0631
200	NA	0.541 ± 0.0740	0.527 ± 0.188

Whole Blood-Plasma Ratio = Nominal whole blood concentration / Measured Plasma concentration

Mean Blood to -Plasma Ratio values presented as Mean ± Standard Deviation

NA: Not Applicable

Method Development, Qualification and Sample Analysis of a Bioanalytical Method for the Determination of M8 in Liver and Kidney Samples Generated from Rat using LC-MS/MS (Study No. IPI/08; 721744-IS17)

This study developed a liquid chromatographic-tandem mass spectrometric (UPLC-MS/MS) bioanalytical method to determine the donidalorsen THA linker-related metabolite M8 (chemical formula = C₄₂H₈₁N₅O₁₂; molecular weight = 847.5882) in liver and kidney samples from rats treated with single SC dose levels of ISIS 721744 at 2 and 10 mg/kg (Study Nos. IPI/06 and IPI/07; Study No. 721744-APK02).

Mean M8 concentrations were below the limit of quantitation in liver and kidney from rats treated with a single SC dose level of ISIS 721744 at 2 mg/kg. Mean M8 concentrations from rats treated with a single SC dose level of ISIS 721744 at 10 mg/kg were 211 ng/g at the 2 hour time point and below the limit of quantitation at the 24 hour time point in the liver and 526 ng/g at the 2 hour time point and 317 ng/g at the 24 hour time point in the kidney. Refer to **Table 6** (Applicant's Table),

Table 6 THA Linker-Related Metabolite M8 in Rat Liver and Kidney (Applicant's Table)

Matrix	Study Phase	Time Point	Dose Level	Mean M8 (ng/g)	Mean M8 (nM)	n	CV (%)
Liver	2	24hr	2 mg/kg	BLQ*	BLQ*	3	N/A
	2	48hr	2 mg/kg	BLQ*	BLQ*	3	N/A
	5	2hr	10 mg/kg	211	248	2	5.7
	5	24hr	10 mg/kg	BLQ*	BLQ*	2	N/A
Kidney	2	24hr	2 mg/kg	BLQ*	BLQ*	3	N/A
	2	48hr	2 mg/kg	BLQ*	BLQ*	3	N/A
	5	2hr	10 mg/kg	526	621	2	27.2
	5	24hr	10 mg/kg	317	373	2	68.1

* Mean value could not be calculated as original results non numeric, BLQ reported for information purposes

N/A Not applicable

Metabolic Profiling and Identification of ISIS 721744 Oligonucleotide-Related Metabolites by HPLC-HRMS in Select Urine Samples from Mice, Monkeys, and Humans (Study No. 727-085, 727-081, ISIS 721744-CS1; 721744-IS10)

This study identified ISIS 721744 oligonucleotide containing metabolites (full-length or chain-shortened) from urine samples at steady state from mice, monkeys, and humans treated with multiple SC doses of ISIS 721744 (i.e., 10 mg/kg dose group from 13-week repeat-dose toxicity study in mice [Study No. 721744-AS01]; 30 mg/kg dose group from 39-week repeat-dose toxicity study in monkeys [Study No. 721744-AS02]; and 80 mg dose group from phase 1 study in humans with dosing every four weeks for four total doses [Study No. ISIS 721744-CS1]) using liquid chromatography with a tandem ultraviolet (UV) detector and high-resolution mass spectrometer (HPLC/UV/HRMS) method.

As described in the study report:

The oligonucleotide portions of conjugated ASO molecules are metabolized by hydrolysis of the phosphodiester bonds via endo-nuclease and/or exo-nuclease (either 5' and/or 3') enzymes. Metabolism of the GalNAc-conjugation component of ISIS 721744 typically occurs by a sequential loss of the three GalNAc sugar moieties (-1, -2, and -3 GalNAc), followed by the loss of a portion, or all the remaining THA linker.^{1,2} Complete loss of the THA linker results in the formation of ISIS 780124, the unconjugated version of ISIS 721744. The metabolism of the two portions of ISIS 721744 (oligonucleotide and GalNAc) may occur simultaneously resulting in many potential metabolites with multiple potential metabolic pathways responsible for a single metabolite compound.

The study report grouped the likely metabolites of ISIS 721744 as follows:

¹Weidolf L, Björkbom A, Dahlén A, Elebring M, Gennemark P, Hölttä M, Janzén D, Li X, Andersson S. Distribution and biotransformation of therapeutic antisense oligonucleotides and conjugates. *Drug Discov Today*. 2021 Oct;26(10):2244-2258.

²Shemesh CS, Yu RZ, Gaus HJ, Greenlee S, Post N, Schmidt K, Migawa MT, Seth PP, Zanardi TA, Prakash TP, Swayze EE, Henry SP, Wang Y. Elucidation of the Biotransformation Pathways of a Galnac3-conjugated Antisense Oligonucleotide in Rats and Monkeys. *Mol Ther Nucleic Acids*. 2016 May 10;5(5):e319.

1. Families I through IV arise from multiple routes of sequential hydrolysis of the GalNAc and THA linker portion of ISIS 721744:
 - a. Family I: Intact GalNAc and linker attached, and loss of the nucleotides at the 3'-end to produce 3' n-X metabolites.
 - b. Family II: Loss of one GalNAc sugar to become ISIS 721744-(GalNAc)₂THA-20-mer, and loss of nucleotides at the 3'-end to produce 3' n-X metabolites.
 - c. Family III: Loss of two GalNAc sugars to become ISIS 721744-(GalNAc)₁THA-20-mer, followed by sequential loss of the nucleotides at the 3'-end to produce 3' n-X metabolites.
 - d. Family IV: Loss of three GalNAc sugars to become ISIS 721744 (GalNAc)₀THA-20-mer, followed by sequential loss of the nucleotides at the 3'-end to produce 3' n-X metabolites.
2. Family V: Hydrolysis of the entire linker of ISIS 721744 to form ISIS 780124 (fully unconjugated ISIS 721744) followed by subsequent endo- and exo-nuclease hydrolysis (3'-deletion and 5'-deletion) to produce 3'- and 5' n-X metabolites.
3. Family VI: Hydrolysis of the entire linker of ISIS 721744 to form ISIS 780124 (fully unconjugated ISIS 721744), while retaining the 5' phosphate group (5'PO ISIS 780124). This metabolite then undergoes endo- and exo-nuclease hydrolysis (5'-deletion) to produce 5' n-X metabolites.

X represents the number of nucleotides that have been removed from the full length ASO.

A diagram of the proposed putative metabolism of donidalorsen is shown in **Figure 5** (Applicant's Figure).

Mouse Urine: ISIS 721744 was the major component (about 53% of total oligonucleotide signal) in mouse urine samples when administered at 10 mg/kg. As described by the Applicant, other oligonucleotide metabolites included the full-length ASO with partial conjugations, fully and partially conjugated donidalorsen with loss of nucleotides from the 3'-end (referred to as 3'-deletion chain-shortened metabolites), and unconjugated donidalorsen with loss of nucleotides from the 5'-end (referred to as 5'-deletion chain-shortened metabolites).

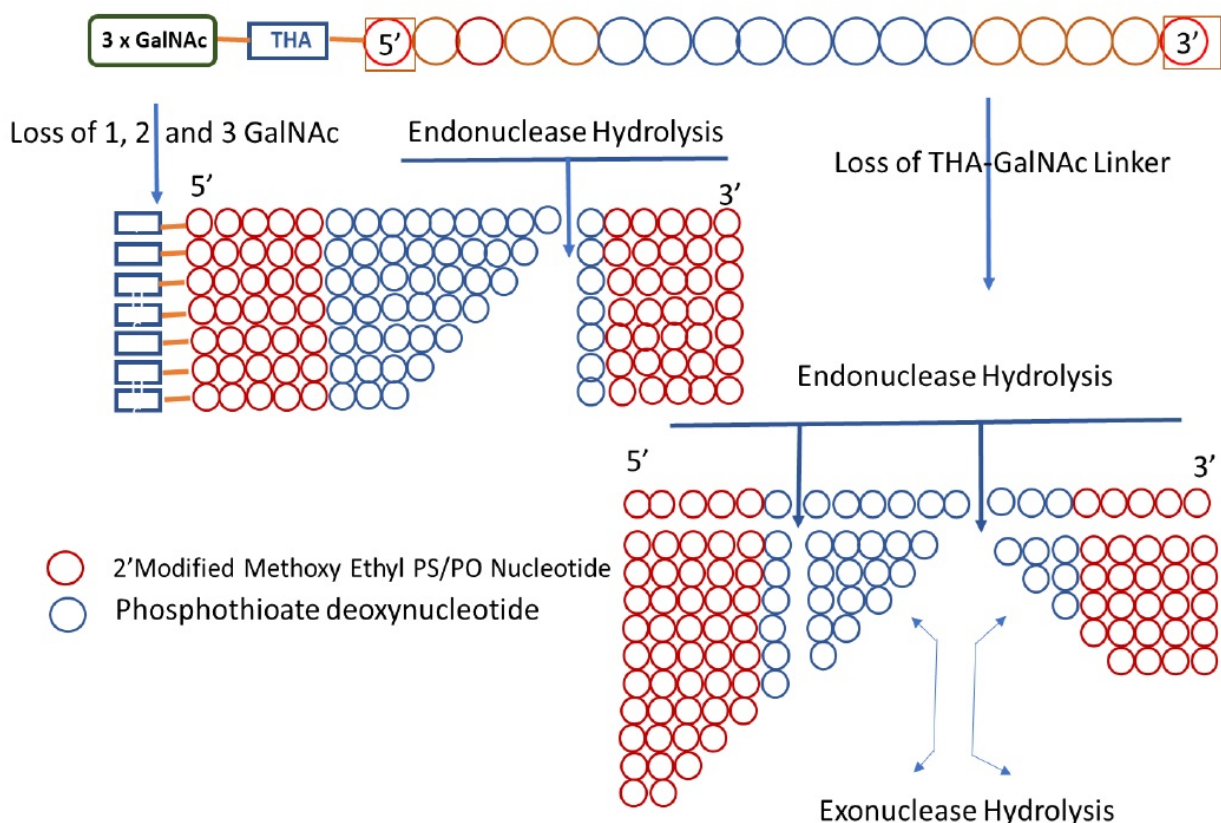
Monkey Urine: ISIS 721744 was the major component (about 41% of total oligonucleotide signal), followed by ISIS 780124 (unconjugated version of ISIS 721744; about 14% of total oligonucleotide signal) in the monkey urine samples when administered at 30 mg/kg. As described by the Applicant, other identified metabolites were 3'-deletion chain-shortened metabolites of donidalorsen (with GalNAc sugar moieties and THA linker attached), as well as metabolites of unconjugated donidalorsen with loss of nucleotides from the 5'-end.

Human Urine: ISIS 780124 (unconjugated version of ISIS 721744) was the major component (about 38% of total oligonucleotide signal) in human urine samples when administered at 80 mg/kg. ISIS 721744 was not detected. As described by the Applicant, the other most abundant metabolites were from unconjugated donidalorsen

with loss of nucleotides from the 5'-end. However, 3'-deletion chain-shortened metabolites of donidalorsen (with GalNAc sugar moieties and THA linker attached) were also seen.

There were no unique metabolites found in humans compared to mice or monkeys.

Figure 5 Diagram of Putative Metabolism of Donidalorsen (Applicant's Figure)



Source: Study No. 721744-IS10, Figure 1

Profiling, Identification, and Quantitation of ISIS 721744 Linker-Related Metabolites by HPLC-MS/MS in Select Plasma, Urine, and Fecal Samples from Mice, Monkeys, and Humans (Study No. 721744-IS12)

This study identified linker-only-related metabolites of ISIS 721744 (no full-length or chain-shortened oligonucleotide components) from plasma (monkey and human), urine (mouse, monkey, and human), and feces (monkey) after SC dosing of ISIS 721744 (i.e., 2 and/or 10 mg/kg dose groups from 13-week repeat-dose toxicity study in mice [Study No. 721744-AS01]; 2 and/or 30 mg/kg dose group from 39-week repeat-dose toxicity study in monkeys [Study No. 721744-AS02]; and 80 mg dose group from phase 1 study in humans with dosing every four weeks for four total doses [Study No. ISIS 721744-CS1]) by a HPLC-MS/MS method. Eleven total linker metabolites (putative linker metabolites M4 to M14) were monitored in these studies.

Six linker metabolites were detected in monkey plasma (M5 and M8 to M12) and three linker metabolites were detected in human plasma (M5, M8, and M12).

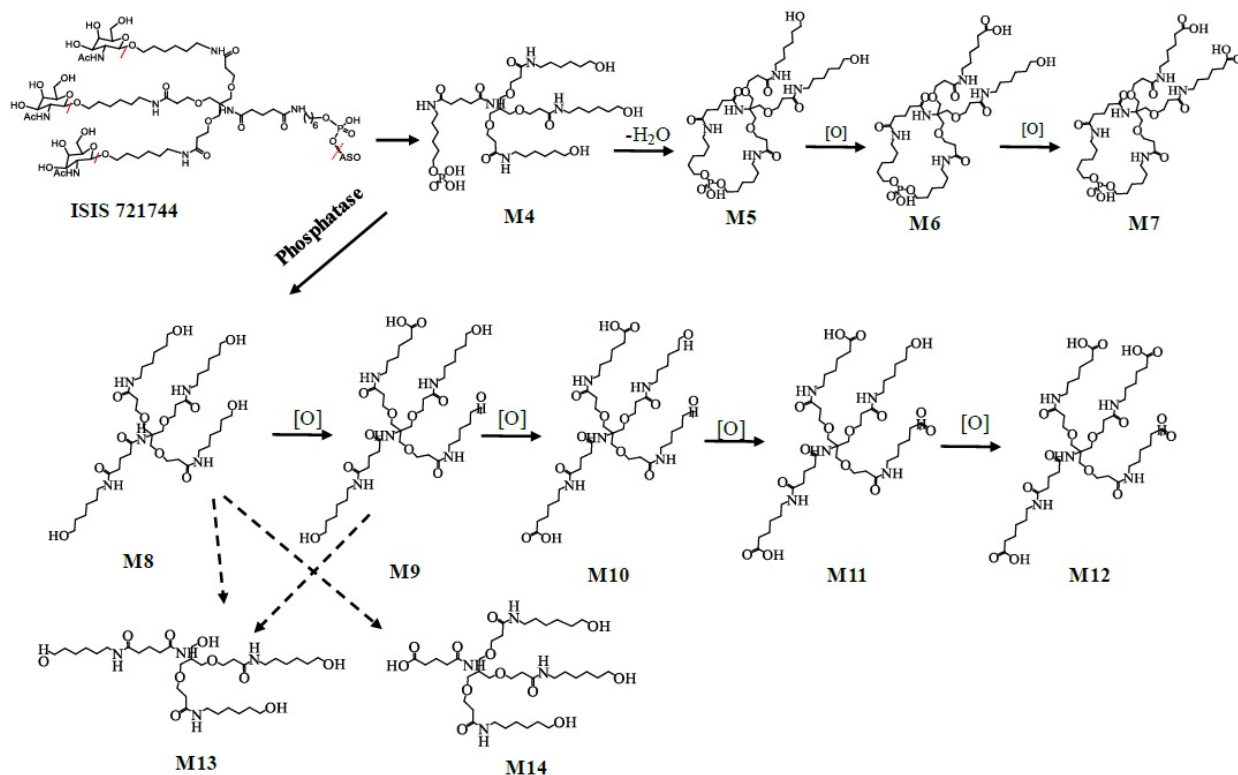
Ten linker metabolites (M5 to M14) were similarly detected in mouse, monkey, and human urine. The linker metabolites with the highest peak area ratios were M8, M5, and M12, which were consistent across the three species.

Ten linker metabolites (M5 to M14) were detected in monkey feces. The linker metabolites with the highest peak area ratios were M8, M10, M11, and M5.

As described by the Applicant: The THA-linker-related metabolism is generally similar across species with oxidative metabolites being formed and eliminated through urine and bile. The overall metabolic pathways for the THA-linker-related metabolites of donidalorsen involve hydrolysis of GalNAc sugars and oligonucleotide moieties to form M4 metabolite, followed by internal cyclization of M4 to form cyclized linker M5 metabolite which would then undergo mono- and di-oxidation to form M6 and M7 metabolites. Dephosphorylation of M4 metabolite resulted in the formation of M8 metabolite followed by sequential oxidation at each branching arm of M8 metabolite to form mono-, di-, tri-, and tetra-oxidation metabolites (M9- M12 metabolites). Additional metabolites resulting from dealkylation (M13 metabolite), or deamination (M14 metabolite) were also detected. The proposed pathway for the metabolism of linker-related moieties of donidalorsen is shown in **Figure 6** (Applicant's Figure).³

³Shemesh CS, Yu RZ, Gaus HJ, Greenlee S, Post N, Schmidt K, Migawa MT, Seth PP, Zanardi TA, Prakash TP, Swayze EE, Henry SP, Wang Y. Elucidation of the Biotransformation Pathways of a Galnac3-conjugated Antisense Oligonucleotide in Rats and Monkeys. *Mol Ther Nucleic Acids*. 2016 May 10;5(5):e319.

Figure 6 Proposed Biotransformation Pathway for the Linker-Related Metabolism of Donidalorsen (Applicant's Figure)



6 General Toxicology

As described in **Section 3.1**, the pivotal GLP-compliant 39-week repeat-dose toxicity study of ISIS 721744 in Cynomolgus monkeys with a 13-week interim necropsy and a 26-week recovery period was previously reviewed (refer to IND 142564 Nonclinical Review dated 08/13/2019). The monkey is considered a pharmacologically relevant species for ISIS 721744 nonclinical evaluations.

Reviewed below is the pivotal GLP-compliant 26-week repeat-dose toxicity study of ISIS 721744 and mouse surrogate ISIS 722059 in mice with a 13-week recovery period. The mouse is not a pharmacologically relevant species for ISIS 721744 nonclinical evaluations. This study, as designed, could assess potential off-target effects of ISIS 721744 and potential on-target effects using the mouse surrogate, ISIS 722059.

6.2 Repeat-Dose Toxicity

Study title: A 26-Week Repeat Dose Toxicity Study of ISIS 721744 in Mice with a 13-Week Recovery

Study no.: 8405032 (721744-AS04)
Study report location: Electronic Document Room (EDR)
Supporting Document Number (SDN) 1
Conducting laboratory and location:  (b) (4)

Date of study initiation: 03/25/2019
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ISIS 721744, bulk test article lot #
CA721744-001, 89.5% purity

ISIS 722059, bulk material API lot #
722059-3, 79.3% purity

Key Study Findings

- In a repeat-dose toxicity study, mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (1, 4, or 12 mg/kg/dose), or mouse surrogate ISIS 722059 (4 mg/kg/dose) Q2W for 26 weeks with a 13-week treatment-free recovery period (vehicle control and 12 mg/kg/dose ISIS 721744 dose groups only).
- There were no unscheduled deaths during the study that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.
- Changes in hematology parameters at the end of the 26-week treatment period included increases in white blood cells, neutrophils, lymphocytes, and/or monocytes in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and at 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period, increases in hematology parameters were no longer seen at 12 mg/kg/dose ISIS 721744.
- Changes in clinical chemistry parameters at the end of the 26-week treatment period included increases in ALT, AST, and/or ALP in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744. Effects on clinical chemistry parameters (including ALT, AST, and ALP) were less clear at 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period, increases in ALT and/or AST were still seen in males and/or females at 12 mg/kg/dose ISIS 721744, while increases in ALP were no longer seen. The toxicological significance of increases in ALT and AST were unclear as histopathology findings in the liver appeared to be partially reversible and levels of unconjugated ISIS 721744 had declined below the lower limit of quantitation.

- Gross pathology findings after the 26-week treatment period with ISIS 721744 were seen in the liver (discoloration in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and enlargement in males at 4 mg/kg/dose ISIS 721744) and inguinal lymph nodes (enlarged in males at 1 and 4 mg/kg/dose ISIS 721744). There were no clear gross pathology findings seen after the 26-week treatment period with mouse surrogate ISIS 722059. Gross pathology findings after the 13-week recovery period for 12 mg/kg/dose ISIS 721744 were noted in the liver (discoloration in males) and subcutaneous injection site (discoloration in males).
- Organ weight changes after the 26-week treatment period with ISIS 721744 included increases in liver weights in males and/or females at 4 and/or 12 mg/kg/dose and increases in spleen weights in males at 12 mg/kg/dose. There were no organ weight changes after the 26-week treatment period with 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period for 12 mg/kg/dose ISIS 721744, liver weights were still increased in males and/or females.
- Histopathology findings after the 26-week treatment period with ISIS 721744 were seen in the liver, skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen. Histopathology findings after the 26-week treatment with mouse surrogate ISIS 722059 were seen in the skin/subcutis, SC dose sites, spleen, and testis.
 - Liver: Minimal or slight centrilobular hepatocellular hypertrophy was noted in males at 1, 4, and 12 mg/kg/dose ISIS 721744 and in one female (moderate) at 12 mg/kg/dose ISIS 721744. This finding correlated with increased mean liver weights at 4 and 12 mg/kg/dose ISIS 721744 and increased liver enzymes (AST, ALT, and/or ALP). Despite a background incidence of hepatocyte vacuolation in vehicle control males, there was an increase in the severity (slight to marked) of hepatocyte vacuolation in males at 4 and 12 mg/kg/dose ISIS 721744, which correlated with gross pathology findings of liver discoloration at all dose levels. These findings were consistent with uptake of ISIS721744.
 - Skin/Subcutis: Increased incidences and/or severity of infiltrates of mononuclear cells, mixed cells, and/or vacuolated macrophages in the skin/subcutis were noted in males and/or females at 4 and/or 12 mg/kg/dose ISIS 721744 and/or ISIS 722059 at 4 mg/kg/dose. Increased incidences and/or severity of vacuolated fibrocytes in the skin/subcutis were noted in males and/or females at 12 mg/kg/dose ISIS 721744 and ISIS 722059 at 4 mg/kg/dose. Slight to moderate inflammation in the skin was noted in females at 4 and 12 mg/kg/dose ISIS 721744. These findings in the skin/subcutis correlated with increased leukocyte counts.
 - SC Dose Sites: Increased incidences and/or severity of infiltrates of vacuolated macrophages, mononuclear cells, and mixed cells, as well as fibrocyte vacuolation at the SC injection sites were noted in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. These findings at the SC injection sites correlated with increased leukocyte counts.

- Inguinal Lymph Nodes: Increased incidences and severities (minimal to marked) of increased lymphocytes in males at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and one female at 12 mg/kg/dose ISIS 721744 were noted in the inguinal lymph node. This finding in the inguinal lymph node correlated with increased leukocyte counts. Amyloid in the inguinal lymph node was noted in one male at 4 mg/kg/dose ISIS 722059.
- Spleen: Increased incidence and/or severity of extramedullary hematopoiesis in the spleen was noted in males and/or females at 1, 4 and/or 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059 and correlated with increased mean spleen weight in males administered 12 mg/kg/dose ISIS 721744.
- Testis: Moderate interstitial cell hyperplasia, minimal sperm stasis, and minimal vacuolation were noted in the testis of males at 4 mg/kg/dose ISIS 722059.
- Histopathology findings after the 13-week recovery period for 12 mg/kg/dose ISIS 721744 were still noted in the liver as hepatocellular vacuolation in males and females, which correlated with increased liver weights. Histopathology findings in the skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen at 12 mg/kg/dose ISIS 721744 were not seen at the end of the recovery period.
- Unconjugated ISIS 721744 was measurable in a dose-dependent manner in the kidney (greater than dose proportional) and liver (dose proportional) after 26 weeks of treatment with ISIS 721744. The mean concentrations of unconjugated ISIS 721744 were similar in the kidney and liver except at 12 mg/kg/dose where mean unconjugated ISIS 721744 concentrations were greater in the kidney than in the liver. Mean unconjugated ISIS 721744 concentrations were higher in females than in males in the kidney but similar in the liver. After the 13-week treatment-free recovery period at 12 mg/kg/dose ISIS 721744, mean unconjugated ISIS 721744 concentrations were below or near the lower limit of quantitation of 0.107 mcg/g, suggesting complete clearance of ISIS 721744.
- Mouse PKK mRNA in mouse liver was reduced by 77% (statistically significant) compared to vehicle control in mice treated with mouse surrogate ISIS 722059 at 4 mg/kg/dose for 26 weeks.
- The NOAEL was 12 mg/kg/dose ISIS 721744, as test article-related findings were considered related to the oligonucleotide uptake.

Methods (Refer to

Table 7, Applicant's Table, for study design)

Doses:	ISIS 721744: 1, 4, or 12 mg/kg/dose ISIS 722059 (mouse surrogate): 4 mg/kg/dose
Frequency of dosing:	Q2W for 26 weeks (Days 1, 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, and 183)
Route of administration:	SC
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.9% Sodium Chloride for Injection, USP
Species/Strain:	Mice/Crl:CD1(ICR)
Number/Sex/Group:	18 (12 treatment + 6 recovery for vehicle control and ISIS 721744 at 12 mg/kg/dose) or 12 (12 treatment + 0 recovery for ISIS 721744 at 1 and 4 mg/kg/dose and ISIS 722059 at 4 mg/kg/dose)
Age:	7 to 8 weeks old at study initiation
Weight:	29.6 to 41.5 g for males and 23.2 to 30.9 g for females at study initiation
Satellite groups:	No
Unique study design:	• Inclusion of 13-week recovery period • Treatment with ISIS 722059 (mouse-specific surrogate ASO targeting mouse PKK mRNA). • PKK mRNA tissue evaluation for ISIS 722059-treated groups • Exploratory cytokine/chemokine analyses
Deviation from study protocol:	Protocol deviations were included in the study report and were not considered to have affected the conduct or integrity of the study.

Table 7 Study Design for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery (Applicant's Table)

Group ^a	Total No. of Animals ^b		No. of Animals/Sex ^b		Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)
	Male	Female	Terminal	Recovery		
1 (Control)	18	18	12	6	0	0
2 ISIS 721744	12	12	12	NA	1	0.1
3 ISIS 721744	12	12	12	NA	4	0.4
4 ISIS 721744	18	18	12	6	12	1.2
5 ISIS 722059 ^c	12	12	12	NA	4	0.4

NA = Not Applicable

a Group 1 was administered vehicle control article only.

b Up to 12 animals/sex/group (dependent on survival) were sacrificed on Day 185 of the dosing phase following dosing on Days 1, 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, and 183. Animals designated for recovery sacrifice (6 animals/sex in Groups 1 and 4) underwent 91 days of recovery following the last day of dose administration, recovery day began Day 185 and animals sacrificed on Day 91 of the recovery phase, Study Day 275.

c ISIS 722059 is a mouse-specific ASO targeting mouse PKK mRNA.

Observations and Results

Mortality

Animals were checked twice daily (AM and PM) for mortality.

There were no unscheduled deaths during the study that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

One male in the vehicle control group (No. M0006) was sacrificed in moribund condition on Day 94 of the treatment period due to a skin lesion in the dorsal cervical area that did not respond to initial treatment with topical antibiotic cream on Day 57 and became worse by Day 93.

One female at 1 mg/kg/dose (No. M0610) was sacrificed in moribund condition on Day 155 of the treatment period due to an injury that was potentially cage-related.

Clinical Signs

Cageside observations were conducted once daily during the treatment and recovery periods. Detailed observations were conducted once during the predose period, once weekly during the treatment period, and once weekly during the recovery period. Dose site(s) were evaluated prior to dosing on each day of dosing, beginning on Day 15.

There were no clinical observations during the study that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Body Weights

Body weights were recorded once during the predose period, weekly during the treatment period, and weekly during the recovery period.

There were no changes in body weight or body weight gain during the study that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Feed Consumption

Quantitative food consumption (grams/animal/day) for each cage was determined weekly during the treatment period and recovery period.

There were no changes in feed consumption during the study that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Ophthalmoscopy

Ophthalmic examinations of dilated eyes using an indirect ophthalmoscope were conducted by an ophthalmologist once during the predose period, once during Week 26 of the treatment period, and during the final week of the recovery period.

There were no ophthalmic findings during the study that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

ECG

Not evaluated

Hematology

Blood was collected for hematology evaluations on Day 185 of the treatment period and Recovery Day 91 of the recovery period. A representative panel of hematology parameters was evaluated.

As shown in **Table 8**, white blood cells, neutrophils, lymphocytes, and/or monocytes were increased at the end of the treatment period (Day 185) in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and at 4 mg/kg/dose ISIS 722059.

- White blood cells were increased in males at 4 and 12 mg/kg/dose ISIS 721744 and in females at 1, 4, and 12 mg/kg/dose ISIS 721744 and in males and females at 4 mg/kg/dose ISIS 722059.
- Neutrophils were increased in males at 1, 4, and 12 mg/kg/dose ISIS 721744 and in females at 12 mg/kg/dose ISIS 721744 and in males and females at 4 mg/kg/dose ISIS 722059.
- Lymphocytes were increased in males at 4 and 12 mg/kg/dose ISIS 721744 and in females at 1, 4, and 12 mg/kg/dose ISIS 721744 and in males and females at 4 mg/kg/dose ISIS 722059.
- Monocytes were increased in males and females at 4 and 12 mg/kg/dose ISIS 721744 and in males and females at 4 mg/kg/dose ISIS 722059.

At the end of the recovery period (Recovery Day 91) at 12 mg/kg/dose ISIS 721744, increases in hematology parameters were no longer evident.

Table 8 Hematology Treatment Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Parameter	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
WBC 10 ³ /mCL	1.91	1.64	3.20	3.49	3.96*	1.65	2.01	2.77	2.93	2.39
% Diff	-	-14.1	67.5	82.7	107.3	-	21.8	67.9	77.6	44.8
tCtrl	-	0.86x	1.68x	1.83x	2.07x	-	1.22x	1.68x	1.78x	1.45x
NEUT 10 ³ /mCL	0.36	0.57	0.71	0.85	0.61	0.32	0.29	0.36	0.52	0.47
% Diff	-	58.3	97.2	136.1	69.4	-	-9.4	12.5	62.5	46.9
tCtrl	-	1.58x	1.97x	2.36x	1.69x	-	0.91x	1.13x	1.63x	1.47x
LYMPH 10 ³ /mCL	1.41	0.97	2.22	2.43	3.14*	1.22	1.55	2.20	2.25	1.74
% Diff	-	-31.2	57.4	72.3	122.7	-	27.0	80.3	84.4	42.6
tCtrl	-	0.69x	1.57x	1.72x	2.23x	-	1.27x	1.80x	1.84x	1.43x
MONO 10 ³ /mCL	0.05	0.05	0.14*	0.10*	0.10*	0.04	0.04	0.09	0.05	0.06
% Diff	-	0.0	180.0	100.0	100.0	-	0.0	125.0	25.0	50.0
tCtrl	-	1.00x	2.80x	2.00x	2.00x	-	1.00x	2.25x	1.25x	1.50x

Abbreviations: % Diff = percent difference from vehicle control; LYMPH = lymphocytes; MONO = monocytes; NEUT = neutrophils; tCtrl = fold change from vehicle control; Veh Cont = vehicle control; WBC = white blood cells

Notes: Applicant's statistical analyses ANOVA and protected t-tests: * = p ≤ 0.05

Clinical Chemistry

Blood was collected for clinical chemistry evaluations on Day 185 of the treatment period and Recovery Day 91 of the recovery period. A representative panel of clinical chemistry parameters was evaluated.

As shown in **Table 9**, ALT, AST, and/or ALP were increased at the end of the treatment period (Day 185) in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744. Effects on clinical chemistry parameters (including ALT, AST, and ALP) were less clear at 4 mg/kg/dose ISIS 722059.

- ALT was increased in males and females at 4 and 12 mg/kg/dose ISIS 721744 and in males at 4 mg/kg/dose ISIS 722059.

- AST was increased in males at 1, 4, and 12 mg/kg/dose ISIS 721744 and in females at 4 and 12 mg/kg/dose ISIS 721744.
- ALP was increased in males at 4 and 12 mg/kg/dose ISIS 721744 and in females at 4 mg/kg/dose ISIS 721744 (no dose response).

As shown in **Table 10**, at the end of the recovery period (Recovery Day 91) for 12 mg/kg/dose ISIS 721744, small increases in ALT and/or AST were still evident in males and/or females, while increases in ALP were no longer evident. The toxicological significance of increases in ALT and AST were unclear as histopathology findings in the liver appeared to be partially reversible and levels of unconjugated ISIS 721744 had declined below the lower limit of quantitation.

Table 9 Clinical Chemistry Treatment Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Parameter	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
ALT (U/L)	30	35	70*	244*	47*	28	24	50	41	26
% Diff	-	16.7	133.3	713.3	56.7	-	-14.3	78.6	46.4	-7.1
tCtrl	-	1.17x	2.33x	8.13x ¹	1.57x	-	0.86x	1.79x	1.46x	0.93x
AST (U/L)	47	62	81	136	56	58	51	79	77	52*
% Diff	-	31.9	72.3	189.4	19.1	-	-12.1	36.2	32.8	-10.3
tCtrl	-	1.32x	1.72x	2.89x ¹	1.19x	-	0.88x	1.36x	1.33x	0.90x
ALP (U/L)	45	36	57	74	46	65	68	79	75	68
% Diff	-	-20.0	26.7	64.4	2.2	-	4.6	21.5	15.4	4.6
tCtrl	-	0.80x	1.27x	1.64x	1.02x	-	1.05x	1.22x	1.15x	1.05x

Abbreviations: % Diff = percent difference from vehicle control; ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses ANOVA and protected t-tests: * = $p \leq 0.05$

¹The study report states the following: One male (Animal M0315) at 12 mg/kg/dose ISIS 721744 had an incidental liver lobe torsion-related liver necrosis associated with markedly higher liver enzyme activities, which were not considered ISIS 721744-related. When the data from this animal are excluded the fold changes from vehicle control for ALT and AST are 2x and 1.5x, respectively.

Table 10 Clinical Chemistry Recovery Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Parameter	Males on Recovery Day 91					Females on Recovery Day 91				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
ALT (U/L)	36	-	-	49	-	41	-	-	60	-
% Diff	-	-	-	36.1	-	-	-	-	46.3	-
tCtrl	-	-	-	1.36x	-	-	-	-	1.46x	-
AST (U/L)	47	-	-	72	-	78	-	-	100	-
% Diff	-	-	-	53.2	-	-	-	-	28.2	-
tCtrl	-	-	-	1.53x	-	-	-	-	1.28x	-
ALP (U/L)	42	-	-	45	-	78	-	-	78	-
% Diff	-	-	-	7.1	-	-	-	-	0	-
tCtrl	-	-	-	1.07x	-	-	-	-	1.00x	-

Abbreviations: % Diff = percent difference from vehicle control; ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Urinalysis

Not evaluated.

Gross Pathology

Scheduled sacrifices for treatment period and recovery period animals were Day 185 and Recovery Day 91 (Day 275), respectively. A complete gross pathology examination occurred at the time of schedule necropsies.

As shown in **Table 11**, gross pathology findings at the end of treatment with ISIS 721744 were seen in the liver and inguinal lymph nodes. There were no clear gross pathology findings seen at the end of treatment with mouse surrogate ISIS 722059.

- Discoloration of the liver was noted in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and correlated with increased incidence and/or severity of hepatocyte vacuolation in males.
- Enlargement of the liver was noted in males at 4 mg/kg/dose ISIS 721744 and correlated with increased liver weights and moderate hepatocyte vacuolation.
- Enlarged inguinal lymph nodes were seen in males at 1 and 4 mg/kg/dose ISIS 721744 and correlated with increased lymphocytes.

As shown in **Table 12**, gross pathology findings at the recovery period sacrifice at 12 mg/kg/dose ISIS 721744 were noted in the liver and subcutaneous injection site.

- Discoloration of the liver was noted in males and correlated with slight hepatocyte vacuolation in one male or hepatocellular adenoma or focus of cellular alteration in the other males.
- Discoloration at the SC injection site was noted in males.

Table 11 Gross Pathology Treatment Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
Liver n =	12	12	12	12	12	12	11	12	12	12
Discolored; entire; tan; collected	0	1	3	4	0	0	0	0	1	0
Discolored; multiple, indistinct; red, tan; collected	0	0	0	1	0	0	0	0	0	0
Discolored; multiple, pinpoint; tan; collected	0	0	0	0	0	0	1	0	0	0
Discolored; multiple, up to 2 mm ² ; tan; collected	0	0	0	0	0	0	0	1	0	0
Discolored; single, up to 10 mm ² ; tan; collected	0	1	0	0	0	0	0	0	0	0
Discolored; single, up to 5 mm ² ; tan; collected	0	1	0	0	0	0	0	0	0	0
Large; present; collected	0	0	2	0	0	0	0	0	0	0
Mass; single; green; firm; cut surface same; collected	0	0	0	0	0	0	0	1	0	0
Mass; single; tan; soft; cut surface-not required; collected	0	0	0	1	0	0	0	0	0	0
Lymph Node, Inguinal n =	12	12	12	12	12	12	11	12	12	12
Large; present; collected	0	1	1	0	0	0	0	0	0	0
Lung n =	12	12	12	12	12	12	11	12	12	12

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
Mass; single; white; firm; cut surface-not required; collected	0	0	0	0	1	0	0	0	0	0
Raised area; single, up to 2 mm ³ ; white; soft; collected	0	0	1	0	0	0	0	0	0	0

Abbreviations: n = number examined; Veh Cont = vehicle control

Table 12 Gross Pathology Recovery Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Organ/Tissue	Males on Recovery Day 91					Females on Recovery Day 91				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
Liver n =	5	-	-	6	-	6	-	-	6	-
Discolored	0	-	-	3	-	1	-	-	1	-
Subcutaneous Injection Site, A n =	5	-	-	6	-	6	-	-	6	-
Discolored	0	-	-	2	-	0	-	-	0	-

Abbreviations: n = number examined; Veh Cont = vehicle control

Organ Weights

Adrenal, brain, gall bladder, heart, kidney, liver with gallbladder, lung with large bronchi, ovary with oviduct, prostate, seminal vesicle, spleen, testis, thymus, and uterus with cervix were weighed on Day 185 of the treatment period and on Recovery Day 91 (Day 275) of the recovery period. Paired organs were weighed together.

Notable organ weight changes at the end of the treatment period sacrifice are shown in **Table 13**. Liver weights (absolute, relative to body weight, and/or relative to brain weight) were increased in males and/or females at 4 and/or 12 mg/kg/dose ISIS 721744. Increased liver weights correlated with gross pathology finding of enlarged liver in two males and histopathology finding of hepatocellular hypertrophy in some males

and one female. Spleen weights (absolute, relative to body weight, and/or relative to brain weight) were increased in males at 12 mg/kg/dose. Increased spleen weight correlated with histopathology finding of increased extramedullary hematopoiesis. There were no organ weight changes at 4 mg/kg/dose ISIS 722059.

Notable organ weight changes at the end of the recovery period sacrifice at 12 mg/kg/dose ISIS 721744 are shown in **Table 14**. Liver weights (absolute, relative to body weight, and/or relative to brain weight) were still increased in males and/or females and correlated with histopathology finding of hepatocyte vacuolation.

Table 13 Organ Weight Treatment Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 (mg/kg/dose)
	0	1	4	12	4	0	1	4	12	4
Liver Weight (g)	2.19	2.28	2.86*	2.84*	2.24	1.56	1.55	1.68	1.68	1.67
% Diff	-	4.33	30.62	29.94	2.42	-	-0.51	7.83	7.99	7.41
tCtrl	-	1.04x	1.31x	1.30x	1.02x	-	0.99x	1.08x	1.08x	1.07x
Liver (% BW)	4.34	4.40	5.05*	5.11*	4.59	3.78	3.98	4.17	4.18	3.94
% Diff	-	1.51	16.52	17.79	5.88	-	5.10	10.12	10.61	4.12
tCtrl	-	1.02x	1.17x	1.18x	1.06x	-	1.05x	1.10x	1.11x	1.04x
Liver (% BrW)	430.4	444.7	553.9	549.1	439.5	297.3	291.9	320.8	315.0	325.3
% Diff	-	3.32	28.69	27.58	2.10	-	-1.82	7.92	5.96	9.42
tCtrl	-	1.03x	1.29x	1.28x	1.02x	-	0.98x	1.08x	1.06x	1.09x
Spleen Weight (g)	0.10	0.12	0.12	0.14*	0.11	0.12	0.14	0.13	0.12	0.13
% Diff	-	14.09	17.26	32.98	2.30	-	15.35	6.24	0.66	9.61
tCtrl	-	1.14x	1.17x	1.33x	1.02x	-	1.15x	1.06x	1.01x	1.10x
Spleen (% BW)	0.21	0.24	0.22	0.25	0.22	0.30	0.36	0.32	0.31	0.32
% Diff	-	12.74	5.68	20.86	4.20	-	21.94	8.04	3.31	6.35
tCtrl	-	1.13x	1.06x	1.21x	1.04x	-	1.22x	1.08x	1.03x	1.06x
Spleen (% BrW)	20.53	23.14	23.77	26.76	20.85	23.24	26.60	24.76	22.99	25.97
% Diff	-	12.75	15.79	30.36	1.60	-	14.43	6.52	-1.11	11.75
tCtrl	-	1.13x	1.16x	1.30x	1.02x	-	1.14x	1.07x	0.99x	1.12x

Abbreviations: % Diff = percent difference from vehicle control; BW = body weight; BrW = brain weight; g = grams; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses * = $p \leq 0.05$

Table 14 Organ Weight Recovery Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

	Males on Recovery Day 91					Females on Recovery Day 91				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
Liver Weight (g)	2.02	-	-	2.20	-	1.44	-	-	1.69	-
% Diff	-	-	-	8.93	-	-	-	-	17.14	-
tCtrl	-	-	-	1.09x	-	-	-	-	1.17x	-
Liver (% BW)	3.86	-	-	4.37	-	2.88	-	-	3.58*	-
% Diff	-	-	-	13.07	-	-	-	-	24.51	-
tCtrl	-	-	-	1.13x	-	-	-	-	1.25x	-
Liver (% BrW)	372.69	-	-	427.58	-	286.51	-	-	327.64	-
% Diff	-	-	-	14.73	-	-	-	-	14.36	-
tCtrl	-	-	-	1.15x	-	-	-	-	1.14x	-

Abbreviations: % Diff = percent difference from vehicle control; BW = body weight; BrW = brain weight; g = grams; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses * = $p \leq 0.05$

Histopathology

Adequate Battery

Yes. The organ/tissue list is shown in **Table 15** (Applicant's Table).

Table 15 Tissue List for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery (Applicant's Table)

Organ/Tissue		Organ/Tissue	
adrenal (2)	W P,E	lymph node (mesenteric)	P,E
aorta	P,E	mammary gland (females)	P,E
bone, femur with bone marrow (articular surface of the distal end to include stifle joint)	P,E	muscle, biceps femoris	P,E
bone, sternum with bone marrow	P,E	optic nerve (2) ^c	P,E
brain	W P,E	ovary (2) ^{a,e}	W P,E
cecum	P,E	oviduct (2) ^a	W P,E
cervix ^a	W P,E	pancreas	P,E
coagulating gland (2) (intact with seminal vesicles)	P,E	pituitary gland	P,E
colon	P,E	prostate	W P,E
dose site(s) ^b	P,E	rectum	P,E
duodenum	P,E	salivary gland (mandibular with sublingual [2	P,E
epididymis (2)	P,E	sciatic nerve	P,E
esophagus	P,E	seminal vesicle	W P,E
eye (2) ^c	P,E	skin/subcutis	P,E
gall bladder ^a	W P,E	spinal cord (cervical, thoracic, and lumbar)	P,E
gut-associated lymphoid tissue (GALT) - (Peyer's Patch)	P,E	spleen	W P,E
Harderian gland ^a	P,E	stomach	P,E
Heart ^{d,e}	W P,E	testis (2) ^{c,e}	W P,E
ileum	P,E	thymus	W P,E
jejunum	P,E	thyroid (2 lobes) with parathyroid	P,E
kidney (2) ^e	W P,E	tongue	P,E
lesions	P,E	trachea	P,E
liver ^a	W P,E	urinary bladder ^d	P,E
lung with large bronchi ^d	W P,E	uterus ^a	W P,E
lymph node (mandibular)	P,E	vagina	P,E
lymph node (inguinal)	P,E		

E = Examined microscopically; P = Processed; W = Weighed.

a Organs weighed together; liver with gall bladder (drained), ovary with oviduct, and uterus with cervix.

b All dose sites were shaved, collected, and documented accordingly.

c Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

d Lungs were infused with formalin via the trachea, and formalin was also infused into the urinary bladder.

e All six animals/sex/group designated for tissue collection for bioanalytical analysis had the right organ collected for histology. All remaining six animals/sex/group had the left and right organ collected for histology.

Peer Review

Yes. The Applicant conducted an independent peer review of the primary histopathology evaluation. As stated in the study report, a retrospective peer review of the liver and testes was conducted by a different Ionis pathologist than the original contemporaneous peer review.

Histological Findings

As shown in **Table 16**, histopathology findings at the end of treatment with ISIS 721744 were seen in the liver, skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen. Histopathology findings at the end of treatment with mouse surrogate ISIS 722059 were seen in the skin/subcutis, SC dose sites, spleen, and testis.

- **Liver:** Minimal or slight centrilobular hepatocellular hypertrophy was noted in males at 1, 4, and 12 mg/kg/dose ISIS 721744 and in one female (moderate) at 12 mg/kg/dose ISIS 721744. This finding correlated with increased mean liver weights at 4 and 12 mg/kg/dose ISIS 721744 and increased liver enzymes (AST, ALT, and/or ALP). Despite a background incidence of hepatocyte vacuolation in vehicle control males, there was an increase in the severity (slight to marked) of hepatocyte vacuolation in males at 4 and 12 mg/kg/dose ISIS 721744, which correlated with gross pathology findings of liver discoloration at all dose levels. These findings correlated with uptake of ISIS 721744 into the liver.
- **Skin/Subcutis:** Increased incidences and/or severity of infiltrates of mononuclear cells, mixed cells, and/or vacuolated macrophages in the skin/subcutis were noted in males and/or females at 4 and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. Increased incidences and/or severity of vacuolated fibrocytes in the skin/subcutis were noted in males and/or females at 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059. Slight to moderate inflammation in the skin was noted in females at 4 and 12 mg/kg/dose ISIS 721744. These findings in the skin/subcutis correlated with increased leukocyte counts.
- **SC Dose Sites:** Increased incidences and/or severity of infiltrates of vacuolated macrophages, mononuclear cells, and mixed cells, as well as fibrocyte vacuolation at the SC injection sites were noted in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. These findings at the SC injection sites correlated with increased leukocyte counts.
- **Inguinal Lymph Nodes:** Increased incidences and severities (minimal to marked) of increased lymphocytes in males at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and one female at 12 mg/kg/dose ISIS 721744 were noted in the inguinal lymph node. This finding in the inguinal lymph node correlated with increased leukocyte counts. Amyloid in the inguinal lymph node was noted in one male at 4 mg/kg/dose ISIS 722059.
- **Spleen:** Increased incidence and/or severity of extramedullary hematopoiesis in the spleen was noted in males and/or females at 1, 4 and/or 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059 and correlated with increased mean spleen weight in males administered 12 mg/kg/dose ISIS 721744.

- Testis: Moderate interstitial cell hyperplasia, minimal sperm stasis, and minimal vacuolation were noted in the testis of males at 4 mg/kg/dose ISIS 722059.

As shown in **Table 17**, histopathology findings at the recovery period sacrifice at 12 mg/kg/dose ISIS 721744 were still noted in the liver as hepatocellular vacuolation in males and females, which correlated with increased liver weights. Histopathology findings in the skin/subcutis, SC dose sites, inguinal lymph nodes, and/or spleen were either not seen or less evident at the end of the recovery period at 12 mg/kg/dose ISIS 721744.

Table 16 Histopathology Treatment Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	1	4	12	4	0	1	4	12	4
Liver n =	12	12	12	12	12	12	11	12	12	12
B-Adenoma, hepatocellular	0	1	0	1	0	0	0	0	0	0
Focus, cellular alteration, basophilic	0	1	0	0	0	0	0	0	0	0
...minimal	0	1	0	0	0	0	0	0	0	0
Hypertrophy, hepatocyte, centrilobular	0	1	2	5	0	0	0	0	1	0
...minimal	0	0	2	3	0	0	0	0	0	0
...slight	0	1	0	2	0	0	0	0	0	0
...moderate	0	0	0	0	0	0	0	0	1	0
Infiltrate, mixed cell	2	1	4	2	0	3	2	5	4	1
...minimal	2	1	4	2	0	3	1	5	4	1
...slight	0	0	0	0	0	0	1	0	0	0
Infiltrate, mononuclear cell	5	5	4	5	8	6	7	6	4	9
...minimal	5	5	4	5	8	6	7	6	4	9
Inflammation	0	0	0	0	0	0	2	1	1	0
...slight	0	0	0	0	0	0	2	1	1	0
Necrosis	0	0	0	3	0	0	3	3	0	0
...minimal	0	0	0	1	0	0	3	1	0	0
...slight	0	0	0	0	0	0	0	1	0	0
...moderate	0	0	0	2	0	0	0	1	0	0

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	1	4	12		4	0	1	4
Vacuolation, hepatocyte	7	6	8	6	7	1	0	1	0	0
...minimal	3	3	1	3	4	1	0	0	0	0
...slight	3	3	3	1	3	0	0	1	0	0
...moderate	1	0	4	1	0	0	0	0	0	0
...marked	0	0	0	1	0	0	0	0	0	0
Skin/Subcutis n =	12	12	12	12	12	12	11	12	12	12
Infiltrate, macrophage, vacuolated	0	0	2	7	4	0	0	0	1	2
...minimal	0	0	1	6	2	0	0	0	0	2
...slight	0	0	1	1	2	0	0	0	1	0
Infiltrate, mixed cell	1	0	2	5	0	5	0	2	5	5
...minimal	0	0	2	2	0	5	0	2	3	4
...slight	1	0	0	3	0	0	0	0	1	1
...moderate	0	0	0	0	0	0	0	0	1	0
Infiltrate, mononuclear cell	2	2	0	2	3	1	0	3	2	2
...minimal	2	2	0	2	2	1	0	2	2	1
...slight	0	0	0	0	1	0	0	1	0	1
Inflammation	0	0	0	0	0	0	0	2	1	0
...slight	0	0	0	0	0	0	0	2	0	0
...moderate	0	0	0	0	0	0	0	0	1	0
Vacuolation, fibrocyte	0	0	0	3	3	0	0	0	1	5
...minimal	0	0	0	2	3	0	0	0	1	4
...slight	0	0	0	1	0	0	0	0	0	1
Subcutaneous Injection Site, A n =	12	12	12	11	10	12	11	12	12	12
Infiltrate, macrophages, vacuolated	0	0	6	4	4	0	2	1	7	6
...minimal	0	0	4	3	4	0	2	1	6	4
...slight	0	0	2	1	0	0	0	0	1	2
Infiltrate, mixed cell	0	0	0	1	0	3	4	2	5	4
...minimal	0	0	0	0	0	3	2	1	4	3
...slight	0	0	0	1	0	0	2	1	1	1

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	1	4	12		4	0	1	4
Infiltrate, mononuclear cell	2	1	1	3	5	1	1	1	0	1
...minimal	2	1	1	2	4	1	0	1	0	0
...slight	0	0	0	1	1	0	1	0	0	1
Vacuolation, fibrocyte	0	0	1	3	5	0	0	0	2	4
...minimal	0	0	1	3	4	0	0	0	2	2
...slight	0	0	0	0	1	0	0	0	0	1
...moderate	0	0	0	0	0	0	0	0	0	1
Subcutaneous Injection Site, B n =	12	12	12	12	12	12	11	12	12	12
Infiltrate, macrophages, vacuolated	0	2	4	4	7	0	2	5	11	11
...minimal	0	1	4	4	7	0	2	2	5	7
...slight	0	1	0	0	0	0	0	2	6	3
...moderate	0	0	0	0	0	0	0	1	0	1
Infiltrate, mixed cell	2	2	2	2	0	4	1	2	3	2
...minimal	2	2	2	2	0	3	1	1	1	1
...slight	0	0	0	0	0	1	0	1	2	0
...moderate	0	0	0	0	0	0	0	0	0	1
Infiltrate, mononuclear cell	1	1	3	2	1	0	1	0	1	3
...minimal	1	1	2	2	1	0	1	0	1	0
...slight	0	0	1	0	0	0	0	0	0	2
...moderate	0	0	0	0	0	0	0	0	0	1
Vacuolation, fibrocyte	0	0	2	8	8	0	1	6	9	10
...minimal	0	0	2	6	8	0	1	4	7	5
...slight	0	0	0	2	0	0	0	2	2	5
Lymph Node, Inguinal n =	12	12	12	12	12	12	11	12	12	12
Amyloid	0	0	0	0	1	0	0	1	0	0
...minimal	0	0	0	0	0	0	0	1	0	0
...moderate	0	0	0	0	1	0	0	0	0	0
Infiltrate, macrophages	0	0	0	1	0	1	0	0	0	0
...slight	0	0	0	1	0	1	0	0	0	0
Inflammation	0	0	0	0	1	0	0	0	0	0

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	1	4	12		4	0	1	4
...slight	0	0	0	0	1	0	0	0	0	0
Lymphocytes, increased	4	6	5	8	3	6	3	2	6	5
...minimal	4	2	1	3	3	5	2	2	4	3
...slight	0	3	3	2	0	1	1	0	1	2
...moderate	0	1	1	1	0	0	0	0	0	0
...marked	0	0	0	2	0	0	0	0	1	0
Spleen n =	12	12	12	12	12	12	11	12	12	12
Hematopoiesis, extramedullary, increased	3	5	7	8	4	3	3	7	5	6
...minimal	3	5	5	4	3	3	3	4	3	4
...slight	0	0	2	4	1	0	0	3	2	1
...moderate	0	0	0	0	0	0	0	0	0	1
Testis n =	12	12	12	12	12	NA	NA	NA	NA	NA
Degeneration, seminiferous tubule	4	0	2	2	1	NA	NA	NA	NA	NA
...minimal	4	0	2	1	0	NA	NA	NA	NA	NA
...slight	0	0	0	1	0	NA	NA	NA	NA	NA
...moderate	0	0	0	0	1	NA	NA	NA	NA	NA
Hyperplasia, interstitial cell	0	0	0	0	1	NA	NA	NA	NA	NA
...moderate	0	0	0	0	1	NA	NA	NA	NA	NA
Stasis, sperm	0	0	1	0	1	NA	NA	NA	NA	NA
...minimal	0	0	1	0	1	NA	NA	NA	NA	NA
Vacuolation	0	1	0	0	3	NA	NA	NA	NA	NA
...minimal	0	1	0	0	3	NA	NA	NA	NA	NA
Epididymis n =	12	12	12	12	12	NA	NA	NA	NA	NA
Debris, cellular, lumen	0	2	2	0	1	NA	NA	NA	NA	NA
...minimal	0	2	2	0	1	NA	NA	NA	NA	NA
Granuloma, sperm	0	0	0	1	0	NA	NA	NA	NA	NA
...minimal	0	0	0	1	0	NA	NA	NA	NA	NA
Hypospermia	1	0	0	1	1	NA	NA	NA	NA	NA
...marked	0	0	0	1	1	NA	NA	NA	NA	NA
...severe	1	0	0	0	0	NA	NA	NA	NA	NA
Infiltrate, mononuclear cell	0	1	1	2	2	NA	NA	NA	NA	NA

Organ/Tissue	Males on Day 185					Females on Day 185				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	1	4	12		4	0	1	4
...minimal	0	1	1	2	1	NA	NA	NA	NA	NA
...slight	0	0	0	0	1	NA	NA	NA	NA	NA
Inflammation	0	0	0	1	0	NA	NA	NA	NA	NA
...minimal	0	0	0	1	0	NA	NA	NA	NA	NA
Lung n =	12	12	12	12	12	12	11	12	12	12
B-Adenoma, bronchiolo- alveolar	0	0	1	0	2	0	0	0	0	0
Hyperplasia, bronchiolo- alveolar	2	0	0	1	0	0	1	0	0	0
...minimal	1	0	0	0	0	0	1	0	0	0
...slight	1	0	0	1	0	0	0	0	0	0
Hyperplasia, epithelium alveolus	0	0	0	0	1	0	0	0	0	0
...slight	0	0	0	0	1	0	0	0	0	0
Infiltrate, macrophages, pigmented	0	0	0	0	1	0	0	0	0	0
...moderate	0	0	0	0	1	0	0	0	0	0
Infiltrate, mononuclear cell	0	0	2	0	0	1	2	0	0	1
...minimal	0	0	0	0	0	1	1	0	0	1
...slight	0	0	2	0	0	0	1	0	0	0
Inflammation	0	0	0	1	0	0	1	0	0	0
...minimal	0	0	0	1	0	0	1	0	0	0
Metaplasia, osseous	0	0	0	0	0	0	0	0	1	0
...minimal	0	0	0	0	0	0	0	0	1	0

Abbreviations: n = number examined; NA = not applicable; Veh Cont = vehicle control

Table 17 Histopathology Recovery Period Findings for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery

Organ/Tissue	Males on Recovery Day 91					Females on Recovery Day 91				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
		0	1	4	12		4	0	1	4
Liver n =	5	-	-	6	-	6	-	-	6	-
B-Adenoma, hepatocellular	1	-	-	1	-	0	-	-	0	-
Focus, cellular alteration, basophilic	0	-	-	2	-	0	-	-	0	-
...minimal	0	-	-	1	-	0	-	-	0	-
...slight	0	-	-	1	-	0	-	-	0	-
Hypertrophy, hepatocyte, centrilobular	0	-	-	0	-	0	-	-	1	-
...minimal	0	-	-	0	-	0	-	-	1	-
Infiltrate, mixed cell	0	-	-	1	-	2	-	-	2	-
...minimal	0	-	-	1	-	1	-	-	2	-
...slight	0	-	-	0	-	1	-	-	0	-
Infiltrate, mononuclear cell	2	-	-	5	-	1	-	-	3	-
...minimal	2	-	-	5	-	1	-	-	3	-
Necrosis	0	-	-	0	-	3	-	-	0	-
...minimal	0	-	-	0	-	2	-	-	0	-
...slight	0	-	-	0	-	1	-	-	0	-
Pigment, Kupffer cell	0	-	-	0	-	0	-	-	2	-
...minimal	0	-	-	0	-	0	-	-	2	-
Vacuolation, hepatocyte	2	-	-	4	-	0	-	-	4	-
...minimal	1	-	-	3	-	0	-	-	2	-
...slight	1	-	-	1	-	0	-	-	2	-
Lung n =	5	-	-	6	-	6	-	-	6	-
B-Adenoma, bronchiolo-alveolar	0	-	-	0	-	1	-	-	0	-
Exudate, lumen, bronchiole	1	-	-	0	-	0	-	-	0	-
...minimal	1	-	-	0	-	0	-	-	0	-
Infiltrate, mononuclear cell	0	-	-	0	-	1	-	-	0	-
...slight	0	-	-	0	-	1	-	-	0	-
Inflammation	0	-	-	0	-	1	-	-	0	-

Organ/Tissue	Males on Recovery Day 91					Females on Recovery Day 91				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	1	4	12		4	0	1	4
...minimal	0	-	-	0	-	1	-	-	0	-
Thrombus	0	-	-	0	-	1	-	-	0	-
...minimal	0	-	-	0	-	1	-	-	0	-
Lymph Node, Inguinal n =	5	-	-	6	-	6	-	-	6	-
Lymphocytes, increased	0	-	-	1	-	0	-	-	0	-
...slight	0	-	-	1	-	0	-	-	0	-
Skin/Subcutis n =	5	-	-	6	-	6	-	-	6	-
Inflammation	0	-	-	0	-	2	-	-	0	-
...minimal	0	-	-	0	-	2	-	-	0	-
Spleen n =	5	-	-	6	-	6	-	-	6	-
Hematopoiesis, extramedullary, increased	2	-	-	1	-	2	-	-	3	-
...minimal	2	-	-	1	-	1	-	-	0	-
...slight	0	-	-	0	-	1	-	-	3	-
Subcutaneous Injection Site, A n =	5	-	-	6	-	5	-	-	6	-
Infiltrate, mononuclear cell	0	-	-	1	-	1	-	-	0	-
...minimal	0	-	-	1	-	1	-	-	0	-
Inflammation	0	-	-	1	-	1	-	-	0	-
...slight	0	-	-	0	-	1	-	-	0	-
...moderate	0	-	-	1	-	0	-	-	0	-
Subcutaneous Injection Site, B n =	5	-	-	6	-	6	-	-	6	-
Infiltrate, mixed cell	0	-	-	1	-	0	-	-	0	-
...slight	0	-	-	1	-	0	-	-	0	-
Inflammation	0	-	-	0	-	1	-	-	0	-
...minimal	0	-	-	0	-	1	-	-	0	-
Testis n =	5	-	-	6	-	NA	-	-	NA	-
Degeneration, seminiferous tubule	1	-	-	0	-	NA	-	-	NA	-
...minimal	1	-	-	0	-	NA	-	-	NA	-
Stasis, sperm	1	-	-	0	-	NA	-	-	NA	-
...minimal	1	-	-	0	-	NA	-	-	NA	-
Vacuolation	0	-	-	1	-	NA	-	-	NA	-

Organ/Tissue	Males on Recovery Day 91					Females on Recovery Day 91				
	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh Cont	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	1	4	12		4	0	1	4
...minimal	0	-	-	1	-	NA	-	-	NA	-
Epididymis n =	5	-	-	6	-	NA	-	-	NA	-
Infiltrate, mixed cell	0	-	-	1	-	NA	-	-	NA	-
...minimal	0	-	-	1	-	NA	-	-	NA	-
Infiltrate, mononuclear cell	2	-	-	0	-	NA	-	-	NA	-
...minimal	1	-	-	0	-	NA	-	-	NA	-
...slight	1	-	-	0	-	NA	-	-	NA	-

Abbreviations: n = number examined; NA = not applicable; Veh Cont = vehicle control

Special Evaluation

Exploratory Cytokine/Chemokine Analysis

Blood was collected for cytokine/chemokine analysis (IFN- γ , IL-1 β , IL-4, IL-5, IL-6, IL-10, IL-13, TNF- α , and MCP-1A) using a multiplex assay kit on Day 185 of the treatment period and Recovery Day 91 of the recovery period.

There were no clear effects of ISIS 721744 or mouse surrogate ISIS 722059 on concentrations of IFN- γ , IL-1 β , IL-4, IL-5, IL-6, IL-10, IL-13, TNF- α , and MCP-1A.

IL-10, IL-13, IL-1 β , IL-4, and IL-5 concentrations were below the limit of quantitation for samples tested from the vehicle control, ISIS 721744-treated, and ISIS 722059-treated groups.

IL-6 concentrations were at or below the limit of quantitation (<29.5 pg/mL) for all samples tested in the vehicle control, ISIS 721744-treated, and ISIS 722059-treated groups except for male No. M0006 in the vehicle control group with an elevated IL-6 concentration (202 pg/mL) on Day 94 (animal was terminated for a non-treatment related injury).

IFN- γ concentrations were low or below the limit of quantitation (<2.87 pg/mL) for most samples tested in the vehicle control, ISIS 721744-treated, and ISIS 722059-treated groups except for female No. M0606 at 1 mg/kg/dose ISIS 721744 with a slight increase in IFN- γ concentration (56.4 pg/mL).

TNF- α concentrations were below the limit of quantitation (<6.60 pg/mL) or just above the limit of quantitation for all samples tested in the vehicle control, ISIS 721744-treated, and ISIS 722059-treated groups.

MCP-1 was measurable at low concentrations in most samples in the vehicle control, ISIS 721744-treated, and ISIS 722059-treated groups with no clear effect of treatment.

Toxicokinetics

Tissue Analysis

Liver samples from vehicle control and ISIS 721744-treated groups and kidney samples from ISIS 721744-treated groups were analyzed for unconjugated ISIS 721744 by a validated UPLC-MS/MS analytical method.

There was no detectable unconjugated ISIS 721744 in the vehicle control liver samples.

As shown in **Table 18** (Applicant's Table), unconjugated ISIS 721744 was measurable in a dose-dependent manner in the kidney (greater than dose proportional) and liver (dose proportional) after 26 weeks of treatment with ISIS 721744. The mean concentrations of unconjugated ISIS 721744 were similar in the kidney and liver except at 12 mg/kg/dose where mean unconjugated ISIS 721744 concentrations were greater in the kidney than in the liver. Mean unconjugated ISIS 721744 concentrations were higher in females than in males in the kidney but similar in the liver. After the 13-week treatment-free recovery period, mean unconjugated ISIS 721744 concentrations at 12 mg/kg/dose ISIS 721744 were below or near the lower limit of quantitation of 0.107 mcg/g, suggesting complete clearance of ISIS 721744 at this dose level.

Table 18 Unconjugated ISIS 721744 Tissue Concentrations for 26-Week Repeat Dose Toxicity Study of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice with a 13-Week Recovery (Applicant's Table)

Matrix	Group	Dose Level (mg/kg)	Number of Doses	Study Day	Time From Last Dose (days)	N Female/Male	Unconjugated ISIS 721744			
							(µg/g) ^a		(µM)	
							Female	Male	Female	Male
Kidney	2	1	14	185	2	6/6	5.41 ± 0.899	2.63 ± 0.529	0.756 ± 0.126	0.367 ± 0.0739
	3	4	14	185	2	6/6	40.0 ± 9.24	22.3 ± 5.52	5.60 ± 1.29	3.12 ± 0.771
	4	12	14	185	2	6/6	177 ± 18.5	86.4 ± 45.7	24.8 ± 2.58	12.1 ± 6.39
	4	12	14	275	91	6/6 ^b	0.254 ± 0.181	0.323 ± 0.602	0.0356 ± 0.0253	0.0452 ± 0.0842
Liver	2	1	14	185	2	6/6	4.12 ± 0.738	6.24 ± 1.31	0.576 ± 0.103	0.873 ± 0.183
	3	4	14	185	2	6/6	20.7 ± 2.83	24.9 ± 5.91	2.90 ± 0.396	3.49 ± 0.826
	4	12	14	185	2	6/6	51.0 ± 20.7	42.6 ± 21.7	7.12 ± 2.89	5.96 ± 3.04
	4	12	14	275	91	6/6 ^c	0.156 ± 0.132	0.0350 ± 0.0859	0.0219 ± 0.0185	0.00490 ± 0.0120

Note: Values are presented as mean ± standard deviation; Lower limit of quantitation (LLOQ) was 0.015 µM/0.107 µg/g

^a Unconjugated ISIS 721744 concentration (µg/g) in tissue was calculated by [(concentration in µM) × [(Molecular Weight of Unconjugated ISIS 721744)/1000]]

(Molecular weight of ISIS 721744 = 7152.9979 g/Mol)

^b 2 male values were below the lower limit of quantitation

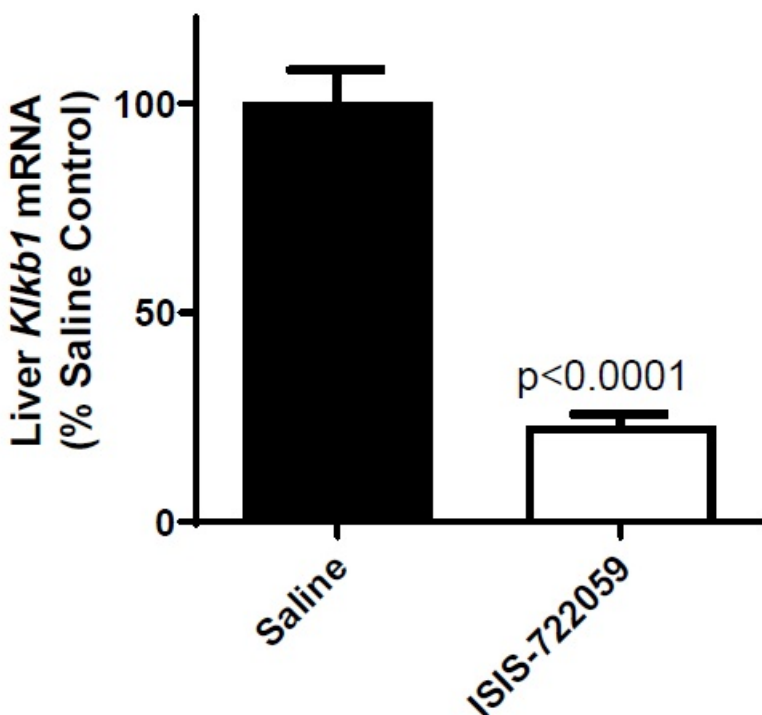
^c 2 female and 4 male values were below the lower limit of quantitation

PKK mRNA Analysis

Liver samples from vehicle control and mouse surrogate ISIS 722059 were collected on Day 185 of the treatment period for PKK mRNA analysis by RT-PCR.

As shown in **Figure 7** (Applicant's Figure), mouse PKK mRNA in mouse liver was reduced by 77% (statistically significant) compared to vehicle control in mice treated with mouse surrogate ISIS 722059 at 4 mg/kg/dose for 26 weeks.

Figure 7 Mouse PKK mRNA in Mouse Liver from 26-Week Repeat Dose Toxicity Study with Mouse Surrogate ISIS 722059 (Applicant's Figure)



Dosing Solution Analysis

The test articles were provided preformulated by the Applicant. The Applicant conducted stability analyses on representative ISIS 721744 test articles within the bracketed concentrations of 0.1 and 200 mg/mL (lot # RP721744-009 and lot # RP721744-012, respectively) by a qualified ion-pair high-performance liquid chromatography with ultraviolet detection and mass spectrometry (IP-HPLC-UV-MS) method.

In the stability analysis for ISIS 721744 at 0.1 mg/mL, degradation impurities were increased >50% in comparison to the ISIS 721744 release results, during the mass spectrum overlay assessment. Upon a more detailed evaluation, the level of degradation impurities was within the bulk drug substance specification limit and the label claim was within the acceptable range of (b) (4) % so the test article was considered suitable for use. There was no change in the impurity profile or stability for ISIS 721744 at 200 mg/mL.

All formulations were within $\pm 15\%$ of the target concentrations, ranging from 94.8% to 109.5% of theoretical, and met the acceptance criteria for study use.

11 Integrated Summary and Safety Evaluation

The Applicant is developing donidalorsen (ISIS 721744) as a prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. ISIS 721744 is a GalNAc-conjugated ASO, which binds to human PKK mRNA to reduce PKK protein production. The GalNAc molecule preferentially delivers ISIS 721744 to the liver by binding to the ASGPR on liver cells.

The nonclinical development program included primary pharmacology (in vitro and in vivo), safety pharmacology (in vivo cardiovascular, respiratory, and CNS in monkeys and in vitro hERG) PK/ADME, repeat-dose toxicity (39-week in monkeys and 13- and 26-week in mice), genotoxicity (in vitro Ames assay, in vitro chromosome aberration assay, and in vivo bone marrow micronucleus assay in mice), carcinogenicity (6-month Tg.rash2 mice), and reproductive and developmental toxicology (combined fertility and development in mice and PPNP in mice) studies. The main nonclinical species used in the pharmacology and toxicology studies were monkeys and mice. The sequence targeted by ISIS 721744 has one mismatch to the binding site in Cynomolgus monkey PKK mRNA (position 19). Despite this mismatch, ISIS 721744 showed dose-dependent inhibition of hepatic target mRNA expression and reductions in PKK protein and activity levels in peripheral circulation of Cynomolgus monkeys. ISIS 721744 does not align perfectly to the mouse PKK gene sequence. As such, in vivo studies in mice evaluated mouse-specific PKK ASOs (either unconjugated ISIS 482584 in the pharmacology studies or conjugated ISIS 722059 in the toxicology studies).

Primary Pharmacology

Donidalorsen (ISIS 721744) is an ASO GalNAc conjugate that causes RNase H1 mediated degradation of PKK mRNA through selective binding to PKK mRNA, which results in reduced production of PKK protein. There is high concordance of the nonclinical pharmacology and clinical efficacy data for inhibition of PKK. PKK is a target of several approved HAE therapies (small molecules and monoclonal antibodies).

In Vitro Pharmacology

Specificity analysis of ISIS 721744 was performed with unconjugated PKK ASO, ISIS 546254, which has the same sequence. ISIS 546254 is perfectly matched to only PKK RNA in human transcriptome (targets site free of meaningful sequence variation). Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

Treatment of HepatoPac® (co-culture system of primary human hepatocytes and stromal cells [mouse fibroblasts]) with ISIS 721744 and ISIS 546254 (unconjugated parent compound of ISIS 721744) resulted in a dose-dependent reduction of PKK mRNA. The IC₅₀ for ISIS 721744 and ISIS 546254 were 0.19 μM and 8.73 μM, respectively, indicating that ISIS 721744 is about 46-fold more potent than ISIS 546254 (likely due to cellular uptake through hepatocyte-specific ASGPR). Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

ISIS 546254 reduced human PKK mRNA levels by greater than 90% in a concentration-dependent manner in human hepatocellular cell lines HepaRG and Hep3b with

corresponding IC_{50} of 0.31 μ M and 0.35 μ M, respectively. The control oligonucleotide ISIS 141923 did not reduce PKK mRNA in either cell line.

In Vivo Pharmacology

ISIS 721744 does not have perfect alignment to the mouse PKK gene sequence. As such, an unconjugated PKK ASO mouse surrogate (i.e., ISIS 482584) was used for in vivo pharmacology studies in mice. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

Male C57Bl/6 albino mice were treated by SC administration with vehicle or unconjugated PKK ASO mouse surrogate ISIS 482584 (5, 10, 20, 40, 80, or 160 mg/kg/week) for 3 weeks. ISIS 482584 inhibited PKK liver mRNA and PKK plasma protein expression in a dose-dependent manner. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

Permeability assays were performed in male C57Bl/6 albino mice treated with unconjugated PKK ASO mouse surrogate ISIS 482584 (80 mg/kg/week) for 3 weeks. Evans Blue tracer was injected into the tail vein of mice and analyzed for its accumulation in the colon tissue. Some mice were also treated intraperitoneally with the angiotensin-converting-enzyme (ACE) inhibitor captopril (1 mg/kg) at the time of Evans Blue tracer injection or with a C1 INH ASO (40 mg/kg/week for 3 weeks) to enhance vascular permeability. ISIS 482584 inhibited basal vascular permeability. Further ISIS 482584 inhibited vascular permeability induced by either captopril or C1 INH ASO. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

Male C57Bl/6 albino mice were treated by SC administration with vehicle or unconjugated PKK ASO mouse surrogate ISIS 482584 (5, 10, 20, 40, 80, or 160 mg/kg/week) for 3 weeks. ISIS 482584 (10 to 160 mg/kg for 3 weeks) reduced basal activated high molecular weight kininogen cleavage in blood of male mice. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

Human KLKB1/PKK transgenic mice were treated by SC administration with ISIS 721744 (0.1, 0.3, 1, 3, or 10 mg/kg) on Day 0 and plasma was collected on Day -1, 3, 7, 14, 28, 42 and 56. A single dose of ISIS 721744 resulted in a dose- and time-dependent reduction of plasma PKK protein that approached 91% at 10 mg/kg on Day 14 after treatment. After reaching the lowest point at Days 7 to 14, plasma PKK levels were fully restored by Day 42 for 1 mg/kg or Day 56 for 3 mg/kg dose. PKK levels were still 20% below baseline levels by Day 56 for 10 mg/kg.

Safety Pharmacology

The safety pharmacology evaluations for the cardiovascular, respiratory, and CNS were combined into one study in Cynomolgus monkeys. Based on cardiovascular and respiratory assessments, there were no test article-related effects on arterial blood pressure parameters (systolic, diastolic, and mean arterial pressure), heart rate, respiratory rate, core body temperature, or ECG parameters (PR interval, QRS duration, QT interval, and RR interval) for up to 24 hours after dosing, or arterial blood gases for

up to 2 hours after dosing, with 12 and 30 mg/kg ISIS 721744. Based on CNS assessments, there were generally no treatment-related effects noted by functional observational battery (FOB) for up to 24 hours after dosing with 12 and 30 mg/kg ISIS 721744. Although, one animal (No. 072104) receiving 12 mg/kg had signs of hunched posture and tremors at 1 hour and 4 hours postdose, which were not evident at 24 hours postdose. Clinical chemistry and hematology parameters for this animal were normal. These findings were not seen in any other animals at this dose (12 mg/kg) or the higher dose (30 mg/kg). Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

In the in vitro hERG assay, ISIS 721744 inhibited hERG current in HEK-293 cells by 0.5% and 1.9% at 30 mcM and 300 mcM, respectively (not statistically significant) versus 2.4% for the vehicle control. The IC₅₀ for the inhibitory effect of ISIS 721744 on hERG potassium current was not calculated but was estimated to be greater than 300 mcM. In general, oligonucleotides are not thought to inhibit the hERG channel.

PK/ADME

The in vitro protein binding of ISIS 721744 at concentrations of 0.5, 5, and 150 mcg/mL was evaluated in the whole plasma from human, Cynomolgus monkey, and mouse by ultrafiltration methodology and a nuclease-dependent hybridization ELISA detection method. ISIS 721744 was highly bound ($\geq 96.81\%$) to whole plasma proteins in human, monkey, and mouse at all three concentrations evaluated. The extent of plasma protein binding of ISIS 721744 was similar across all species. Refer to the IND 142564 Nonclinical Review dated 08/13/2019.

The in vitro mean blood to plasma ratio of ISIS 721744 was between 0.630 and 0.824 in human, 0.468 and 0.566 in monkey, and 0.501 and 0.661 in rat suggesting that ISIS 721744 distributes mostly in the plasma compartment across the concentrations tested in all three species.

Based on metabolic profiling and identification of ISIS 721744 oligonucleotide-related metabolites in urine from mice, monkeys, and humans, the Applicant described that the primary route of elimination of donidalorsen is initial rapid hydrolysis of the GalNAc conjugate in tissues, followed by slow nuclease-mediated metabolism of the unconjugated oligonucleotide, and subsequent excretion of chain-shortened metabolites, as well as full-length oligonucleotides, albeit minor in urine and feces. There were no unique metabolites found in humans compared to animal species.

Based on metabolic profiling, identification, and quantitation of ISIS 721744 linker-related metabolites in plasma, urine, and/or fecal samples from mice, monkeys, and/or humans, the Applicant determined that the GalNAc moiety is hydrolyzed intracellularly following uptake into tissues, and the THA linker undergoes extensive oxidative metabolism, and is nearly completely eliminated via biliary excretion, and to a lesser extent, renal excretion, within 24 hours after administration.

Repeat-Dose Toxicity

In the pivotal GLP-compliant chronic toxicity study in *Cynomolgus* monkeys (pharmacologically relevant species), monkeys received SC doses of ISIS 721744 at 0, 2, 6, 12, or 30 mg/kg/dose. The monkeys were dosed for 39-weeks (dosing on Days 1, 5, 9, and 14 [loading doses] and once weekly thereafter) with a 13-week interim sacrifice (monkeys at 30 mg/kg/dose were dosed only until the interim sacrifice). A 26-week treatment-free recovery period was included after both the 13-week interim dosing interval (30 mg/kg/dose) and the 39-week dosing interval (0, 2, 6, 12 mg/kg/dose) to assess potential reversibility of any observed effects. Refer to Nonclinical Primary Review dated 08/13/2019 for details. In brief, the main test article-related findings included thrombocytopenia in two animals (12 and 30 mg/kg/dose) and histopathology findings of basophilic granules in various organs/tissues (including kidneys, liver, lymph nodes, and injection site) that in the absence of correlating histopathology were considered consistent with oligonucleotide uptake and not considered adverse. There were dose-dependent decreases in hepatic PKK mRNA expression and dose-dependent and time-dependent decreases in PKK plasma protein after treatment with ISIS 721744 at ≥ 2 mg/kg/dose at the interim and/or terminal sacrifices. Both hepatic PKK mRNA expression and plasma protein increased by the end of the recovery periods. Tissue concentrations of unconjugated ISIS 721744 in liver or kidney increased in a dose-dependent manner. The NOAEL was 6 mg/kg/week based on thrombocytopenia in two monkeys at the higher dose levels of 12 and 30 mg/kg/dose. The NOAEL corresponded to a C_{max} of 6.57 mcg/mL and an AUC_{0-48hr} of 37.6 mcg*hr/mL on Day 273.

In the pivotal chronic toxicity study in mice (not a pharmacologically relevant species), mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (1, 4, or 12 mg/kg/dose), or mouse surrogate ISIS 722059 (4 mg/kg/dose) Q2W for 26 weeks with a 13-week treatment-free recovery period (vehicle control and 12 mg/kg/dose ISIS 721744 dose groups only). Changes in hematology parameters at the end of the 26-week treatment period included increases in white blood cells, neutrophils, lymphocytes, and/or monocytes in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and at 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period, increases in hematology parameters were no longer seen at 12 mg/kg/dose ISIS 721744. Changes in clinical chemistry parameters at the end of the 26-week treatment period included increases in ALT, AST, and/or ALP in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744. Effects on clinical chemistry parameters (including ALT, AST, and ALP) were less clear at 4 mg/kg/dose ISIS 722059. At the end of the 13-week recovery period, increases in ALT and/or AST were still seen in males and/or females at 12 mg/kg/dose ISIS 721744, while increases in ALP were no longer seen. Histopathology findings after the 26-week treatment period with ISIS 721744 were seen in the liver, skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen. Histopathology findings after the 26-week treatment with mouse surrogate ISIS 722059 were seen in the skin/subcutis, SC dose sites, spleen, and testis.

- Liver: Minimal or slight centrilobular hepatocellular hypertrophy was noted in males at 1, 4, and 12 mg/kg/dose ISIS 721744 and in one female (moderate) at

12 mg/kg/dose ISIS 721744. This finding correlated with increased mean liver weights at 4 and 12 mg/kg/dose ISIS 721744 and increased liver enzymes (AST, ALT, and/or ALP). Despite a background incidence of hepatocyte vacuolation in vehicle control males, there was an increase in the severity (slight to marked) of hepatocyte vacuolation in males at 4 and 12 mg/kg/dose ISIS 721744, which correlated with gross pathology findings of liver discoloration at all dose levels.

- Skin/Subcutis: Increased incidences and/or severity of infiltrates of mononuclear cells, mixed cells, and/or vacuolated macrophages in the skin/subcutis were noted in males and/or females at 4 and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. Increased incidences and/or severity of vacuolated fibrocytes in the skin/subcutis were noted in males and/or females at 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059. Slight to moderate inflammation in the skin was noted in females at 4 and 12 mg/kg/dose ISIS 721744. These findings in the skin/subcutis correlated with increased leukocyte counts.
- SC Dose Sites: Increased incidences and/or severity of infiltrates of vacuolated macrophages, mononuclear cells, and mixed cells, as well as fibrocyte vacuolation at the SC injection sites were noted in males and/or females at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and/or 4 mg/kg/dose ISIS 722059. These findings at the SC injection sites correlated with increased leukocyte counts.
- Inguinal Lymph Nodes: Increased incidences and severities (minimal to marked) of increased lymphocytes in males at 1, 4, and/or 12 mg/kg/dose ISIS 721744 and one female at 12 mg/kg/dose ISIS 721744 were noted in the inguinal lymph node. This finding in the inguinal lymph node correlated with increased leukocyte counts. Amyloid in the inguinal lymph node was noted in one male at 4 mg/kg/dose ISIS 722059.
- Spleen: Increased incidence and/or severity of extramedullary hematopoiesis in the spleen was noted in males and/or females at 1, 4 and/or 12 mg/kg/dose ISIS 721744 and 4 mg/kg/dose ISIS 722059 and correlated with increased mean spleen weight in males administered 12 mg/kg/dose ISIS 721744.
- Testis: Moderate interstitial cell hyperplasia, minimal sperm stasis, and minimal vacuolation were noted in the testis of males at 4 mg/kg/dose ISIS 722059.

Histopathology findings after the 13-week recovery period for 12 mg/kg/dose ISIS 721744 were still noted in the liver as hepatocellular vacuolation in males and females, which correlated with increased liver weights. Histopathology findings in the skin/subcutis, SC dose sites, inguinal lymph nodes, and spleen at 12 mg/kg/dose ISIS 721744 were not seen at the end of the recovery period.

Unconjugated ISIS 721744 was measurable in a dose-dependent manner in the kidney (greater than dose proportional) and liver (dose proportional) after 26 weeks of treatment with ISIS 721744. The mean concentrations of unconjugated ISIS 721744 were similar in the kidney and liver except at 12 mg/kg/dose where mean unconjugated ISIS 721744 concentrations were greater in the kidney than in the liver. Mean unconjugated ISIS 721744 concentrations were higher in females than in males in the kidney but similar in the liver. After the 13-week treatment-free recovery period at 12

mg/kg/dose ISIS 721744, mean unconjugated ISIS 721744 concentrations were below or near the lower limit of quantitation of 0.107 mcg/g, suggesting complete clearance of ISIS 721744.

Mouse PKK mRNA in mouse liver was reduced by 77% (statistically significant) compared to vehicle control in mice treated with mouse surrogate ISIS 722059 at 4 mg/kg/dose for 26 weeks.

The NOAEL was 12 mg/kg/dose ISIS 721744, as test article-related findings were considered related to the oligonucleotide uptake.

Conclusions

The nonclinical pharmacology and toxicology studies were judged to be adequate to support the safety of donidalorsen for marketing. There are no outstanding concerns from the nonclinical perspective.

Recommendation: From the nonclinical perspective, the NDA is recommended for approval.

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/s/

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03/25/2025 10:56:15 AM

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I concur

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 219407
Supporting document/s: 1 and 21
Applicant's letter date: 08/21/2024 and 02/21/2025
CDER stamp date: 08/21/2024 and 02/21/2025
Product: Donidalorsen (ISIS 721744)
Pharmacologic Class: Prekallikrein (PKK)-targeted *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO)
Indication: Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older
Applicant: Ionis Pharmaceuticals Inc.
Clinical Division: Division of Pulmonology, Allergy, and Critical Care (DPACC)
PharmTox Division: Division of Pharmacology Toxicology for Immunology and Inflammation (DPT-II)
Reviewer: Eleni Salicru, PhD
Supervisor/Team Leader: Timothy Robison, PhD, DABT
Acting Clinical Division: Banu Karimi Shah, MD
Director:
Project Manager: Cindy Chee, PharmD

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Ionis Pharmaceuticals Inc. (Applicant) submitted a 505(b)(1) New Drug Application (NDA) for subcutaneous (SC) administration of donidalorsen (ISIS 721744) as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older. ISIS 721744 is an *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO), which targets human prekallikrein (PKK). The GalNAc molecule preferentially delivers ISIS 721744 to the liver by binding to the asialoglycoprotein receptor (ASGPR) on liver cells.

This review focuses on the evaluation of the GLP-compliant reproductive and developmental toxicology studies with ISIS 721744 (i.e., combined fertility and developmental toxicity study in mice and pre- and postnatal development [PPND] study in mice). ISIS 721744 does not have perfect alignment to the mouse PKK gene sequence. As such, ISIS 721744 is not pharmacologically active in mice and the mouse surrogate ISIS 722059, which targets mouse PKK, was included in both studies in mice to assess potential toxicities related to the intended pharmacology of PKK inhibition. This review also provides a summary of the GLP-compliant reproductive and developmental toxicology studies (i.e., combined fertility and developmental toxicity study in mice and embryofetal development [EFD] study in rabbits) with the unconjugated parent compound of ISIS 721744 (i.e., ISIS 546254) and/or its unconjugated mouse surrogate (ISIS 482584) which targets mouse PKK to assess potential toxicities related to the intended pharmacology of PKK inhibition.

1.2 Brief Discussion of Nonclinical Findings

In the combined fertility and developmental toxicity study, mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (1, 4, or 10 mg/kg/week), or mouse surrogate ISIS 722059 (4 mg/kg/week). Males were dosed once weekly during the pre-mating, mating, and post-mating phases until euthanasia (about 10 weeks total). Females were dosed weekly during the pre-mating and mating phases and then every other day during the gestation period from Gestation Day (GD) 0 to GD 16 (encompassing the periods of implantation and organogenesis). Since ISIS 722059 statistically decreased PKK mRNA expression in the liver of male and female mice by 80% and 75%, respectively. Unconjugated ISIS 721744 concentrations in the placenta (up to 0.31 mcg/g) were >10% lower than in the liver (i.e., 3 to 43 mcg/g for 1 to 10 mg/kg/week ISIS 721744 in females on GD 18). SC administration of ISIS 721744 (up to 10 mg/kg/week) or mouse surrogate ISIS 722059 (4 mg/kg/week) did not affect fertility, pregnancy, or embryofetal development in mice.

In a combined fertility and developmental toxicity study in mice, SC administration of the unconjugated parent compound of ISIS 721744 (i.e., ISIS 546254) at dose levels up to 84 mg/kg/week or unconjugated mouse surrogate (i.e., ISIS 482584) at 84 mg/kg/week did not affect fertility, pregnancy, or embryofetal development.

In an EFD study in rabbits, SC administration of the unconjugated parent compound of ISIS 721744 (i.e., ISIS 546254) at dose levels up to 75 mg/kg/week every other day from GD 6 to GD 18 did not result in any fetal external findings, visceral variations or malformations, or skeletal variations or malformations attributed to treatment.

In the PPND toxicity study, F₀ female mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (5, 10, or 20 mg/kg/week), or mouse surrogate ISIS 722059 (5 mg/kg/week) every other day from GD 6 to GD 18 and weekly from Lactation Day (LD) 1 to LD 20 (the treatment period encompassed organogenesis through weaning). Since ISIS 721744 is not active in mice, the mouse-surrogate, ISIS 722059, was included in the study to assess the potential effects related to the target inhibition. ISIS 722059 statistically decreased PKK mRNA expression in the liver of F₀ female mice by 82% on LD 22. ISIS 721744 was present in milk of lactating F₀ females at 10 and 20 mg/kg/week, although the drug is unlikely to be adsorbed systemically in F₁ pups. SC administration of ISIS 721744 (up to 20 mg/kg/week) or mouse surrogate ISIS 722059 (5 mg/kg/week) did not affect pregnancy in F₀ females or prenatal and postnatal development (including behavioral, fertility, and reproduction function) in F₁ offspring.

Conclusion: The reproductive and developmental toxicology studies with ISIS 721744 (and mouse surrogate ISIS 722059) were adequate to support the safety of ISIS 721744 for marketing. There are no outstanding concerns from the nonclinical perspective related to reproductive and developmental toxicology.

1.3 Recommendations

1.3.1 Approvability

Refer to main Pharmacology/Toxicology NDA Review and Evaluation.

1.3.2 Additional Nonclinical Recommendations

There are no additional nonclinical recommendations related to the evaluation of reproductive and developmental toxicity.

1.3.3 Labeling

Nonclinical sections of the product label are evaluated in a separate review.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
2304692-48-4 (free acid)
2304701-45-7 (sodium salt)

Trade Name
Dawnzera

Generic Name

Donidalorsen

Code Name

ISIS 721744

Chemical Name (IUPAC)

all-P-ambo-5'-O-[[[(6-{5-[(tris{3-[6-(2-acetamido-2-deoxy-β-D-galactopyranosyloxy)hexylamino]-3-oxopropoxymethyl})methyl]amino-5-oxopentanamido}hexyl)]phospho}-2'-O-(2-methoxyethyl)-5-methyl-P-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioguanlyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methylcytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-deoxy-P-thioguanlyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-P-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-P-thioguanlyl-(3'-O→5'-O)-2'-deoxy-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenosine, icosasodium salt

Molecular Formula/Molecular Weight

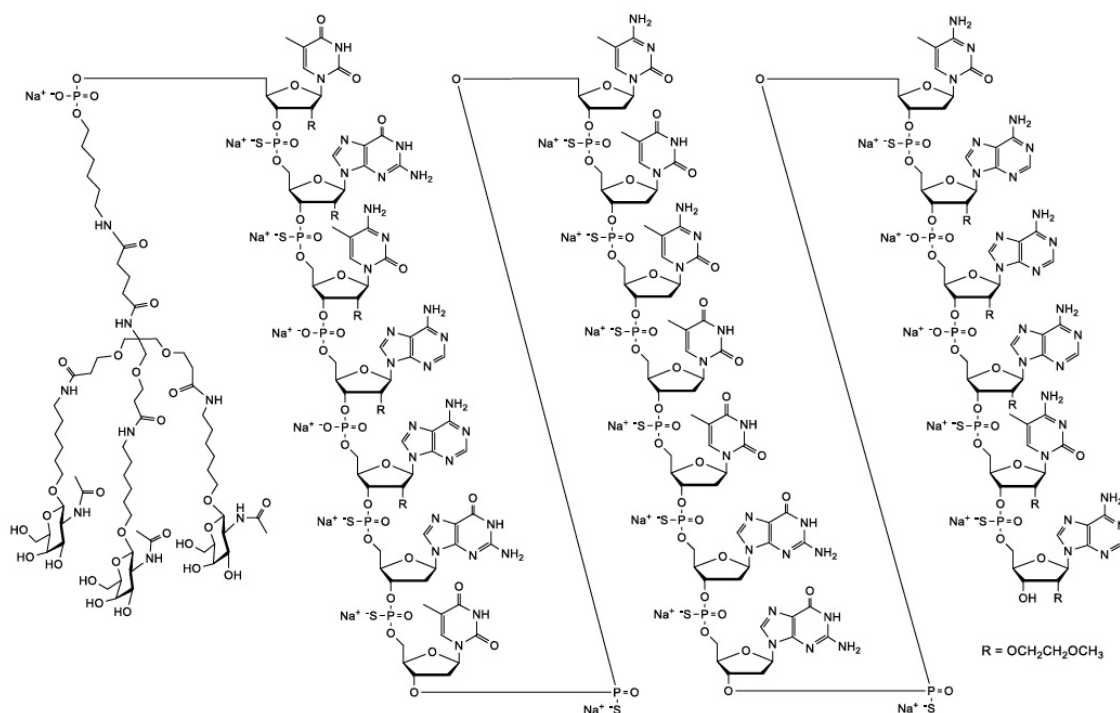
 $C_{296}H_{435}N_{83}O_{151}P_{20}S_{15}$ (free acid) $C_{296}H_{415}N_{83}O_{151}P_{20}S_{15}Na_{20}$ (sodium salt)

8672.66 Da (free acid)

9112.27 Da (sodium salt)

Structure or Biochemical Description

The structure of donidalorsen sodium is shown in **Figure 1** (Applicant's Figure).

Figure 1 Structure of Donidalorsen Sodium (Applicant's Figure)

Pharmacologic Class
 PKK-targeted GalNAc-conjugated ASO

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 142564 (active): Donidalorsen (ISIS 721744) by Ionis Pharmaceuticals Inc. for prophylaxis to prevent attacks of HAE in Center for Drug Evaluation and Research/Office of New Drugs/Office of Immunology and Inflammation/Division of Pulmonology, Allergy, and Critical Care (CDER/OND/OII/DPACC)

2.3 Drug Formulation

The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The qualitative and quantitative drug product formulation is shown in **Table 1** (Applicant's Table). The drug product is packaged in a staked needle glass prefilled syringe (PFS) closed with a (b) (4) rubber plunger stopper. The PFS is assembled into an autoinjector for the final drug product presentation. The autoinjector delivers a single dose of 80 mg donidalorsen.

Table 1 Donidalorsen Drug Product Formulation (Applicant's Table)

Ingredients	Quality Standards ^j	Quantity per Autoinjector	Quantity per Unit Dose (0.8mL)	Pharmaceutical Function
Donidalorsen ^a	Professed ^b	(b) (4) mg (equivalent to (b) (4) mg as sodium salt)	80 mg (equivalent to 84 mg as sodium salt)	Active
Sodium dihydrogen phosphate (b) (4)	USP-NF	(b) (4)		
Disodium hydrogen phosphate (b) (4)	USP-NF			
Sodium chloride	USP-NF			
Hydrochloric acid ^f	USP-NF	QS ^{g, h}	QS ^h	pH adjustment
Sodium hydroxide ^f	USP-NF	QS ^h	QS ^h	pH adjustment
Water for Injection (WFI)	USP-NF	QS to (b) (4) mL ⁱ	QS to 0.8 mL	Vehicle

^a Donidalorsen drug substance is supplied as the sodium salt. The label claim is expressed as the free acid concentration

^b Per Section 3.2.S.4.1

(b) (4)

ⁱ Added (b) (4) for pH adjustment

^g QS: quantity sufficient

^h Ingredient may be added to adjust pH; therefore, the quantity is variable

ⁱ To ensure that no less than 0.8 mL is dosed upon injection, a (b) (4) mL fill is targeted

^j USP - United States Pharmacopoeia; NF - National Formulary

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product formulation. All the excipients are within levels found in other FDA-approved SC drug products.

2.6 Proposed Clinical Population and Dosing Regimen

Donidalorsen is indicated for prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. The recommended dosage is 80 mg administered SC (b) (4). A dosing interval of (b) (4) may be considered in some patients.

2.7 Regulatory Background

Provided below is the regulatory background for the ISIS 721744 reproductive and developmental toxicology program. In brief, the Agency agreed that an EFD study of ISIS 721744 in rabbits was not needed. The following is excerpted from the Type B End of Phase 2 (EOP2) Meeting Minutes dated 10/18/2021 capturing discussions from the teleconference between the Applicant and the Agency on 08/30/2021.

Question 2

Does the Agency agree to a waiver from the requirement to conduct an embryo-fetal development study of ISIS 721744 in rabbits?

FDA Response:

Pending review of the available, ongoing, and planned reproductive and developmental toxicity studies with ISIS 721744 and ISIS 546254, unconjugated parent compound of ISIS 721744, (and the mouse surrogates ISIS 722059 and ISIS 482584 [unconjugated]), we agree that an embryo-fetal development study of ISIS 721744 in rabbits is not needed.

Refer to the ICH M3(R2) Guidance regarding the timing of reproductive toxicology studies and the inclusion of women of childbearing potential in clinical studies.

The ongoing fertility and early embryonic development toxicity study in CD-1 mice should be available prior to the initiation of the phase 3 clinical trials with ISIS 721744. The planned pre and postnatal development study in CD-1 mice can be conducted in parallel with phase 3 trials and provided with a potential NDA.

Discussion for FDA Response to Question 2:

There was no discussion held for FDA Response to Question 2.

3 Studies Submitted

3.1 Studies Reviewed

A Combined Fertility and Developmental Toxicity Study of ISIS 721744 in Mice via Subcutaneous Administration (Study No. 727-134; Applicant No. 721744-AS06)
GLP-compliant

Study for Toxic Effects of ISIS 721744 on Pre- and Postnatal Development, Including Maternal Function in Mice (Study No. 727-142; Applicant No. 721744-AS09)
GLP-compliant

A Combined Fertility and Developmental Toxicity Study of ISIS 546254 and ISIS 482584 Administered by Subcutaneous Injection in CD-1 Mice (Study No. (b) (4) 090.60; Applicant No. 546254-AS04) – SUMMARY REVIEW
GLP-compliant

An Assessment of Effects of ISIS 546254 on Embryo-Fetal Development in New Zealand White Rabbits (Study No. (b) (4) 090.61; Applicant No. 546254-AS05) – SUMMARY REVIEW
GLP-compliant

Reviewer's Note: ISIS 546254 is the unconjugated parent compound of ISIS 721744 and ISIS 482584 is the unconjugated mouse surrogate, which targets mouse PKK.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A Combined Fertility and Developmental Toxicity Study of ISIS 721744 in Mice via Subcutaneous Administration

Study no.: 727-134 (Applicant No. 721744-AS06)
 Study report location: Electronic Document Room (EDR)
 Supporting Document Number (SDN) 1
 Conducting laboratory and location: (b) (4)

Date of study initiation: 11/10/2020
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: ISIS 721744 bulk test article, lot # CA721744-001, and 89.5% purity

ISIS 722059 (mouse surrogate) bulk test article, lot # 722059-4, and 88.2% purity

Key Study Findings

- In a combined fertility and developmental toxicity study, mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (1, 4, or 10 mg/kg/week), or mouse surrogate ISIS 722059 (4 mg/kg/week). Males were dosed once weekly during the premating (beginning 28 days prior to mating), mating, and postmating phases (10 weeks total). Females were dosed beginning 14 days prior to mating. Females were dosed weekly (Days 1, 8, 15, and 22, as needed) during the premating phase and through the mating phase until positive evidence of copulation was observed, and every other day during the gestation period from GD 0 to GD 16 (i.e., GDs 0, 2, 4, 6, 8, 10, 12, 14, and 16). Unmated females continued to be dosed weekly until termination (following the last GD 18 necropsies).
- There were no estrous cycle changes (i.e., mean number of cycles and mean cycle length) related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.
- Percent abnormal sperm was increased at 10 mg/kg/week ISIS 721744 (15.6%, statistically significant) and 4 mg/kg/week ISIS 722059 (13.4%), compared to the vehicle control (10.1%), and outside of the conducting laboratory historical control ranges of 3.4% to 10.2%. It did not appear that an increase in percent

abnormal sperm affected pregnancy indices at 10 mg/kg/week ISIS 721744 or 4 mg/kg/week ISIS 722059.

- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on male or female mating performance.
- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on the number of corpora lutea, implantations, preimplantation and postimplantation losses, or live fetuses.
- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on fetal body weights.
- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on fetal external, visceral, or skeletal variations or malformations (i.e., no fetal anomalies) in mice.
- Unconjugated ISIS 721744 concentrations in the parental liver of males and females increased with increasing dose of drug. Unconjugated ISIS 721744 concentrations in the fetal liver were below the limit of quantitation in all ISIS 721744-treated groups. Notably, unconjugated ISIS 721744 concentrations in the placenta (up to 0.31 mcg/g) and testes (up to 1.61 mcg/g) were much lower than in the liver (i.e., 3 to 43 mcg/g for 1 to 10 mg/kg/week ISIS 721744 in F₀ females on GD 18 and 7 to 47 mcg/g for 1 to 10 mg/kg/week for males on Day 73). It was unclear if ISIS 721744 or the unconjugated version of ISIS 721744 crossed the placenta to reach fetal tissues.
- Treatment of F₀ male and female mice with mouse surrogate ISIS 722059 at 4 mg/kg/week statistically decreased hepatic mRNA expression of Klkb1/Pkk by 80% and 75%, respectively.
- The NOAEL for ISIS 721744 on male and female fertility and mating performance, as well as developmental toxicity, was the high dose of 10 mg/kg/week.

Methods

Doses:	<u>ISIS 721744</u> 1, 4, or 10 mg/kg/week
	<u>ISIS 722059 (mouse surrogate)</u> 4 mg/kg/week
Frequency of dosing:	Males: once weekly for 10 weeks Females: once weekly for 2 weeks during pre mating and mating phases and every other day from GD 0 to GD 16.
	See study design below
Dose volume:	15 mL/kg for weekly dosing 4.29 mL/kg for every other day dosing
Route of administration:	SC
Formulation/Vehicle:	0.9% Sodium Chloride for Injection, USP
Species/Strain:	Mice/Crl:CD1® (ICR)
Number/Sex/Group:	Main Study: 25 TK Study: 6
Satellite groups:	Yes. TK groups
Study design:	Males were dosed once weekly beginning 28 days prior to mating (pre mating phase) and through the mating and postmating phases until euthanasia (Day 73). Females were dosed beginning 14 days prior to mating. Females were dosed weekly (Days 1, 8, 15, and 22, as needed) during the pre mating phase and through the mating phase until positive evidence of copulation was observed, and every other day during the implantation and gestation periods from GD 0 to GD 16 (i.e., GDs 0, 2, 4, 6, 8, 10, 12, 14, and 16). Unmated females continued to be dosed weekly until termination (following the last GD 18 necropsies).
Deviation from study protocol:	Refer to Table 2 (Applicant's Table). Deviations from the study protocol were described in the study report. None of the deviations appeared to have affected the integrity, results, or conclusions of the study.

Table 2 Study Design for Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Group No.	Test Material	Dose Level ^a (mg/kg/week)	Dose Volume ^a (mL/kg)	Dose Concentration (mg/mL)	Main Study	
					No. of Males	No. of Females
1	Control Article	0	15/4.29	0	25	25
2	ISIS 721744	1	15/4.29	0.07	25	25
3	ISIS 721744	4	15/4.29	0.27	25	25
4	ISIS 721744	10	15/4.29	0.67	25	25
5	ISIS 722059 ^b	4	15/4.29	0.27	25	25

Group No.	Test Material	Dose Level ^a (mg/kg/week)	Dose Volume ^a (mL/kg)	Dose Concentration (mg/mL)	Toxicokinetic	
					No. of Males	No. of Females
6	Control Article	0	15/4.29	0	6	6
7	ISIS 721744	1	15/4.29	0.07	6	6
8	ISIS 721744	4	15/4.29	0.27	6	6
9	ISIS 721744	10	15/4.29	0.67	6	6
10	ISIS 722059 ^b	4	15/4.29	0.27	6	6

^a Males were dosed once weekly throughout the study. Females were dosed once weekly during the pre-mating and mating phases and qod during gestation. The weekly dose (mg/kg/week) for females during gestation was equivalent to 3.5-times each dose (mg/kg/dose). Doses were based on the most recent body weight measurement. A dose volume of 15 mL/kg was used for weekly doses and 4.29 mL/kg for was used for qod dosing.

^b Mouse specific oligonucleotide.

Observations and Results

Mortality

Mortality/cageside observations were made at least twice daily (morning and afternoon) beginning upon arrival through termination/release.

There were two unscheduled deaths during the study. One male (No. 2021) at 1 mg/kg/week ISIS 721744 was euthanized on Day 61 due to severe penile prolapse and decreased urine output. One toxicokinetic (TK) female (No. 7505) at 1 mg/kg/week ISIS 721744 was euthanized on GD 12 due to pregnancy complications (red discharge from the vagina and red material present in the cage bedding). These unscheduled deaths were considered incidental and unrelated to treatment with ISIS 721744 as there were no deaths in higher dose groups.

All other animals survived to scheduled termination.

Clinical Signs

Detailed clinical observations were made prior to randomization and daily during treatment (predose and about 1.5 hours postdose on dosing days).

There were no clinical sign observations attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Body Weight

Males

Males were weighed at receipt, prior to randomization, and twice weekly during the pre-mating and post-mating phases.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on body weight in males during the study.

Females

Females were weighed at receipt, prior to randomization, and twice weekly prior to mating. Upon the beginning of the mating phase, females were weighed once weekly or until mating was confirmed (body weight data for the mating phase was not included in the study report). Upon verification of mating, females were weighed on the presumed GDs 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on body weight in females during the pre-mating phase of the study.

As shown in **Table 3**, mean gestational body weight was increased beginning on GD 10 through GD 18 in females treated with 4 and 10 mg/kg/week ISIS 721744 (+5% to +15% difference from vehicle control). Mean gestational body weight was increased beginning on GD 16 through GD 18 in females treated with 4 mg/kg/week ISIS 722059 (+8% to +9% difference from vehicle control). These increases in mean gestational body weight corresponded with increases in mean gestational body weight gain. The increases in mean gestational body weight gain were not considered adverse. A comparison of the body weight changes between GD 0 and GD 16 (drug treatment period) is shown in **Table 4**.

Table 3 Mean Gestational Body Weight in Females from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

	GD 0	GD 2	GD 4	GD 6	GD 8	GD 10	GD 12	GD 14	GD 16	GD 18
Vehicle Control										
GBW (g)	29.23	29.61	30.58	31.78	33.26	35.09	39.14	43.5	48.96	54.91
% Diff	-	-	-	-	-	-	-	-	-	-
1 mg/kg/week ISIS 721744										
GBW (g)	30.36	30.67	32.05	32.89	34.31	36.33	40.46	45.46	52.25	57.91
% Diff	3.88	3.58	4.81	3.49	3.16	3.53	3.38	4.52	6.71	5.46

	GD 0	GD 2	GD 4	GD 6	GD 8	GD 10	GD 12	GD 14	GD 16	GD 18
4 mg/kg/ week ISIS 721744										
GBW (g)	30.36	30.80	32.11	33.04	34.70	37.00*	41.63*	46.83*	54.30**	61.81**
% Diff	3.88	4.01	5.01	3.96	4.35	5.43	6.38	7.68	10.91	12.57
10 mg/kg/ week ISIS 721744										
GBW (g)	30.42	30.84	31.92	33.07	34.93	37.26*	42.27**	47.52**	55.20**	63.38**
% Diff	4.07	4.15	4.37	4.07	5.04	6.18	8.02	9.26	12.75	15.43
4 mg/kg/ week ISIS 722059										
GBW (g)	29.97	30.39	31.70	32.90	34.35	36.64	41.22	46.33	53.26*	59.42
% Diff	2.54	2.63	3.66	3.53	3.29	4.41	5.33	6.53	8.77	8.21

Abbreviations: % Diff = percent difference from vehicle control; g = grams; GBW = gestational body weight; GD = Gestation Day

Notes: Applicant's statistical analyses Anova & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$

Table 4 Body Weight Change from Gestation Day 0 to Gestation Day 16 in Females from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Parameter	Vehicle Control	ISIS 721744 1 mg/kg/week	ISIS 721744 4 mg/kg/week	ISIS 721744 10 mg/kg/week	ISIS 722059 4 mg/kg/week
GD 0 BW (g)	29.23	30.36	30.36	30.42	29.97
GD16 BW (g)	48.96	52.25	54.30	52.20	53.26
BW Change GD 0 to GD 16 (g)	19.73	21.89	23.94	21.78	23.29
BW Change as % of GD 0 BW	67.50	72.10	78.85	71.60	77.71
% Vehicle Control	100.00	106.82	116.82	106.07	115.13

Abbreviations: BW = body weight; g = grams; GD = Gestation Day

Feed Consumption

Qualitative food consumption was recorded weekly prior to mating. Food consumption was not recorded during the mating phase. Following the mating phase, qualitative food consumption for males was recorded on a weekly basis until euthanasia. Qualitative food consumption for females was recorded on the corresponding gestation body weight days.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on food consumption during the study.

Estrous Cyclicity

Females were examined daily for estrous cyclicity at least 10 days prior to initiation of treatment and until evidence of copulation was observed or the cohabitation period ended.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on estrous cyclicity (i.e., mean number of cycles and mean cycle length).

Sperm Analysis

At the time of euthanasia, the right testis and epididymis were used for sperm analysis (i.e., sperm motility, sperm count/concentration, and sperm morphology).

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on sperm motility or sperm count/concentration.

As shown in **Table 5** (Applicant's Table), percent abnormal sperm was increased at 10 mg/kg/week ISIS 721744 (15.6%, statistically significant) and 4 mg/kg/week ISIS 722059 (13.4%) compared to the vehicle control (10.1%). These percent abnormal sperm mean values were outside the conducting laboratory historical control ranges of 3.4% to 10.2%. As described in the study report: These differences were mostly driven by a few animals at 10 mg/kg/week ISIS 721744 (up to 36%) and 4 mg/kg/week ISIS 722059 (up to 27.7%) that had individual percent abnormal sperm slightly higher than the concurrent control (up to 24.5%). It did not appear that an increase in percent abnormal sperm affected pregnancy indices at 10 mg/kg/week ISIS 721744 or 4 mg/kg/week ISIS 722059.

Table 5 Sperm Evaluation from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Endpoint		0 mg/kg/week	1 mg/kg/week ISIS 721744	4 mg/kg/week ISIS 721744	10 mg/kg/week ISIS 721744	4 mg/kg/week ISIS 722059
Sperm % Motility [g]	Mean	49.8	48.4	51.5	44.6	48.6
	SD	21.89	18.55	23.03	24.12	21.93
	N	25	24	25	25	25
Total Sperm Count per Cauda Epididymis x 10 ⁸ [g1]	Mean	0.269	0.281	0.276	0.265	0.264
	SD	0.062	0.054	0.052	0.064	0.063
	N	25	24	25	25	25
Sperm Concentration per gram Cauda Epididymis x 10 ⁸ [g1]	Mean	18.458	17.350	16.783	18.497	17.050
	SD	8.152	4.696	4.311	11.441	5.390
	N	25	24	25	25	25
% Abnormal [g]	Mean	10.05	9.10	10.55	15.58 b	13.43
	SD	5.155	4.006	3.916	8.502	6.391
	N	25	24	25	25	25

Notes: Applicant's statistical analyses [g] - Anova & Dunnett(Arcsine); [g1] - Anova & Dunnett; b = p < 0.01

Hematology

Blood was collected for hematology evaluations from the first set of 6 animals/sex/group at necropsy (i.e., Day 73 for males and GD 18 for females). A representative panel of hematology parameters was evaluated.

As shown in **Table 6**, treatment with 1, 4, and 10 mg/kg/week ISIS 721744 and 4 mg/kg/week ISIS 722059 decreased RBC (-4% to -9% difference from vehicle control), hemoglobin (-6% to -11% difference from vehicle control), hematocrit (-5% to -9% difference from vehicle control), and reticulocytes (-13% to -50% difference from vehicle control) in males and females. These changes were indicative of decreased RBC production.

Table 6 Hematology from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

	Males (Day 73)					Females (GD 18)				
	Veh Cont	ISIS 721744 mg/kg/week			ISIS 722059 mg/kg/week	Veh Cont	ISIS 721744 mg/kg/week			ISIS 722059 mg/kg/week
	0	1	4	10	4	0	1	4	10	4
RBC 10⁶/mcL	11.19	10.44	10.42	10.26	10.19	9.51	8.76	8.72	8.99	9.12
% Diff	-	-6.7	-6.8	-8.3	-8.9	-	-7.9	-8.3	-5.4	-4.0
tCtrl	-	0.93	0.93	0.92	0.91	-	0.92	0.92	0.95	0.96
HGB g/dL	17.16	16.13	15.73	15.60	15.87	15.17	13.92	13.58	14.3	14.24
% Diff	-	-6.0	-8.3	-9.1	-7.5	-	-8.2	-10.5	-5.7	-6.1
tCtrl	-	0.94	0.92	0.91	0.92	-	0.92	0.9	0.94	0.94
HCT %	60.26	56.87	55.9	54.88	57.07	54.03	49.15	48.72	50.78	50.12
% Diff	-	-5.6	-7.2	-8.9	-5.3	-	-9.0	-9.8	-6.0	-7.2
tCtrl	-	0.94	0.93	0.91	0.95	-	0.91	0.9	0.94	0.93
RETIC 10⁹/L	357.8	309.8	291.4*	285.2*	278.7*	199.8	150.5	99.2	107.8	110.7
% Diff	-	-13.4	-18.6	-20.3	-22.1	-	-24.7	-50.3	-46.1	-44.6
tCtrl	-	0.87	0.81	0.8	0.78	-	0.75	0.5	0.54	0.55

Abbreviations: % Diff = percent difference from vehicle control; GD = Gestation Day; HCT = hematocrit; HGB = hemoglobin; RBC = red blood cells; RETIC = reticulocytes; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses Anova & Dunnett: * = p ≤ 0.05

Clinical Chemistry

Blood was collected for clinical chemistry evaluations from the second set of 6 animals/sex/group at necropsy (i.e., Day 73 for males and GD 18 for females). A representative panel of clinical chemistry parameters was evaluated.

As shown in **Table 7**, treatment with 4 and 10 mg/kg/week ISIS 721744 increased AST (1.6x and 1.9x of control, respectively) and ALT (2.7x and 3.0x of control, respectively) in males suggestive of an effect on the liver. Other changes included a decrease in albumin at 4 and 10 mg/kg/week ISIS 721744 in males, an increase in cholesterol at 1,

4, and 10 mg/kg/week ISIS 721744 in males, and a decrease in triglycerides at 4 and 10 mg/kg/week ISIS 721744 in males, 1, 4, and 10 mg/kg/week ISIS 721744 in females, and 4 mg/kg/week ISIS 722059 in females. Most of these changes appear to be consistent with the uptake of ISIS 721744 into the liver.

Table 7 Clinical Chemistry from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

	Males (Day 73)					Females (GD 18)				
	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
	0	1	4	10	4	0	1	4	10	4
AST (U/L)	45.7	56.5	72.2	84.8*	47.8	84.5	68.0	80.2	78.7	61.5
% Diff	-	23.6	58.0	85.6	4.6	-	-19.5	-5.1	-6.9	-27.2
tCtrl	-	1.24	1.58	1.86	1.05	-	0.80	0.95	0.93	0.73
ALT (U/L)	35.8	50.2	96.5*	107.0**	42.8	41.7	34.0	45.5	47.7	28.5
% Diff	-	40.2	169.6	198.9	19.6	-	-18.5	9.1	14.4	-31.7
tCtrl	-	1.40	2.69	2.99	1.20	-	0.82	1.09	1.14	0.68
ALB (g/dL)	3.22	3.2	2.95**	2.98*	3.08	2.37	2.50	2.48	2.38	2.62
% Diff	-	-0.6	-8.4	-7.5	-4.3	-	5.5	4.6	0.4	10.5
tCtrl	-	0.99	0.92	0.93	0.96	-	1.06	1.05	1.01	1.11
CHOL (mg/dL)	160.7	207.2	206.3	197.7	159.5	79.2	79.0	89.8	76.7	98.5
% Diff	-	28.9	28.4	23.0	-0.7	-	-0.3	13.4	-3.2	24.4
tCtrl	-	1.29	1.28	1.23	0.99	-	1.00	1.13	0.97	1.24
TRIG (mg/dL)	204.7	200.3	159.5	159.0	218.3	415.5	312.5	249.5	158.0	345.8
% Diff	-	-2.1	-22.1	-22.3	6.6	-	-24.8	-40.0	-62.0	-16.8
tCtrl	-	0.98	0.78	0.78	1.07	-	0.75	0.60	0.38	0.83
ALP (U/L)	62.3	61.7	69.0	84.3	62.3	59.0	63.3	77.8	66.2	60.8
% Diff	-	-1.0	10.8	35.3	0.0	-	7.3	31.9	12.2	3.1
tCtrl	-	0.99	1.11	1.35	1.00	-	1.07	1.32	1.12	1.03

Abbreviations: ALB = albumin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; CHOL = cholesterol; % Diff = percent difference from vehicle control; GD = Gestation Day; tCtrl = fold change from vehicle control; TRIG = triglycerides; Veh Cont = vehicle control
Applicant's statistical analyses Anova & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$ or Kruskal-Wallis & Dunn: * = $p \leq 0.05$; ** = $p \leq 0.01$

Toxicokinetics

Tissue Bioanalysis

Parental (liver, testis, and placenta) and fetal (liver) tissue were analyzed using a validated ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) procedure to determine unconjugated ISIS 721744 concentrations.

Parental and fetal tissue concentrations of unconjugated ISIS 721744 are shown in **Table 8** (Applicant's Table). Unconjugated ISIS 721744 concentrations in the parental liver of vehicle control males and females were below the limit of quantitation except for one female which had a liver exposure of 1.72 mcg/g. Unconjugated ISIS 721744 concentrations in the parental liver of males and females increased with increase in ISIS 721744 dose. Unconjugated ISIS 721744 concentrations in the fetal liver were below the limit of quantitation in all ISIS 721744-treated groups. Unconjugated ISIS 721744 concentrations in the placenta were below the limit of quantitation at 1 mg/kg/week ISIS 721744 and at 0.03 and 0.31 mcg/g at 4 and 10 mg/kg/week ISIS 721744, respectively. Unconjugated ISIS 721744 concentrations in the testes were below the limit of quantitation at 1 mg/kg/week ISIS 721744 and at 0.25 and 1.61 mcg/g at 4 and 10 mg/kg/week ISIS 721744, respectively. Notably, unconjugated ISIS 721744 concentrations in the placenta and testes were much lower than in the liver (3 to 43 mcg/g for 1 to 10 mg/kg/week ISIS 721744 in females on GD 18 and 7 to 47 mcg/g for 1 to 10 mg/kg/week ISIS 721744 in males on Day 73). The uptake of ISIS 721744 by the liver was promoted by the GalNAc conjugate, which is subsequently cleaved.

Table 8 Parental and Fetal Tissue Exposure from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Group No.	ISIS 721744 Dose Level ^a (mg/kg/week)	Interval	Gender	Number of Doses	N	Unconjugated ISIS 721744 Tissue Concentration (µg/g)			
						Liver	Fetal Liver	Placenta	Testes
6	0	Day 73	Male	11	6	BLQ	NA	NA	NA
7	1	Day 73	Male	11	6	7.41 ±1.207	NA	NA	BLQ
8	4	Day 73	Male	11	6	22.50 ±5.848	NA	NA	0.25 ±0.135
9	10	Day 73	Male	11	6	46.82 ±14.692	NA	NA	1.61 ±0.421
6	0	GD 18	Female	12 to 13	6	0.29 ^c ±0.702	NA	NA	NA
7	1	GD 18	Female	12	5 ^b	3.35 ±1.428	BLQ	BLQ	NA
8	4	GD 18	Female	12 to 13	6	24.95 ±8.781	BLQ	0.03 ±0.061	NA
9	10	GD 18	Female	12 to 13	6	43.42 ±19.502	BLQ	0.31 ±0.106	NA

Note: The lower limit of quantitation (LLOQ) for the assay was 0.107 µg/g for all evaluated tissues. All values below the lower limit of quantitation (BLQ) were treated as zero for the statistical summary analysis. Values are presented as mean ± standard deviation.

^a Males were dosed once weekly throughout the study. Females were dosed once weekly during the pre-mating and mating phases and every other day during gestation. The weekly dose (mg/kg/week) was equivalent to 3.5 times each dose (mg/kg/dose).

^b The Group 7 N for maternal liver was 5 and the N for fetal liver and placenta was 3.

^c One control animal had a liver exposure of 1.72 µg/g, while the other 5 animals were BLQ.

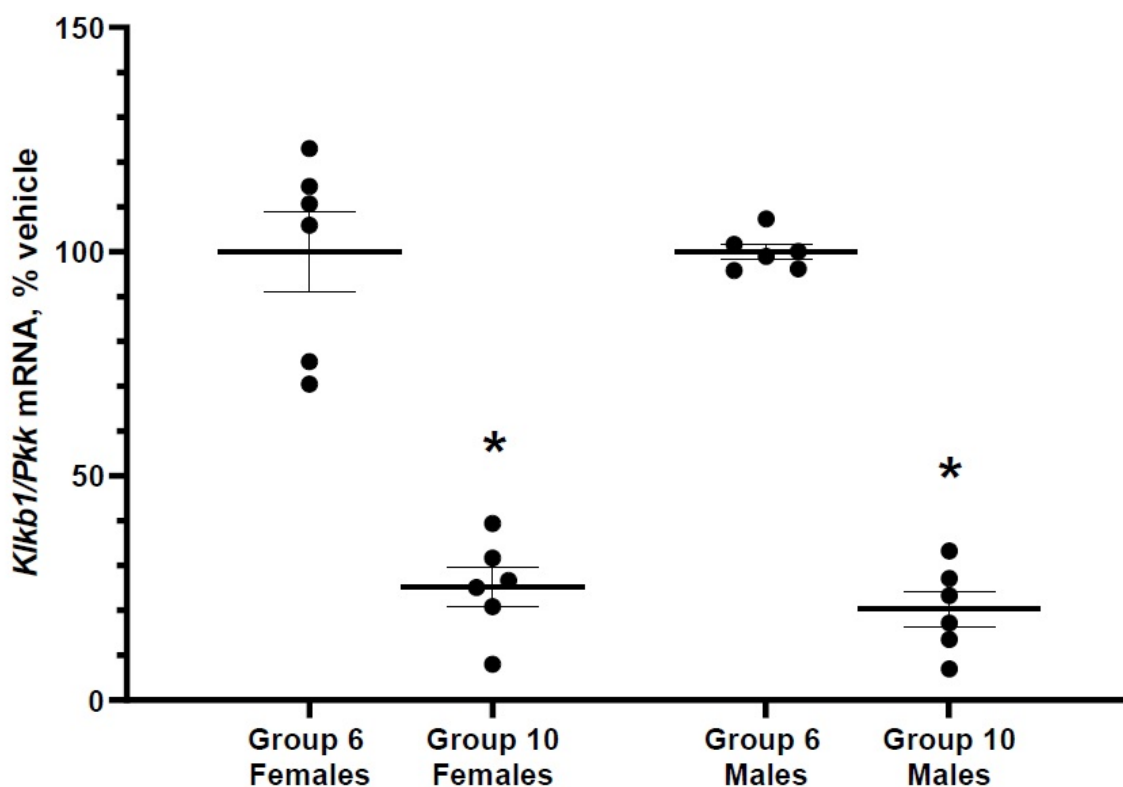
GD = Gestation Day; NA = Not Applicable; N = Number; BLQ = Below the lower limit of quantitation

Target mRNA

Liver samples were collected from males on Day 73 and females on GD 18 from the vehicle control and 4 mg/kg/week ISIS 722059 groups for RNA extraction and Klkb1/Pkk mRNA RT-qPCR analysis.

As shown in **Figure 2** (Applicant's Figure), treatment of male and female mice with mouse surrogate ISIS 722059 at 4 mg/kg/week statistically decreased hepatic mRNA expression of Klkb1/Pkk by 80% and 75%, respectively.

Figure 2 Hepatic Klkb1/Pkk mRNA Expression Following Administration of Mouse Surrogate ISIS 722059 from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Figure)



Average and SEM for both females and males. * Significantly different from saline (group 6) using two-tailed unpaired t-test ($p < 0.0001$)

Dosing Solution Analysis

Samples were collected for concentration and stability analyses from the middle stratum of dose concentrations for ISIS 721744 at 0.07, 0.27, and 0.67 mg/mL and mouse surrogate ISIS 722059 at 0.27 mg/mL. The concentration results were within the acceptance criterion of (b) (4) % indicating that the dosing solutions were prepared at the correct concentrations.

Necropsy

Scheduled euthanasia and necropsy for males was on Day 73 (about 48 hours after the last dose). Scheduled euthanasia and necropsy for mated females was on presumed GD 18 (about 48 hours following the last dose) and for unmated females was on Day 47 following completion of all GD 18 necropsies.

The tissues/organs that were weighed, collected, processed, and evaluated at the time of necropsy are shown in **Table 9** (Applicant's Table).

Table 9 Tissues/Organs Weighed, Collected, Processed, and Evaluated from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Organ	Weigh	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Animal ID	-	X	-	-
Epididymis, left (fixed in Bouin's fixative)	X	X	-	-
Epididymis, right	X	X	-	-
Gland, prostate	X	X	-	-
Gland, seminal vesicles with coagulating gland	X	X (2)	-	-
Kidneys	X (2)	X (2)	-	-
Liver	X	X	-	-
Macroscopic abnormalities	-	X	-	-
Ovaries with oviduct	X (2)	X (2)	-	-
Spleen	X	X	-	-
Testis, left (fixed in Bouin's fixative)	X	X	X	X
Testis, right	X	X	-	-
Uterus/Cervix	X	X	-	-
Vagina	-	X	-	-
X = Procedure to be conducted. - = Not applicable. (1) = one side. (2) = both sides.				

Organ Weights

Organ weights were collected for testis (left and right separately), epididymis (left and right separately), kidneys (paired), liver, seminal vesicles, spleen, and prostate gland for males. Gravid uterine weight and organ weights for kidneys, liver, ovaries with oviduct (paired), and spleen were recorded for females.

As shown in **Table 10**, there were increases in mean liver, kidney, and/or spleen weights in males and/or females after treatment with ISIS 721744 and/or mouse surrogate ISIS 722059. Mean liver weight was increased in males and females at 4 and 10 mg/kg/week ISIS 721744 and 4 mg/kg/week ISIS 722059. Mean kidney weight was increased in females at 10 mg/kg/week ISIS 721744 and 4 mg/kg/week ISIS 722059. Mean spleen weight was increased in males at 10 mg/kg/week ISIS 721744. Increased liver and kidney weights were attributed to uptake of ISIS 721744 and ISIS 722059.

Table 10 Mean Organ Weight from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

	Males					Females				
	Veh Cont	ISIS 721744 mg/kg/week			ISIS 722059 mg/kg/week	Veh Cont	ISIS 721744 mg/kg/week			ISIS 722059 mg/kg/week
	0	1	4	10	4	0	1	4	10	4
Liver Weight (g)	2.11	2.25	2.34**	2.41**	2.33**	2.73	2.97	3.03*	3.20**	2.98*
% Diff	-	6.62	10.68	14.04	10.17	-	8.93	10.94	17.41	9.37
tCtrl	-	1.07	1.11	1.14	1.10	-	1.09	1.11	1.17	1.09
Kidney Weight (g)	0.66	0.70	0.72	0.70	0.72	0.42	0.46	0.46	0.51**	0.47**
% Diff	-	6.54	8.83	6.44	9.15	-	9.02	8.77	20.16	11.28
tCtrl	-	1.07	1.09	1.06	1.09	-	1.09	1.09	1.20	1.11
Spleen Weight (g)	0.09	0.10	0.10	0.11**	0.09	0.11	0.13	0.12	0.14	0.12
% Diff	-	9.44	9.45	16.19	-0.39	-	11.10	7.13	20.57	8.34
tCtrl	-	1.09	1.09	1.16	1.00	-	1.11	1.07	1.21	1.08

Abbreviations: % Diff = percent difference from vehicle control; g = grams; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses Anova & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$ or Kruskal-Wallis & Dunn: ** = $p \leq 0.01$

There was no significant effect on mean gravid uterine weights after treatment with ISIS 721744 at 1, 4, and 10 mg/kg/week (19.30, 22.25, and 23.53 grams, respectively) or mouse surrogate ISIS 722059 at 4 mg/kg/week (20.61 grams) compared to the vehicle control (18.60 grams).

Gross Pathology

A complete necropsy and gross pathology evaluation was performed on all animals, including a uterine examination for females.

There were no observations noted by gross pathology evaluation that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Histopathology

The testis was processed for histopathology evaluation.

There were no histopathology changes noted in the testis that were considered related to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Mating

As described in the study report:

Mating began on Day 29 for males and Day 15 for females. One female was housed with one male from the same treatment group in the cage of the male. Positive evidence of copulation was established by daily inspection for a copulatory plug in situ or vaginal lavage for sperm. The day in which positive evidence of copulation was observed was considered GD 0. After mating, each female was returned to an individual cage. The maximum mating phase was 14 days.

As shown in **Table 11** and **Table 12** (Applicant's Tables), the male and female mating index was 100% for the vehicle control group as well as for the ISIS 721744 and mouse surrogate ISIS 722059 groups. Further, the male and female fertility and pregnancy indices were 92% in ISIS 721744 and mouse surrogate ISIS 722059 groups compared to 88% for the vehicle control group. As shown in **Table 12** (Applicant's Table), there were 23 pregnancies in each of the ISIS 721744 groups and the mouse surrogate ISIS 722059 group compared to 22 pregnancies in the vehicle control group.

Table 11 Mating Performance for Males from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Sex: Male		0	1	4	10	4
Day(s) Relative to Pairing (Litter: A)		mg/kg/week	mg/kg/week	mg/kg/week	mg/kg/week	mg/kg/week
			ISIS	ISIS	ISIS	ISIS
			721744	721744	721744	722059
Group Size - Males		25	25	25	25	25
Paired - Males	N+ve	25	25	25	25	25
Mated Males	N+ve	25	25	25	25	25
Male Impregnated a Female	N+ve	22	23	23	23	23
Male Mating Index [f]	%	100.0	100.0	100.0	100.0	100.0
	ProA	25/25	25/25	25/25	25/25	25/25
Male Fertility Index [f]	%	88.0	92.0	92.0	92.0	92.0
	ProA	22/25	23/25	23/25	23/25	23/25
Male Pregnancy Index [f]	%	88.0	92.0	92.0	92.0	92.0
	ProA	22/25	23/25	23/25	23/25	23/25

Table 12 Mating Performance for Females from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Sex: Female		0	1	4	10	4
Day(s) Relative to Pairing (Litter: A)		mg/kg/week	mg/kg/week	mg/kg/week	mg/kg/week	mg/kg/week
			ISIS 721744	ISIS 721744	ISIS 721744	ISIS 722059
Group Size - Females		25	25	25	25	25
Paired - Females	N+ve	25	25	25	25	25
Mated Females	N+ve	25	25	25	25	25
Pregnant	N+ve	22	23	23	23	23
Pre-coital Interval (Days) [k]	Mean	1.8	2.2	2.9 *	2.3	2.4
	SD	1.0	1.6	1.7	1.1	2.4
	N	25	25	25	25	25
Pregnant No Confirmed Mating	N+ve	0	0	0	0	0
Confirmed Mating Days 1-7	N+ve	25	25	24	25	23
	%	100.0	100.0	96.0	100.0	92.0
Confirmed Mating Days 8-14	N+ve	0	0	1	0	2
	%	0.0	0.0	4.0	0.0	8.0
Female Mating Index [f]	%	100.0	100.0	100.0	100.0	100.0
	ProA	25/25	25/25	25/25	25/25	25/25
Female Fertility Index [f]	%	88.0	92.0	92.0	92.0	92.0
	ProA	22/25	23/25	23/25	23/25	23/25
Female Pregnancy Index [f]	%	88.0	92.0	92.0	92.0	92.0
	ProA	22/25	23/25	23/25	23/25	23/25

Uterine and Ovarian Examination

On GD 18, the gross pathology evaluation included an examination of the uterus and ovaries for number of corpora lutea, implantations, early and late resorptions, and live and dead fetuses.

As shown in **Table 13**, there were no changes in number of corpora lutea, implantations, preimplantation and postimplantation losses, or live fetuses that were attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Table 13 Uterine and Ovarian Examinations from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Parameter	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/ week)
	0	1	4	10	4
Female with Live Fetuses N	22	23	23	23	23
%	95.5	95.7	100.0	100.0	100.0
Number of Corpora Lutea Mean	15.7	16.0	15.6	15.7	15.3
% Diff	-	1.7	-0.8	-0.2	-3
Number of Implantations Mean	13.2	13.8	14.3	14.1	13.5
% Diff	-	4.2	8.5	6.5	1.9
Preimplantation Loss (%) Mean	17.15	15.73	7.72	11.36	12.80
% Diff	-	-8.27	-54.96	-33.77	-25.33

Parameter	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
	0	1	4	10	4
Total Number of Resorptions Mean	1.7	1.7	1.4	1.1	1.1
% Diff	-	-4.3	-16.9	-37.1	-34.6
Number of Early Resorptions Mean	1.6	1.4	1.3	0.9	0.9
% Diff	-	-12.5	-20.7	-42.6	-42.6
Number of Late Resorptions Mean	0.1	0.3	0.2	0.2	0.2
% Diff	-	91.3	27.5	27.5	59.4
Total Number of Fetuses Mean	12.0	12.7	12.9	13.0	12.3
% Diff	-	5.3	7.2	7.5	2.5
Number of Live Fetuses Mean	11.5	12.1	12.9	13.0	12.3
% Diff	-	5.9	12.7	13.5	7.8
Number of Live Male Fetuses Mean	6.2	5.9	6.3	6.0	5.9
% Diff	-	-5.1	1.3	-3.6	-4.3
Number of Dead Fetuses Mean	0.0	0.0	0.0	0.0	0.0
% Diff	-	-100	-100	-100	-100
Postimplantation Loss (%) Mean	16.26	15.41	9.78	7.38	8.80
% Diff	-	-5.21	-39.82	-54.62	-45.88
Live Male Fetus/Litter (%) Mean	55.67	48.62	48.25	43.58	46.39
% Diff	-	-12.67	-13.33	-21.72	-16.67
Number of Live Female Fetuses Mean	5.2	6.3	6.7	7.0	6.4
% Diff	-	19.8	27.3	33.9	23.1

Abbreviations: % Diff = percent difference from vehicle control; N = number; Veh Cont = vehicle control

Notes: Applicant's statistical analyses Ancova/Anova & Dunnett: ** = $p \leq 0.01$

Fetal Evaluations

Each fetus was sexed, weighed, and evaluated for external, visceral, and skeletal malformations and variations.

Fetal Sex

The fetal sex ratios (% males per litter) were comparable between 1, 4, and 10 mg/kg/week ISIS 721744 (i.e., 48.62%, 48.25%, and 43.58%, respectively), mouse surrogate ISIS 722059 (i.e., 46.39%) and the vehicle control group (i.e., 55.67%).

Fetal Weights

As shown in **Table 14**, mean fetal body weight (all, males, and females) was statistically higher at 10 mg/kg/week ISIS 721744 compared to the vehicle control group. Since the weights were within historical control ranges for the conducting laboratory, the differences were not considered related to ISIS 721744.

Overall, there were no changes in fetal body weights attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Table 14 Litter Weights from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Parameter	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
	0	1	4	10	4
Mean Fetal Weight All (g)	1.355	1.354	1.415	1.476**	1.393
Mean Fetal Weight Males (g)	1.368	1.373	1.441	1.493**	1.414
Mean Fetal Weight Females (g)	1.335	1.331	1.389	1.449**	1.371

Abbreviations: g = grams; Veh Cont = vehicle control

Notes: Applicant's statistical analyses Ancova/Anova & Dunnett: ** = $p \leq 0.01$

Fetal External Variations and Malformations

There were no fetal external variations in ISIS 721744- or mouse surrogate ISIS 722059-treated groups.

As shown in **Table 15**, fetal external malformation of cleft palate malformation was noted in three fetuses from two litters at 4 mg/kg/week ISIS 722059 at a greater percentage than in the historical control data for the conducting laboratory (1.1% vs. 0.8% fetuses, respectively and 8.7% vs. 4.8% litters, respectively). One control fetus was observed with cleft palate. The increased incidence of cleft palate for fetuses in the 4 mg/kg/week ISIS 722059 group was considered unlikely to be treatment-related.

Table 15 Fetal External Observations from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Parameter		Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
		0	1	4	10	4
Mouth	Number of Fetuses Examined:	252	279	297	299	284
	Number of Fetuses Evaluated:	253	279	297	299	284
	Number of Litters Evaluated:	21	22	23	23	23
Palate, Cleft Palate - Malformation	Fetuses N(%)	1(0.4)	0(0.0)	1(0.3)	0(0.0)	3(1.1)
	Litters N(%)	1(4.8)	0(0.0)	1(4.3)	0(0.0)	2(8.7)

Abbreviations: Veh Cont = vehicle control

Fetal Visceral Variations and Malformations

Only one visceral variation or malformation was noted in ISIS 721744- or ISIS 722059-treated groups with higher incidence than in the vehicle control group. Diaphragmatic hernia malformation was noted in one fetus from one litter at 4 mg/kg/week ISIS 722059 (0.7% fetuses and 4.3% litters). Due to the low incidence, this malformation was not considered related to treatment with mouse surrogate ISIS 722059.

Overall, there were no fetal visceral variations or malformations attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Fetal Skeletal Variations and Malformations

Fetal skeletal variations or malformations noted in ISIS 721744- or mouse surrogate ISIS 722059-treated groups with higher incidence than in the vehicle control group are shown in **Table 16**.

There were no treatment-related skeletal malformations or variations (e.g., high background incidence, no dose-response).

Table 16 Fetal Skeletal Variations and Malformations from Combined Fertility and Developmental Toxicity Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Finding		Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
		0	1	4	10	4
	Number of Fetuses Examined:	252	279	297	299	284
	Number of Fetuses Evaluated:	253	279	297	299	284
	Number of Litters Evaluated:	21	22	23	23	23
Pelvic Girdle						
Ilium, Left, Misaligned - Variation	Fetuses N(%)	0(0.0)	1(0.7)	0(0.0)	0(0.0)	2(1.4)
	Litters N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	2(8.7)
Ilium, Right, Misaligned – Variation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)	0(0.0)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
Rib						
Rib, 1 or more, Incomplete ossification - Variation	Fetuses N(%)	0(0.0)	1(0.7)	1(0.7)	0(0.0)	0(0.0)
	Litters N(%)	0(0.0)	1(4.5)	1(4.3)	0(0.0)	0(0.0)

Finding		Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
		0	1	4	10	4
	Number of Fetuses Examined:	252	279	297	299	284
	Number of Fetuses Evaluated:	253	279	297	299	284
	Number of Litters Evaluated:	21	22	23	23	23
Skull						
Exoccipital, Both, Fused - Malformation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
Exoccipital, Right, Fused - Malformation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
Palatine, Both, Split - Malformation	Fetuses N(%)	1(0.8)	0(0.0)	0(0.0)	0(0.0)	2(1.4)
	Litters N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)	2(8.7)
Suture bone, Supernumerary site - Variation	Fetuses N(%)	38(30.2)	26(18.4)	50(33.6)	37(24.5)	38(27.0)
	Litters N(%)	15(71.4)	15(68.2)	18(78.3)	15(68.2)	16(69.6)
Sternebra						
Sternebra, 1 or more, Branched - Variation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
Sternebra, 1 or more, Fused - Variation	Fetuses N(%)	0(0.0)	2(1.4)	3(2.0)	1(0.7)	0(0.0)
	Litters N(%)	0(0.0)	2(9.1)	3(13.0)	1(4.5)	0(0.0)
Sternebra, 1 or more, Misaligned - Variation	Fetuses N(%)	4(3.2)	5(3.5)	6(4.0)	6(4.0)	3(2.1)
	Litters N(%)	4(19.0)	4(18.2)	5(21.7)	4(18.2)	3(13.0)
Sternebra, 1 or more, Unossified - Variation	Fetuses N(%)	1(0.8)	0(0.0)	0(0.0)	0(0.0)	1(0.7)
	Litters N(%)	1(4.8)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
Sternebra, 1 or more, Isolated ossification site - Variation	Fetuses N(%)	1(0.8)	5(3.5)	2(1.3)	3(2.0)	2(1.4)
	Litters N(%)	1(4.8)	4(18.2)	2(8.7)	2(9.1)	2(8.7)
Supernumerary rib						
Cervical, 1 or more, Full - Variation	Fetuses N(%)	0(0.0)	0(0.0)	1(0.7)	0(0.0)	1(0.7)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)	1(4.3)
Thoracolumbar, 1 or more, Full - Variation	Fetuses N(%)	11(8.7)	10(7.1)	11(7.4)	20(13.2)	16(11.3)

Finding		Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
		0	1	4	10	4
	Number of Fetuses Examined:	252	279	297	299	284
	Number of Fetuses Evaluated:	253	279	297	299	284
	Number of Litters Evaluated:	21	22	23	23	23
	Litters N(%)	6(28.6)	6(27.3)	8(34.8)	8(36.4)	8(34.8)
Thoracolumbar, 1 or more, Short - Variation	Fetuses N(%)	9(7.1)	21(14.9)	19(12.8)	20(13.2)	14(9.9)
	Litters N(%)	6(28.6)	11(50.0)	11(47.8)	10(45.5)	11(47.8)
Vertebra						
Cervical arch, 1 or more, Fused - Malformation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)	0(0.0)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
Cervical arch, 1 or more, Split - Variation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.3)	0(0.0)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	2(9.1)	0(0.0)
Thoracic centrum, 1 or more, Small - Variation	Fetuses N(%)	0(0.0)	0(0.0)	1(0.7)	0(0.0)	0(0.0)
	Litters N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)	0(0.0)
Thoracic vertebra, 1 or more, Absent - Malformation	Fetuses N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.4)
	Litters N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
Thoracic vertebra, 1 or more, Fused - Malformation	Fetuses N(%)	0(0.0)	3(2.1)	2(1.3)	0(0.0)	4(2.8)
	Litters N(%)	0(0.0)	3(13.6)	2(8.7)	0(0.0)	3(13.0)

Abbreviations: Veh Cont = vehicle control

9.3 Prenatal and Postnatal Development

Study title: Study for Toxic Effects of ISIS 721744 on Pre- and Postnatal Development, Including Maternal Function in Mice

Study no.: 727-142 (Applicant No. 721744-AS09)
 Study report location: EDR SDN 1
 Conducting laboratory and location:  (b) (4)

Date of study initiation: 01/11/2023
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: ISIS 721744 bulk test article, lot # CA721744-003, 92.6% purity
 ISIS 722059 bulk test article, lot # 722059-4, 88.2% purity

Key Study Findings

- In a PPND toxicity study, F₀ female mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (5, 10, or 20 mg/kg/week), or mouse surrogate ISIS 722059 (5 mg/kg/week) every other day from GD 6 to GD 18 (GDs 6, 8, 10, 12, 14, 16, and 18) and weekly from LD 1 to LD 20 (LDs 1, 7, 13, and 20).
- There were no deaths in any of the F₀ females attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.
- AST (3x and 2x of control, respectively) and ALT (5x and 4x of control, respectively) were increased in F₀ females on LD 22 at 10 and 20 mg/kg/week ISIS 721744 that was consistent with uptake into the liver.
- Mean ISIS 721744 concentrations in milk from F₀ females was below the limit of quantitation for the vehicle control and 5 mg/kg/week ISIS 721744 groups and 7.63 and 24.63 ng/mL at 10 and 20 mg/kg/week ISIS 721744, respectively.
- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on parturition parameters (gestation index, live newborn pups/litter, live birth index, uterine implantation sites, and gestation length).
- Kidney, liver, and/or spleen weights (absolute and/or relative to terminal body weight) on LD 22 were increased in F₀ females treated with ISIS 721744 at 5, 10, and/or 20 mg/kg/week. Increases of kidney and liver weights were consistent with uptake of ISIS 721744.
- Unconjugated ISIS 721744 concentrations in the kidney and liver of F₀ females on LD 22 increased with ISIS 721744 dose, which was consistent with uptake of ISIS 721744.
- Treatment of F₀ females with mouse surrogate ISIS 722059 at 5 mg/kg/week statistically decreased hepatic mRNA expression of Klkb1/Pkk by 82% on LD 22.

- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pups (preweaning) based on evaluation of survival, clinical signs, body weights, sex ratios, physical development, and gross pathology.
- There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on the F₁ generation based on evaluation of survival, clinical signs, sexual maturation, body weights, food consumption, behavioral evaluations (motor activity, Morris Water Maze, acoustic startle response), estrous cyclicity, mating/reproductive/fertility performance indices, GD 13 corpora lutea/uterine implantation data, organ weights, and gross pathology evaluations.
- The NOAEL for maternal toxicity and the PPND study was the high dose of 20 mg/kg/week ISIS 721744.

Methods

Doses:	<u>ISIS 721744</u> 5, 10, or 20 mg/kg/week
	<u>ISIS 722059 (mouse surrogate)</u> 5 mg/kg/week
Frequency of dosing:	Every other day from GD 6 to GD 18 Weekly from LD 1 to LD 20
	See study design below
Dose volume:	15 mL/kg for weekly dosing 4.29 mL/kg for every other day dosing
Route of administration:	SC
Formulation/Vehicle:	0.9% Sodium Chloride for Injection, USP
Species/Strain:	Mouse/CD-1® IGS/Crl:CD1 (ICR)
Number/Sex/Group:	32 F ₀ female 26 selected F ₁
Satellite groups:	No
Study design:	Dosing in parental (F ₀) mated female mice began on GD 6 and continued every other day through GD 18 (GD 6, 8, 10, 12, 14, 16, and 18). During lactation, dosing began on LD 1 and continued once weekly through LD 20 (LD 1, 7, 13, and 20).
Deviation from study protocol:	Refer to Table 17 (Applicant's Table) Deviations from the study protocol were described in the study report. None of the deviations appeared to have affected the integrity, results, or conclusions of the study.

Table 17 Study Design for Prenatal and Postnatal Development Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Group No.	Test Article	Dose Level ^a (mg/kg/week)	Dose Concentration (mg/mL)	Dose Volume ^{a, b} (mL/kg)	No. of Animals		
					F ₀ Female	Selected F ₁	
						Male	Female
1	Control Article	0	0	4.29/15	32	26	26
2	ISIS 721744	5	0.33	4.29/15	32	26	26
3	ISIS 721744	10	0.67	4.29/15	32	26	26
4	ISIS 721744	20	1.33	4.29/15	32	26	26
5	ISIS 722059 ^c	5	0.33	4.29/15	32	26	26

^a Based on the most recent body weight measurement.

^b F₀ animals were dosed every other day (qod) during gestation starting on GD 6 and weekly during lactation. The weekly dose (mg/kg/week) was equivalent to 3.5 times each dose (mg/kg/dose) based on the most recent body weight measurement. A dose volume of 4.29 mL/kg was used for qod dosing and a dose volume of 15 mL/kg was used for weekly doses.

^c Mouse specific oligonucleotide.

Observations and Results

F₀ Dams

F₀ Survival:

Mortality/cageside observations for F₀ females were conducted at least twice daily (morning and afternoon) beginning upon arrival through termination/release.

There were no deaths in any of the F₀ females attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

Two F₀ females in the vehicle control group (Nos. 1508 and 1513) were found dead on LD 17 and LD 15, respectively. There were no clinical or gross pathology findings for either animal.

One F₀ female at 5 mg/kg/week ISIS 721744 (No. 2515) was found dead on LD 15. There were no clinical or gross pathology findings for this animal. Since two F₀ females were in the vehicle control group, and no animals treated with higher doses of ISIS 721744-were found dead, this death was considered incidental and unrelated to treatment with ISIS 721744.

Two F₀ females at 5 mg/kg/week ISIS 721744 (Nos. 2503 and 2529) were euthanized on LD 4 and LD 1, respectively, due to no surviving pups (i.e., total litter loss). As described in the study report, Animal No. 2503 delivered one pup which was found dead on PND 3 and Animal No. 2529 delivered three pups which were found dead on PND 0.

F₀ Clinical Signs:

Cageside observations for F₀ females were conducted predose on dosing days and once daily on non-dosing days. Detailed clinical observations for F₀ females were conducted daily (about 1.5 hours postdose on dosing days).

There were no clinical sign observations in F₀ females attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

F₀ Body Weight:

Body weights for F₀ females were recorded once prior to dosing and on GDs 0, 4, 6, 8, 10, 12, 14, 16, and 18 and LDs 1, 4, 7, 10, 14, 17, and 21.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on body weight in F₀ females.

F₀ Feed Consumption:

Quantitative food consumption for F₀ females was measured on the corresponding body weight days.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on food consumption in F₀ females.

F₀ Hematology:

Blood from F₀ females was collected for hematology evaluations on LD 22. A representative panel of hematology parameters was evaluated.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on hematology parameters in F₀ females on LD 22.

F₀ Clinical Chemistry

Blood from F₀ females was collected for clinical chemistry evaluations on LD 22. A representative panel of clinical chemistry parameters was evaluated.

As shown in **Table 18**, AST (3x and 2x of control, respectively) and ALT (5x and 4x of control, respectively) were statistically increased in F₀ females on LD 22 at 10 and 20 mg/kg/week ISIS 721744, respectively.

There were no effects of mouse surrogate ISIS 722059 on clinical chemistry parameters in F₀ females on LD 22.

Table 18 Clinical Chemistry for F₀ Females from Prenatal and Postnatal Development Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Parameter	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
	0	5	10	20	5
AST (U/L)	58.6	69.5	179.4**	137.6*	79.7
% Diff	-	18.6	206.1	134.8	36.0
tCtrl	-	1.19	3.06	2.35	1.36
ALT (U/L)	42.5	55.8	220.2**	148.6**	58.3

Parameter	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
	0	5	10	20	5
% Diff	-	31.3	418.1	249.6	37.2
tCtrl	-	1.31	5.18	3.50	1.37
GLUC (mg/dL)	384.9	325.7*	294.1**	312.3*	397.8
% Diff	-	-15.4	-23.6	-18.9	3.4
tCtrl	-	0.85	0.76	0.81	1.03
CHOL (mg/dL)	153.4	185.1	163.8	191.3*	139.8
% Diff	-	20.7	6.8	24.7	-8.9
tCtrl	-	1.21	1.07	1.25	0.91

Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase; CHOL = cholesterol; % Diff = percent difference from vehicle control; GLUC = glucose; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses Anova & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$ or Kruskal-Wallis & Dunn: * = $p \leq 0.05$; ** = $p \leq 0.01$

F₀ Milk Bioanalysis:

Milk was collected from F₀ females (6/group) on LD 15 for measurement of ISIS 721744 concentration by a hybridization enzyme-linked immunosorbent assay (HELISA) method (lower limit of quantitation [0.6 nM]).

Mean ISIS 721744 concentrations in milk from F₀ females were below the limit of quantitation for the vehicle control and 5 mg/kg/week ISIS 721744 groups and 7.63 and 24.63 ng/mL at 10 and 20 mg/kg/week ISIS 721744.

F₀ Pregnancy and Parturition:

Toward the end of the gestation period (GD 18), F₀ females were examined twice daily for signs of parturition.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on parturition parameters (gestation index, live newborn pups/litter, live birth index, uterine implantation sites, and gestation length). For instance, the mean gestation length was comparable between the vehicle control and ISIS 721744 and mouse surrogate ISIS 722059 groups (about 19 days), as was the mean number of implantation sites (about 13 to 14).

F₀ Necropsy Observation:

Scheduled euthanasia and terminal necropsy for F₀ females occurred on LD 22. Gross pathology evaluation included examination of the pituitary gland, kidney, liver, ovary, spleen, vagina, and uterus/cervix. Liver, kidney, and spleen weights were recorded.

There were no gross pathology lesions in the organs evaluated that were attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059 in F₀ females on LD 22.

As shown in **Table 19**, kidney, liver, and spleen weights on LD 22 were increased in F₀ females treated with ISIS 721744. Kidney weight (absolute and relative to terminal body weight) was statistically increased at 10 and 20 mg/kg/week ISIS 721744 (+8% to +10% difference from vehicle control, respectively). Liver weight was statistically increased for absolute weights at 10 and 20 mg/kg/week ISIS 721744 (+18% and +22% difference from vehicle control, respectively) and relative to terminal body weight at 5, 10, and 20 mg/kg/week ISIS 721744 (+10%, +19%, and +23% difference from vehicle control, respectively). Spleen weight (relative to terminal body weight) was statistically increased at 20 mg/kg/week ISIS 721744 (+19% difference from vehicle control). Increased liver and kidney weights were consistent with the uptake of ISIS 721744.

There were no effects of ISIS 722059 on organ weights on LD 22 in F₀ females.

Table 19 Organ Weights for F₀ Females from Prenatal and Postnatal Development Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice

Organ	Veh Cont	ISIS 721744 (mg/kg/week)			ISIS 722059 (mg/kg/week)
	0	5	10	20	5
Kidney Weight (g)	0.49	0.52	0.54*	0.54*	0.48
%Diff	-	5.50	8.39	9.91	-2.65
tCtrl	-	1.05	1.08	1.10	0.97
Kidney (% BW)	1.34	1.38	1.46**	1.47**	1.32
%Diff	-	3.44	8.93	9.69	-1.68
tCtrl	-	1.03	1.09	1.10	0.98
Liver/Gallbladder Weight (g)	2.62	2.93	3.10**	3.20**	2.69
%Diff	-	11.81	18.38	22.39	2.72
tCtrl	-	1.12	1.18	1.22	1.03
Liver/Gallbladder (% BW)	7.04	7.77*	8.41**	8.65**	7.32
%Diff	-	10.34	19.33	22.75	3.91
tCtrl	-	1.10	1.19	1.23	1.04
Spleen Weight (g)	0.13	0.14	0.14	0.16	0.13
%Diff	-	2.32	4.84	16.86	-2.46
tCtrl	-	1.02	1.05	1.17	0.98
Spleen (% BW)	0.36	0.37	0.38	0.43*	0.36
%Diff	-	1.59	5.59	18.26	-1.32
tCtrl	-	1.02	1.06	1.18	0.99

Abbreviations: BW = body weight; % Diff = percent difference from vehicle control; g = grams; tCtrl = fold change from vehicle control; Veh Cont = vehicle control

Notes: Applicant's statistical analyses Anova & Dunnett: * = p ≤ 0.05; ** = p ≤ 0.01

F₀ TK:

F₀ Tissue Bioanalysis

Kidney and liver tissue from F₀ females on LD 22 were analyzed using a validated UHPLC-MS/MS procedure to determine unconjugated ISIS 721744 concentrations.

As shown in **Table 20** (Applicant's Table), unconjugated ISIS 721744 concentrations in the kidney and liver of F₀ females on LD 22 were increased with increase in ISIS 721744 dose.

Table 20 Tissue Exposure from Prenatal and Postnatal Development Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Table)

Group No.	ISIS 721744 Dose Level ^a (mg/kg/week)	Interval	Gender	Number of Doses	N	Unconjugated ISIS 721744 Tissue Concentration (µg/g)	
						Kidney	Liver
2	5	LD 22	Female	11	10	60.63 ± 15.089	26.49 ± 10.266
3	10	LD 22	Female	11	10	171.70 ± 38.482	45.05 ± 13.036
4	20	LD 22	Female	11	10	437.50 ± 86.002	78.09 ± 15.557

Note: The lower limit of quantitation (LLOQ) for the assay was 0.107 µg/g for all evaluated tissues. Values are presented as mean ± standard deviation.

LD = Lactation Day; N = Number.

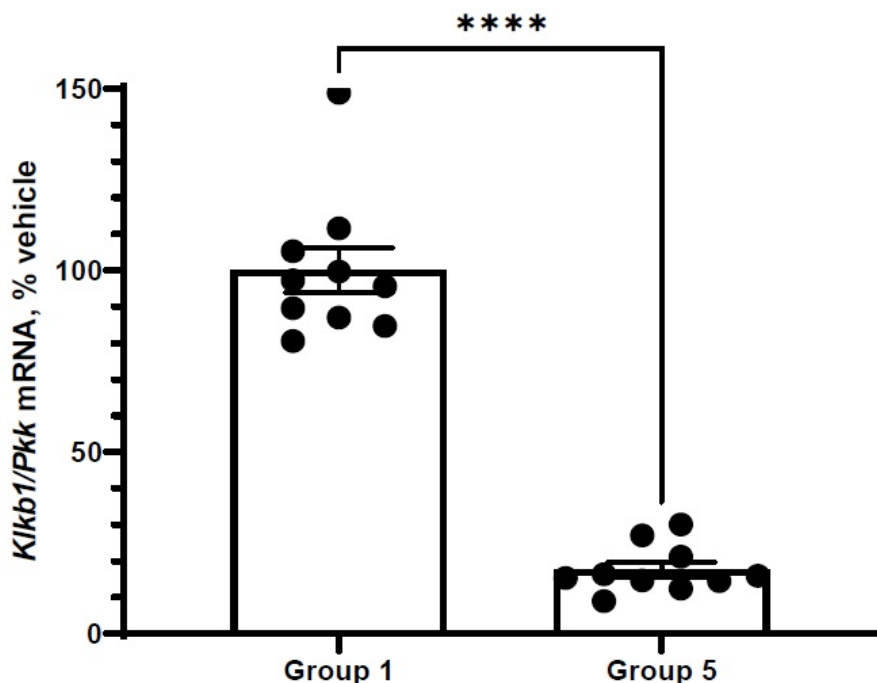
^aFemales were dosed every other day during gestation starting on GD 6 and weekly during lactation. The weekly dose (mg/kg/week) was equivalent to 3.5 times each dose (mg/kg/dose).

F₀ Target mRNA

Liver samples were collected from F₀ females at scheduled necropsy (LD 22) from the vehicle control and 5 mg/kg/week ISIS 722059 groups for RNA extraction and Klkb1/Pkk mRNA RT-qPCR analysis.

As shown in **Figure 3** (Applicant's Figure), treatment of F₀ females with mouse surrogate ISIS 722059 at 5 mg/kg/week statistically decreased hepatic mRNA expression of Klkb1/Pkk by 82% on LD 22.

Figure 3 Hepatic Kikb1/Pkk mRNA Expression Following Administration of Mouse Surrogate ISIS 722059 from Prenatal and Postnatal Development Study with Subcutaneous Administration of ISIS 721744 and Mouse Surrogate ISIS 722059 in Mice (Applicant's Figure)



Average and SEM indicated for both males and females. **** Significant difference from saline (Group 6) using two-tailed unpaired t-test ($p < 0.0001$)

Dosing Solution Analysis:

Concentration verification of each dose formulation was documented by the Applicant and provided in the study report.

The concentration results were within the acceptance criterion of (b) (4) % indicating that the dosing solutions were prepared at the correct concentrations.

F₁ Pups (Prewaning)

F₁ pups were checked for litter size (total number live born pups and partially cannibalized pups), number of live born pups, number of pups per sex, clinical signs, body weights, gross abnormalities, and physical development.

F₁ Pup Survival (Prewaning):

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pup survival from PND 1 to PND 4 precull or from PND 4 postcull to PND 21.

F₁ Pup Sex Ratios (Prewaning)

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pup sex ratios (% male pups/litter).

F₁ Pup Clinical Signs (Prewaning):

Detailed clinical observations were conducted on F₁ pups daily from PND 1 to PND 28.

There were no clinical sign observations attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pups from PND 1 to 21 (preweaning) and PND 28 (postweaning).

F₁ Pup Body Weight (Prewaning):

F₁ pups were weighed on PNDs 1, 4, 7, 10, 14, 17, and 21 (preweaning) and PND 28 (postweaning).

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pup body weight from PND 1 to PND 21 (preweaning) and PND 28 (postweaning).

F₁ Pup Physical Development (Prewaning):

Each F₁ pup was evaluated for pinna detachment (unfolding of the pinna) on PND 3. If pups did not have both pinna detached on PND 3 they were observed daily until detachment was complete.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pup pinna detachment.

Each F₁ pup was evaluated for eye opening on PND 12. If pups did not have both eyes open on PND 12 they were observed daily until eye opening was detected.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pup eye opening.

Each F₁ pup was evaluated for auditory startle response on PND 21.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pup auditory startle response.

F₁ Pup Gross Pathology:

F₁ pups were examined externally on PNDs 1, 4, 7, 10, 14, 17, and 21 (preweaning) and PND 28 (postweaning).

There were no gross findings attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059 on F₁ pups found dead/euthanized early during lactation, PND 4 culled pups (examined externally), and on PND 28 (postweaning).

Selected F₁ Generation (Postweaning)

As described in the study report: After weaning, selected F₁ animals were observed for clinical signs, body weights, body weight gains during gestation, food consumption, organ weights, and anatomical pathology including a uterine examination. The selected

F₁ animals were also evaluated for growth, sexual maturation, behavior, reproductive performance, and fertility.

F₁ Generation Mortality (Postweaning):

There were no deaths in the F₁ generation during the postweaning period attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

F₁ Generation Clinical Signs (Postweaning):

Clinical observations were conducted for males weekly from PND 28 to PND 129 (day of scheduled euthanasia) and for females weekly from PND 28 through the pre mating phase and then on GDs 0, 3, 7, 10, and 13.

There were no clinical sign observations in F₁ generation males or females during the postweaning period or gestation period in F₁ females that were attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

F₁ Generation Body Weight (Postweaning):

Body weights were recorded for F₁ males weekly from PND 28 until termination and on the day of scheduled euthanasia (PND 129). Body weights were recorded for F₁ females weekly from PND 28 until positive evidence of copulation and during gestation on GDs 0, 3, 7, 10, and 13.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on body weight in F₁ generation males or females during the postweaning period or the gestation period in F₁ females.

Feed consumption (Postweaning):

Change in quantitative food consumption was measured on the corresponding body weight days.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on food consumption in F₁ generation males or females during the postweaning period or the gestation period in F₁ females.

F₁ Sexual Maturation (Postweaning):

Selected F₁ females were evaluated for vaginal opening beginning on PND 21. Selected F₁ Males were evaluated for preputial separation beginning on PND 22.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on sexual maturation as evaluated by vaginal opening in F₁ females or preputial separation in F₁ males.

F₁ Generation Neurological Assessment (Postweaning):

Motor activity (i.e., basic movement, fine movement, rearing, and total distance) was assessed for F₁ generation males and females on PND 37.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on motor activity in F₁ generation males or females on PND 37.

Auditory startle habituation was conducted for F₁ generation males and females from PNDs 65 to 70.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on auditory startle habituation in F₁ generation males or females from PNDs 65 to 70.

The Morris Water Maze was conducted for F₁ generation males and females from PND 70 to 100.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on learning and memory by Morris Water Maze analysis.

F₁ Generation Reproduction (Postweaning):

As described in the study report: When the selected F₁ animals reached a minimum of 90 days of age, one male and one female from the same treatment group were placed together for mating. Mating took place in the cage of the male. Daily inspection for a copulatory plug in situ or vaginal lavage for sperm was performed daily on females during the cohabitation period. The day on which evidence of mating was observed was designated as GD 0. Upon evidence of mating, the female was removed from the cage of the male and individually housed. Estrous cycle determinations were made on F₁ females during mating and until evidence of copulation was observed or the cohabitation period ended.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on the estrous cycle in F₁ females during the mating phase.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on reproductive performance in F₁ males or females (mating, fertility, or pregnancy indices; pre-coital interval).

F₁ Generation Gross Pathology (Postweaning):

There were no gross pathology findings in F₁ generation males or females attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

F₁ Generation Organ Weights (Postweaning):

Organ weights for epididymis, kidney, liver, ovary, prostate gland, seminal vesicles, spleen, and testis were collected (paired organs were weighed together).

There were no effects on organ weights in F₁ generation males or females attributed to treatment with ISIS 721744 or mouse surrogate ISIS 722059.

F₁ Uterine Examination (Postweaning):

On GD 13, uterine examination for the mean number of corpora lutea, implantation sites, live embryos, resorptions, and pre- and post-implantation loss indices was performed for F₁ females.

There were no effects of ISIS 721744 or mouse surrogate ISIS 722059 on mean number of corpora lutea, implantation sites, live embryos, resorptions, or pre- and post-implantation loss indices in F₁ females.

11 Integrated Summary and Safety Evaluation

ISIS 721744 is a GalNAc-conjugated ASO, which targets human PKK. The GalNAc molecule preferentially delivers ISIS 721744 to the liver by binding to the ASGPR on liver cells. SC administration of ISIS 721744 is proposed as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older.

This review evaluates the GLP-compliant reproductive and developmental toxicology studies with ISIS 721744 (i.e., combined fertility and developmental toxicity study in mice and PPNP study in mice) to support the safety of ISIS 721744 for marketing approval. ISIS 721744 does not have perfect alignment to the mouse PKK gene sequence. As such, ISIS 721744 is not pharmacologically active in mice and the mouse surrogate ISIS 722059, which targets mouse PKK, was included in both studies in mice to assess potential toxicities related to the intended pharmacology of PKK inhibition.

This review also provides a summary of the GLP-compliant reproductive and developmental toxicology studies with ISIS 546254 (i.e., combined fertility and developmental toxicity study in mice and embryofetal development [EFD] study in rabbits). ISIS 546254 is the unconjugated parent compound of ISIS 721744, which has the same sequence as ISIS 721744 (i.e., 5'-TGCAAGTCTCTTGGCAAACA-3'), but with a uniform backbone of phosphorothioate diester internucleotide linkages instead of a mixed backbone like ISIS 721744. Based on specificity analysis against the human transcriptome, ISIS 546254 is completely aligned to only PKK RNA. ISIS 546254 does not have perfect alignment with the mouse PKK mRNA. Since ISIS 546254 is not pharmacologically active in mice, the unconjugated mouse surrogate ISIS 482584, which targets mouse PKK, was included in the combined fertility and developmental toxicity study in mice to assess potential toxicities related to the intended pharmacology of PKK inhibition. Refer to summaries below.

Fertility and Early Embryonic Development

In the combined fertility and developmental toxicity study, mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (1, 4, or 10 mg/kg/week), or mouse surrogate ISIS 722059 (4 mg/kg/week). Males were dosed once weekly during the pre-mating, mating, and post-mating phases until euthanasia (about 10 weeks total). Females were dosed weekly during the pre-mating and mating phases and then every other day during the gestation period from GD 0 to GD 16 (encompassing the periods of implantation and organogenesis). Since ISIS 721744 is not active in mice, the mouse-surrogate ISIS 722059 was included in the

study to assess the potential effects related to the target inhibition. ISIS 722059 statistically decreased PKK mRNA expression in the liver of male and female mice by 80% and 75%, respectively. Notably, unconjugated ISIS 721744 concentrations in the placenta (up to 0.31 mcg/g) were >10% lower than in the liver (i.e., 3 to 43 mcg/g for 1 to 10 mg/kg/week ISIS 721744 in females on GD 18). SC administration of ISIS 721744 (up to 10 mg/kg/week) or mouse surrogate ISIS 722059 (4 mg/kg/week) did not affect fertility, pregnancy, or embryofetal development. It was unclear if ISIS 721744 or the unconjugated version of ISIS 721744 crossed the placenta to reach fetal tissues.

A summary of the GLP-compliant combined fertility and developmental toxicity study with ISIS 546254 (unconjugated parent compound of ISIS 721744, which has the same sequence as ISIS 721744) and unconjugated mouse surrogate ISIS 482584 (Study No. (b) (4) 090.60; Applicant No. 546254-AS04) is provided here.

- CD-1 mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 546254 (10.5, 42, or 84 mg/kg/week), or mouse surrogate ISIS 482584 (84 mg/kg/week). As described in the study report, animals were dosed every other day and received three or four doses per week; thus, the weekly dose (mg/kg/week) was equivalent to 3.5 times the single mg/kg/dose. Males were dosed every other day during the pre-mating (beginning 4 weeks prior to mating), mating, and post-mating phases (10 weeks total). Females were dosed every other day for two weeks prior to mating, during the mating phase, and throughout GD 15 (encompassing the period of organogenesis).
- There was one unscheduled death during the study. One male at 84 mg/kg/week ISIS 482584 was euthanized on Day 49 due to declining clinical condition (lack of skin turgor and progressive face swelling). The animal was noted with a SC hemorrhagic mass on top of the skull, which was considered incidental, likely of traumatic origin. There were no remarkable gross findings in the other tissues and organs that were examined. The unscheduled death was considered incidental and unrelated to treatment with mouse surrogate ISIS 482584. All other animals survived to scheduled termination.
- Body weight decreases were noted in females on GD 18 at 10.5 and 84 mg/kg/week ISIS 546254 and 84 mg/kg/week ISIS 482584 (less than 10% difference from vehicle control). The decreases were not considered adverse.
- Decreases (percent difference from vehicle control) in hematology parameters were noted at 42 and/or 84 mg/kg/week ISIS 546254 in males on Day 71 for RBC (up to -14%), hemoglobin (up to -23%), hematocrit (up to -21%), mean corpuscular volume (MCV) (up to -9%), mean corpuscular hemoglobin (MCH) (up to -11%), and platelets (up to -26%) and females on GD 18 for platelets (up to -21%).
- Decreases (percent difference from vehicle control) in hematology parameters were noted at 84 mg/kg/week ISIS 482584 in males on Day 71 for hemoglobin (-18%), hematocrit (-14%), MCV (-14%), MCH (-18%), and platelets (-54%) and in females on GD 18 for platelets (-54%).
- Increases (percent difference from vehicle control) in hematology parameters were noted at 42 and/or 84 mg/kg/week ISIS 546254 in males on Day 71 for

- RBC distribution width (up to +16%), white blood cells (WBC) (up to +63%), neutrophils (up to +58%), and monocytes (up to +207%) and in females on GD 18 for lymphocytes (up to +41%) and monocytes (up to +160%).
- Increases (percent difference from vehicle control) in hematology parameters were noted at 84 mg/kg/week ISIS 482584 in males on Day 71 for RBC distribution (+23%), WBC (+112%), neutrophils (+172%), lymphocytes (+98%), and monocytes (+223%) and in females on GD 18 for WBC (+65%), neutrophils (+77%), lymphocytes (+53%), and monocytes (+276%).
 - Decreases (percent difference from vehicle control) in clinical chemistry parameters were noted at 10.5, 42, and/or 84 mg/kg/week ISIS 546254 in males on Day 71 for total protein (up to -11%), albumin (up to -20%), A/G (up to -20%) and in females on GD 18 for triglycerides (up to -54%) and creatine kinase (up to -51%).
 - Decreases (percent difference from vehicle control) in clinical chemistry parameters were noted at 84 mg/kg/week ISIS 482584 in males on Day 71 for albumin (-9%), A/G (-15%), triglycerides (-44%), total cholesterol (-40%) and in females on GD 18 for triglycerides (-59%) and creatine kinase (-65%).
 - Increases (percent difference from vehicle control) in clinical chemistry parameters associated with liver weight changes in most cases were noted at 42 and/or 84 mg/kg/week ISIS 546254 in males on Day 71 for ALT (up to +627%), AST (up to +309%), and ALP (+101%).
 - Increases (percent difference from vehicle control) in clinical chemistry parameters associated with liver weight changes were noted at 84 mg/kg/week ISIS 482584 in males on Day 71 for ALT (+380%) and AST (up to +402%).
 - Increases (percent difference from vehicle control) in organ weights were noted at 10.5, 42, and/or 84 mg/kg/week ISIS 546254 in males for liver (up to +32%) and spleen (up to +38%) and in females for liver (up to +15%) and spleen (+49%).
 - Increases (percent difference from vehicle control) in organ weights were noted at 84 mg/kg/week ISIS 482584 in males for liver (+29%) and spleen (+117%) and in females for spleen (+93%).
 - Decreases (percent difference from vehicle control) in organ weights were noted at 84 mg/kg/week ISIS 546254 and 84 mg/kg/week ISIS 482584 in males for prostate with seminal vesicle (-21% and -31%, respectively).
 - There were no effects of ISIS 546254 or mouse surrogate ISIS 482584 on estrous cyclicity.
 - There were no effects of ISIS 546254 or mouse surrogate ISIS 482584 on fertility (mating index) or fecundity (pregnancy index).
 - At the time of Cesarean section, mean gravid uterus weights were decreased at 10.5, 42, and 84 mg/kg/week ISIS 546254 (-11%, -5%, and -7% difference from vehicle control respectively; not statistically significant) and at 84 mg/kg/week ISIS 482584 (-24% difference from vehicle control; statistically significant).
 - The number of live fetuses was decreased at 10.5, 42, and 84 mg/kg/week ISIS 546254 (12 live fetuses in each group; statistically significant only at 10.5 mg/kg/week ISIS 546254) and at 84 mg/kg/week ISIS 482584 (11 live fetuses; statistically significant) compared to the vehicle control (14 live fetuses). This is

not likely biologically significant. In addition, the fetal viability percentage at 84 mg/kg/week ISIS 482584 was statistically decreased compared to the vehicle control (86% vs. 96%).

- There were no effects of ISIS 546254 on mean fetal weight (male or female) although at 84 mg/kg/week ISIS 482584 mean fetal weight was decreased for males and females (-11% and -10% difference from vehicle control, respectively; statistically significant in females only).
- The number of early resorptions was increased at 10.5, 42, and 84 mg/kg/week ISIS 546254 (1 in each group; not statistically significant) and at 84 mg/kg/week ISIS 482584 (1; statistically significant) compared to the vehicle control (0). This is not likely biologically significant. The percent early resorptions/animal was increased at 10.5, 42, and 84 mg/kg/week ISIS 546254 and at 84 mg/kg/week ISIS 482584 compared to the vehicle control. The embryo-fetal mortality index was increased at 10.5, 42, and 84 mg/kg/week ISIS 546254 and at 84 mg/kg/week ISIS 482584 compared to the vehicle control. As described in the study report: Although these changes were noticeable and statistically significant in some groups, they were consistent with what is noted in the control animals in these types of studies, therefore, a clear relationship to either test article cannot be established.
- Overall, there were no fetal external findings, visceral variations or malformations, or skeletal variations or malformations attributed to treatment with ISIS 546254 or mouse surrogate ISIS 482584 in mice.
- There were no effects of ISIS 546254 or mouse surrogate ISIS 482584 on sperm motility, morphology, or sperm count.
- ISIS 546254 concentrations in the parental liver of vehicle control males and females were below the limit of quantitation. ISIS 546254 concentrations in the parental liver of males and females increased with ISIS 546254 dose with higher concentrations in male mice than female mice. ISIS 546254 concentrations in the fetal liver were below the limit of quantitation in all ISIS 546254-treated groups. ISIS 546254 concentrations in the placenta were below the limit of quantitation, 6.73, and 22.3 mcg/g with doses of 10.5, 42, and 84 mg/kg/week ISIS 546254, respectively. Notably, ISIS 546254 concentrations in the placenta were lower than in the liver (52 to 612 mcg/g for 10.5 to 84 mg/kg/week ISIS 546254 in males and females).
- Treatment of male and female mice with mouse surrogate ISIS 482584 at 84 mg/kg/week statistically decreased hepatic mRNA expression of Klkb1/Pkk by 94% and 87%, respectively.
- Conclusion: SC administration of ISIS 546254 (up to 84 mg/kg/week) or mouse surrogate ISIS 482584 (84 mg/kg/week) did not affect fertility, pregnancy, or embryofetal development in mice.

Embryofetal Development

It was agreed upon at the time of the EOP2 meeting that an EFD study in rabbits with ISIS 721744 would not be needed (refer to **Section 2.7**). This was based on the availability of an EFD study in rabbits with ISIS 546254 (unconjugated parent compound of ISIS 721744, which has the same sequence as ISIS 721744). A summary of the

GLP-compliant EFD study in rabbits with ISIS 546254 (Study No. (b) (4) 090.61; Applicant No. 546254-AS05) is provided here.

- Pregnant New Zealand white rabbits were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP) or ISIS 546254 (12.5, 25, or 75 mg/kg/week) every other day from GD 6 to GD 18 (i.e., GDs 6, 8, 10, 12, 14, 16, and 18) encompassing the period of organogenesis. As stated in the study report, animals were dosed every other day, thus, the weekly dose (mg/kg/week) was equivalent to 3.5 times the single mg/kg dose.
- There were no unscheduled deaths during the study.
- Food consumption was decreased beginning on GD18 to GD 19 at 12.5, 25, and 75 mg/kg/week (-9%, -5%, and -31% difference from vehicle control, respectively). The decrease was more consistent at 75 mg/kg/week and was statistically decreased from the vehicle control between GD 25 and GD 27 (up to -41% difference from vehicle control). The decrease in food consumption did not appear to affect body weight or body weight gain.
- Changes in hematology parameters in females on GD 28 at 12.5, 25, and/or 75 mg/kg/week included decreases (percent difference from vehicle control) in RBC (up to -16%), hemoglobin (up to -16%), hematocrit (up to -16%), and basophils (up to -48%) and increases in RBC distribution width (up to +7%), WBC (up to +27%), neutrophils (up to +52%), and lymphocytes (up to +27%)
- Changes in clinical chemistry parameters in females on GD 28 at 25 and/or 75 mg/kg/week included decreases (percent difference from vehicle control) for albumin (up to -9%) and ALP (up to -45%) and increases (percent difference from vehicle control) for triglycerides (up to +87%).
- Organ weight changes in females necropsied on GD 28 were noted in the liver, kidneys, and spleen as follows:
 - Liver weight (absolute and relative to body weight) was statistically increased at 25 mg/kg/week (+16% and +18% difference from vehicle control, respectively) and 75 mg/kg/week (+40% and +39% difference from vehicle control, respectively)
 - Kidney weight (absolute and relative to body weight) was statistically increased at 75 mg/kg/week (+15% and +14% difference from vehicle control, respectively)
 - Increased liver and kidney weights were consistent with the uptake of ISIS 546254.
 - Spleen weight (absolute and relative to body weight) was statistically increased at 75 mg/kg/week (+45% and +44% difference from vehicle control, respectively)
- ISIS 546254 concentrations in the maternal liver of vehicle control females on GD 28 were below the limit of quantitation. ISIS 546254 concentrations increased in the maternal liver of females on GD 20 (TK animals) and GD 28 with increase in ISIS 546254 dose, with concentrations on GD 28 about half of the concentrations on GD 20 (TK animals). ISIS 546254 concentrations in the fetal liver were below the limit of quantitation in all ISIS 546254-treated groups. ISIS 546254 concentrations in the placenta were below the limit of quantitation at 12.5

mg/kg/week ISIS 546254 and at <10% of maternal exposures at 25 and 75 mg/kg/week ISIS 546254.

- There was no effect of ISIS 546254 on fecundity as the fecundity index was similar between 12.5, 25, and 75 mg/kg/week (88%, 100%, and 84%, respectively) and the vehicle control (91%).
- There were no effects of ISIS 546254 on gravid uterine weight, the number of corpora lutea, the number of implantations, the implantation index, percent of early or late resorptions, live fetuses, the sex ratio, or fetal viability.
- Mean fetal weight appeared to decrease at 75 mg/kg/week for males (-12.3% difference from vehicle control; statistically significant), females (-10.4% difference from vehicle control), and combined (-11.6% difference from vehicle control; statistically significant)
- The embryo-fetal mortality index at 12.5, 25, and 75 mg/kg/week (5.0%, 5.7%, and 6.7%, respectively) was increased (not statistically significant) compared to the vehicle control (1.3%). The percent of dead fetuses/implantation site at 12.5, 25, and 75 mg/kg/week (1.3%, 0.3%, and 5.8%, respectively) was increased (statistically significant at 75 mg/kg/week only) compared to the vehicle control (0%). As stated in the study report, although these changes were noticeable and statistically significant at 75 mg/kg/week, they were consistent with what is noted in the vehicle control animals in these types of studies, therefore, a clear relationship to test article cannot be established.
- Overall, there were no fetal external findings, visceral variations or malformations, or skeletal variations or malformations attributed to treatment with ISIS 546254 in rabbits.

Prenatal and Postnatal Development

In the PPND toxicity study, F₀ female mice were treated by SC administration with vehicle control (0.9% Sodium Chloride for Injection, USP), ISIS 721744 (5, 10, or 20 mg/kg/week), or mouse surrogate ISIS 722059 (5 mg/kg/week) every other day from GD 6 to GD 18 and weekly from Lactation Day (LD) 1 to LD 20 (the treatment period encompassed organogenesis through weaning). Since ISIS 721744 is not active in mice, the mouse-surrogate ISIS 722059 was included in the study to assess the potential effects related to the target inhibition. ISIS 722059 statistically decreased PKK mRNA expression in the liver of F₀ female mice by 82% on LD 22. SC administration of ISIS 721744 (up to 20 mg/kg/week) or mouse surrogate ISIS 722059 (5 mg/kg/week) did not affect pregnancy in F₀ females or prenatal and postnatal development (including behavioral, fertility, and reproduction function) in F₁ offspring. ISIS 721744 was present in milk of lactating F₀ females at 10 and 20 mg/kg/week.

Safety Evaluation:

In the combined fertility and developmental toxicity study in mice, SC administration of ISIS 721744 (up to 10 mg/kg/week) or mouse surrogate ISIS 722059 (4 mg/kg/week) did not affect fertility, pregnancy, or embryofetal development. In the PPND toxicity study in mice, SC administration of ISIS 721744 (up to 20 mg/kg/week) or mouse surrogate ISIS 722059 (5 mg/kg/week) did not affect pregnancy in F₀ females or prenatal and postnatal development (including behavioral, fertility, and reproduction

function) in F₁ offspring. The reproductive and developmental toxicology studies with ISIS 721744 did not provide TK of ISIS 721744 from blood (e.g., AUC and C_{max} exposures) for which to calculate exposure margins between the animal and clinical studies.

In a combined fertility and developmental toxicity study in mice, SC administration of ISIS 546254 (up to 84 mg/kg/week), unconjugated parent compound of ISIS 721744, or unconjugated mouse surrogate ISIS 482584 (84 mg/kg/week) did not affect fertility, pregnancy, or embryofetal development. In an EFD study in rabbits, SC administration of ISIS 546254 (up to 75 mg/kg/week) every other day from GD 6 to GD 18 did not result in any fetal external findings, visceral variations or malformations, or skeletal variations or malformations attributed to treatment with ISIS 546254.

Conclusion: The reproductive and developmental toxicology studies were judged to be adequate to support the safety of ISIS 721744 for marketing. There are no outstanding concerns from the nonclinical perspective with respect to the reproductive toxicity assessment.

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/s/

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I concur

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 219407

Supporting document/s: 1, 8, and 10

Applicant's letter date: 08/21/2024, 10/25/2024, and 11/04/2024

CDER stamp date: 08/21/2024, 10/25/2024, and 11/04/2024

Product: Donidalorsen (ISIS 721744)

Pharmacologic Class: Prekallikrein (PKK)-targeted *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO)

Indication: Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older

Applicant: Ionis Pharmaceuticals Inc.

Clinical Review Division: Division of Pulmonology, Allergy, and Critical Care (DPACC)

Pharm/Tox Division: Division of Pharmacology Toxicology for Immunology and Inflammation (DPT-II)

Reviewer: Eleni Salicru, PhD

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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Ionis Pharmaceuticals Inc. (Applicant) submitted a 505(b)(1) New Drug Application (NDA) for subcutaneous (SC) administration of donidalorsen (ISIS 721744) as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older. ISIS 721744 is an *N*-acetyl galactosamine (GalNAc)-conjugated antisense oligonucleotide (ASO), which targets prekallikrein (PKK). The GalNAc molecule preferentially delivers ISIS 721744 to the liver.

This review focuses on the evaluation of the 6-month carcinogenicity study of ISIS 721744, as well as the mouse surrogate, ISIS 722059, by SC injection in CByB6F1-Tg(HRAS)2Jic Hemizygous Transgenic mice (Tg.rasH2 mice). The mouse is not a pharmacologically relevant species with ISIS 721744 and this study primarily assessed off-target toxicity. As such, the mouse surrogate, ISIS 722059, was included to assess the effect of the intended pharmacology to inhibit synthesis of PKK. The Executive Carcinogenicity Assessment Committee (ECAC) concurred with the doses for the study in male mice but could not recommend doses for female mice (refer to Carcinogenicity Special Protocol Assessment-2 [SPA-2] agreement dated 11/16/2022).

Of note, a two-year carcinogenicity study in Sprague Dawley (SD) rats is in progress. The Division previously agreed that it would be acceptable to submit a summary of the in-life data for the 2-year carcinogenicity study in rats at the time of the NDA submission and to submit the final report as a post marketing requirement (PMR) (Refer to IND 142564 pre-NDA Meeting Preliminary Comments dated 04/10/2024). Per the Applicant, at the time of the NDA submission, the 2-year rat study was at >Week 74 of treatment. The final study report is expected to be available around Quarter 4 (Q4) of 2025.

This review also provides an evaluation of the genotoxicity studies with ISIS 721744.

1.2 Brief Discussion of Nonclinical Findings

The genotoxic potential of ISIS 721744 was evaluated in the Good Laboratory Practice (GLP)-compliant in vitro Ames assay, in vitro chromosome aberration assay, and in vivo mammalian micronucleus assay in mice. The carcinogenic potential of ISIS 721744 (off-target) and mouse surrogate ISIS 722059 (intended pharmacology to inhibit PKK synthesis) was evaluated in the GLP-compliant 6-month carcinogenicity study in Tg.rasH2 mice.

The in vitro Ames assay, using the plate incorporation method, was conducted to evaluate the potential mutagenicity of ISIS 721744 (15 to 5000 mcg/plate) in *S. typhimurium* TA98, TA100, TA1535 and TA1537, and *E. coli* WP2uvrA tester strains (with or without 10% S9 mix for metabolic activation). There was no increase in the number of revertant colonies in any tester strain, at any ISIS 721744 concentration up to 5000 µg/plate, with or without metabolic activation. ISIS 721744 was negative for

mutagenicity up to 5000 µg/plate with or without metabolic activation. Refer to the IND 142564 Nonclinical Primary Review dated 08/13/2019 for further information.

The in vitro chromosome aberration assay was conducted to evaluate the clastogenic potential of ISIS 721744 (62.5 to 500 mcg/mL) in Chinese hamster lung (CHL) cells for 6 hours (with or without 2% S9 mix for metabolic activation) and for 22 hours (without S9 mix). No cytotoxicity was noted at up to 500 mcg/mL ISIS 721744 after 6 hours with or without 2% S9 mix and 22 hours without S9 mix. There was no significant increase in the number of metaphases with structural aberrations up to 500 mcg/mL ISIS 721744 after 6 hours (with or without 2% S9 mix) and 22 hours (without S9 mix). ISIS 721744 was negative for clastogenicity up to 500 mcg/mL with or without S9 in cultured CHL cells. Refer to the IND 142564 Nonclinical Primary Review dated 08/13/2019 for further information.

The in vivo mammalian micronucleus assay was conducted to evaluate the potential clastogenicity of ISIS 721744 (500, 1000, and 2000 mg/kg SC) administered on Days 1 and 3 (48 hours postdose on Day 1) to ICR mice. Mice were sacrificed on Day 4 (24 hours postdose on Day 3) for bone marrow collection. There was no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes (MNPCEs) at any dose of ISIS 721744 tested compared to the vehicle control (negative control) group. ISIS 721744 was negative in the in vivo bone marrow micronucleus assay in mice. Refer to **Section 7** for further information.

Conclusion: Overall, ISIS 721744 was not genotoxic in a complete battery of genotoxicity assays.

The 6-month study in Tg.rasH2 mice was conducted to evaluate the carcinogenic potential of ISIS 721744 (off-target) and mouse surrogate ISIS 722059 (intended pharmacology to inhibit PKK synthesis). Tg.rasH2 mice (n = 25/sex/group) were administered vehicle control (0.9% sodium chloride for injection, USP), ISIS 721744 (5, 10, or 20 mg/kg/dose for males and 10, 20, or 60 mg/kg/dose for females), or mouse surrogate ISIS 722059 (10 mg/kg/dose) every two weeks (Q2W) for up to 6 months by SC injection. A positive control group was also included in which Tg.rasH2 mice (10/sex) were administered urethane (500 mg/kg/dose) via intraperitoneal (IP) injection on Days 1, 3, and 5. A maximum tolerated dose (MTD) was achieved at the high dose of 20 mg/kg Q2W in males based on histopathology findings in the liver and elevations of glutamate dehydrogenase [GLDH]. The high dose of 60 mg/kg Q2W in females did not meet the criteria for a MTD.

There was no statistically significant increase or decrease in mortality dose response across the vehicle control and ISIS 721744-treated groups, in either male or female mice. There was a statistically significant decrease in mortality in the male 5 mg/kg/dose group (low dose) compared to the vehicle control group.

Effects on clinical chemistry included increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and GLDH in males at 10 and 20 mg/kg/dose ISIS

721744 and females at 60 mg/kg/dose ISIS 721744, which correlated with histopathology findings in the liver.

Neoplastic histopathology findings of hepatocellular adenoma in the liver were seen in 1/25 males at 5 mg/kg/dose ISIS 721744, 2/25 males at 20 mg/kg/dose ISIS 721744, and 1/25 males at 10 mg/kg/dose ISIS 722059. The adenomas at 20 mg/kg/dose were accompanied by several non-neoplastic findings in the liver (see below). Based on the FDA Statistical Review and Evaluation, there was no statistically significant dose response relationship across the vehicle control and the ISIS 721744-treated groups in any tumor types in either male or female mice. The pairwise comparisons also showed no tumor types with a statistically significant increase in tumor incidences in ISIS 721744- or ISIS 722059-treated groups, when compared to the vehicle control group in either male or female mice.

Non-neoplastic histopathology findings in ISIS-721744-treated and/or ISIS-722059 mice were noted in the **liver** (microvesicular hepatocellular vacuolation, basophilic pigmented macrophages, hepatocellular single cell necrosis, karyocytomegaly, and/or basophilic focus of cellular alteration); **kidney** (epithelial vacuolation and epithelial basophilic granules); **injection site** (macrophage granular vacuolation); **urinary bladder** (urothelial vacuolation); and the **axillary and/or mesenteric lymph nodes** (macrophage vacuolation). All histopathological findings were attributed to or a consequence of oligonucleotide uptake.

The positive control (urethane) group resulted in neoplastic findings consistent with known effects of urethane administration¹ and confirmed the carcinogenic responsiveness of the Tg.rash2 model.

The mouse is not a pharmacologically relevant species for ISIS 721744, so the study included one dose group treated with mouse surrogate ISIS 722059. Hepatic PKK mRNA expression was statistically reduced in male and female mice treated with 10 mg/kg/dose ISIS 722059 Q2W for 26 weeks by 94% and 79%, respectively, compared to controls.

Conclusion: Treatment with ISIS 721744 resulted in hepatocellular adenoma in 2/25 males at 20 mg/kg/dose ISIS 721744, which was not statistically significant based on FDA Statistical Review and Evaluation. The ECAC concluded that the carcinogenicity study was adequate for male mice and negative for drug-related tumors. However, the carcinogenicity study was inadequate for female mice because a MTD was not achieved and no other valid basis to support the high dose was discernable.

¹National Toxicology Program. 15th Report on Carcinogens [Internet]. Research Triangle Park (NC): National Toxicology Program; 2021 Dec 21. Urethane: CAS No. 51-79-6. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK590786/>

O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-P-thioadenylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-P-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)adenosine, icosasodium salt

Molecular Formula/Molecular Weight

C₂₉₆H₄₃₅N₈₃O₁₅₁P₂₀S₁₅ (free acid)

C₂₉₆H₄₁₅N₈₃O₁₅₁P₂₀S₁₅Na₂₀ (sodium salt)

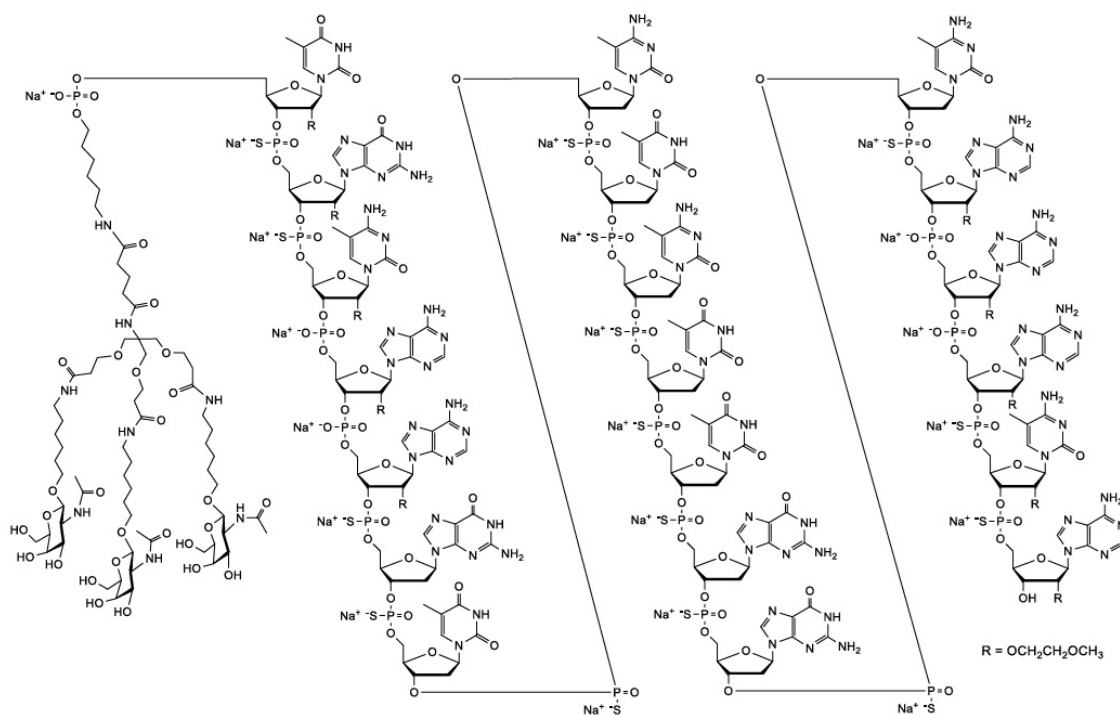
8672.66 Da (free acid)

9112.27 Da (sodium salt)

Structure or Biochemical Description

The structure of donidalorsen sodium is shown in **Figure 1** (Applicant's Figure).

Figure 1 Structure of Donidalorsen Sodium (Applicant's Figure)



Pharmacologic Class

PKK-targeted GalNAc-conjugated ASO

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 142564 (active): Donidalorsen (ISIS 721744) by Ionis Pharmaceuticals Inc. for prophylaxis to prevent attacks of HAE in Center for Drug Evaluation and Research/Office of New Drugs/Office of Immunology and Inflammation/Division of Pulmonology, Allergy, and Critical Care (CDER/OND/OII/DPACC)

2.3 Drug Formulation

The donidalorsen drug product is a sterile, single-dose, ready-to-use parenteral solution for SC administration. The qualitative and quantitative drug product formulation is shown in **Table 1** (Applicant's Table). The drug product is packaged in a staked needle glass prefilled syringe (PFS) closed with a (b) (4) rubber plunger stopper. The PFS is assembled into an autoinjector for the final drug product presentation. The autoinjector delivers a single dose of 80 mg donidalorsen.

Table 1 Donidalorsen Drug Product Formulation (Applicant's Table)

Ingredients	Quality Standards ^j	Quantity per Autoinjector	Quantity per Unit Dose (0.8mL)	Pharmaceutical Function
Donidalorsen ^a	Professed ^b	(b) (4) mg (equivalent to (b) (4) mg as sodium salt)	80 mg (equivalent to 84 mg as sodium salt)	Active
Sodium dihydrogen phosphate (b) (4)	USP-NF	(b) (4)		
Disodium hydrogen phosphate (b) (4)	USP-NF			
Sodium chloride	USP-NF			
Hydrochloric acid ^f	USP-NF	QS ^{g, h}	QS ^h	pH adjustment
Sodium hydroxide ^f	USP-NF	QS ^h	QS ^h	pH adjustment
Water for Injection (WFI)	USP-NF	QS to (b) (4) mL ⁱ	QS to 0.8 mL	Vehicle

^a Donidalorsen drug substance is supplied as the sodium salt. The label claim is expressed as the free acid concentration

^b Per [Section 3.2.S.4.1](#)

(b) (4)

ⁱ Added (b) (4) for pH adjustment

^g QS: quantity sufficient

^h Ingredient may be added to adjust pH; therefore, the quantity is variable

ⁱ To ensure that no less than 0.8 mL is dosed upon injection, a (b) (4) mL fill is targeted

^j USP - United States Pharmacopoeia; NF - National Formulary

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product formulation. All the excipients are within levels found in other FDA-approved SC drug products.

2.6 Proposed Clinical Population and Dosing Regimen

Donidalorsen is indicated for prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older. The recommended dosage is 80 mg administered SC (b) (4). A dosing interval of (b) (4) may be considered in some patients.

2.7 Regulatory Background

At the pre-NDA meeting, the Division agreed that it would be acceptable to submit the report of the 6-month carcinogenicity study in Tg.rasH2 mice and a summary of the in-life data for the 2-year carcinogenicity study in rats at the time of the NDA submission and to submit the final report as a PMR (Refer to IND 142564 pre-NDA Meeting Preliminary Comments dated 04/10/2024). This replaced comments conveyed from the End of Phase 2 (EOP2) meeting in which the Applicant was requested to provide reports for the 6-month carcinogenicity study in Tg.rasH2 mice and the 2-year carcinogenicity study in rats at the time of an NDA (Refer to IND 142564 EOP2 Meeting Minutes dated 10/18/2021).

In the EOP2 meeting package, the Applicant had indicated their plan to conduct a 6-month carcinogenicity study in Tg.rasH2 mice and to seek a waiver for a 2-year rat carcinogenicity study for ISIS 721744. Based on input from the ECAC, the conduct of a 2-year carcinogenicity study in rats could not be waived. As such, it was recommended that the protocols for the 6-month carcinogenicity study in Tg.rasH2 mice and the 2-year carcinogenicity study in rats, along with supporting dose range-finding (DRF) studies, be submitted for evaluation by the ECAC (Refer to IND 142564 EOP2 Meeting Minutes dated 10/18/2021).

The carcinogenicity SPA-2 for a 6-month study in Tg.rasH2 mice with ISIS 721744 and mouse surrogate ISIS 722059 was submitted on 10/12/2022 (SDN 36). The 14-week DRF study in Tg.rasH2 and proposed protocol were reviewed (refer to Nonclinical Primary Review dated 12/12/2022) and presented to the ECAC on 11/15/2022.

ECAC Recommendations and Conclusions for 6-Month Study in Tg.rasH2 Mice

- The Committee concurred with doses of 0 (0.9% sodium chloride for injection, USP), 5, 10, and 20 mg/kg Q2W via SC injection in male mice with the high dose based on a MTD (i.e., incidences of karyocytomegaly and increases of ALT, AST, and alkaline phosphatase (ALP) in male mice at higher doses in the 14-week DRF study). The mid and low doses are based on adequate dose spacing.
- For female mice, the Committee could not recommend doses for the proposed study, as no acceptable basis for dose selection could be identified. If the Sponsor would like ECAC concurrence for dose selection, a new DRF study should be conducted to identify an acceptable high dose as outlined in ICH S1C(R2) or ICH S1B(R1).
- The Committee noted that the 6-month carcinogenicity study will be performed in a different facility than used in the 14-week DRF study. The carcinogenicity study may not be acceptable if toxicity is significantly different due to this change such that dose selection would have been altered.
- The Committee noted that the planned ophthalmic examinations in the proposed carcinogenicity study are not needed by FDA.
- If there are survival issues during the study, the Sponsor should contact the Review Division before any changes are made.

Refer to IND 142564 ECAC Protocol Minutes dated 11/16/2022 for more details.

3 Studies Submitted

3.1 Studies Reviewed

Mammalian Micronucleus Assay in Bone Marrow Erythrocytes Following Subcutaneous Injection of ISIS 721744 to ICR Mice (Study No. G120005; Applicant No. 721744-AS05)
GLP-compliant

A 6-Month Study of ISIS 721744 by Subcutaneous Bolus Injection in CByB6F1-Tg(HRAS)^{2Jic} Hemizygous Transgenic Mouse (Study No. 727-140; Applicant No. 721744-AS07)
GLP-compliant

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 142564	Eleni Salicru, PhD	08/13/2019	Nonclinical Primary Review Pharmacology/Toxicology IND 30-day safety assessment and evaluation of primary pharmacology, safety pharmacology (cardiovascular, respiratory, and central nervous system in monkeys), pharmacokinetic/ADME, repeat-dose toxicology (39-week toxicity in monkeys with a 13-week interim necropsy and 26-week recovery), and genetic toxicology (in vitro bacterial reverse mutation assay and in vitro chromosome aberration assay in CHL cells) studies with ISIS 721744
IND 142564	Robeena Aziz	11/16/2022	ECAC Protocol Minutes SPA-2 carcinogenicity protocol review of 6-

Application	Reviewer	Date in DARRTS	Notes
			month carcinogenicity study in Tg.rasH2 mice with ISIS 721744 and mouse surrogate ISIS 722059
IND 142564	Eleni Salicru, PhD	12/12/2022	Nonclinical Primary Review SPA-2 carcinogenicity protocol review of 14-week repeat-dose toxicity study of ISIS 721744 and mouse surrogate ISIS 722059 in Tg.rasH2 mice to support dose selection for 6-month carcinogenicity study in Tg.rasH2 mice with ISIS 721744 and mouse surrogate ISIS 722059
NDA 219407	Malick Mbodj, PhD	12/16/2024	FDA Statistical Review and Evaluation of 6-month carcinogenicity study in Tg.rasH2 mice with ISIS 721744 and mouse surrogate ISIS 722059

7 Genetic Toxicology

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mammalian Micronucleus Assay in Bone Marrow Erythrocytes Following Subcutaneous Injection of ISIS 721744 to ICR Mice

Study no: G120005 (721744-AS05)
Study report location: NDA 219407 Electronic Document Room (EDR) Supporting Document Number (SDN) 1

Conducting laboratory and location:

(b) (4)

Date of study initiation: 05/28/2020
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: ISIS 721744, formulated test article lot # RP721744-013 (bulk test article lot # CA721744-002), formulated test article 93.8% purity (bulk test article 92.5% purity)

Key Study Findings

The *in vivo* mammalian micronucleus assay was conducted to evaluate potential clastogenicity of ISIS 721744 (500, 1000, and 2000 mg/kg SC) administered on Days 1 and 3 (48 hours postdose on Day 1) to ICR male mice. Mice were sacrificed on Day 4 (24 hours postdose on Day 3) for bone marrow collection. There was no statistically significant increase in the frequencies of MNPCEs at any dose of ISIS 721744 tested compared to the vehicle control (negative control) group. ISIS 721744 was negative for clastogenic potential in the *in vivo* bone marrow micronucleus assay in mice.

Methods (**Table 2**, Applicant's Table)

Doses in definitive study:	500, 1000, or 2000 mg/kg ISIS 721744
Frequency of dosing:	Twice: Day 1 (0 hour) and Day 3 (48 hours)
Route of administration:	SC
Dose volume:	10 mL/kg
Formulation/Vehicle:	Sterile 0.9% sodium chloride
Species/Strain/Substrain:	Mouse/ICR/CrI/Ori:CD1
Number/Sex/Group:	6 male mice/group (no female mice)
Satellite groups:	No
Basis of dose selection:	Dose selection was based on a DRF study in male and female mice (4/sex/group) administered 500, 1000, and 2000 mg/kg ISIS 721744 by SC injection (Study No. P120003). There were no mortality, abnormal clinical signs, or body weight changes in either male or female mice at ISIS 721744 doses up to 2000 mg/kg (limit dose for studies of 14 days or less). As such, the ISIS 721744 doses for the definitive micronucleus study were chosen to be 500, 1000, and 2000 mg/kg (limit dose according to OECD TG No. 474 and ICH S2(R1)).
Negative control:	Sterile 0.9% sodium chloride
Positive control:	Cyclophosphamide monohydrate: 70 mg/kg IP (10 mL/kg dose volume)

Table 2 Study Design for In Vivo Mammalian Micronucleus Assay in Bone Marrow Erythrocytes Following Subcutaneous Injection of ISIS 721744 to ICR Mice (Applicant's Table)

Group	Treatment	Sex	No. of Animals	Animal IDs	Volume (mL/kg)	Dose (mg/kg) ^a	Dosing Date	Route ^b
1	Vehicle	Male	6	1 - 6	10	0	Day 1, 3	SC
2	T1	Male	6	7 - 12	10	500	Day 1, 3	SC
3	T2	Male	6	13 - 18	10	1000	Day 1, 3	SC
4	T3	Male	6	19 - 24	10	2000	Day 1, 3	SC
5	Positive	Male	6	25 - 30	10	70	Day 3	IP

^a Mice were dosed with either vehicle, T1, T2 or T3 on Days 1 and 3 (a total of two occasions). The positive control was administered once by intraperitoneal injection on Day 3.

^b SC: Subcutaneous injection, IP: Intraperitoneal injection

Study Validity

The high dose of 2000 mg/kg ISIS 721744 was based on a DRF study in mice and considered a limit dose according to OECD TG No. 474 and ICH S2(R1). As such, dosing of ISIS 721744 was considered adequate for the micronucleus assessment.

Adequate numbers of polychromatic erythrocytes (PCEs) per animal (i.e., 4000) were scored for the presence of micronuclei.

The incidence of MNPCEs in the vehicle control (negative control) was within the range of historical control data.

A positive control group (cyclophosphamide monohydrate) was included in the definitive study. The incidence of MNPCEs in the positive control was significantly increased compared to the negative control.

Results

There was no mortality during the study.

There were no abnormal clinical signs during the study.

There were no body weight changes during the study.

The mean incidence of MNPCEs based on examining 4000 PCEs per animal were 4.80, 6.00, 6.80, 5.00, and 92.80 for vehicle control, 500 mg/kg ISIS 721744, 1000 mg/kg ISIS 721744, 2000 mg/kg ISIS 721744, and positive control, respectively. There was no statistically significant increase in the incidence of MNPCEs at any dose of ISIS 721744 compared to the vehicle control. The increase in the incidence of MNPCEs for the positive control was statistically significant compared to the vehicle control.

The mean ratio of PCEs to total erythrocytes (i.e., $\text{PCEs}/[\text{PCEs} + \text{normochromatic erythrocytes (NCEs)}]$) based on examining 500 erythrocytes per animal were 0.53, 0.59, 0.52, 0.65, and 0.54 for vehicle control, 500 mg/kg ISIS 721744, 1000 mg/kg ISIS 721744, 2000 mg/kg ISIS 721744, and positive control, respectively. There was no statistically significant difference in the mean $\text{PCEs}/(\text{PCEs} + \text{NCEs})$ at any dose of ISIS 721744 or the positive control compared to the vehicle control, used as an indicator of cytotoxicity.

Liver samples from three mice/group were analyzed for tissue concentrations of unconjugated ISIS 721744 on Day 4 (24 hours postdose on Day 3). As shown in **Table 3** (Applicant's Table), unconjugated ISIS 721744 concentrations were increased in the ISIS 721744-treated groups compared to the vehicle control group with group mean ranging from 502 mcg/g to 774 mcg/g for ISIS 721744 doses ranging from 500 to 2000 mg/kg.

Table 3 Unconjugated ISIS 721744 Concentrations in Mouse Liver Samples Collected from In Vivo Mammalian Micronucleus Assay in Bone Marrow Erythrocytes Following Subcutaneous Injection of ISIS 721744 to ICR Mice (Applicant's Table)

Group	Animal ID	Study Day	Time Point	Gender	Dose Level	Unconjugated ISIS 721744 Concentration (µg/g)	Group Mean (µg/g)	SD
Vehicle	1	4	24 hr	M	0 mg/kg	<10.0	NA	NA
Vehicle	2	4	24 hr	M	0 mg/kg	<10.0		
Vehicle	3	4	24 hr	M	0 mg/kg	<10.0		
T1	7	4	24 hr	M	500 mg/kg	497	502	115
T1	8	4	24 hr	M	500 mg/kg	618		
T1	9	4	24 hr	M	500 mg/kg	389		
T2	13	4	24 hr	M	1000 mg/kg	346	515	156
T2	14	4	24 hr	M	1000 mg/kg	548		
T2	15	4	24 hr	M	1000 mg/kg	652		
T3	19	4	24 hr	M	2000 mg/kg	745	774	184
T3	20	4	24 hr	M	2000 mg/kg	971		
T3	21	4	24 hr	M	2000 mg/kg	606		

NA = not applicable; SD = standard deviation

8 Carcinogenicity

Study title: A 6-Month Study of ISIS 721744 by Subcutaneous Bolus Injection in CByB6F1-Tg(HRAS)^{2Jic} Hemizygous Transgenic Mouse

Study no.: 727-140 (721744-AS07)
 Study report location: NDA 219407 EDR SDN 1
 Conducting laboratory and location:  (b) (4)

Reviewer's Note: The conducting laboratory and location are different than that used for the 14-week DRF study in Tg.rasH2 mice.

Date of study initiation: 12/21/2022
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: ISIS 721744, lot # RP721744-018 concentration stock solution from bulk test material lot # CA721744-003, 92.6% purity

Mouse surrogate ISIS 722059, lot # RP722059-005 ready to use dosing solution from bulk test material lot # 722059-4, 88.2% purity

CAC concurrence: Based on the 14-week DRF study in Tg.rasH2 mice, the Sponsor proposed ISIS 721744 doses of 0 (vehicle control), 5, 10, and 20 mg/kg/dose Q2W for male mice and 0 (vehicle control), 10, 20, and 40 mg/kg/dose Q2W for female mice. The ECAC agreed with the ISIS 721744 doses for male mice with the high dose based on a MTD and the mid and low doses based on adequate dose spacing. The ECAC could not recommend ISIS 721744 doses for female mice, as no acceptable basis for dose selection could be identified in the 14-week DRF study in Tg.rasH2 mice. Refer to IND 142564 Nonclinical Review dated 12/12/2022 and IND 142564 ECAC Protocol Minutes dated 11/16/2022.

Key Study Findings

- Tg.rashH2 mice (n = 25/sex/group) were administered vehicle control (0.9% sodium chloride for injection, USP), ISIS 721744 (5, 10, or 20 mg/kg/dose for males and 10, 20, or 60 mg/kg/dose for females), or mouse surrogate ISIS 722059 (10 mg/kg/dose) Q2W for up to 6 months by SC injection. A positive control group was also included in which Tg.rashH2 mice (10/sex) were administered urethane (500 mg/kg/dose) via IP injection on Days 1, 3, and 5. The mouse was not a pharmacologically relevant species with ISIS 721744 and this study evaluated off-target effects. The mouse surrogate ISIS 722059 was included to assess inhibition of PKK and potential on-target effects.
- There was no statistically significant increase or decrease in mortality dose response across the vehicle control and ISIS 721744-treated groups, in either male or female mice. The pairwise comparisons showed a statistically significant decrease in mortality in the male 5 mg/kg/dose group (low dose) compared to the vehicle control group (p = 0.0160). The pairwise comparisons showed no statistically significant increase or decrease in mortality between the mouse surrogate ISIS 722059 group and the vehicle control group, in either male or female mice.
- There were no ISIS 721744-related or mouse surrogate ISIS 722059-related effects on body weights.
- Effects on clinical chemistry included increases in AST, ALT, and GLDH in males at 10 and 20 mg/kg/dose ISIS 721744 and females at 60 mg/kg/dose ISIS 721744, which correlated with histopathology findings in the liver.
- A MTD was achieved at the high dose of 20 mg/kg Q2W in males based on histopathology findings in the liver and elevations of GLDH. The high dose of 60 mg/kg Q2W in females did not meet the criteria for a MTD.
- Gross pathology findings included liver mass in one male at 5 mg/kg/dose ISIS 721744 and one male at 20 mg/kg/dose ISIS 721744, which correlated with neoplastic histopathology findings of hepatocellular adenoma in the liver of both mice.
- Neoplastic histopathology findings included hepatocellular adenoma in the liver in 1/25 males at 5 mg/kg/dose ISIS 721744, 2/25 males at 20 mg/kg/dose ISIS 721744, and 1/25 males at 10 mg/kg/dose ISIS 722059. The hepatocellular adenoma seen in the males at 5 and 20 mg/kg/dose ISIS 721744 were accompanied by increases in AST and ALT, gross pathology findings in the liver, and non-neoplastic histopathology findings in the liver of microvesicular hepatocellular vacuolation, basophilic focus of cellular alteration, hepatocellular single cell necrosis, and/or basophilic pigmented macrophage. There were no correlating gross pathology or non-neoplastic histopathology findings for the male at 10 mg/kg/dose ISIS 722059.

- Non-neoplastic histopathology findings were noted in the **liver** (microvesicular hepatocellular vacuolation, basophilic pigmented macrophages, hepatocellular single cell necrosis, karyocytomegaly, and/or basophilic focus of cellular alteration in males at 5, 10, and/or 20 mg/kg/dose ISIS 721744, and/or females at 10, 20, and/or 60 mg/kg/dose ISIS 721744, and/or males and/or females at 10 mg/kg/dose ISIS 722059); **kidney** (epithelial vacuolation in females at 60 mg/kg/dose ISIS 721744 and epithelial basophilic granules in males at 20 mg/kg/dose ISIS 721744 and females at 20 and 60 mg/kg/dose ISIS 721744); **injection site** (macrophage granular vacuolation in males and females at all ISIS 721744 doses and in males and females at 10 mg/kg/dose ISIS 722059); **urinary bladder** (urothelial vacuolation in males at 10 and 20 mg/kg/dose ISIS 721744 and females at 20 and 60 mg/kg/dose ISIS 721744); and the **axillary and/or mesenteric lymph nodes** (macrophage vacuolation in females at 60 mg/kg/dose ISIS 721744). The histopathology findings were attributed to or a consequence of oligonucleotide uptake.
- The positive control (urethane) group confirmed the carcinogenic responsiveness of the Tg.rasH2 model. Urethane treatment resulted in a decrease in body weight in both male and female mice and a statistically significant increase in unscheduled deaths (i.e., decrease in survival) compared to the vehicle control group in both male and female mice. Neoplastic findings included hemangiosarcoma (whole body in male and female mice), malignant lymphoma (hemolymphoreticular tissue in male mice), and bronchioloalveolar adenoma and carcinoma (lungs in male and female mice). These findings are consistent with known effects of urethane administration.¹
- Toxicokinetics were not evaluated.
- ISIS 721744 concentrations in the kidney and liver of males and females were generally increased with increasing ISIS 721744 dose following 6-months of treatment with ISIS 721744.
- Hepatic PKK mRNA expression was statistically reduced in male and female mice treated with 10 mg/kg/dose ISIS 722059 Q2W for 26 weeks by 94% and 79%, respectively, compared to controls.

Adequacy of Carcinogenicity Study

- As described above, the ECAC concurred with the doses for the study in male mice but could not recommend doses for female mice (refer to Carcinogenicity SPA-2 agreement dated 11/16/2022). The Applicant did not conduct a new DRF study to identify an acceptable high dose or seek ECAC concurrence for their final dose selection in female mice. The ECAC concluded that the carcinogenicity study was adequate for male mice but inadequate for female mice because a

MTD was not achieved and no other valid basis to support the high dose was discernable.

- There were no test article-related body weight changes or other adverse effects that limited the duration of the study that were suggestive of the MTD being exceeded in males receiving up to 20 mg/kg/dose ISIS 721744 and females receiving up to 60 mg/kg/dose ISIS 721744.
- The duration of treatment was adequate (i.e., up to 6 months for males and females).

Appropriateness of Test Models

The Tg.rasH2 mouse is considered an appropriate test model for evaluating the carcinogenic potential of pharmaceuticals per *ICH Guidance for Industry S1B Testing for Carcinogenicity of Pharmaceuticals*.

Evaluation of Tumor Findings

Test Articles

There was no statistically significant increase in tumor incidences in ISIS 721744- or ISIS 722059-treated groups, when compared to the vehicle control group in either male or female mice. Neoplastic histopathology findings included hepatocellular adenoma in the liver in 1/25 males at 5 mg/kg/dose ISIS 721744, 2/25 males at 20 mg/kg/dose ISIS 721744, and 1/25 males at 10 mg/kg/dose ISIS 722059. The hepatocellular adenoma noted in 2/25 males (8%) at 20 mg/kg/dose ISIS 721744 exceeded the testing laboratory Tg.rasH2 mouse historical control incidence (0/643 grand total male mice examined [0%]; 1/643 grand total female mice examined [0.2%] or up to 4% per study [1/25 female mice]).

Positive Control

There were statistically significant increases in tumor incidences of hemangiosarcoma (whole body), malignant lymphoma in the hemolymphoreticular tissue, and bronchioloalveolar adenoma and/or carcinoma in the lungs for the positive control group (urethane), when compared to the vehicle control group, in either male or female mice, which were expected findings for this known carcinogen.¹

Methods (**Table 4**, Applicant's Table)Doses: ISIS 721744Males: 0 (vehicle control), 5, 10, or 20
mg/kg/doseFemales: 0 (vehicle control), 10, 20, or 60
mg/kg/doseMouse Surrogate ISIS 722059

10 mg/kg/dose

Positive Control (Urethane)

500 mg/kg/dose

Frequency of dosing: Vehicle Control, ISIS 721744, and Mouse
Surrogate ISIS 722059
Q2W for 6 monthsPositive Control (Urethane)

Days 1, 3, and 5

Dose volume: 10 mL/kg

Route of administration: Vehicle Control, ISIS 721744, and Mouse
Surrogate ISIS 722059
SC injectionPositive Control (Urethane)

IP injection

Formulation/Vehicle: 0.9% sodium chloride for injection, USP

Basis of dose selection: Dose selection was based on results from the 14-week DRF study in Tg.rasH2 mice.

The Sponsor conducted a non-GLP-compliant 14-week repeat-dose DRF study in TgRasH2 mice treated by the SC route of administration with vehicle control (0.9% sodium chloride for injection, USP [saline]), ISIS 721744 (6, 18, or 36 mg/kg/dose), or mouse surrogate ISIS 722059 (6 mg/kg/dose) Q2W (Study No. 721744-AS07P) to evaluate potential toxicity and to provide the dose selection for the 6-month carcinogenicity study in TgRasH2 mice.

The mouse is not a pharmacologically relevant species with ISIS 721744 and this study primarily assessed off-target toxicity. Although, the murine surrogate product ISIS 722059 assessed the effect of the intended pharmacology to inhibit synthesis of PKK. Animal numbers were noted to be low (n = 6/sex/group). The histopathology examination was limited (i.e., sternum bone marrow and bone, heart, injection sites, kidneys, liver, lung, spleen, testes, thymus, gross lesions, and tissue masses).

Key study findings are summarized as follows:

Test article-related effects on clinical chemistry parameters were noted as follows:

- Increases in ALP in males at 6, 18, and 36 mg/kg/dose (1.2x, 1.4x, and 1.6x over control, respectively; dose-dependent; statistically significant at ≥ 18 mg/kg/dose) and females at 36 mg/kg/dose (1.2x; statistically significant; dose-dependent).
- Increases in AST in males at 6, 18, and 36 mg/kg/dose (1.3x, 3.5x, and 5.4x over control, respectively; dose-dependent; statistically significant at ≥ 18 mg/kg/dose) and females at 6, 18, and 36 mg/kg/dose (1.6x, 1.1x, and 1.2x over

control, respectively; not dose-dependent; not statistically significant).

- Increases in ALT in males at 6, 18, and 36 mg/kg/dose (1.2x, 4.5x, and 5.1x over control, respectively; dose-dependent; statistically significant at ≥ 18 mg/kg/dose) and females at 6, 18, and 36 mg/kg/dose (1.8x, 1.3x, and 1.6x over control, respectively; not dose-dependent; not statistically significant).
- As described in the study report, dose-dependent increases in ALP, AST, and ALT in males correlated with histopathology findings of minimal hepatocyte karyocytomegaly in the liver. In the females, mild increases in ALP, AST, and ALT were generally not dose-dependent and did not correlate with histopathology findings in the liver. ALP was significantly increased for females at 36 mg/kg/day, although the increase (1.2-fold) was not toxicologically significant.

ISIS 721744-related histopathology findings were noted in the kidney, liver, and injection site(s) as follows:

- In the kidneys, histopathology findings in males and females at ≥ 18 mg/kg/dose included minimal accumulation of small basophilic granules in the tubular epithelium.
- In the liver, histopathology findings in males at ≥ 6 mg/kg/dose included minimal karyocytomegaly (described as an increase in hepatocyte nuclear size and cytoplasmic volume). Karyocytomegaly reflects hepatocyte polyploidy that occurs when there is duplication of nuclear material in the absence of cytokinesis. Changes can consist of increased numbers of diploid nuclei per hepatocyte

or an increase in the ploidy level of a single hepatocyte nucleus. Spontaneous occurrence of karyocytomegaly is commonly seen in aging rodents and is not typically associated with increased liver weight. This aging change, often referred to as polyploidy, is more common in mice than in rats (NTP). As described in the study report, this finding correlated with increases in AST, ALT, and ALP. There were no comparable histopathological findings in the liver for female drug-treated groups, although ALP activity was increased for high dose females. Increases of ALT, AST, and/or ALP in male drug-treated groups and the high dose female group, as well as karyocytomegaly in male drug-treated groups, were attributed to the uptake and accumulation of the test article in hepatocytes. There were no comparable histopathological findings in the liver for female drug-treated groups, although higher concentrations of unconjugated ISIS 721744 were measured in the livers of females compared to males.

- At the skin injection site, histopathology findings in males at ≥ 6 mg/kg/dose and females at ≥ 18 mg/kg/dose included minimal to mild numbers of macrophages with vacuolated or granular cytoplasm. As described in the study report, when comparing males and females of similar dose groups, the injection site finding in males was more distinct and easily observed on microscopic examination while in females vacuolated/granular macrophages were more difficult to detect.
- As described in the study report, the findings within the liver, kidneys, and macrophages at the injection site were likely secondary to uptake and accumulation of the ASO. Because they

were not associated with evidence of cellular degeneration or necrosis, these findings were not considered adverse.

For male and female mice that received the mouse surrogate, ISIS 722059, at 6 mg/kg/dose, there were no changes of body weight and clinical pathology parameters. Further, there were no notable histopathological findings.

The high dose selection with ISIS 721744 at 20 mg/kg/dose in male mice was based upon observations of karyocytomegaly and increases of ALT, AST, and ALP in male mice at 18 mg/kg/dose in the 14-week DRF study.

For female mice there were no histopathological findings in the liver and there were minimal generally non-dose-related elevations of ALT, AST, and ALP. There were higher concentrations of unconjugated ISIS 721744 in the liver of females compared to males. A MTD was not identified for females.

The testing laboratory was different between the 14-week dose range finding study and 26-week carcinogenicity study.

Vehicle, frequency of dosing, and route of administration were identical between the 14-week dose range finding study and 26-week carcinogenicity study.

Species/Strain: Mice/CByB6F1-Tg(HRAS)2Jic (hemizygous)
[RasH2]

Number/Sex/Group: 25 for 0 (vehicle control), ISIS 721744, and
mouse surrogate ISIS 722059 groups

Age: About 9 weeks old at initiation of dosing

Animal housing: 10 for positive control (urethane)
As stated in the protocol: Upon receipt, males
may be individually housed, and females will
be pair-housed, when possible (females may
be housed 2 to 3/cage during acclimation and
in-life depending on study design). The caging
was solid bottom cages with nonaromatic
bedding.

Paradigm for dietary restriction: Food was available ad libitum. No dietary
restriction paradigm was used. Block Lab Diet®
Certified Rodent Diet #5CR4 (PMI Nutrition
International, Inc.) was available ad libitum
(except during designated procedures). Tap
water was freely available to each animal via
an automatic watering system (except during
designated procedures).

Dual control employed: No

Interim sacrifice: No

Satellite groups: No

Deviation from study protocol: Deviations from the study protocol were listed
in the final study report and were not
considered to have impacted the overall
integrity of the study or the interpretation of the
study results and conclusions.

Table 4 Study Design for 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Group No.	Test Article	Dose Level (mg/kg/q2w)	Dose Concentration (mg/mL)	Main Study	
				No. of Males	No. of Females
1	Vehicle ^a	0	0	25	25
2	ISIS 721744 ^a	5	0.5	25	-
3		10	1	25	25
4		20	2	25	25
5		60	6	-	25
6		ISIS 722059 ^c	10	1	25
7	Positive Control (Urethane) ^b	500	50	10	10

No. = Number; - = Not Applicable; The designation mg/kg/q2w may also be referred to as mg/kg/dose. Dose volume was 10 mL/kg based on the most recent body weight measurement.

^a Vehicle control and ISIS 721744 were administered subcutaneously once every two weeks (q2w).

^b Intraperitoneal injection on Days 1, 3, and 5.

^c Mouse surrogate group; dosed in the same manner as vehicle and ISIS 721744 (q2w).

Observations and Results

Mortality

Observations for mortality/morbidity occurred at least twice daily (morning and afternoon) beginning upon arrival through termination.

The number of male and female mice that experienced early deaths (i.e., unscheduled euthanasia or found dead) versus those that survived to terminal necropsy is shown in **Table 5**.

An FDA Statistical Review and Evaluation by Malick Mbodj, PhD dated 12/16/2024 analyzed intercurrent mortality data using the Kaplan-Meier product limit method. Further, the dose response relationship and homogeneity of survival distributions were tested for the treatment groups using the Likelihood ratio test and the Log-Rank test.

Based on the FDA Statistical Review and Evaluation:

- There was no statistically significant increase or decrease in mortality dose response across the vehicle control and ISIS 721744-treated groups, in either male or female mice. The pairwise comparisons showed a statistically significant decrease in mortality in the male 5 mg/kg/dose group (low dose) compared to the vehicle control group ($p = 0.0160$).
- The pairwise comparisons showed no statistically significant increase or decrease in mortality between the mouse surrogate ISIS 722059 group and the vehicle control group, in either male or female mice.
- The positive control group had a statistically significant increase in unscheduled deaths compared to the vehicle control group ($p < 0.0001$) in both male and female mice.

Table 5 Early Deaths versus Survival in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

Test Article	Dose Level (mg/kg/dose)	Males		Females	
		# of Early Deaths ^{1,2}	# at Terminal Necropsy ²	# of Early Deaths ^{1,2}	# at Terminal Necropsy ²
Vehicle Control	0	4/25 (16%)	21/25 (84%)	0/25 (0%)	25/25 (100%)
ISIS 721744	5	0/25 (0%)	25/25 (100%)	-	-
ISIS 721744	10	2/25 (8%)	23/25 (92%)	2/25 (8%)	23/25 (92%)
ISIS 721744	20	4/25 (16%)	21/25 (84%)	1/25 (4%)	24/25 (96%)
ISIS 721744	60	-	-	0/25 (0%)	25/25 (100%)
ISIS 722059	10	3/25 (12%)	22/25 (88%)	2/25 (8%)	23/25 (92%)
Positive Control (Urethane)	500	10/10 (100%)	0/10 (0%)	10/10 (100%)	0/10 (0%)

Abbreviations: # = number

Notes:

¹Number of early deaths = unscheduled euthanasia or found dead (there were no accidental deaths reported during the study)

²Numbers in parentheses represent the percent when compared to the number of main study animals at the start of the study (i.e., 25 animals/sex/group for vehicle control, ISIS 721744, and mouse surrogate ISIS 722059 groups and 10 animals/sex/group for positive control [urethane] group)

Kaplan-Meier survival curves for vehicle control, ISIS 721744, and mouse surrogate ISIS 722059 male and female groups are shown in **Figure 2** and **Figure 3** (FDA Statistical Reviewer's Figures), respectively.

Figure 2 Kaplan-Meier Survival Curves for Males in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (FDA Statistical Reviewer's Figure)

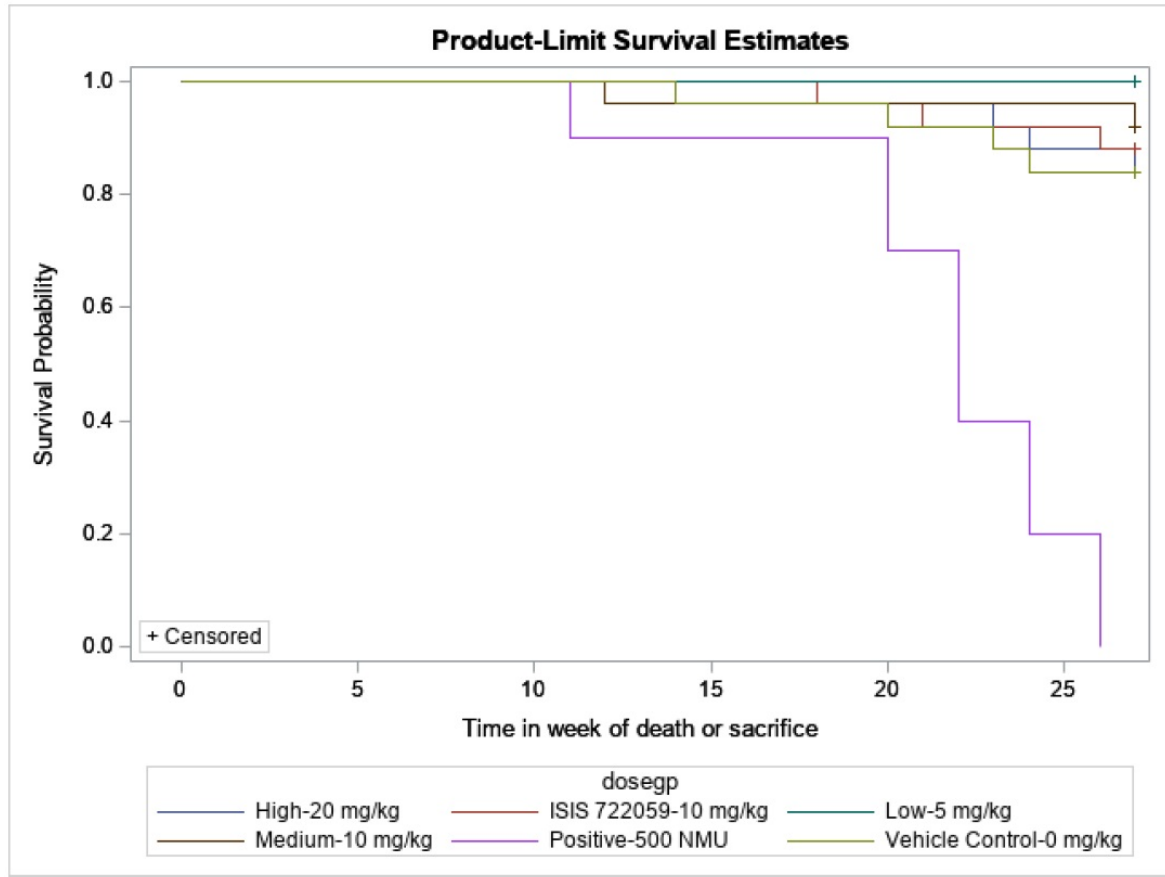
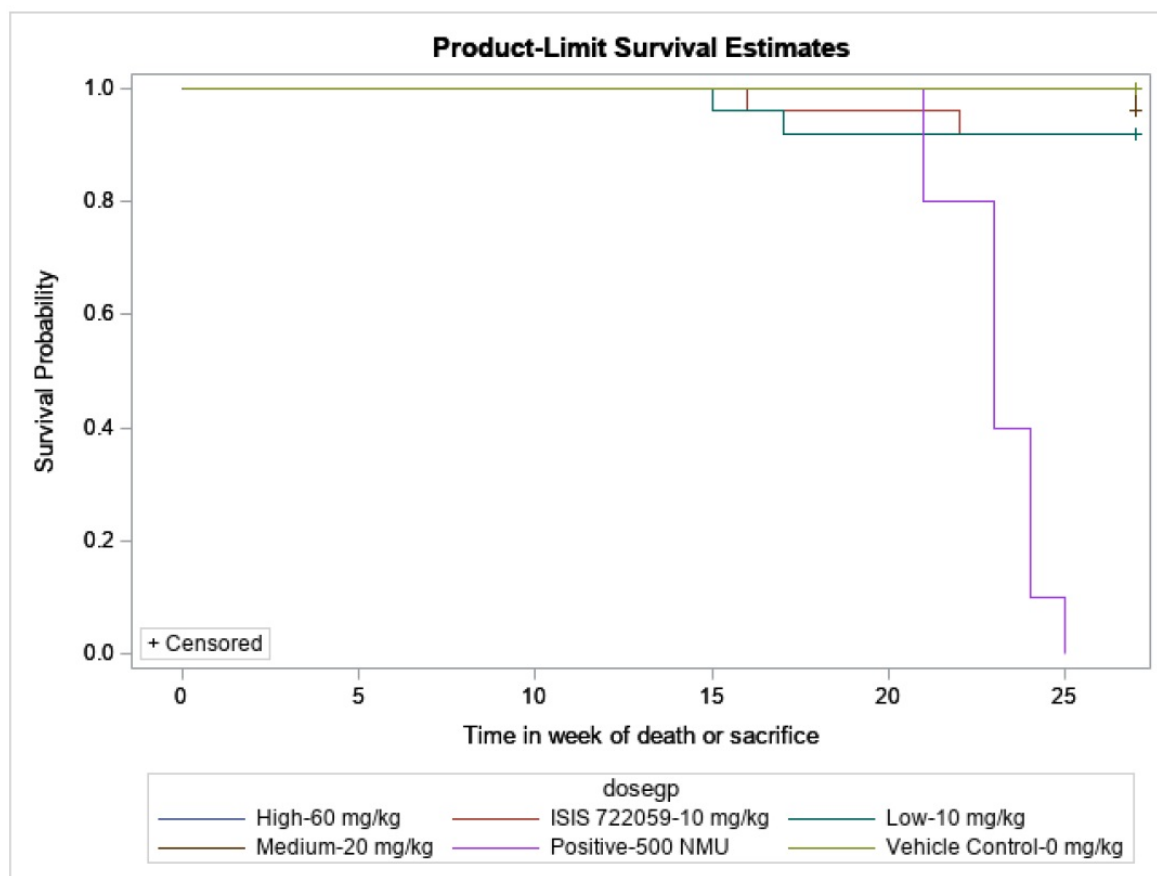


Figure 3 Kaplan-Meier Survival Curves for Females in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (FDA Statistical Reviewer's Figure)



In cases of early deaths for ISIS-721744-treated and ISIS-722059-treated mice, where the cause of death could be determined, causes of death were malignant mesothelioma, hemangiosarcoma, squamous cell carcinoma, and testicular hemorrhage. These causes of death were either of low incidence, similar in incidence between vehicle control and drug-treated groups, or did not exhibit a dose-response relationship across groups (**Table 6**, Applicant's Table).

As described in the study report, bronchioloalveolar carcinoma was the most common cause of early death in positive control (urethane) mice; other causes of early death included hemangiosarcoma and lymphoma.

Table 6 Individual Early Deaths in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (Positive Control Group Excluded) (Applicant's Table)

Animal Number/Sex	Dose Level (mg/kg/q2w)	Test Material	Fate/Animal Disposition	Fate Day	Cause of Death/Euthanasia
1003/M	0	ISIS 721744	UE	161	Malignant mesothelioma
1009/M	0	ISIS 721744	FD	140	Hemangiosarcoma
1012/M	0	ISIS 721744	UE	92	Squamous cell carcinoma
1014/M	0	ISIS 721744	UE	164	Undetermined
3004/M	10	ISIS 721744	UE	183	Hemangiosarcoma
3010/M	10	ISIS 721744	FD	84	Undetermined
4004/M	20	ISIS 721744	FD	94	Undetermined
4011/M	20	ISIS 721744	FD	162	Undetermined
4017/M	20	ISIS 721744	FD	159	Undetermined
4020/M	20	ISIS 721744	FD	183	Undetermined
6002/M	10	ISIS 722059	FD	147	Testicular hemorrhage
6003/M	10	ISIS 722059	FD	124	Malignant mesothelioma
6009/M	10	ISIS 722059	FD	177	Hemangiosarcoma
3523/F	10	ISIS 721744	FD	119	Undetermined
3524/F	10	ISIS 721744	FD	101	Hemangiosarcoma
4520/F	20	ISIS 721744	FD	183	Undetermined
6505	10	ISIS 722059	FD	154	Squamous cell carcinoma
6524	10	ISIS 722059	FD	112	Undetermined

M-Male; F-Female; FD-Found Dead; UE-Unscheduled Euthanasia.

Clinical Signs

Cageside observations occurred at least twice daily (morning and afternoon) beginning upon arrival through termination. Detailed clinical observations (including but not limited to evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, unusual behavior) occurred at predose and at about 4 hours postdose on dosing days and once weekly on non-dosing weeks. The injection site(s) was evaluated prior to dosing on each day of dosing beginning on Day 29 for any signs of irritation, discoloration, and/or swelling. Palpation of tissue masses occurred weekly throughout the study.

There were no ISIS 721744-related or mouse surrogate ISIS 722059-related clinical signs or palpable masses.

Body Weights

Individual body weights were collected once after arrival, Day -1, and once weekly during the study.

As shown in **Table 7**, there were no ISIS 721744-related or mouse surrogate ISIS 722059-related effects on absolute body weights compared to concurrent controls. The

body weight curves for ISIS 721744-treated and ISIS 722059-treated groups were generally similar to the control groups for males and females (Figure 4 and Figure 5, respectively).

Table 7 Body Weight Changes in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

	Males					Females				
	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)
	0	5	10	20	10	0	10	20	60	10
Day -1	23.61	23.66	24.19	23.55	24.09	19.36	19.14	19.69	19.25	19.47
Day 182	40.54	41.67	41.28	40.19	40.52	25.61	26.30	26.79	26.30	26.68
Absolute BW (% Change from Day 182 Control)	0.00	2.79	1.83	-0.86	-0.05	0.00	2.69	4.61	2.69	4.18

Abbreviations: BW = body weight; Veh = vehicle

Figure 4 Mean Body Weight Curves in Males in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

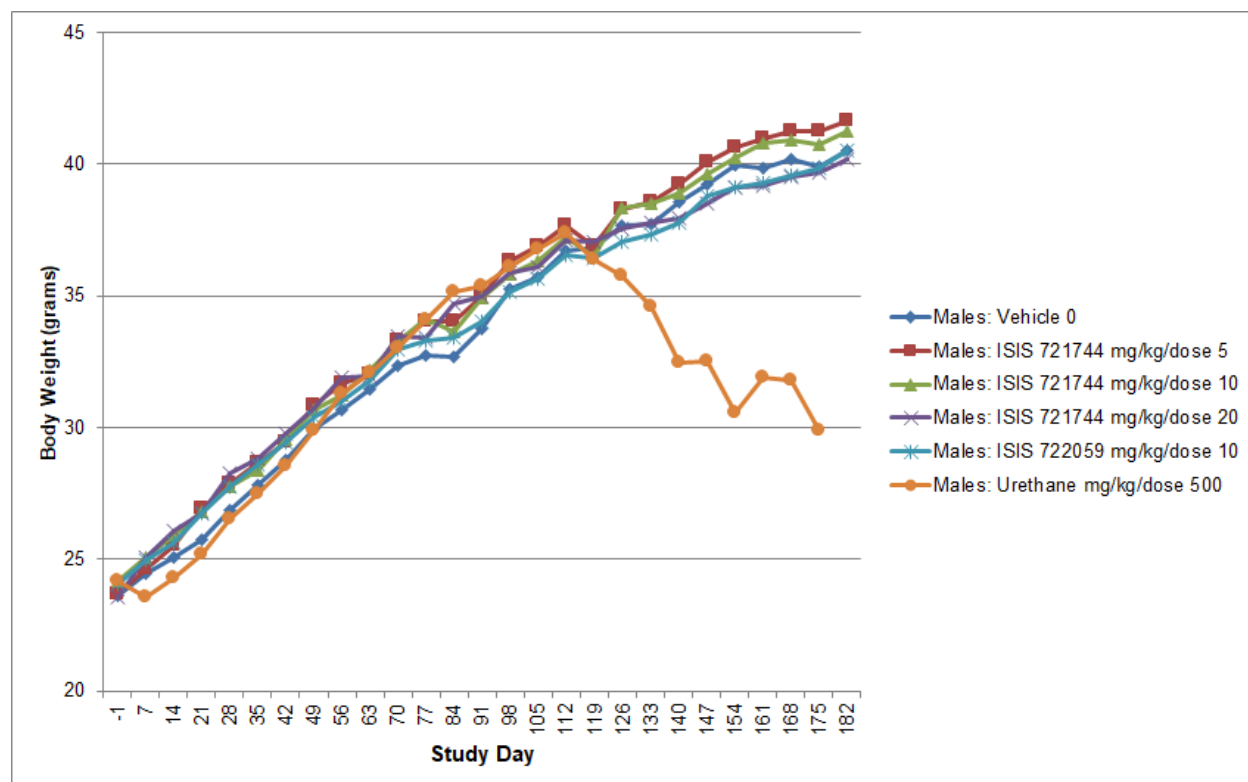
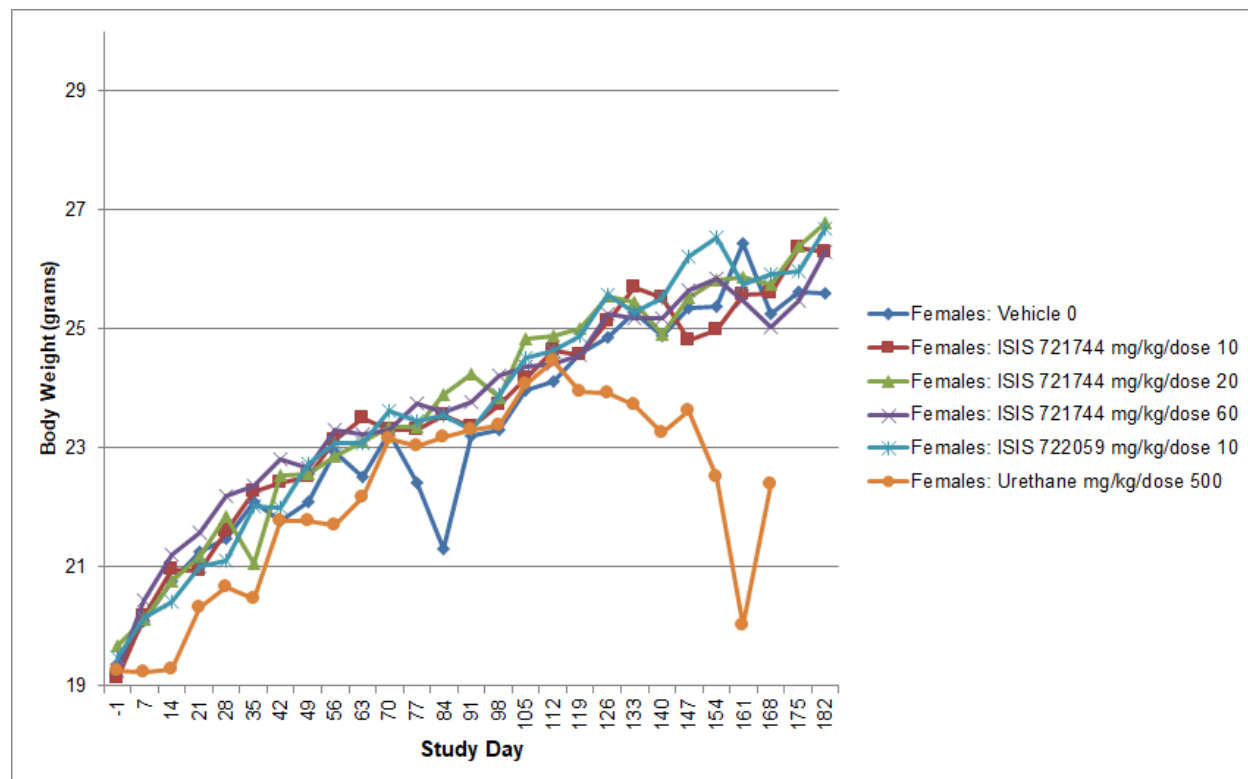


Figure 5 Mean Body Weight Curves in Females in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice



Feed Consumption

Food consumption was quantitatively measured once weekly for the first 14 weeks and every 2 weeks thereafter.

There were no ISIS 721744-related or mouse surrogate ISIS 722059-related effects on food consumption.

Clinical Chemistry

Blood was taken for clinical chemistry evaluations at termination (Day 185). A representative panel of clinical chemistry parameters was evaluated.

There were five vehicle control male mice (Nos. 1004, 1006, 1008, 1011, and 1018) and three vehicle control female mice (Nos. 1511, 1516, and 1523) with large AST and ALT values compared to the other mice in the vehicle control groups, which skewed the group means higher. Taking this into account, there were still several males at 10 and 20 mg/kg/dose ISIS 721744 and females at 60 mg/kg/dose ISIS 721744 with minimal to mild increases in AST and ALT compared to the vehicle control groups. Males at 10 and

20 mg/kg/dose ISIS 721744 and females at 60 mg/kg/dose ISIS 721744 also had statistically significant increases in GLDH compared to vehicle control animals.

Increases in AST, ALT, and GLDH were indicative of effects on the liver. As described in the study report, these increases correlated with histopathology findings related to uptake of oligonucleotide that included hepatocellular vacuolation/basophilic granule accumulation in macrophages and evidence of ongoing degeneration/hepatocyte turnover (single cell necrosis and karyocytomegaly) in males, as well as with histopathology findings of single cell necrosis (60 mg/kg/dose ISIS 721744) and basophilic pigmented macrophages (20 and 60 mg/kg/dose ISIS 721744) in the liver in females that were attributed to uptake of oligonucleotide. The clinical chemistry changes may also correlate to neoplastic findings of hepatocellular adenomas in two males at 20 mg/kg/dose ISIS 721744 (Nos. 4006 and 4016).

Triglycerides were statistically decreased in males and females at all ISIS 721744 doses and in females at 10 mg/kg/dose ISIS 722059. Cholesterol was increased in males at all ISIS 721744 doses and at 10 mg/kg/dose ISIS 722059 and in females at 20 and 60 mg/kg/dose ISIS 721744.

Total protein and albumin were decreased in males at 20 mg/kg/dose ISIS 721744 and females at 60 mg/kg/dose ISIS 721744.

Table 8 Clinical Chemistry Findings in 6-Month Subcutaneous Carcinogenicity Study in Tg.rash2 Mice

	Males					Females				
	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)
	0	5	10	20	10	0	10	20	60	10
AST (U/L)	97.4	61.6	102.0	129.1**	49.2	78.2	59.9	56.3	92.4**	53.3
% Change Control	-	-36.8	4.7	32.5	-49.5	0	-23.4	-28.0	18.2	-31.8
Fold Change	-	0.63	1.05	1.32	0.51	-	0.77	0.72	1.18	0.68
ALT (U/L)	153.5	71.6	129.2*	154.1**	56.2	72.7	49.5	42.3	88.1**	42.8
% Change Control	0	-53.4	-15.8	0.4	-63.4	0	-31.9	-41.8	21.2	-41.1
Fold Change	-	0.47	0.84	1.00	0.37	-	0.68	0.58	1.21	0.59
GLDH (U/L)	10.31	17.03	26.39**	47.12**	11.59	8.12	7.48	8.37	12.29**	6.58

	Males					Females				
	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)
		0	5	10	20		10	0	10	20
% Change Control	0	65.2	156.0	357.0	12.4	0	-7.9	3.1	51.4	-19.0
Fold Change	-	1.65	2.56	4.57	1.12	-	0.92	1.03	1.51	0.81
TRIG (mg/dL)	266.3	198.5**	193.4**	171.2**	236.9	141.5	109.7**	110.2**	88.7**	105.8**
% Change Control	0	-25.5	-27.4	-35.7	-11.0	0	-22.5	-22.1	-37.3	-25.2
Fold Change	-	0.75	0.73	0.64	0.89	-	0.78	0.78	0.63	0.75
CHOL (mg/dL)	174.0	228.1	217.4	236.6	234.4	128.1	136.0	156.1	144.4	131.7
% Change Control	0	31.1	24.9	36.0	34.7	0	6.2	21.9	12.7	2.8
Fold Change	-	1.31	1.25	1.36	1.35	-	1.06	1.22	1.13	1.03
TPROT (g/dL)	6.24	6.17	6.08	5.90	6.24	5.64	5.76	5.52	5.36*	5.70
% Change Control	0	-1.1	-2.6	-5.4	0.0	0	2.1	-2.1	-5.0	1.1
Fold Change	-	0.99	0.97	0.95	1.00	-	1.02	0.98	0.95	1.01
ALB (g/dL)	3.48	3.36	3.30**	3.18**	3.47	3.40	3.45	3.31	3.23**	3.49
% Change Control	0	-3.4	-5.2	-8.6	-0.3	0	1.5	-2.6	-5.0	2.6
Fold Change	-	0.97	0.95	0.92	1.00	-	1.01	0.98	0.95	1.03
ALP (U/L)	68.1	77.3	92.7**	108.1**	62.6	105.2	114.6	110.0	113.3	96.8
% Change Control	0	13.5	36.1	58.7	-8.1	0	8.9	4.6	7.7	-8.0
Fold Change	-	1.14	1.36	1.59	0.92	-	1.09	1.05	1.08	0.92
GLUC (mg/dL)	345.9	282.7**	296.6	279.9**	321.1	350.7	308.8	298.2	254.3**	329.2
% Change Control	0	-18.3	-14.3	-19.1	-7.2	0	-11.9	-15.0	-27.5	-6.1

	Males					Females				
	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)	Veh	ISIS 721744 (mg/kg/dose)			ISIS 722059 (mg/kg/dose)
	0	5	10	20	10	0	10	20	60	10
Fold Change	-	0.82	0.86	0.81	0.93	-	0.88	0.85	0.73	0.94
CREAT (mg/dL)	0.26	0.20**	0.23	0.21*	0.22	0.17	0.17	0.18	0.13*	0.19
% Change Control	0	-23.1	-11.5	-19.2	-15.4	0	0.0	5.9	-23.5	11.8
Fold Change	-	0.78	0.87	0.78	0.83	-	1.02	1.03	0.79	1.10
UREAN (mg/dL)	18.1	15.9*	18.6	17.4	16.7	14.7	13.5	13.5	13.0*	12.9*
% Change Control	0	-12.2	2.8	-3.9	-7.7	0	-8.2	-8.2	-11.6	-12.2
Fold Change	-	0.88	1.03	0.96	0.92	-	0.91	0.92	0.88	0.87
TBIL (mg/dL)	0.144	0.161	0.162	0.148	0.170**	0.146	0.162	0.143	0.169*	0.164
% Change Control	0	11.8	12.5	2.8	18.1	0	11.0	-2.1	15.8	12.3
Fold Change	-	1.12	1.12	1.02	1.18	-	1.11	0.98	1.15	1.12

Abbreviations: ALB = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CHOL = cholesterol; CREAT = creatinine; GLDH = glutamate dehydrogenase; GLUC = glucose; TBIL = total bilirubin; TPROT = total protein; TRIG = triglycerides; UREAN = urea nitrogen; Veh = vehicle control

Notes: Applicant's statistical analyses of Kruskal-Wallis & Dunn * = $p \leq 0.05$ ** = $p \leq 0.01$ or Anova & Dunnett * = $p \leq 0.05$ ** = $p \leq 0.01$

Gross Pathology

Mice were euthanized on Day 185 (about 48 hours after the Day 183 dose) and subjected to a complete necropsy evaluation, including an external evaluation of palpable masses.

Liver mass was noted in one male at 5 mg/kg/dose ISIS 721744 (No. 2019) and one male at 20 mg/kg/dose ISIS 721744 (No. 4016), which correlated with neoplastic histopathology finding of hepatocellular adenoma in both mice. Spleen mass was noted in one male at 20 mg/kg/dose ISIS 721744. Refer to **Table 9**.

Gross pathology findings in the positive control (urethane) group included masses, nodules, pale discoloration, and pale foci in the lungs, masses and nodules in the spleen, and mass and enlargement in the thymus. Refer to **Table 9**.

Table 9 Gross Pathology Terminal Findings in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

Tissue Finding	Males						Females					
	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	+ mg/kg/dose	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	+ mg/kg/dose
	0	5	10	20	10	500	0	10	20	60	10	500
LIVER n =	25	25	25	25	25	10	25	25	25	25	25	10
Focus, pale	0	0	0	1	0	0	0	0	0	0	0	0
Focus, dark	0	0	0	0	1	0	0	0	0	0	0	0
Mass	0	1	0	1	0	0	0	0	0	0	0	0
LUNG n =	25	25	25	25	25	10	25	25	25	25	25	10
Mass	0	0	0	0	0	6	0	0	0	0	0	9
Nodule	0	0	0	0	0	2	0	1	0	1	1	4
Discoloration, pale	0	0	0	0	0	0	0	0	0	0	0	1
Focus, pale	0	0	0	0	0	1	0	1	0	0	0	0
OVARY n =	NA	NA	NA	NA	NA	NA	25	25	25	25	25	10
Cyst, dark	NA	NA	NA	NA	NA	NA	0	0	0	0	0	3
SITE, INJECTION 1 n =	25	25	25	25	25	10	25	25	25	25	25	10
Discoloration, dark	0	0	0	1	0	0	0	0	0	0	0	0
SPLEEN n =	25	25	25	25	25	10	25	25	25	25	25	10
Nodule	0	0	0	1	0	3	0	0	0	0	0	3
Mass	0	0	0	1	0	1	0	0	0	0	0	6
Discoloration, dark	0	0	0	1	0	0	0	3	0	1	1	0
THYMUS n =	25	25	25	25	25	10	25	25	25	25	25	10
Enlargement	0	0	0	0	0	1	0	0	0	0	0	0
Mass	0	0	0	0	0	1	0	0	0	0	0	0

Abbreviations: + = positive control urethane; n = number examined; Veh = vehicle control

Histopathology

The tissues collected at the time of necropsy and evaluated by histopathology are shown in **Table 10** (Applicant's Table). The list of organs and tissues evaluated by microscopy was adequate.

Peer Review

Yes. The Applicant conducted a pathology peer review. The study report states: The Pathology Peer Review of this study was performed under non-GLP conditions. However, the test site followed established SOPs and standard industry practices in conducting the pathology peer review. Any differences of opinion between the study and

peer review pathologists were resolved and agreement on terminology and diagnoses was achieved. The signed final pathology report represents the consensus of the study and peer review pathologists.

Table 10 Tissue List for 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Organ	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Animal ID	X	-	-
Artery, aorta	X	X	X
Body cavity, nasal (Processing: levels 2 and 4)	X	X	X
Bone, femur	X (1)	X (1)	X (1)
Bone marrow, sternum	X	X	X
Bone, sternum	X	X	X
Brain (8 levels, including olfactory bulbs, which are left <i>in situ</i>)	X	X	X
Epididymis	X (2) ^a	X (2)	X (2)
Esophagus	X	X	X
Eye	X (2) ^a	X (2)	X (2)
Gallbladder	X	X	X
Gland, adrenal	X (2)	X (2)	X (2)
Gland, clitoral	X (2)	X (2)	X (2)
Gland, coagulating	X (2)	-	-
Gland, Harderian	X (2)	X (2)	X (2)
Gland, lacrimal	X (2)	X (2)	X (2)
Gland, mammary (females)	X	X	X
Gland, parathyroid	X (2)	X (2)	X (2)
Gland, pituitary	X	X	X
Gland, preputial	X (2)	X (2)	X (2)
Gland, prostate	X	X	X
Gland, salivary, parotid	X (2)	X (2)	X (2)
Gland, salivary, sublingual	X (2)	X (2)	X (2)
Gland, salivary, mandibular	X (2)	X (2)	X (2)
Gland, seminal vesicle	X (2)	X (2)	X (2)
Gland, thyroid	X (2)	X (2)	X (2)
Gland, Zymbal's	X (2)	X (2)	X (2)
Heart	X	X	X
Joint, femorotibial	X (1)	X (1)	X (1)
Kidney	X (2)	X (1)	X (1)
Large intestine, cecum	X	X	X
Large intestine, colon	X	X	X
Large intestine, rectum	X	X	X
Larynx	X	X	X
Liver	X	X	X
Lung	X	X	X
Lymph node(s) draining administration site(s): Axillary	X (2)	X (2)	X (2)
Lymph node, mandibular	X (2)	X (2)	X (2)
Lymph node, mesenteric	X	X	X
Muscle, skeletal	X (2)	X (2)	X (2)

Organ	Macroscopic Evaluation and Collection	Histology Processing	Microscopic Evaluation
Nerve, optic	X (2) ^a	X (2)	X (2)
Nerve, sciatic	X (2)	X (2)	X (2)
Ovary	X (2)	X (2)	X (2)
Oviduct	X (2)	X (2)	X (2)
Pancreas	X	X	X
All dose site, injection subcutaneous	X	X	X
Skin	X	X	X
Small intestine, duodenum	X	X	X
Small intestine, ileum	X	X	X
Small intestine, jejunum	X	X	X
Spinal cord, cervical	X	X	X
Spinal cord, thoracic	X	X	X
Spinal cord, lumbar	X	X	X
Spleen	X	X	X
Stomach	X	X	X
Testis	X (2) ^a	X (2)	X (2)
Thymus	X	X	X
Tongue	X	X	X
Trachea	X	X	X
Ureter	X (2)	-	-
Urinary bladder	X	X	X
Uterus/cervix	X	X	X
Vagina	X	X	X
Macroscopic abnormalities ^b	X	X	X

X = Procedure to be conducted. - = Not applicable. (1) = one side. (2) = both sides.

^a Eyes and optic nerves will be preserved in Davidson's fixative. Testes and epididymides will be preserved in modified Davidson's fixative.

^b Macroscopic abnormalities in the tissues listed, and in any other tissues, will be sampled, processed and evaluated histologically.

Historical Control Provided for Tumor Incidence:

The final study report (SDN 1, 08/21/2024) referenced the testing facility's (i.e., (b) (4)) historical control data for cause of death, neoplastic lesions, and non-neoplastic lesions from 6 month studies in Tg.rash2 mice (from (b) (4)) but it did not appear that these data were included in the final study report. As such, in an information request (IR) dated 10/28/2024, the Applicant was asked to submit these historical control data to the NDA (or indicate their location in the NDA if they were already submitted). In their response to the IR (SDN 10, 11/04/2024), the Applicant indicated that the historical control data had not been previously submitted and therefore provided the testing facility's historical control data for Tg.rash2 mice, as requested.

Neoplastic

An FDA Statistical Review and Evaluation by Malick Mbodj, PhD dated 12/16/2024 analyzed the tumor data for dose response relationship across the vehicle control group and the treated groups, as well as the pairwise comparisons of vehicle control group with each of the treated groups. Following the recommendations by FDA statisticians for the carcinogenicity study design and data analysis, the FDA Statistician applied multiple

testing adjustments and used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons. A tumor is defined as a rare tumor if the published spontaneous rate or the spontaneous rate of the vehicle control of the tumor is less than 1%, and a common tumor is defined as one with tumor rate greater than or equal to 1%.

Test Articles

As shown in **Table 12**, the incidence of combined multicentric hemangiosarcoma was greater in all male ISIS 721744-treated groups (up to 3/25 or 12% at 20 mg/kg/dose) compared to the male vehicle control group (1/25 or 4%). However, based on the FDA Statistical Review and Evaluation, this increase in hemangiosarcoma incidence was not statistically significant. Based on the testing laboratory Tg.rasH2 mouse historical control data, male and female mice developed multicentric hemangiosarcoma (38/643 grand total male mice examined [6%] or up to 16.7% per study; 39/643 grand total female mice examined [6%] or up to 12% per study). The highest incidence of hemangiosarcoma noted as 3/25 males (12%) at 20 mg/kg/dose ISIS 721744 was within range of the testing laboratory historical control data.

As shown in **Table 12**, hepatocellular adenoma in the liver was not observed in the male (or female) vehicle control group(s), but was seen in males at 5 and 20 mg/kg/dose ISIS 721744 and 10 mg/kg/dose ISIS 722059, as follows:

- Male No. 2019 at 5 mg/kg/dose ISIS 721744 (1/25; 4%).
Male No. 2019 had correlating gross pathology finding of liver mass and non-neoplastic histopathology finding in the liver of minimal microvesicular hepatocellular vacuolation. Male No. 2019 also had elevated AST (i.e., 112 U/L) and ALT (i.e., 272 U/L) compared to other males at 5 mg/kg/dose ISIS 721744 and males in the vehicle control group.
- Male Nos. 4006 and 4016 at 20 mg/kg/dose ISIS 721744 (2/25; 8%).
Male No. 4006 had correlating gross pathology finding of pale focus in the liver and non-neoplastic histopathology findings in the liver of moderate basophilic focus of cellular alteration, minimal hepatocellular single cell necrosis, and minimal basophilic pigmented macrophage. Male No. 4006 also had elevated AST (i.e., 120 U/L) and ALT (i.e., 126 U/L).

Male No. 4016 had correlating gross pathology finding of liver mass and non-neoplastic histopathology findings in the liver of moderate microvesicular hepatocellular vacuolation, minimal hepatocellular single cell necrosis, and minimal basophilic pigmented macrophage. Male No. 4016 also had elevated AST (i.e., 183 U/L) and ALT (i.e., 257 U/L).
- Male No. 6016 at 10 mg/kg/dose ISIS 722059 (1/25; 4%).
Male No. 6016 had no correlating gross pathology or non-neoplastic histopathology findings in the liver.

Based on the testing laboratory Tg.rashH2 mouse historical control data, hepatocellular adenoma in the liver was not observed in male mice (0/643 grand total male mice examined [0%]) and only observed in one female mouse (1/643 grand total female mice examined [0.2%] or up to 4% per study [1/25 female mice]). The hepatocellular adenoma noted in 2/25 males (8%) at 20 mg/kg/dose ISIS 721744 exceeded the incidence from the testing laboratory historical control data. The findings of adenoma at 20 mg/kg/dose were accompanied by several non-neoplastic findings in the liver.

The observed incidence of hepatocellular adenoma in the present study for male mice at 20 mg/kg/dose was at the upper end or exceeded the published range (**Table 11**). The historical control data from the testing laboratory would take precedence. This neoplastic finding does occur at a low spontaneous incidence in male rashH2 transgenic mice.

Table 11 Incidences of Hepatocellular Adenoma from the Published Literature

Source	Mean	Range	
		Minimum	Maximum
Nambiar et al., Toxicologic Pathology 40: 614-623, 2012 (Pfizer)	Males, 1.8% Females, 0%	Males, 0% Females, 0%	Males, 9% Females, 0%
Paranjpe et al., Int. J. Toxicology 32(1):48-57, 2013 (BioReliance)	Males, 0.28% Females, 0%	Males, 0% Females, 0%	Males, 4% Females, 0%
Charles River, 2017	Males, 0.9% Females, 0%		
Laast et al., Labcorp STP, 43rd Annual Symposium, 2024	Males, 0.8% (=12/1525) Females, 0%		

Based on the FDA Statistical Review and Evaluation:

- There was no statistically significant dose response relationship across the vehicle control and the ISIS 721744-treated groups in any tumor types in either male or female mice. The pairwise comparisons also showed no tumor types with a statistically significant increase in tumor incidences in ISIS 721744- or ISIS 722059-treated groups, when compared to the vehicle control group in either male or female mice.

Positive Control

Multicentric neoplasms noted in the positive control group included hemangiosarcoma in 7/10 males (70%) and 9/10 females (90%), malignant lymphoma in 2/10 males (20%), and histiocytic sarcoma in 1/10 males (10%). Other neoplasms noted in the positive control group included malignant bronchioloalveolar carcinoma in the lungs in

8/10 males (80%) and 10/10 females (100%), multiple bronchioloalveolar adenoma in the lungs in 10/10 males (100%) and 8/10 females (80%), malignant bronchioloalveolar carcinoma in the thymus in 1/10 females (10%), and cortical adenoma in the adrenal gland in 1/10 females (10%).

Based on FDA Statistical Review and Evaluation, the pairwise comparisons showed statistically significant increases in the positive control group for the incidences of:

- hemangiosarcoma in the whole body in both male and female mice (7/10 and 9/10, respectively), when compared to male and female mice in the vehicle control groups (1/25 and 1/25, respectively) (p-values < 0.0001);
 - hemangiosarcoma in the spleen in both male and female mice (7/10 and 9/10, respectively), when compared to male and female mice in the vehicle control groups (1/25 and 0/25, respectively) (p-values < 0.0001);
 - hemangiosarcoma in the lung in male mice (2/10), when compared to male mice in the vehicle control group (0/25) (p-value = 0.0483);
- malignant lymphoma in the hemolymphoreticular tissue in male mice (2/10), when compared to male mice in the vehicle control group (0/25) (p-value = 0.0483);
- bronchioloalveolar carcinoma in the lungs in both male and female mice (8/10 and 10/10, respectively) when compared to male and female mice in the vehicle control groups (0/25 and 0/25, respectively) (p-values < 0.0001); and bronchioloalveolar adenoma in the lung in both male and female mice (10/10 and 8/10, respectively), when compared to male and female mice in the vehicle control groups (1/25 and 0/25, respectively) (p-values < 0.0001).

Table 12 Neoplastic Histopathology Terminal Findings in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

Tissue Finding	Males						Females						
	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	+ mg/kg/dose	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	+ mg/kg/dose	
		0	5	10				20	10	500			0
WHOLE BODY													
Hemangiosarcoma (COMBINED MULTI-CENTRIC)	1	2	2	3	1	7	1	1	0	1	0	9	
LIVER n =	25	25	25	25	25	10	25	25	25	25	25	10	
Hepatocellular adenoma; benign	0	1	0	2	1	0	0	0	0	-	0	0	

Abbreviations: + = positive control urethane; n = number examined; Veh = vehicle control

Non-neoplastic

Non-neoplastic histopathology findings at the time of the terminal sacrifice were noted in the liver, kidney, injection site, urinary bladder, and lymph nodes (axillary and mesenteric). Refer to **Table 13**.

Liver

Microvesicular hepatocellular vacuolation (minimal to moderate) in the liver was noted in males at all ISIS 721744 doses and in one female at 20 mg/kg/dose ISIS 721744. The finding (minimal to mild) was seen in males at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Basophilic pigmented macrophages (minimal to moderate) in the liver were noted in males at 10 and 20 mg/kg/dose ISIS 721744 and females at 20 and 60 mg/kg/dose ISIS 721744. The finding (minimal) was noted in males and females at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Hepatocellular single cell necrosis (minimal) in the liver was seen in males at 10 and 20 mg/kg/dose ISIS 721744 and females at 60 mg/kg/dose ISIS 721744. The finding (minimal) was seen in one male at 10 mg/kg/dose ISIS 722059.

Karyocytomegaly (minimal to mild) in the liver was seen in males at 10 and 20 mg/kg/dose ISIS 721744. The finding (minimal) was seen in one male at 10 mg/kg/dose ISIS 722059.

Basophilic focus of cellular alteration (minimal to moderate) in the liver was seen in males at 10 and 20 mg/kg/dose ISIS 721744. The finding (minimal) was seen in one female at 10 mg/kg/dose ISIS 722059.

Kidney (Right)

Epithelial vacuolation (minimal to mild) in the kidney was noted in females at 60 mg/kg/dose ISIS 721744. The finding was not seen at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Epithelial basophilic granules (minimal to moderate) in the kidney were noted in males at 20 mg/kg/dose ISIS 721744 and females at 20 and 60 mg/kg/dose ISIS 721744. The finding was not noted at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Injection Site

Macrophage granular vacuolation (minimal to mild) at the injection site was noted in males and females at all ISIS 721744 doses. The finding (minimal to mild) was seen in males and females at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Urinary Bladder

Urothelial vacuolation (minimal to moderate) in the urinary bladder was noted in males at 10 and 20 mg/kg/dose ISIS 721744 and females at 20 and 60 mg/kg/dose ISIS 721744. The finding was not seen at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Lymph Nodes

Macrophage vacuolation (minimal to mild) in the lymph nodes (axillary and/or mesenteric) was noted in females at 60 mg/kg/dose ISIS 721744. The finding was not seen at 10 mg/kg/dose ISIS 722059. This finding was attributed to oligonucleotide uptake.

Table 13 Non-Neoplastic Histopathology Terminal Findings in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice

	Males					Females				
	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/dose
	0	5	10	20	10	0	10	20	60	10
LIVER n =	25	25	25	25	25	25	25	25	25	25
Vacuolation; hepatocellular, microvesicular	0	18	20	18	7	0	0	1	0	0
...minimal	0	10	12	9	6	0	0	1	0	0
...mild	0	8	7	4	1	0	0	0	0	0
...moderate	0	0	1	5	0	0	0	0	0	0
Pigmented macrophage; basophilic	0	0	5	22	6	0	0	3	25	3
...minimal	0	0	5	17	6	0	0	3	3	3
...mild	0	0	0	5	0	0	0	0	15	0
...moderate	0	0	0	0	0	0	0	0	7	0
Single cell necrosis; hepatocellular	0	0	1	8	1	0	0	0	12	0
...minimal	0	0	1	8	1	0	0	0	12	0
Karyocytomegaly	0	0	2	15	1	0	0	0	0	0
...minimal	0	0	2	13	1	0	0	0	0	0
...mild	0	0	0	2	0	0	0	0	0	0
Focus of cellular alteration, basophilic	1	0	3	3	0	0	0	0	0	1
...minimal	1	0	1	0	0	0	0	0	0	1
...mild	0	0	2	1	0	0	0	0	0	0
...moderate	0	0	0	2	0	0	0	0	0	0

	Males					Females				
	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose	Veh	ISIS 721744 mg/kg/dose			ISIS 722059 mg/kg/ dose
		0	5	10	20		10	0	10	20
KIDNEY, RIGHT n =	25	25	25	25	25	25	25	25	25	25
Vacuolation; epithelial	0	0	0	0	0	0	0	0	25	0
...minimal	0	0	0	0	0	0	0	0	23	0
...mild	0	0	0	0	0	0	0	0	2	0
Basophilic granules; epithelial	0	0	0	3	0	0	0	14	25	0
...minimal	0	0	0	3	0	0	0	14	3	0
...mild	0	0	0	0	0	0	0	0	21	0
...moderate	0	0	0	0	0	0	0	0	1	0
SITE, INJECTION n =	25	25	25	25	25	25	25	25	25	25
Vacuolation; granular, macrophage	0	3	3	5	13	0	14	7	20	17
...minimal	0	3	3	5	13	0	13	6	16	13
...mild	0	0	0	0	0	0	1	1	4	4
URINARY BLADDER n =	25	24	25	25	25	25	24	25	25	25
Vacuolation; urothelial	0	0	5	16	0	0	0	11	24	0
...minimal	0	0	5	7	0	0	0	11	7	0
...mild	0	0	0	4	0	0	0	0	12	0
...moderate	0	0	0	5	0	0	0	0	5	0
Hyperplasia; angiomatous	0	1	0	0	0	3	4	4	6	2
...minimal	0	1	0	0	0	3	4	3	6	2
...mild	0	0	0	0	0	0	0	1	0	0
LYMPH NODE, AXILLARY n =	25	24	23	24	25	24	25	24	25	25
Vacuolation; macrophage	0	0	0	0	0	0	0	0	21	0
...minimal	0	0	0	0	0	0	0	0	17	0
...mild	0	0	0	0	0	0	0	0	4	0
LYMPH NODE, MESENTERIC n =	24	24	25	25	25	25	25	25	25	25
Vacuolation; macrophage	0	0	0	0	0	0	0	0	16	0
...minimal	0	0	0	0	0	0	0	0	16	0

Abbreviations: n = number examined; Veh = vehicle control

The most prominent, non-neoplastic histopathology findings at termination in positive control (urethane)-treated groups included lung adhesion in males (n = 6) and females (n = 4), erythrophagocytosis in the axillary lymph node in males (n = 4) and females (n = 2), hematocyst in the ovary in females (n = 3), stromal hyperplasia in the spleen in females (n = 2), and angiomatous hyperplasia in the uterus/cervix in females (n = 2).

Toxicokinetics

The toxicokinetics of ISIS 721744 was not evaluated in this study.

Tissue Concentration Analysis

Portions of the kidney (left) and liver (left lobe) from 10 animals/sex/group were used for tissue concentration analysis since these organs are the major organs of distribution for oligonucleotides.

As shown in **Table 14** (Applicant's Table), ISIS 721744 concentrations in the kidney and liver of males and females were generally increased with increasing ISIS 721744 dose following 6-months of treatment with ISIS 721744.

Table 14 Mean Tissue Concentrations of ISIS 721744 in Kidney and Liver in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Table)

Matrix	Group	Dose Level (mg/kg/q2w)	Number of Doses	Study Day	ISIS 721744 Concentrations (µg/g)					
					N	Female	N	Male	N	Combined
Kidney	1	0	14	185	10	NA	10	NA	20	NA
	2	5	14	185	10	NA	10	34.4 ± 6.5	NA	NA
	3	10	14	185	10	88.8 ± 35.1	10	64.53 ± 20.0	20	76.7 ± 30.5
	4	20	14	185	10	143.6 ± 70.1	10	95.3 ± 32.5	10	119.4 ± 58.7
	5	60	14	185	10	519.2 ± 127.9	10	NA	NA	NA
Liver	1	0	14	185	10	BLQ	10	BLQ	20	BLQ
	2	5	14	185	10	NA	10	30.1 ± 5.9	NA	NA
	3	10	14	185	10	34.5 ± 17.6	10	40.3 ± 16.7	20	37.4 ± 17.0
	4	20	14	185	10	30.6 ± 22.1	10	59.0 ± 15.3	20	44.8 ± 23.5
	5	60	14	185	10	91.5 ± 15.9	10	NA	NA	NA

BLQ = below the lower limit of quantitation (LLOQ= 0.107 µg/g).

NA- Not Applicable

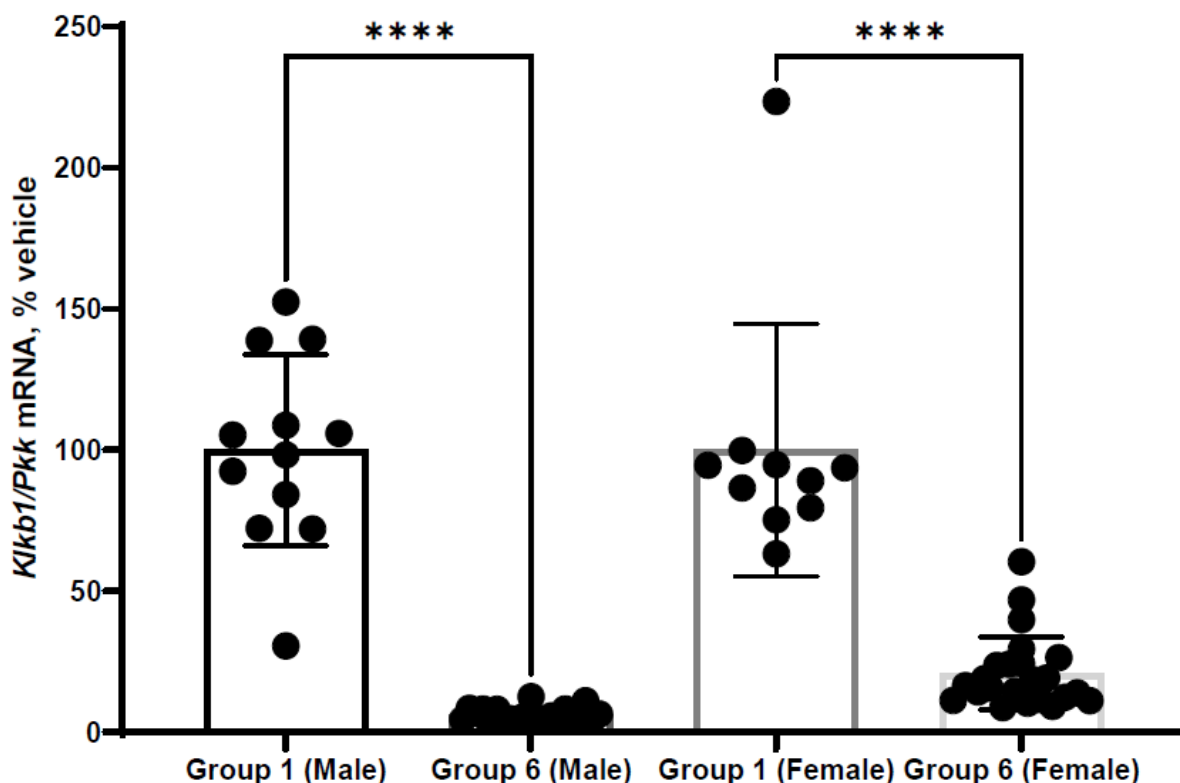
Values are presented as mean ± standard deviation (SD). For the purpose of calculating mean and SD, all BLQ values were set to zero. Mean concentrations that were BLQ are presented as BLQ, and the corresponding SD was not calculated.

Target mRNA Analysis

Liver tissue from the first 10 surviving animals/sex from the vehicle control group and all surviving animals from the mouse surrogate ISIS 722059-treated group were used for target mRNA analysis.

As shown in **Figure 6** (Applicant's Figure), hepatic PKK mRNA expression was statistically reduced in male and female mice treated with 10 mg/kg/dose mouse surrogate ISIS 722059 Q2W for 26 weeks by 94% and 79%, respectively, compared to controls.

Figure 6 Hepatic PKK mRNA Expression in Mice Treated with ISIS 722059 in 6-Month Subcutaneous Carcinogenicity Study in Tg.rasH2 Mice (Applicant's Figure)



Average and STDEV indicated for both males and females. **** Significantly different from saline (Group 6) using two-tailed unpaired t-test ($p < 0.0001$).

Dosing Solution Analysis

As described and included in the study report: Concentration verification of each ISIS 721744 and mouse surrogate ISIS 722059 dose formulation was documented by the Applicant and communicated to the Testing Facility in the form of a GLP Analytical Summary Report. Representative samples which bracketed the range of ISIS 721744 and mouse surrogate 722059 concentrations were assessed for stability. No homogeneity analysis of ISIS 721744 or mouse surrogate ISIS 722059 was performed as they form a true solution in aqueous buffers.

The concentrations of the ISIS 721744 formulations (0.5, 1, 2, and 6 mg/mL) and mouse surrogate ISIS 722059 formulation (1 mg/mL) were within acceptable ranges (94.4% to 99.3% of label claim and 106.6% of label claim, respectively) at test article release. Further, ISIS 721744 formulations which bracketed those used on study (0.1 and 200 mg/mL) were stable for 42 months (results within 98.2 to 99.8% of the label claim) and mouse surrogate ISIS 722059 formulations which bracketed that used on study (0.1 to

15 mg/mL) were stable for 12 months (results within 101.6 to 107.3% of label claim), which confirmed the stability of the formulated test articles during the dosing period.

11 Integrated Summary and Safety Evaluation

The Applicant submitted a 505(b)(1) NDA for SC administration of donidalorsen (ISIS 721744), an ASO which targets PKK, as 80 mg (b) (4) (or 80 mg (b) (4) in some patients) prophylaxis to prevent attacks of HAE in adult and pediatric patients 12 years of age and older.

ISIS 721744 was not genotoxic in a complete battery of genotoxicity assays (in vitro Ames assay, in vitro chromosome aberration assay, in vivo mammalian micronucleus assay).

The Applicant conducted a 6-month study in Tg.rasH2 mice to evaluate the carcinogenic potential of ISIS 721744 (off-target) and mouse surrogate ISIS 722059 (intended pharmacology to inhibit PKK synthesis). Tg.rasH2 mice (n = 25/sex/group) were administered vehicle control (0.9% sodium chloride for injection, USP), ISIS 721744 (5, 10, or 20 mg/kg/dose for males and 10, 20, or 60 mg/kg/dose for females), or mouse surrogate ISIS 722059 (10 mg/kg/dose) Q2W for up to 6 months by SC injection. A positive control group was also included in which Tg.rasH2 mice (10/sex) were administered urethane (500 mg/kg/dose) via intraperitoneal (IP) injection on Days 1, 3, and 5. Based on the 14-week DRF study in Tg.rasH2 (refer to Nonclinical Primary Review dated 12/12/2022), the ECAC concurred with doses in male mice (high dose based on a MTD) but could not recommend doses in female mice (no acceptable basis for dose selection could be identified) (Refer to IND 142564 ECAC Protocol Minutes dated 11/16/2022 for additional details).

There was no statistically significant increase or decrease in mortality dose response across the vehicle control and ISIS 721744-treated groups, in either male or female mice. There was a statistically significant decrease in mortality in the male 5 mg/kg/dose group (low dose) compared to the vehicle control group.

Effects on clinical chemistry included increases in AST, ALT, and GLDH in males at 10 and 20 mg/kg/dose ISIS 721744 and females at 60 mg/kg/dose ISIS 721744, which correlated with histopathology findings in the liver.

A MTD was achieved at the high dose of 20 mg/kg Q2W in males based on histopathology findings in the liver and elevations of GLDH. The high dose of 60 mg/kg Q2W in females did not meet the criteria for a MTD.

Neoplastic histopathology findings included hepatocellular adenoma in the liver in 1/25 males at 5 mg/kg/dose ISIS 721744, 2/25 males at 20 mg/kg/dose ISIS 721744, and 1/25 males at 10 mg/kg/dose ISIS 722059. Although the tumor incidence was not statistically significant, the hepatocellular adenoma noted in 2/25 males (8%) at 20 mg/kg/dose ISIS 721744 exceeded the testing laboratory historical control incidence (0/643 grand total male mice examined [0%]; 1/643 grand total female mice examined

[0.2%] or up to 4% per study). Based on the FDA Statistical Review and Evaluation, there was no statistically significant dose response relationship across the vehicle control and the ISIS 721744-treated groups in any tumor types in either male or female mice. The pairwise comparisons also showed no tumor types with a statistically significant increase in tumor incidences in ISIS 721744- or ISIS 722059-treated groups, when compared to the vehicle control group in either male or female mice.

Non-neoplastic histopathology findings were noted in the **liver** (microvesicular hepatocellular vacuolation, basophilic pigmented macrophages, hepatocellular single cell necrosis, karyocytomegaly, and/or basophilic focus of cellular alteration); **kidney** (epithelial vacuolation and epithelial basophilic granules); **injection site** (macrophage granular vacuolation); **urinary bladder** (urothelial vacuolation); and the **axillary and/or mesenteric lymph nodes** (macrophage vacuolation). All histopathology findings were attributed to or a consequence of oligonucleotide uptake.

The positive control (urethane) group resulted in findings consistent with known effects of urethane administration¹ and confirmed the carcinogenic responsiveness of the Tg.rasH2 model.

Toxicokinetics were not evaluated in the 6-month carcinogenicity study in Tg.rasH2. Drug concentrations in the liver and kidneys generally increased with increasing dose.

The mouse is not a pharmacologically relevant species for ISIS 721744, so the study included one dose group treated with mouse surrogate ISIS 722059. Hepatic PKK mRNA expression was statistically reduced in male and female mice treated with 10 mg/kg/dose ISIS 722059 Q2W for 26 weeks by 94% and 79%, respectively, compared to controls.

The results of the 6-month carcinogenicity study in Tg.rasH2 mice carcinogenicity study were presented to the ECAC (refer to meeting minutes dated 12/12/2024). The ECAC concluded that the carcinogenicity study was adequate for male mice and negative for drug-related tumors. However, the carcinogenicity study was inadequate for female mice because an MTD was not achieved and no other valid basis to support the HD was discernable.

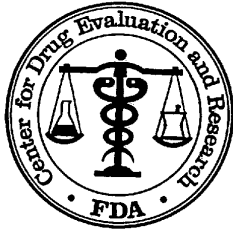
Reviewer's Note: Based on this nonclinical review, and the pending results of the 2-year carcinogenicity study in male and female rats as a post marketing requirement, another 6-month study in female mice will not be requested at this time.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

ELENI M SALICRU
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TIMOTHY W ROBISON
12/19/2024 10:06:31 AM
I concur



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number:	NDA 219407
Drug Name:	Donidalorsen (ISIS 721744)
Indication(s):	Prophylaxis to prevent attacks of hereditary angioedema (HAE) in adult and pediatric patients 12 years of age and older
Studies	Six-Month Study of ISIS 721744 by Subcutaneous Bolus Injection in CByB6F1-Tg(HRAS)2Jic Hemizygous Transgenic Mouse
Applicant:	Ionis Pharmaceuticals, Inc. 2855 Gazelle Court Carlsbad, CA, USA
Test Facility:	(b) (4) (b) (4)
Documents Reviewed:	Electronic submission: Submitted on August 21, 2024, via SDN 0001 Electronic data: Submitted on October 25, 2024, via SDN 0008
Review Priority:	Standard
Biometrics Division:	Division of Biometrics -VI
Statistical Reviewer:	Malick Mbodj, Ph.D.
Concurring Reviewer:	Mohammad A. Rahman, Ph.D.
Medical Division:	Division of Pharmacology-Toxicology for Immunology & Inflammation
Reviewing Pharmacologist:	Eleni Salicru, Ph.D.
Project Manager:	Chee, Cindy C.
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1. Background

In this submission the sponsor included a carcinogenicity study report in transgenic mice. This study was intended to assess the carcinogenic potential of the test article donidalorsen (ISIS 721744), when given once every other week via subcutaneous injection for up to 6-months in CByB6F1-Tg (HRAS)²Jic mice. In addition, since ISIS 721744 was not homologous to mouse PKK mRNA, a mouse surrogate, ISIS 722059, was included to evaluate carcinogenicity potential related to PKK inhibition. Results of this review have been discussed with the reviewing pharmacologist Dr. Salicru.

In this review the phrase "dose response relationship" (trend) refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as dose increases.

2. Mouse Study

In this study two separate experiments were conducted, one in male mice and one in female mice. In each of these two experiments, one hundred and ten Tg rasH2 transgenic mice were assigned randomly to one of the five groups which included three treated groups, one vehicle control group and one positive control group in equal size of 25 animals except the positive control group which had 10 animals. The dose levels for the treated groups were 5, 10, and 20 mg/kg/dose, for males and, 10, 20, and 60 mg/kg/dose, for females as indicated in Table 1. In this review these dose groups would be referred to as the low, medium, and high dose group, respectively. In addition, since ISIS 721744 was not homologous to mouse PKK mRNA, a group of 25 mice (mouse surrogate, ISIS 722059), was included to evaluate carcinogenicity potential related to PKK inhibition. The vehicle control group received the vehicle 0.9% Sodium Chloride for Injection, USP (also known as Saline) administered subcutaneously once every two weeks in the same manner as the treated groups. The positive control was administered with an intraperitoneal injection (Urethane) at 500 mg/kg/dose on Days 1, 3, and 5. This group was included to verify sensitivity of the test system to detect carcinogenicity effect.

Table 1: Experimental Design in Mouse Study

Group Name	Males			Females		
	Group NO.	Dose Level (mg/kg/dose)	Number of Animal Males	Group NO.	Dose Level (mg/kg/dose)	Number of Animal Females
Vehicle Control	1	0	25	1	0	25
Low	2	5	25	3	10	25
Medium	3	10	25	4	20	25
High	4	20	25	5	60	25
ISIS 722059	6	10	25	6	10	25
Positive control	7	500	10	7	500	10

The positive control was administered with intraperitoneal injection on Days 1, 3, and 5.

All animals were euthanized during week 27, (Groups 1-6)

During the study period, all animals were observed for morbidity, mortality, injury, and the availability of food and water at least twice daily (morning and afternoon) beginning upon arrival throughout the study, and all animals were given a detailed clinical examination within 3 days of the first dose administration and 4 ± 0.5 hours postdose on dosing days, once weekly on non-dosing weeks. Any animal showing signs of severe debility or intoxication, and if determined to be moribund or suffering excessively was euthanized. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, unusual behavior, and palpation of tissue masses were done weekly throughout the

study. Histopathological examinations were performed on all animals found dead or killed moribund or sacrificed at the end of the experiment. Body weights for all animals were measured and recorded once after arrival, Day-1 and once weekly during the study. The food consumption recorded once weekly for the first 14 weeks, every 2 weeks thereafter.

2.1. Sponsor's analyses

2.1.1. Survival analysis

Intercurrent mortality data were analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using the log-rank test. If this overall test was significant ($p < 0.05$), a follow up analysis was performed where each treated group was compared to the vehicle control group using the log-rank test. Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests.

Sponsor's findings:

Sponsor's analysis showed the numbers of mice surviving to their terminal necropsy were 21 (84%), 25 (100%), 23 (92%), 21 (84%), and 22 (88%) in the vehicle control group, low, medium, high, and ISIS 722059 groups, respectively in male mice, and 25 (100%), 23 (92%), 24 (96%), 25 (100%), and 23 (92%), respectively, in female mice. The sponsor's report concluded that, there was no statistically significant increase or decrease in mortality dose response across the vehicle control group and the three treated groups, in either male or female mice. The pairwise comparisons, also showed no statistically significant increase or decrease in mortality between the individual treated groups, the ISIS 722059 group, and the vehicle control group in either male or female mice.

2.1.2. Tumor data analysis (Peto and Poly-k Method)

Tumor incidence data were analyzed using both survival-adjusted and unadjusted tests. The unadjusted tests were based on the incidences and numbers of sites examined for each tumor type. The Cochran-Armitage test was used to test dose response, and Fisher's exact test was used to compare each treated group with the control group. Trend test was applied for all control and treated groups able to be arranged in a strictly increasing dose level. The survival-adjusted test was conducted according to the prevalence/mortality methods described by Peto et al.

Survival adjusted analysis was also performed using the Poly-k method to assess prevalence of tumors (Bailer and Portier, 1988) (Bieler and Williams, 1993). The survival-adjusted rates based on the risk weights were displayed. The tests included both overall trend and pair-wise comparisons of each treated group with the vehicle control. All p-values are reported using upper-tailed tests.

Adjustment for the multiplicity:

For multiplicity adjustment, the sponsor used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

Sponsor's findings:

The sponsor's report concluded that, the donidalorsen (ISIS 721744) or ISIS 722059 does not have carcinogenic activity or potential when administered daily for 26 weeks subcutaneously at doses up to 20 mg/kg/dose for males and up to 60 mg/kg/dose for females.

2.2 Reviewer's analyses

This reviewer independently performed the survival data analyses, tumor data analyses and additional analyses suggested by the reviewing pharmacologist. Data used in this reviewer's analyses were provided by the sponsor electronically on October 25, 2024, via SDN 0008.

2.2.1 Survival analysis

In the reviewer's analysis, intercurrent mortality data were analyzed using the Kaplan-Meier product limit method. The Kaplan-Meier's curve was presented graphically. The dose response relationship and homogeneity of survival distributions were tested for the treatment groups using the Likelihood ratio test and the Log-Rank test. The intercurrent mortality data are given in Tables 1A and 1B in the appendix. The Kaplan-Meier curve for survival rate is given in Figures 1A and 1B in the appendix. Results of the tests for dose response relationship and homogeneity of survivals are given in Tables 2A and 2B in the appendix.

Reviewer's findings:

This reviewer's analysis showed the numbers of male mice surviving to their terminal necropsy were 21 (84%), 25 (100%), 23 (92%), 21 (84%), and 22 (88%) in the vehicle control group, low, medium, high, and ISIS 722059 groups, respectively in male mice, and 25 (100%), 23 (92%), 24 (96%), 25 (100%), and 23(92%), in vehicle control, low, medium, high, and ISIS 722059 groups, respectively, in female mice. This reviewer's analysis showed no statistically significant increase or decrease in mortality dose response across the vehicle control group and the three ISIS 721744 treated groups, in either male or female mice. The pairwise comparisons, showed a statistically significant decrease in mortality in the male's low dose group when compared to the vehicle control group, ($p=0.0160$).

Also, the pairwise comparisons, showed no statistically significant increase or decrease in mortality between the ISIS 722059 group, and the vehicle control group, in either male or female mice.

This reviewer's analysis also, showed that the positive control group had a statistically significant increase in unscheduled death compared to the vehicle control group, ($p<0.0001$) in both male and female mice.

2.2.2. Tumor data analysis

In the reviewer's analysis, the tumor data were analyzed for dose response relationship across vehicle control group and the treated groups, as well as the pairwise comparisons of vehicle control group with each of the treated groups using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice with development of the tumor type being tested gets a score of $s_h = 1$. An animal that dies at Week w_h without development of the given tumor type before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}}\right)^k < 1$. The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal with score $s_h = 1$ can be considered as a whole animal, while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the

study or if each animal develops the given tumor being tested, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise comparison) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k. For this study rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of the data. Based on the intent to treat (ITT) principle Wmax was considered as 26.

For the calculation of p-values, if there were less than 10 tumor bearing animals across all treatment groups for a given tumor type, the exact tests based on the discrete permutation distribution were used, with dose levels (0, 5, 10, and 20 mg/kg/dose, for males and, 0, 10, 20, and 60 mg/kg/dose, for females) as scores, and asymptotic tests were used for tumor types with higher incidences. The tumor rate and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for male mice and female mice, respectively.

Multiple testing adjustments:

Following the recommendations by FDA statisticians for the carcinogenicity study design and data analysis, this reviewer used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

A tumor is defined as a rare tumor if the published spontaneous rate or the spontaneous rate of the vehicle control of the tumor is less than 1%, and a common tumor is defined as one with tumor rate greater than or equal to 1%.

Reviewer's findings:

Following the multiple testing adjustment method described above, this reviewer's analyses showed no statistically significant dose response relationship across the vehicle control and the ISIS 721744 treated groups in any tumor types in either male or female mice. The pairwise comparisons also showed no tumor types with a statistically significant increase in tumor incidences in donidalorsen (ISIS 721744) or ISIS 722059 treated groups, when compared to the vehicle control group in either male or female mice.

The pairwise comparisons showed statistically significant increases in the positive control group for the incidences of bronchioloalveolar carcinoma, bronchioloalveolar adenoma, in lung and hemangiosarcoma in spleen, when compared to the vehicle control group in both male and female mice (p-values <0.0001, <0.0001, <0.0001 for males, and <0.0001, <0.0001, <0.0001, for females, respectively). Also, the pairwise comparisons showed statistically significant increases in the positive control group for the incidences of hemangiosarcoma in the lung, and lymphoma, malignant in the hemolymphoreticular tissue in male mice (p-values=0.0483, and =0.0483, respectively).

3. Summary

In this submission the sponsor included a carcinogenicity study report in transgenic mice. This study was intended to assess the carcinogenic potential of the test article donidalorsen (ISIS 721744), when given once every other week via subcutaneous injection for up to 6-months in CByB6F1-Tg (HRAS)2Jic mice. In addition, since ISIS 721744 was not homologous to mouse PKK mRNA, a mouse surrogate, ISIS 722059, was included to evaluate carcinogenicity potential related to PKK inhibition.

One hundred and ten Tg rasH2 transgenic male mice were assigned randomly to one of the five groups which included three treated groups, one vehicle control group and one positive control group in equal size of 25

animals except the positive control group which had 10 animals. The dose levels for treated groups were 5, 10, and 20 mg/kg, for males and, 10, 20, and 60 mg/kg, for females as indicated in Table 1. In this review these dose groups would be referred to as the low, medium, and high dose group, respectively. In addition, since ISIS 721744 was not homologous to mouse PKK mRNA, a group of 25 mice (mouse surrogate, ISIS 722059), was included to evaluate carcinogenicity potential related to PKK inhibition. The vehicle control group received the vehicle 0.9% Sodium Chloride for Injection, USP (also known as Saline) administered subcutaneously once every two weeks in the same manner as the treated groups. The positive control was administered with an intraperitoneal injection (Urethane) at 500 mg/kg/dose on Days 1, 3, and 5. This group was included to verify sensitivity of the test system to detect carcinogenicity effect.

This reviewer's analysis showed that the numbers of male mice surviving to their terminal necropsy were 21 (84%), 25 (100%), 23 (92%), 21 (84%), and 22 (88%) in the vehicle control group, low, medium, high, and ISIS 722059 groups, respectively in male mice, and 25 (100%), 23 (92%), 24 (96%), 25 (100%), and 23(92%), in vehicle control, low, medium, high, and ISIS 722059 groups, respectively, in female mice. This reviewer's analysis showed that no statistically significant increase or decrease in mortality dose response across the vehicle control group and the three ISIS 721744 treated groups. The pairwise comparisons, showed a statistically significant decrease in mortality in the male's low dose group when compared to the vehicle control group with p-value = 0.0160.

Also, the pairwise comparisons, showed no statistically significant increase or decrease in mortality between the ISIS 722059 group, and the vehicle control group, in either male or female mice.

This reviewer's analysis showed that the positive control group had a statistically significant increase in unscheduled death compared to the vehicle control group, (p<0.0001) in both male and female mice.

For tumor data, following the multiple testing adjustment method described above, this reviewer's analyses showed no statistically significant dose response relationship across the vehicle control and the ISIS 721744 treated groups in any tumor types in either male or female mice. The pairwise comparisons also showed no tumor types with a statistically significant increase in tumor incidences in donidalorsen (ISIS 721744) or ISIS 722059 treated groups, when compared to the vehicle control group in either male or female mice.

The pairwise comparisons showed statistically significant increases in the positive control group for the incidences of bronchioloalveolar carcinoma, bronchioloalveolar adenoma, in lung and hemangiosarcoma in spleen, when compared to the vehicle control group in both male and female mice (p-values <0.0001, <0.0001, <0.0001 for males, and <0.0001, <0.0001, <0.0001, for females, respectively). Also, the pairwise comparisons showed statistically significant increases in the positive control group for the incidences of hemangiosarcoma in the lung, and lymphoma, malignant in the hemolymphoreticular tissue in male mice (p-values=0.0483, and =0.0483, respectively).

Malick Mbodj, Ph.D.
Mathematical Statistician

Concur: Mohammad A Rahman, Ph.D. DD, Acting Team Leader, DBVI

cc:

Archival deonidalorsen (ISIS 721744)

Dr. Tsong Dr. Rahman
Dr. Salicru Cindy C, Chee

4. Appendix

Table 1A: Intercurrent Mortality Rate
Male Mice

Week	0mg/kg/dose Vehicle Cont.		5 mg/kg/dose Low		10 mg/kg/dose Med		20 mg/kg/dose High		10 mg/kg/dose ISIS 722059		500 mg/kg/dose Positive Cont.	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 13	.	.	.	.	1	4.00	.	.	.	.	1	10.00
14 - 27	4	16.00	.	.	1	8.00	4	16.00	3	12.00	9	100.00
Ter. Sac.	21	84.00	25	100.00	23	92.00	21	84.00	22	88.00	.	.
Total	25	100.00	25	100.00	25	100.00	25	100.00	25	100.00	10	100.00

Table 1A: Intercurrent Mortality Rate
Female Mice

Week	0mg/kg/dose Vehicle Cont.		10 mg/kg/dose Low		20 mg/kg/dose Med		60 mg/kg/dose High		10 mg/kg/dose ISIS 722059		500 mg/kg/dose Positive Cont.	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 13	.	.	.	.	.	.	.	.	.	.	.	.
14 - 27	.	.	2	8.00	1	4.00	.	.	2	8.00	10	100.00
Ter. Sac.	25	100.00	23	92.00	24	96.00	25	100.00	23	92.00	.	.
Total	25	100.00	25	100.00	25	100.00	25	100.00	25	100.00	10	100.00

Table 2A: Intercurrent Mortality Comparison for Male Mice

Test Statistics	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High	P-value for Vehicle Cont. vs ISIS 722059	P-value for Vehicle Cont. vs Positive Cont.
Dose-Response (Likelihood Ratio)	0.5965	0.0160*	0.3845	0.9689	0.6745	<0.0001*
Homogeneity (Log-Rank)	0.1902	0.0390*	0.3885	0.9686	0.6749	<0.0001*

* = statistically significant at the 0.05 significance level.

Table 2B: Intercurrent Mortality Comparison for Female Mice

Test Statistics	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High	P-value for Vehicle Cont. vs ISIS 722059	P-value for Vehicle Cont. vs Positive Cont.
Dose-Response (Likelihood Ratio)	0.4463	0.0935	0.2390	.	0.0935	<0.0001*
Homogeneity (Log-Rank)	0.2814	0.1531	0.3173	.	0.1531	<0.0001*

* = statistically significant at the 0.05 significance level.

Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Male Mice Using Poly-3 Test.

Organ Name	Tumor Name	0 mg Vehicle Cont (N=25) P - Trend	5 mg Low (N=25) P - VC vs. L	10 mg Med (N=25) P - VC vs. M	20 mg High (N=25) P - VC vs. H	10 mg ISIS 722059 (N=25) P - VC vs. IS	500 mg Positive Cont.(N=10) P - VC vs. PC
Bone, Femur	Hemangiosarcoma	0/25 (23) 0.5000	0/25 (25) NC	1/25 (24) 0.5106	0/25 (24) NC	0/25 (24) NC	1/10 (7) 0.2333
Epididymis	Hemangiosarcoma	0/25 (23) 0.7604	1/25 (25) 0.5208	0/25 (24) NC	0/25 (24) NC	0/25 (24) NC	0/10 (6) NC
Gland, Harderian	Adenoma	2/25 (23) 0.6892	0/25 (25) 1.0000	1/25 (24) 0.8908	1/25 (24) 0.8908	0/25 (24) 1.0000	2/10 (7) 0.2245
Hemolymphoreticular Tissue	Histiocytic Sarcoma	0/25 (23) NC	0/25 (25) NC	0/25 (24) NC	0/25 (24) NC	0/25 (24) NC	1/10 (6) 0.2069
	Lymphoma, Malignant	0/25 (23) NC	0/25 (25) NC	0/25 (24) NC	0/25 (24) NC	0/25 (24) NC	2/10 (7) 0.0483*
Liver	Hemangiosarcoma	0/25 (23) 0.5000	0/25 (25) NC	1/25 (24) 0.5106	0/25 (24) NC	0/25 (24) NC	1/10 (7) 0.2333
	Hepatocellular Adenoma	0/25 (23) 0.1088	1/25 (25) 0.5208	0/25 (24) NC	2/25 (24) 0.2553	1/25 (24) 0.5106	0/10 (6) NC
Lung	Bronchioloalveolar Adenoma	4/25 (23) 0.7674	3/25 (25) 0.8253	6/25 (24) 0.3907	2/25 (24) 0.9154	1/25 (24) 0.9781	10/10 (10) <0.0001*
	Bronchioloalveolar Carcinoma	0/25 (23) NC	0/25 (25) NC	0/25 (24) NC	0/25 (24) NC	0/25 (24) NC	8/10 (9) <0.0001*
	Hemangiosarcoma	0/25 (23) NC	0/25 (25) NC	0/25 (24) NC	0/25 (24) NC	0/25 (24) NC	2/10 (7) 0.0483*
Site, Injection	Hemangiosarcoma	0/25 (23) 0.2500	0/25 (25) NC	0/25 (24) NC	1/25 (24) 0.5106	0/25 (24) NC	0 (0) NC
Skin	Hemangiosarcoma	0/25 (23) 0.5000	0/25 (25) NC	1/25 (24) 0.5106	0/25 (24) NC	0/25 (24) NC	0/10 (6) NC
Spleen	Hemangiosarcoma	1/25 (24) 0.2949	1/25 (25) 0.7653	0/25 (24) 1.0000	2/25 (24) 0.5000	0/25 (24) 1.0000	7/10 (9) 0.0001*
Stomach	Squamous Cell Carcinoma	1/25 (24) 1.0000	0/24 (24) 1.0000	0/25 (24) 1.0000	0/24 (23) 1.0000	0/25 (24) 1.0000	0/10 (6) 1.0000
Testis	Hemangiosarcoma	0/25 (23) NC	0/25 (25) NC	0/25 (24) NC	0/25 (24) NC	1/25 (24) 0.5106	0/10 (6) NC
Whole Body	Hemangiosarcoma	1/25 (24) 0.1890	2/25 (25) 0.5156	2/25 (24) 0.5000	3/25 (24) 0.3043	1/25 (24) 0.7553	7/10 (9) 0.0001*

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable

* = statistically significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Female Mice Using Poly-3 Test.

Organ Name	Tumor Name	0 mg Vehicle Cont (N=25) P - Trend	10 mg Low (N=25) P - VC vs. L	20 mg Med (N=25) P - VC vs. M	60 mg High(N=25) P - VC vs. H	10 mg ISIS 722059(N=25) P - VC vs. IS	500 mg Positive Cont (N=10) P - VC vs. PC
Bone, Femur	Hemangiosarcoma	0/25 (25) 0.2551	0/25 (23) NC	0/25 (25) NC	1/25 (25) 0.5000	0/25 (24) NC	0/10 (7) NC
Gland, Adrenal	Cortical Adenoma	0/25 (25) NC	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	1/10 (8) 0.2424
Gland, Harderian	Adenoma	1/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	1/10 (7) 0.3952
Lung	Bronchioloalveolar Adenoma	2/25 (25) 0.9849	1/25 (23) 0.8670	0/25 (25) 1.0000	0/25 (25) 1.0000	1/25 (24) 0.8752	8/10 (9) <0.0001*
	Bronchioloalveolar Carcinoma	0/25 (25) NC	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	10/10 (10) <0.0001*
Skin	Lymphangiosarcoma	0/25 (25) 0.5102	0/25 (23) NC	1/25 (25) 0.5000	0/25 (25) NC	0/25 (24) NC	0/10 (7) NC
	Squamous Cell Papilloma	0/25 (25) NC	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	1/10 (7) 0.2188
Spleen	Hemangiosarcoma	0/25 (25) 0.7475	1/25 (24) 0.4898	0/25 (25) NC	0/25 (25) NC	0/25 (24) NC	9/10 (10) <0.0001*
Stomach	Squamous Cell Carcinoma	0/25 (25) NC	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	1/25 (24) 0.4898	0/10 (7) NC
Thymus	Thymoma, Benign	0/25 (25) 0.5155	0/24 (22) NC	1/25 (25) 0.5000	0/25 (25) NC	0/25 (24) NC	0/10 (7) NC
Urinary Bladder	Hemangiosarcoma	1/25 (25) 1.0000	0/24 (22) 1.0000	0/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/10 (7) 1.0000
Uterus/Cervix	Hemangiosarcoma	0/25 (25) NC	0/25 (23) NC	0/25 (25) NC	0/25 (25) NC	0/24 (23) NC	2/10 (8) 0.0530
Whole Body	Hemangiosarcoma	1/25 (25) 0.5995	1/25 (24) 0.7449	0/25 (25) 1.0000	1/25 (25) 0.7551	0/25 (24) 1.0000	9/10 (10) <0.0001*

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable

* = statistically significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

Figure 1A: Kaplan-Meier Survival Curves for Male Mice

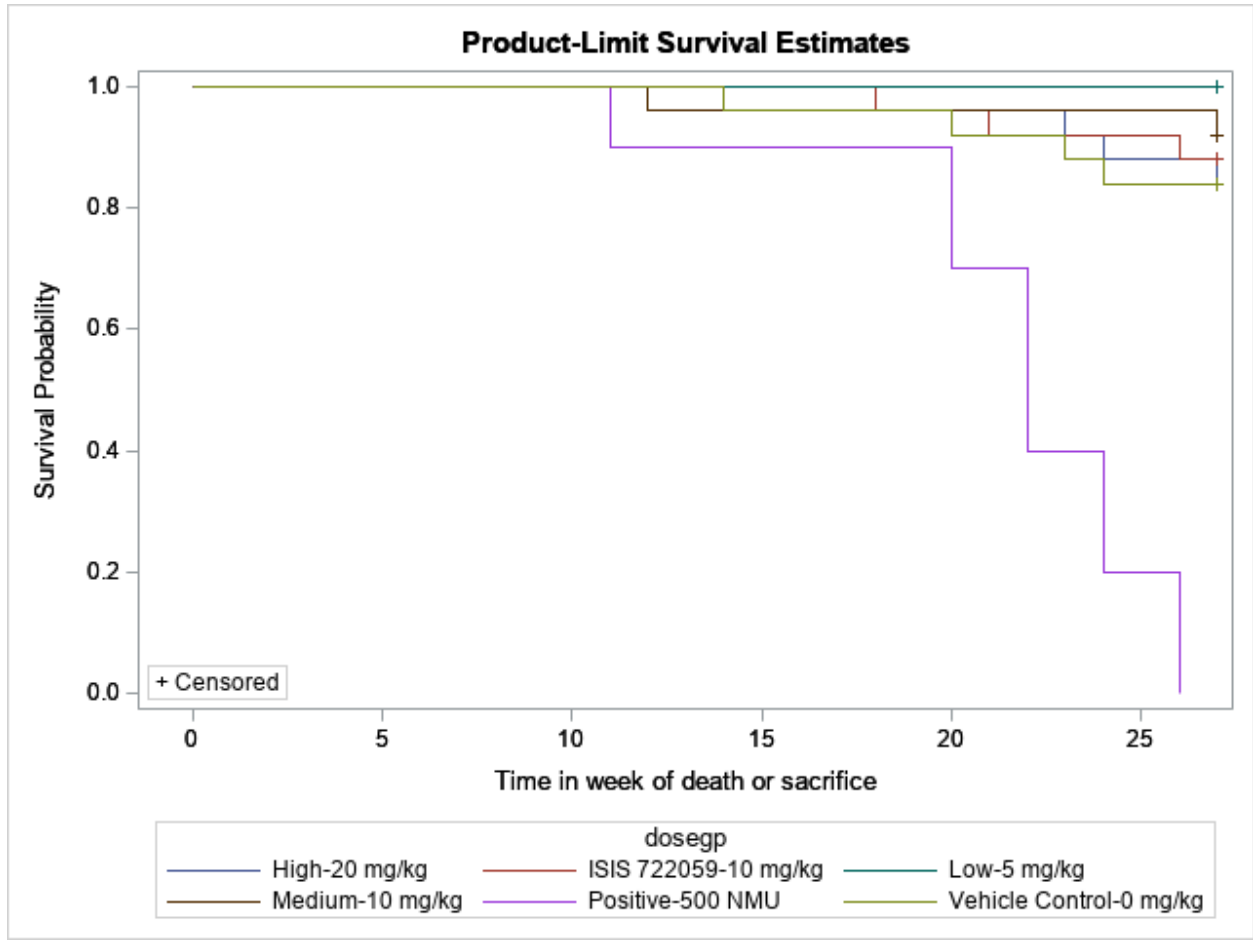
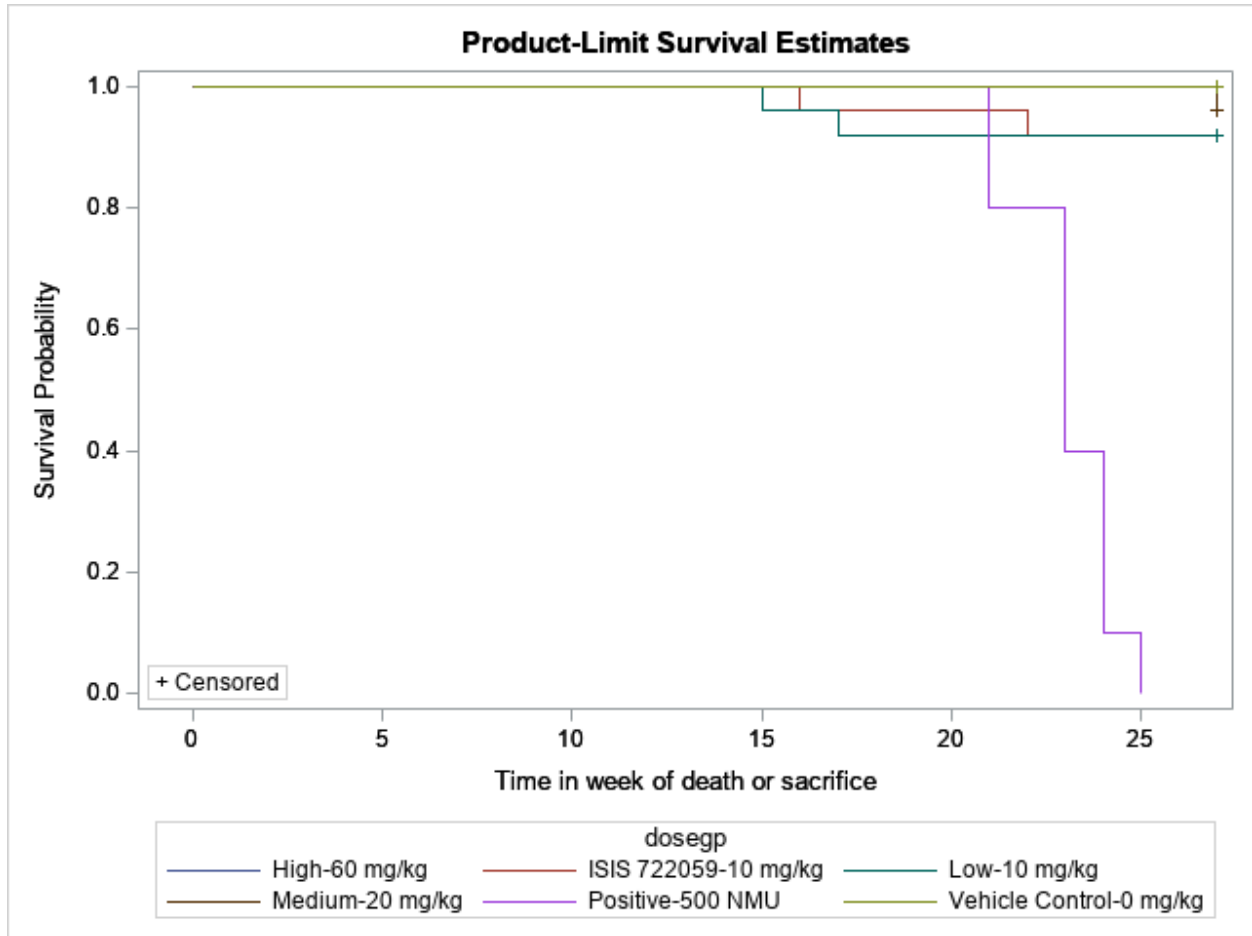


Figure 1B: Kaplan-Meier Survival Curves for Female Mice



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