

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**21172Orig1s000**

**NON-CLINICAL REVIEW(S)**

## Tertiary Pharmacology Review

**By:** Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**NDA:** 211172

**Submission date:** November 6, 2017

**Drug:** inotersen

**Applicant:** Ionis Pharmaceuticals, Inc.

**Indication:** Treatment of adults with hereditary transthyretin-mediated amyloidosis with polyneuropathy

**Reviewing Division:** Division of Neurology Products

### **Discussion:**

Inotersen is a transthyretin-directed antisense oligonucleotide.

The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. Both found the information sufficient to support approval.

The carcinogenic potential of inotersen (and a species-specific surrogate) was assessed in a 6-month transgenic rasH2 mouse study. No drug-related tumors were noted. A 2-year carcinogenicity study in rats is recommended as a post-marketing requirement.

**Conclusions:** I agree that this NDA can be approved from a pharm/tox perspective and that the rat carcinogenicity study can be a post-marketing requirement.

Comments on labeling were provided separately.

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/s/  
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PAUL C BROWN  
10/04/2018

**MEMORANDUM**

**DEPARTMENT OF HEALTH & HUMAN SERVICES  
Public Health Service  
Food and Drug Administration**

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**Division of Neurology Products (HFD-120)  
Center for Drug Evaluation and Research**

Date: October 3, 2018  
From: Lois M. Freed, Ph.D.  
Supervisory Pharmacologist

Subject: NDA 211-172 (inotersen)

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NDA 211-172 was submitted by Ionis Pharmaceuticals to support approval of inotersen for the treatment of adults with hereditary transthyretin-mediated amyloidosis with polyneuropathy. The application was received on November 6, 2017. The sponsor was notified on January 4, 2018, that the NDA was filed for Priority review, with no potential filing review issues identified. On April 23, 2018, the sponsor submitted a major amendment, resulting in an extension of the goal date to October 6, 2018.

To support clinical development and an NDA for inotersen, the sponsor conducted a full battery of nonclinical studies, except for the lack of a 2-year carcinogenicity study in rat. The nonclinical studies were reviewed by Dr. Hawver (Pharmacology/Toxicology NDA Review and Evaluation, NDA 211172, David B. Hawver, Ph.D., September 19, 2018). Based on his review, Dr. Hawver has concluded that the nonclinical data are adequate to support approval, with a post-marketing requirement (PMR) for a 2-year carcinogenicity study in rat.

Selected nonclinical data are summarized briefly below; a detailed description and evaluation of all the nonclinical studies are provided in Dr. Hawver's review.

**Summary and Conclusions**

**Pharmacology**

Inotersen (ISIS 420915) is a 20-nucleotide 2'-MOE antisense oligonucleotide that binds wild type and mutant transthyretin (TTR) mRNA, resulting in a reduction in production of the TTR protein. Inotersen is pharmacologically active in cynomolgus monkey but not rodent or rabbit. In vitro (human and monkey hepatocytes) and in vivo (monkey and transgenic mouse expressing the human TTR Ile84Ser mutation) studies documented decreases in TTR mRNA and protein levels following repeat-dose subcutaneous (SC) administration of inotersen.

Because TTR is involved with stabilization (binds and prevents clearance) of retinal binding protein (RBP) and transport of thyroxine, effects on RBP and thyroid hormone levels were assessed in selected nonclinical studies.

No adverse effects of inotersen were observed on a core battery of safety pharmacology parameters in CNS in CD-1 mouse (CNS) or cynomolgus monkey (cardiovascular and respiratory) following acute SC doses of up to 300 or 40 mg/kg, respectively.

### **PK/TK/ADME**

The majority of the PK/TK/ADME data were collected as part of the pivotal toxicity studies in CD-1 mouse, Sprague-Dawley rat, and cynomolgus monkey. An in vitro study to evaluate plasma protein binding in human and cynomolgus monkey indicated >94% binding at 150 µg/mL in both species.

### **Toxicology**

Pivotal toxicity studies were conducted in CD-1 mouse (13-week [30-day interim] + 13-week recovery, 26-week + 13-week recovery), Sprague-Dawley rat (26-week), and cynomolgus monkey (13-week + 13-week recovery, 39-week [26-week interim] + 26-week recovery). In all species, inotersen was administered by subcutaneous injection.

Mouse: in the 13-week study, inotersen was administered at doses of 0, 4, 12, 40, and 100 mg/kg on Days 1, 3, 5, and 7 and weekly thereafter; a separate group received 40 mg/kg of a species-specific surrogate (ISIS 401724) according to the same schedule. Animals were sacrificed after 30 days (6/sex/group) or 13 weeks of dosing (10/sex/group) or after a 13-week recovery period (6/sex at 0, 12, and 100 mg/kg). The most notable finding was an ~2-fold increase in ALT in HDM, although hepatocellular degeneration or necrosis was not detected. Pharmacological activity of the surrogate was confirmed by a 70% decrease in liver TTR mRNA at the end of the 13-week dosing period; no unique toxicities were observed with the surrogate. Basophilic granules, indicating accumulation of ASO, were observed in multiple tissues (e.g., kidney, liver, and lymph node) in main-study and, to a lesser extent, in recovery animals. Plasma exposure was not assessed; kidney and liver concentrations indicated dose-related uptake of inotersen.

In the 26-week study, inotersen was administered at doses of 0, 3, 10, 40, and 80 mg/kg on Days 1, 3, 5, and 7 and weekly thereafter. Animals were sacrificed at the end of the 26-week dosing period (12/sex/group) or the 13-week recovery period (6/sex at 0, 40 and 80 mg/kg). Increases in ALT and AST were observed at 40 (3-fold in males) and 80 (2.9- and 1.5-fold in males and females, respectively) mg/kg; hepatocellular degeneration or necrosis was not detected. Basophilic granules, indicating accumulation of ASO, were observed in multiple tissues (e.g., kidney, liver, lymph node, choroid plexus of brain) in main-study and, to a lesser extent, in recovery animals. No effects were observed on cytokine/chemokine parameters. Plasma exposure was not assessed; kidney and liver concentrations indicated a less than dose-proportional uptake of inotersen.

Rat: in the 26-week study (10/sex/group), inotersen was administered at doses of 0, 5, 15, and 40 mg/kg on Days 1, 5, 10, and 14 and weekly thereafter; a separate group received a species-specific surrogate (ISIS 594799) at 15 mg/kg according to the same schedule. One HDM was sacrificed moribund on Day 161; no cause of death was identified. In survivors, clinical signs of poor condition were observed primarily at the mid (inotersen and surrogate) and high doses; reduced body weight gain and body weight (relative to controls), associated with reduced food consumption, were observed at all doses of inotersen and with the surrogate. Clinical pathology

findings included small decreases in platelets (27-19 and 29-31% in males and females at the mid and high dose of inotersen, respectively) and urinalysis findings. Effects on urinalysis parameters consisted of decreases in creatinine (16-hr) at the high dose of inotersen and the surrogate (40-46% at Week 26), increases in protein (16-hr) at the mid and high dose of inotersen (2- and 5.2-fold at Week 26, respectively) and with the surrogate (1.7-fold at Week 26), and increases in the protein:creatinine ratio at all sampling times at the mid and high dose of inotersen (2.2-2.8 and 2.4-10.7 fold, respectively) and with the surrogate (1.7-2.7 fold).

Microscopic changes were evident at all doses of inotersen and with the surrogate. Vacuolated/granular macrophages (reflecting ASO accumulation) and mononuclear cell infiltration (reflecting pro-inflammatory effects) were detected in multiple organs, including kidney, liver, heart valves, and injection site. In liver, individual hepatocyte necrosis was detected in 2 MDF and 1 HDF receiving inotersen. Kidney findings that correlated with the changes in urinalysis parameters consisted of increased cellularity of the glomeruli and increased glomerular matrix in males and females at the mid and high dose of inotersen and with the surrogate (summarized in the table below; doses in mg/kg).

FINDING	INOTERSEN								ISIS 594799		
	MALE				FEMALE				M	F	
	0	5	15	40	0	5	15	40	15		
↑ cellularity, glomeruli											
minimal	0/10	0/10	0/10	2/10	0/10	0/10	2/10	2/10	0/10	2/10	
mild	0/10	0/10	2/10	1/10	0/10	0/10	1/10	0/10	1/10	0/10	
<b>Total</b>	<b>0/10</b>	<b>0/10</b>	<b>2/10</b>	<b>3/10</b>	<b>0/10</b>	<b>0/10</b>	<b>3/10</b>	<b>2/10</b>	<b>1/10</b>	<b>2/10</b>	
↑ glomerular matrix											
minimal	0/10	0/10	1/10	2/10	0/10	0/10	2/10	1/10	2/10	2/10	
mild	0/10	0/10	2/10	3/10	0/10	0/10	2/10	1/10	1/10	0/10	
moderate	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	
<b>Total</b>	<b>0/10</b>	<b>0/10</b>	<b>3/10</b>	<b>5/10</b>	<b>0/10</b>	<b>0/10</b>	<b>5/10</b>	<b>2/10</b>	<b>3/10</b>	<b>2/10</b>	

Increases in trabecular bone (femur, sternum) were observed in males and females at all doses of inotersen and the surrogate. Microscopic changes in female reproductive organs consisted of interstitial cell hypertrophy/hyperplasia of the ovary (all doses of inotersen and the surrogate) and increased endometrial thickness of the uterus (mid and high doses of inotersen and the surrogate).

Plasma (only for MDM and HDM) and tissue (kidney, liver) toxicokinetic analysis indicated dose-proportional increases in plasma inotersen exposure and less than dose-proportional increases in tissue concentrations. Pharmacological activity of the surrogate was demonstrated by decreases in liver TTR mRNA (34%); effects on RBP4 were not assessed.

Monkey: in the 13-week study, inotersen was administered at doses of 0, 4, 8, 12, and 40 mg/kg on Days 1, 3, 5, and 7 and weekly thereafter. Animals were sacrificed after 30 (2/sex/group) or 93 days (3/sex/group) of dosing or after a 13-week recovery period (2/sex for 0, 12, and 40 mg/kg). There were no drug-related deaths, clinical signs, or effects on body weight or food consumption. A small (1.2-fold) increase in APTT was observed at the high dose at 1-4 hours post dose on Day 1. Increases in complement split product Bb were observed at the mid and high doses, on Day 1 (~2 and 12-fold over baseline) and Day 93 (~2-7 fold at the high dose).

Microscopic changes consistent with ASO accumulation were observed in multiple tissues (including choroid plexus of brain). The primary toxicity was in kidney; proximal tubular degeneration/regeneration was detected at the high dose in males and females on Day 93. Plasma and tissue (kidney, liver) toxicokinetic analysis indicated greater than dose-proportional increases in plasma exposure but a less than dose-proportional increase in tissue concentrations. Pharmacological activity of inotersen was demonstrated by dose-related decreases in liver TTR mRNA levels (8-70%).

In the 39-week study, inotersen was administered at doses of 0, 3, 6, 10, and 20 mg/kg once weekly. Animals were sacrificed after 26 (3/sex/group) or 39 weeks (4/sex/group) of dosing or after a 26-week recovery period (2/sex for C and HD). Two males (10 and 20 mg/kg) were sacrificed prematurely (Day 74 or 151) because of “marked” thrombocytopenia, with associated anemia and hemorrhage in multiple organs. The HDM received a dosing holiday (Days 94-127) during which the platelet count increased; however, upon re-dosing, platelet count again decreased, accompanied by petechial hemorrhages (Day 147), and the animal was sacrificed on Day 151. Mean platelet count was not affected; however, both animals sacrificed moribund had platelet counts  $\leq 3 \times 10^3/\mu\text{L}$ . ALT was slightly increased in both males and females at the high dose (1.4-2.5 fold), and transient increases (at 4 hrs post dose on Day 1) in complement split product Bb were observed at 10 and 20 mg/kg. After 26 or 39 weeks of dosing, microscopic changes consistent with ASO accumulation (basophilic granules) were observed primarily in lymph nodes (histiocytes), kidney (proximal tubular epithelial cells), and liver (Kupffer cells); there was no evidence of degeneration or necrosis. At the 26-week sacrifice, microscopic evidence of vasculitis was observed in two females (6 and 10 mg/kg); the findings were described by the study pathologist as follows:

“...distinctly different perivascular lesion of infiltration of neutrophils, macrophages, and lymphocytes, sometimes 20 or more cell layers thick, surrounding blood vessels in a variety of organs, including the pancreas, gallbladder, intestine, and reproductive tract. The lesions were characterized by infiltration of minimal to moderate numbers of the mixed inflammatory cell population into the tunica adventitia and outer segments of the tunica media. In some cases, the tunica media was thickened relative to control animals. At no time were inflammatory cells present in the inner layers of the tunica media nor was there any necrosis or hemorrhage in the tunica media or tunica intima. The endothelium was unaffected and histologically unremarkable.”

Because of the lack of similar findings at the high dose, the study pathologist concluded that “...the lesion is most likely idiopathic in nature and associated with inflammatory process not solely related to administration...” of drug. Similar findings were observed in two mid-dose animals (male and female) at the end of the 39-week dosing period; the study pathologist concluded that the lesions were “...not due directly to administration...” of drug. The study pathologist also concluded that the thrombocytopenia resulting in the moribund sacrifice in the two affected animals “...was not considered to be directly related to the administration [of drug] and is best regarded as an idiosyncratic response.”

At the end of the recovery period, the only notable finding was the presence of basophilic granules in lymph node and kidney in HDM.

Plasma and tissue (kidney, liver) toxicokinetic analysis indicated dose-proportional increases in inotersen exposure (except at the high dose) and a less than dose-proportional increase in tissue concentrations. Pharmacological activity was documented by decreases in liver TTR mRNA at 26 (20, 30, and 79% at 6, 10, and 20 mg/kg, respectively) and 39 (10, 30, 49, and 60% at 3, 6, 10, and 20 mg/kg, respectively) weeks and in plasma TTR (20, 40, and 60% at 6, 10, and 20 mg/kg, respectively, at 26 and 39 weeks) and RBP4 (25 and 60% at 10 and 20 mg/kg, respectively, at 26 and 39 weeks).

### **Reproductive and Developmental Toxicology**

A full battery of reproductive and development studies was conducted in CD-1 mouse and New Zealand White rabbit. Plasma inotersen, RBP, and thyroid hormone levels were not quantitated in any of the studies.

In the combined fertility and embryofetal development study in mouse, inotersen (0, 3, 15, or 25 mg/kg) or a species-specific surrogate (15 mg/kg) was administered SC at doses every other day, prior to and during the mating period and continuing in females through gestation day (GD) 15. No adverse effects on fertility or embryofetal development were observed. Assessed on GD 17, maternal liver concentrations were 52.5, 244, and 384  $\mu\text{g/g}$  at the low, mid, and high doses, respectively; placental concentrations were 3.78, 5.47, and 14.77  $\mu\text{g/g}$ , respectively; concentrations were <LOQ in fetal liver at all doses. Pharmacological activity of the surrogate was demonstrated by a decrease in liver TTR mRNA (60%).

In a preliminary embryofetal development study in rabbit, inotersen (0, 5, 15, or 30 mg/kg SC) administered every other day throughout gestation (GDs 6-18) resulted in decreases in body weight (or body weight loss) in dams (primarily mid and high doses), premature delivery (mid dose), and reduced fetal body weight (all doses). (The sponsor attributed the premature delivery to inotersen because, according to the sponsor, premature delivery or abortion, associated with reduced food consumption and body weight, were observed in previous studies in rabbit at the same high dose.) In the pivotal embryofetal development study in rabbit, SC administration of inotersen (0, 2.5, 5, or 15 mg/kg) every other day throughout gestation (GDs 6-18), the only drug-related effect was a decrease in fetal body weight (~13%) at the high dose. In high-dose dams, body weight was not affected during early gestation (<GD 15), but body weight loss was reported on GDs 15-18 (19.6 gm), GDs 18-20 (27.3 gm), and GDs 20-23 (2.3 gm). Overall body weight gain in dams was similar among groups. When assayed 2 or 10 days after the last dose, fetal liver levels of inotersen were <LOQ at all doses; inotersen was detectable in placenta (17.9  $\mu\text{g/g}$ ) only at the high dose, 2 days after the last dose (maternal liver concentration at the same sampling time was 345  $\mu\text{g/g}$  at the high dose).

In the pre- and postnatal development study in mouse, inotersen (0, 2.9, 11.4, or 22.9 mg/kg) or a species-specific surrogate (11.4 mg/kg) was administered SC every other day during gestation (GDs 6-17) and weekly (approximately 0, 10, 40, and 80 mg/kg) during lactation (lactation days [LD] 0-20). No adverse developmental effects were observed in the offspring. Inotersen was detected in milk at all doses (0.0121-0.701  $\mu\text{g/g}$ , using a “non-validated method”) on LD 13. Pharmacological activity of the surrogate was demonstrated by a decrease (60%) in liver TTR mRNA.

### **Genetic Toxicology**

Inotersen was negative in an adequate standard battery of in vitro (Ames, chromosomal aberration in Chinese hamster lung cells) and in vivo (CD-1 mouse micronucleus at single doses up to 2000 mg/kg SC) assays.

### **Carcinogenicity**

In transgenic (TgRasH2) mice, subcutaneous administration of inotersen (0, 10, 30, or 80 mg/kg) or a species-specific surrogate (ISIS 401724; 30 mg/kg) weekly for 26 weeks resulted in no drug-related increase in tumors. Pharmacological activity of the surrogate was demonstrated by a decrease (65-70%) in liver TTR mRNA after 26 weeks of dosing.

A 2-year carcinogenicity study in rat has not been submitted. A study protocol was submitted on October 27, 2015, for Special Protocol Assessment and was reviewed by the division and the Executive CAC committee (Minutes dated December 2, 2015). The sponsor has been informed that there will be a PMR for this study should the NDA be approved (Memorandum of Late Cycle Meeting Minutes, August 29, 2018).

### **Conclusion and Recommendation**

The nonclinical data submitted for inotersen are adequate to support approval of the NDA, with a PMR for a 2-year carcinogenicity study in rat.

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LOIS M FREED  
10/03/2018

**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: 211172  
Supporting document: 1  
Applicant's letter date: November 6, 2017  
CDER stamp date: November 6, 2017  
Product: Tegsedi (Inotersen)  
Indication: Treatment of hereditary transthyretin amyloidosis  
Applicant: Ionis Pharmaceuticals, Inc.  
Review Division: Neurology Products  
Reviewer: David B. Hawver, Ph.D.  
Supervisor: Lois M. Freed, Ph.D.  
Division Director: Billy Dunn, M.D.  
Project Manager: Fannie Choy, R.Ph.

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 211172 are owned by Ionis Pharmaceuticals, Inc. or are data for which Ionis Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 211172 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 are for descriptive purposes only and are not relied upon for approval of NDA 211172.

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# 1 Executive Summary

## 1.1 Introduction

Inotersen is a 20-base 2'-methoxyethyl-modified phosphorothioate antisense oligonucleotide (ASO) designed to bind specifically to a sequence within the 3'-untranslated region of the human transthyretin (hTTR) mRNA, resulting in degradation of the mRNA by RNase H1 and reduced synthesis of TTR protein in liver cells. The proposed indication is "for the treatment of adult patients with hereditary TTR amyloidosis with polyneuropathy (hATTR-PN) (b) (4)

(b) (4) " Patients with hATTR-PN have mutations in the hTTR gene that destabilize the protein's normal tetrameric conformation and allow the TTR protein to misfold into amyloidogenic conformations that accumulate in the peripheral nervous system (and multiple other organs), causing damage. The proposed therapeutic dosing regimen is subcutaneous injection of 284 mg inotersen (300 mg inotersen sodium salt) (b) (4) once weekly (b) (4)

## 1.2 Brief Discussion of Nonclinical Findings

In vitro studies in HepG2 cells and isolated hepatocytes and in vivo studies in transgenic hTTR I184S mice and cynomolgus monkey demonstrated that inotersen can selectively reduce levels of the intended target, hTTR mRNA, in liver cells. In the monkey studies, reduction of hTTR mRNA levels was associated with substantial (~60-80%) reductions in plasma levels of TTR protein. Plasma levels of RBP4 protein (which transports Vitamin A from the liver to peripheral tissues in a complex with TTR) were also reduced, but no adverse effects were associated with the reductions in TTR or RBP4.

In the chronic toxicity studies of inotersen in mouse, rat, and monkey, accumulation of basophilic granules (consistent with drug-related material) was observed in liver, kidney, lymph nodes, injection sites, and other organs, along with associated inflammatory responses typically seen with administration of ASOs. In the 26-week rat study, adverse kidney toxicity was observed in the two highest dose groups, characterized by increases in urine protein/creatinine and albumin/creatinine ratios, glomerular cellularity, and glomerular matrix. Degeneration/regeneration of proximal tubular epithelial cells was observed in the 13-week monkey study. In the 39-week monkey study, severe thrombocytopenia associated with petechiae, bruising, and internal hemorrhages was observed within the first 11-14 weeks of dosing in two animals (one in each of the two highest dose groups), necessitating early euthanasia. In the same study, five other animals across the three lowest dose groups showed perivascular mixed cell infiltration in multiple organs, associated with increases in anti-drug antibody, CRP, IL-6, MIP-1 $\beta$ , TNF $\alpha$ , and serum IgG and IgM; and (in 4/5 animals) with moderate reductions in platelet counts (49-70%, compared to baseline). The cause of the thrombocytopenia observed in monkeys is unknown, but similar effects have been observed in humans with inotersen and other oligonucleotide therapies (see Clinical Pharmacology Review of NDA 211172 dated June 25, 2018, and Chi, Gatti, and Papoian, 2017, Drug Discovery Today 22(5):823-833).

Safety margins based on AUCs at the no observed adverse effect levels (NOAELs) for severe thrombocytopenia in monkeys and kidney toxicity in rats were  $\leq$  ~2-fold.

Key findings from a standard battery of reproductive and developmental toxicity studies in mouse and rabbit included premature delivery and reductions in maternal and fetal body weights in the high dose group in the rabbit embryofetal development study (NOAEL = 17.5 mg/kg/week).

### 1.3 Recommendations

#### 1.3.1 Approvability

The nonclinical data submitted adequately support the approval of inotersen for the treatment of adult patients with hereditary transthyretin amyloidosis with polyneuropathy.

#### 1.3.2 Additional Nonclinical Recommendations

A two-year carcinogenicity study of inotersen in rat should be conducted as a post-marketing requirement.

#### 1.3.3 Labeling

The sponsor's proposed labeling for the nonclinical sections should be revised as specified below:

##### 8.1 Pregnancy

###### Risk Summary

(b) (4)

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

###### Data

###### *Animal Data*

(b) (4)

(b) (4)

[Redacted] (b) (4)

8.2 Lactation  
Risk Summary

[Redacted] (b) (4)

There is no information regarding the presence of TRADENAME in human milk, the effects on the breastfed infant, or the effects on milk production. The development and health benefits of breastfeeding should be considered along with the mother's clinical need for TRADENAME and any potential adverse effects on the breastfed infant from TRADENAME or from the underlying maternal condition.

[Redacted] (b) (4)

[Redacted] (b) (4)

12 Clinical Pharmacology  
12.1 Mechanism of Action

TRADENAME is a [Redacted] (b) (4) antisense oligonucleotide [Redacted] (b) (4)

[Redacted] (b) (4)

[Redacted] (b) (4)

.....  
13 Nonclinical Toxicology  
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

[Redacted] (b) (4)

[Redacted] (b) (4)

## 2 Drug Information

### 2.1 Drug

#### CAS Registry Number

1432726-13-0

#### Generic Name

Inotersen (b) (4) Injection

#### Proprietary Name

Tegsedi

#### Code Name

ISIS 420915

#### Chemical Name

2'-O-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-*P*-thioguanilyl-(3'-O→5'-O)-2'-deoxy-*P*-thioguanilyl-(3'-O→5'-O)-2'-deoxy-*P*-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-*P*-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-*P*-thioadenilyl-(3'-O→5'-O)-2'-deoxy-5-methyl-*P*-thiocytidylyl-(3'-O→5'-O)-2'-deoxy-*P*-thioadenilyl-(3'-O→5'-O)-2'-deoxy-*P*-thiothymidylyl-(3'-O→5'-O)-2'-deoxy-*P*-thioguanilyl-(3'-O→5'-O)-2'-deoxy-*P*-thioadenilyl-(3'-O→5'-O)-2'-deoxy-*P*-thioadenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-*P*-thioadenilyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'-O→5'-O)-2'-O-(2-methoxyethyl)-5-methylcytidine, nonadeca sodium salt.

#### Molecular Formula

C<sub>230</sub>H<sub>299</sub>N<sub>69</sub>O<sub>121</sub>P<sub>19</sub>S<sub>19</sub>Na<sub>19</sub>

#### Molecular Weight

7600 (b) (4) Da (nonadecasodium salt)

#### Biochemical Description

5'-MeUMeCMeUMeUGGTTA<sup>Me</sup>CATGAAA<sup>Me</sup>UMeC<sup>Me</sup>C<sup>Me</sup>C<sup>Me</sup>C-3'

Inotersen sodium is comprised of 20 nucleotides connected via 19 phosphorothioate linkages that are fully ionized as the sodium salt. Underlined letters are 2'-O-(2-methoxyethyl)ribonucleotides; non-underlined letters are 2'-deoxyribonucleotides; all pyrimidines are 5-methylated; all linkages are phosphorothioates.

The absolute configuration of each 2-deoxy-D-ribose unit is (1*R*, 3*S*, 4*R*). The absolute configuration of each 2-O-(2-methoxyethyl)-D-ribose unit is (1*R*, 2*R*, 3*R*, 4*R*). The absolute configuration at each phosphorus atom is undefined; therefore, inotersen is a mixture of 2<sup>19</sup> diastereoisomers.

Pharmacologic Class

Inotersen is a 2'-O-(2-methoxyethyl) antisense oligonucleotide inhibitor of mutant and wild-type human transthyretin production.

**2.2 Relevant INDs, NDAs, BLAs, and DMFs**

IND 113968 Inotersen for treatment of Familial Amyloid Polyneuropathy

**2.3 Drug Formulation**

Inotersen sodium is formulated as a sterile solution for subcutaneous injection, containing 189 mg/mL drug substance in a pre-filled syringe designed to deliver 1.5 mL (284 mg inotersen free acid in 1.5 mL; 300 mg inotersen sodium). The quantities of inotersen sodium and other components of the solution are listed in the sponsor's Table 1 below:

**Table 1 Composition of Inotersen Solution for Injection, 284 mg/1.5 mL (189 mg/mL)**

Component	Quantity (mg/mL)	Function	Reference to Standard
Inotersen sodium <sup>1</sup>	(b) (4)	Active substance	Professed specification <sup>4</sup>
Sodium hydroxide	(b) (4)	pH modifier	USP, Ph.Eur.
Hydrochloric acid	(b) (4)	pH modifier	USP, Ph.Eur.
Water for Injection	(b) (4)		USP, Ph.Eur.

Note

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

<sup>4</sup> Details of the specification of the active ingredient are provided in [CTD Section 3.2.S.4.1](#)

(section 2.3.P.1 of *Quality Overall Summary*)

**2.4 Comments on Novel Excipients**

No novel excipients are present in the drug product.

**2.5 Comments on Impurities/Degradants of Concern**

No impurities or degradants of concern have been identified. Specification limits were proposed for (b) (4) based on the absence of increased toxicity in mice administered a formulation of ISIS 420915 with these impurities added, compared to mice given ISIS 420915 alone, once weekly for 13 weeks (Study 420915-AS15). However, the number of animals used in that study (N=6/sex/group) was insufficient to provide adequate qualification of these impurities. Following discussion with FDA experts, it was concluded that the proposed specification limits for these impurities are not likely to present a safety concern, and, therefore, are acceptable.

## 2.6 Proposed Clinical Population and Dosing Regimen

Inotersen sodium is to be administered to adult patients with hereditary transthyretin amyloidosis with polyneuropathy via subcutaneous injection of 300 mg in 1.5 mL solution (284 mg free base) once weekly, [REDACTED] (b) (4)

## 2.7 Regulatory Background

A Pre-IND meeting was held on March 8, 2012, to discuss IND 113968 ISIS 420915 for the treatment of familial amyloid polyneuropathy. As reflected in the meeting minutes dated April 2, 2012, the sponsor was advised to provide justification for not including a group administered a pharmacologically active murine surrogate ASO in the pivotal 26-week toxicity study in mouse; to use a murine surrogate in the planned pre- and postnatal development study and carcinogenicity study in mouse; to conduct a standard battery of genotoxicity studies of ISIS 420915; and that final decisions on whether carcinogenicity studies would be needed in two species and whether they may submitted post approval would depend on review of all relevant nonclinical and clinical data and further justification that may be provided by the sponsor.

In the May Proceed Letter dated December 6, 2012, the sponsor was advised to revise the Clinical Investigator's Brochure "to indicate that the substantial reductions (62-99%) in platelets observed in individual monkeys at 6, 10, and 20 mg/kg/week, and the related hemorrhages in several organs that necessitated early sacrifice in two animals, were clearly drug-related;" to "include a discussion of the risk of thrombocytopenia, based on these findings and on published reports of clinical trials suggesting that thrombocytopenia '...appears to be a class effect of oligonucleotide phosphorothioates';" and to provide a table summarizing the safety margins between the NOAELs in the pivotal nonclinical studies and the maximum recommended human dose.

In the Minutes of the Executive Carcinogenicity Assessment Committee Meeting held on December 1, 2015, the Committee recommended doses of 0 (saline), 10, 30, and 100 mg/kg/week SC ISIS 401724 (the mouse surrogate of inotersen) for the 6-month carcinogenicity study in Tg.rasH2 mouse. The Committee concurred [REDACTED] (b) (4) [REDACTED] for the 2-year carcinogenicity study in rat.

On June 6, 2016, the sponsor was informed via email that specific revisions needed to be made in the informed consent forms and the Clinical Investigator's Brochure to more accurately describe the drug-related platelet reductions observed in monkeys that were associated with internal hemorrhage and early sacrifice in two animals.

As reflected in the written responses dated April 5, 2017, the sponsor was informed that the 2-year carcinogenicity study in rat may be conducted post approval, that nonclinical studies completed prior to December 2016 would not need to be submitted in SEND format, and that electronic datasets would be needed for each carcinogenicity study.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

##### Pharmacology

*In Vitro* Characterization of Human Transthyretin ASO ISIS 420915  
(Study 420915-NP01)

*In Vivo* Characterization of ISIS 420915  
(Study 420915-NP02)

A 12-Week Pharmacology Study of ISIS 420915 Administered by Subcutaneous Injection in Cynomolgus Monkeys  
(Study 420915-NP03)

##### Safety Pharmacology

Evaluation of the Effects of ISIS 420915 on Neurobehavior (Irwin's Test) and Body Temperature in Mice Following a Single Subcutaneous Administration  
(Study 420915-AS03)

A Cardiovascular and Respiratory Safety Pharmacology Study of ISIS 420915 by Subcutaneous Administration in Telemetered Cynomolgus Monkeys  
(Study 420915-AS06)

Effect of ISIS 420915 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells  
(Study 420915-IS01)

##### Pharmacokinetics

ISIS 420915: Pharmacokinetic Study of ISIS 420915 in Mice  
(Study 420915-APK01)

[<sup>3</sup>H]-ISIS 420915: Pharmacokinetics, Distribution, Metabolism, Excretion and Mass Balance of Radioactivity in Rats Following a Single Subcutaneous Bolus Injection  
(Study 420915-APK02)

*In Vitro* Evaluation of the Extent of Plasma Protein Binding of ISIS 420915 in Human and Monkey Plasma by Ultrafiltration  
(Study 420915-IS04)

Identification and Profiling of ISIS 420915 and its Associated Metabolites by Ion-Pair HPLC Electro spray/Mass Spectrometry (IP-HPLC-ES/MS) in Selected Available Plasma, Tissue and Urine Samples from Mice, Monkeys, and Humans  
(Study 420915-IS09)

Assessment of ISIS 420915 As an Inhibitor or a Substrate of Human BCRP, P-gp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 and BSEP Mediated Transport

(Study 420915-IS06)

*In Vitro* Assessment of Cytochrome P450 Induction Potential of ISIS 420915 in Plated Cryopreserved Human Primary Hepatocytes  
(Study 420915-IS07)

*In Vitro* Assessment of Cytochrome P450 Inhibition Potential of ISIS 420915 in Cultured Cryopreserved Human Primary Hepatocytes  
(Study 420915-IS08)

Partial Validation of an HPLC Method for the Determination of ISIS 420915 in Mouse Tissue Homogenate  
(Study 420915-MV01)

Validation of a Hybridization ELISA Method for the Determination of ISIS 420915 in Monkey Plasma  
(Study 420915-MV02)

Validation of a HPLC Method for the Determination of ISIS 420915 in Cynomolgus Monkey Tissue Homogenate  
(Study 420915-MV03)

Partial Validation of an HPLC Method for the Determination of ISIS 420915 in Rabbit Tissue Homogenate  
(Study 420915-MV06)

Validation of an ELISA Method for the Detection of Anti-ISIS 420915 Antibodies in Monkey Serum  
(Study 420915-MV08)

Qualification of an ELISA Method for the Detection of Anti-ISIS 420915 in Mouse Serum  
(Study 420915-MV09)

Partial Validation of a Hybridization ELISA Method for the Determination of ISIS 420915 in Monkey Plasma  
(Study 420915-MV10)

Validation of a Hybridization ELISA Method for the Determination of ISIS 420915 in Mouse Plasma  
(Study 420915-MV12)

Validation of a Hybridization ELISA Method for the Quantitation of ISIS 420915 in Rat Plasma  
(Study 420915-MV14)

Validation of an HPLC Method for the Determination of ISIS 420915 in Rat Tissue  
(Study 420915-MV15)

Partial Validation of an HPLC Method for the Determination of ISIS 420915 in Mouse Tissue  
(Study 420915-MV17)

### **Repeat-Dose Toxicology**

A 13-Week Repeated Subcutaneous Dose Toxicity Study of ISIS 420915 and ISIS 401724 in Mice with a 4-Week Interim Necropsy and a 13-Week Recovery  
(Study 420915-AS01)

A 13-Week Repeat Dose Toxicity Study of ISIS 420915 and ISIS 401724 in CByB6F1-Tg(HRAS)2Jic Wild Type and Hemizygous Transgenic Mice  
(Study 420915-AS12P; draft report reviewed by David B. Hawver, Ph.D., under IND 113968, February 11, 2016)

26-Week Repeat-Dose Subcutaneous Toxicity Study of ISIS 420915 in CD-1 Mice with a 13-Week Recovery  
(Study 420915-AS07)

A 26-Week Repeat Dose Toxicity Study of ISIS 420915 and ISIS 594799 in Rats  
(Study 420915-AS11; draft report reviewed by David B. Hawver, Ph.D., under IND 113968, February 11, 2016)

A 13-Week Repeated Subcutaneous Dose Toxicity Study of ISIS 420915 in Cynomolgus Monkeys with a 4-Week Interim Necropsy and a 13-Week Recovery  
(Study 420915-AS02)

A 39-Week Repeat Subcutaneous Dose Toxicity Study of ISIS 420915 in Cynomolgus Monkeys with a 26-Week Interim Necropsy and a 26-Week Recovery  
(Study 420915-AS08)

### **Genetic Toxicology**

Evaluation of ISIS 420915 in the Bacterial Reverse Mutation Assay  
(Study 420915-IS02)

The Effect of ISIS 420915 on the *In Vitro* Induction of Chromosome Aberrations in Chinese Hamster Lung (CHL) Cells  
(Study 420915-IS03)

In Vivo Mammalian Micronucleus Assessment in Bone Marrow Erythrocytes Following Treatment with ISIS 420915 in ICR Mice  
(Study 420915-AS05)

**Carcinogenicity**

A 26-Week Subcutaneous Carcinogenicity Study of ISIS 420915 and ISIS 401724 in CByB6F1-Tg(HRAS)2Jic Hemizygous Transgenic Mice (Study 420915-AS12)

**Reproductive and Developmental Toxicology**

Study of Fertility and Embryo-Fetal Development in Mice with ISIS 420915 and ISIS 401724 Administered Subcutaneously (Study 420915-AS09)

Embryo-Fetal Development Study of ISIS 420915 by Subcutaneous Administration in Rabbits (Study 420915-AS10)

Pre and Postnatal Development of ISIS 420915 and ISIS 401724 via Subcutaneous Administration Including Maternal Function in CD-1 Mice (Study 420915-AS14)

**Other**

Evaluation of ISIS 420915 and ISIS 401724 in the Mouse Influenza Host Resistance Model: Viral Clearance, TDAR, and Biological Mediators (Study 420915-AS16)

Assessment of ISIS 420915 to Directly Activate Human Platelets (Study 420915-IS10)

A 13-Week Repeat Dose Toxicity and Impurity Qualification Study of GSK2998728 (ISIS 420915) in CD-1 Mice (Study 420915-AS15)

**3.2 Studies Not Reviewed**

None

**3.3 Previous Reviews Referenced**

Nonclinical Review of IND 113968, David B. Hawver, Ph.D., February 11, 2016

## 4 Pharmacology

### 4.1 Primary Pharmacology

#### ***In Vitro* Characterization of Human Transthyretin ASO ISIS 420915**

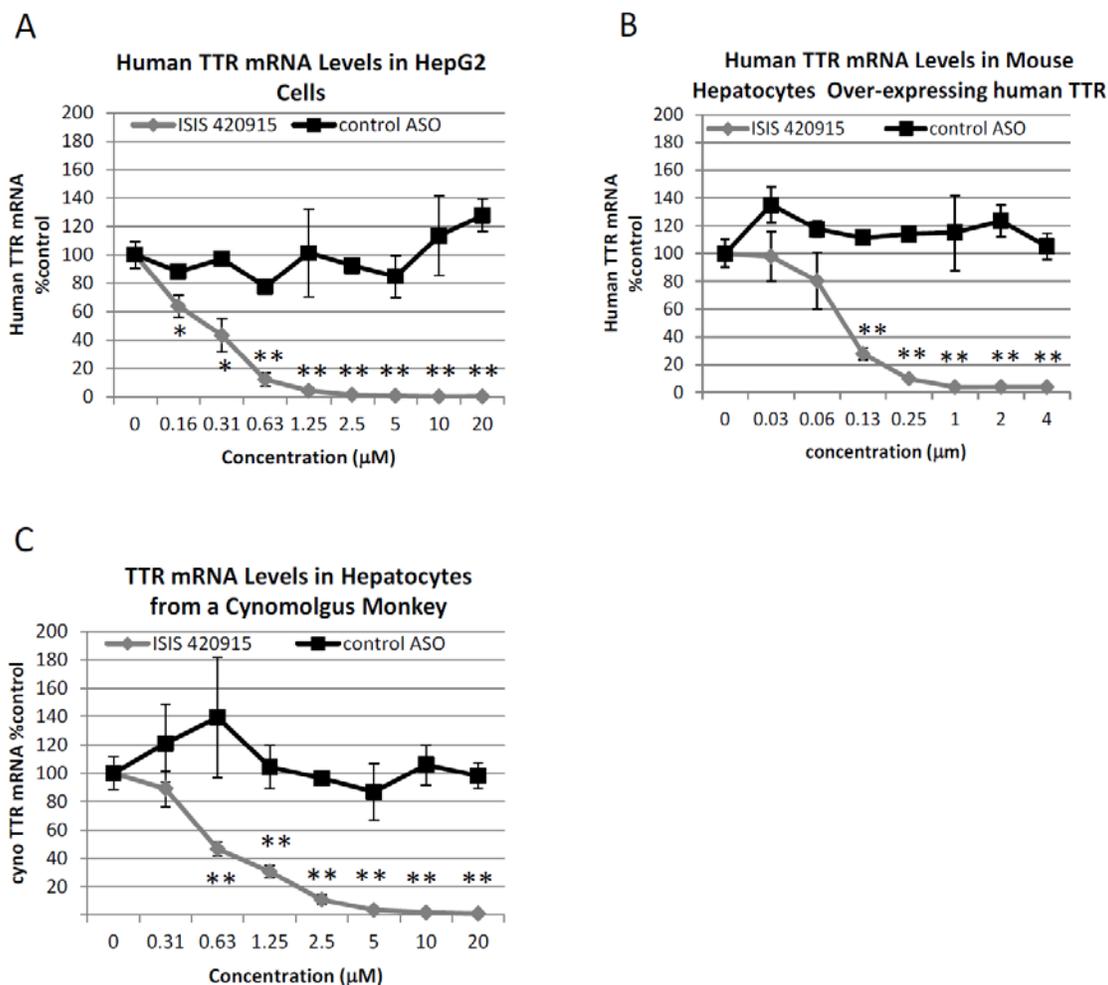
(IONIS Study 420915-NP01; IONIS Pharmaceuticals, Inc., Carlsbad, CA; initiated October 13, 2009; Final Report dated September 26, 2017; non-GLP; non-QA)

ISIS 420915 is a 2'-methoxyethyl-modified ASO complementary to a 20-base sequence within the human TTR mRNA. Specific binding of ISIS 420915 to human TTR mRNA leads to degradation of the mRNA by RNase H1 and reduced synthesis of TTR protein. Two additional 2'-MOE modified ASOs (ISIS 129700 and ISIS 141923) were used as negative controls in these studies, as they do not target any known mRNA transcripts.

In the first experiment, HepG2 cells (a human hepatocellular carcinoma-derived cell line) were incubated for 24 hours at 37 °C following electroporation with 0, 156, 313, 625, 1250, 2500, 5000, 10000, or 20000 nM ISIS 420915 or ISIS 129700; total RNA and TTR mRNA levels were assessed using Ribogreen and RT-PCR, respectively. Human TTR mRNA levels were concentration-dependently reduced (compared to untransfected controls, after normalization to total RNA), with  $IC_{50} = 0.2 \mu\text{M}$  and maximum effect of >95% reduction at  $\geq 1.25 \mu\text{M}$  ISIS 420915. No antisense activity was observed with the control ASO.

In the second experiment, freshly isolated hepatocytes from a female transgenic mouse expressing human TTR were incubated for 16 hours at 37 °C following electroporation with 0, 31.25, 62.6, 125, 250, 1000, 2000, or 4000 nM ISIS 420915 or ISIS 141923; total RNA and TTR mRNA levels were assessed using Ribogreen and RT-PCR, respectively. Human TTR mRNA levels were concentration-dependently reduced (compared to untransfected controls, after normalization to total RNA), with  $IC_{50} = 0.1 \mu\text{M}$  and maximum effect of >95% reduction at  $\geq 1 \mu\text{M}$  ISIS 420915. No antisense activity was observed with the control ASO.

In the third experiment, freshly isolated hepatocytes from a male cynomolgus monkey were incubated for 24 hours at 37 °C following electroporation with 0, 156, 313, 625, 1250, 2500, 5000, 10000, or 20000 nM ISIS 420915 or ISIS 129700; total RNA and TTR mRNA levels were assessed using Ribogreen and RT-PCR, respectively. Monkey TTR mRNA levels were concentration-dependently reduced (compared to untransfected controls, after normalization to total RNA), with  $IC_{50} = 0.8 \mu\text{M}$  and maximum effect of >95% reduction at  $\geq 5 \mu\text{M}$  ISIS 420915. No antisense activity was observed with the control ASO.



**Figure 1 Concentration-Dependent Reduction in TTR MRNA upon ISIS 420915 Treatment**

ISIS 420915 was evaluated for its ability to reduce TTR mRNA levels in HepG2 cells (A); primary hepatocytes isolated from human TTR transgenic mouse (B); and primary hepatocytes from a cynomolgus monkey (C). Oligonucleotides were transfected into the cells using electroporation. Twenty-four (24) hours after transfection total cellular RNA was isolated and the amount of TTR mRNA present was quantitated using a RT-qPCR. Results represent the mean  $\pm$  standard deviation as percentages relative to UTC (N = 1 for HepG2 cells and TG hepatocytes; N = 1 for Cyno hepatocytes).

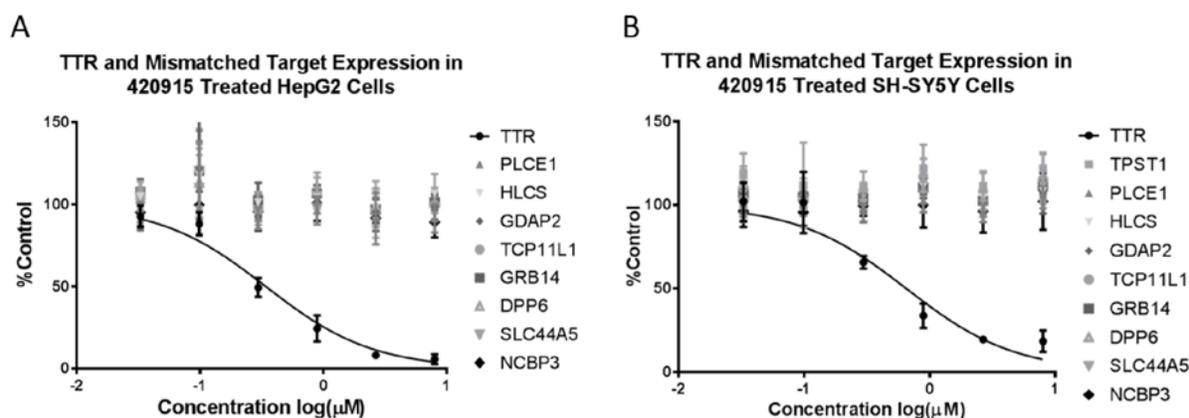
\*P < 0.05      \*\*P < 0.01 when compared to UTC

(page 15 of Study Report)

Potential off-target mRNAs were identified by bioinformatic analysis of the human transcriptome (GRCh38.p6, GCF\_000001405.32 RefSeq assembly) using Bowtie v0.12.2 to search for RNA sequences having  $\leq 3$  mismatches to the ISIS 420915 sequence (sequences with  $\geq 4$  mismatches have been shown to have almost no antisense activity). Two genes were identified whose primary transcripts included only 2 mismatches to ISIS 420915, while 50 genes were identified whose transcripts included 3 mismatches. This list of 52 potential off-target mRNAs was further narrowed down by querying an online database from 53 human tissue samples for expression of the 16 mRNAs containing sequences with  $\leq 3$  mismatches and  $\geq 10$  contiguous matches with

ISIS 420915 (in addition to the TTR sequence, which had 0 mismatches and 20 contiguous matches). An additional database of RNA sequences from normal tissues was queried for expression of 3 of these 16 that did not have results in the original dataset. Of these 16 potential off-target genes, 5 were not expressed in tissues relevant to antisense activity and two did not have expressed isoforms that overlapped with mismatched binding site of ISIS 420915, leaving a set of 9 that were further examined for susceptibility to ISIS 420915 antisense activity.

HepG2 cells and SH-SY5Y (human neuroblastoma) cells were incubated for 24 hours at 37 °C following electroporation with 0, 0.03, 0.10, 0.30, 0.89, 2.67, or 8.00  $\mu\text{M}$  ISIS 420915; total RNA and all 9 mismatched potential off-target mRNA levels were assessed using Ribogreen and RT-qPCR, respectively. As shown in Figure 4 below, transfection with ISIS 420915 resulted in no reduction in any of the mismatched mRNAs in either cell line tested, whereas TTR mRNA was concentration-dependently reduced.



**Figure 4 Specificity of ISIS 420915 in Human Cells**

The effect of ISIS 420915 on mismatched transcripts was evaluated in two human cell lines; human HepG2 hepatocellular carcinoma cells (A); and human SH-SY5Y neuroblastoma cells (B). Oligonucleotides were transfected into the cells using electroporation. Twenty-four (24) hours after transfection total cellular RNA was isolated and the amount of TTR mRNA and mismatched target mRNA present was quantitated using a RT-qPCR. Results represent the mean  $\pm$  standard deviation as percentages relative to UTC (N = 1).

(page 17 of Study Report)

Finally, the potential impact of single nucleotide polymorphisms (SNPs) in the TTR gene sequence corresponding to the ISIS 420915 binding site on the TTR mRNA. Four annotated SNPs were found in the NCBI dbSNP database within the targeted sequence. However, the minor allele frequency for each was below 0.00005, suggesting that very few patients are likely to show reduced effectiveness of ISIS 420915 due to SNP-related mismatches.

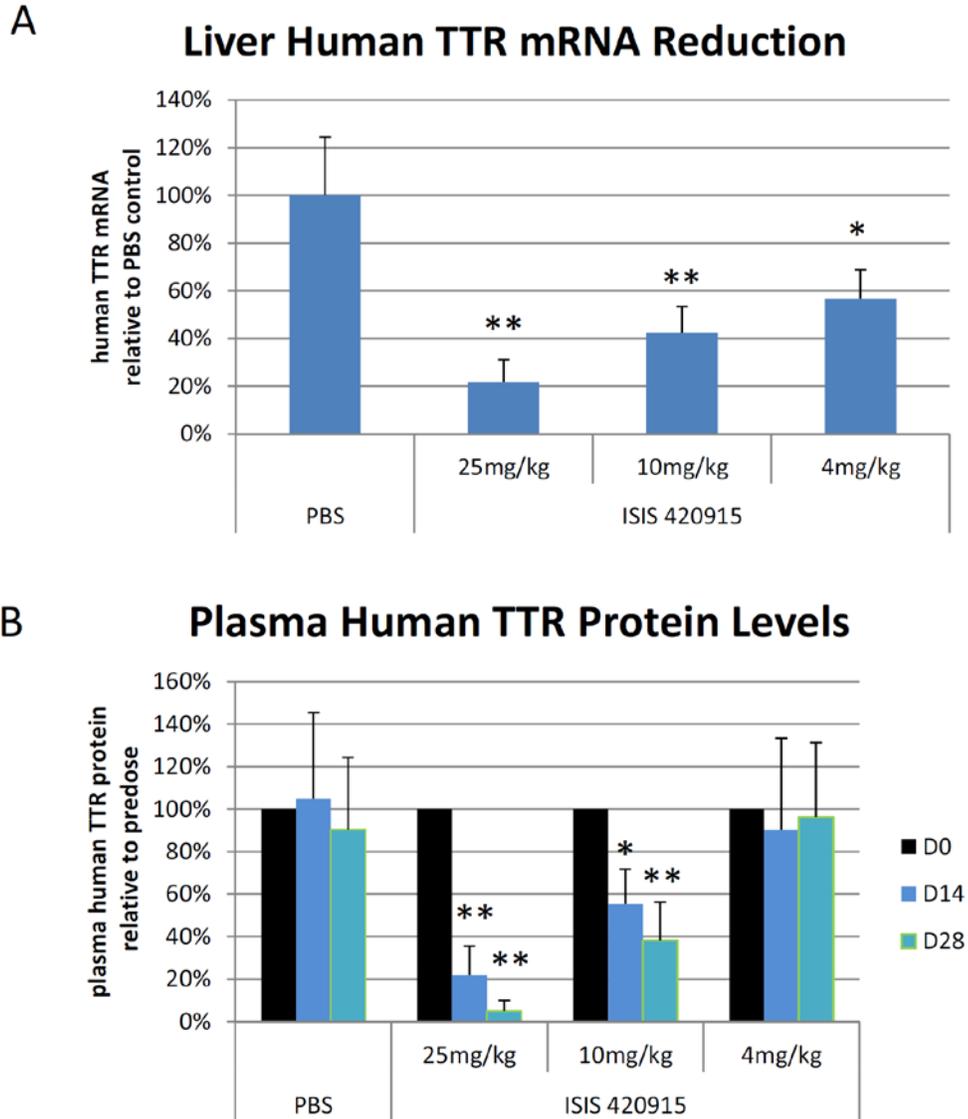
These experiments demonstrated the specific antisense activity of ISIS 420915 against TTR mRNA expressed in human liver carcinoma and neuroblastoma cell lines and in isolated hepatocytes from an hTTR transgenic mouse and a cynomolgus monkey.

**Dose Response Study of ISIS 420915 in TTR Transgenic Mice**

(A brief report of this preliminary study was included as Appendix 1 of the Final Report of Ionis Study 420915-NP02; Ionis Pharmaceuticals, Inc., Carlsbad, CA; dosing initiated November February 27, 2009; non-GLP; non-QA; ISIS 420915 Lot Number 420915-3 at 125 mg/mL in PBS)

Male hTTR Ile84Ser transgenic mice (N=4/group; 4-8 weeks old at initiation of dosing) were administered vehicle (PBS) or ISIS 420915 (4, 10, or 25 mg/kg/dose; 8, 20, or 50 mg/kg/week) via SC injection twice weekly for 4 weeks. The site of administration was not specified. Livers were isolated ~48 hours after the final dose for determination of human TTR mRNA and total mRNA using RT-PCR and RiboGreen, respectively. Plasma was collected from each animal at baseline, 2 weeks postdose, and at sacrifice for determination of human TTR protein levels using an ELISA.

As shown in Figure 1 below, SC administration of ISIS 420915 resulted in dose-dependent reductions in liver hTTR mRNA and plasma hTTR protein, with maximal reductions of ~80% and ~95%, respectively, compared to controls. Significant reductions in plasma hTTR protein were observed by Day 14. While the number of animals in each group was too small to support the reliability of individual data points, the dose-dependence, time-dependence, relatively small error bars, and consistency among the two endpoints measured provide reasonable evidence of the expected drug effect.



**Figure 1 Human TTR mRNA and Protein Reduction after ISIS 420915 Treatment in Transgenic Mice**

Human TTR Ile84Ser transgenic mice were treated twice a week for 4 weeks with 25, 10, or 4 mg/kg ISIS 420915 via subcutaneous injection.

(A) hTTR mRNA levels in liver were quantified by Q-RT-PCR (TaqMan).

(B) Plasma TTR levels were determined by an in-house established ELISA method specific for human TTR. Results represent the mean ± standard deviation (N = 4).

\* P < 0.05 and \*\* P < 0.01 when compared to PBS control group

(page 15 of Study Report 420915-NP02)

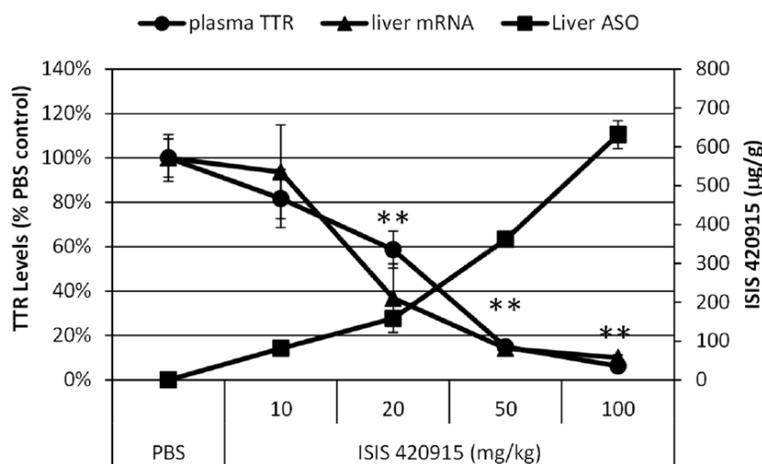
### ***In Vivo* Characterization of ISIS 420915**

(Ionis Study 420915-NP02; Ionis Pharmaceuticals, Inc., Carlsbad, CA; dosing initiated November 17, 2011; Final Report dated September 26, 2017; non-GLP; non-QA; ISIS 420915 Lot Number 420915-125-Q119-118 at 125 mg/mL in PBS)

Male hTTR Ile84Ser transgenic mice (N=4/group; 4-8 weeks old at initiation of dosing) were administered vehicle (PBS) or ISIS 420915 (5, 10, 25, or 50 mg/kg/dose; 10, 20, 50, or 100 mg/kg/week) via SC injection twice weekly for 4 weeks (dose-response phase); or a single dose of ISIS 420915 (100 mg/kg) followed by sacrifice at 1, 2, 3, 4, 7, 14, 21, or 28 days postdose, compared to a vehicle control group given PBS on Day 0 (duration of action study). The site of administration was not specified. Livers were isolated for determination of human TTR mRNA and total mRNA using RT-PCR and RiboGreen, respectively, and ISIS 420915 concentrations using capillary gel electrophoresis with UV detection at 260 nm. Plasma was collected from each animal at sacrifice for determination of human TTR protein levels. Body weight, serum chemistry, organ weight, and histopathology (dose-response phase only) were evaluated but not included in this study report.

SC administration of ISIS 420915 as a single dose of 100 mg/kg or twice weekly for 4 weeks at up to 100 mg/kg/week “was well-tolerated with no dramatic effect on body weights, organ weights, serum chemistry, or histopathology” (page 8 of Study Report; supporting data not provided).

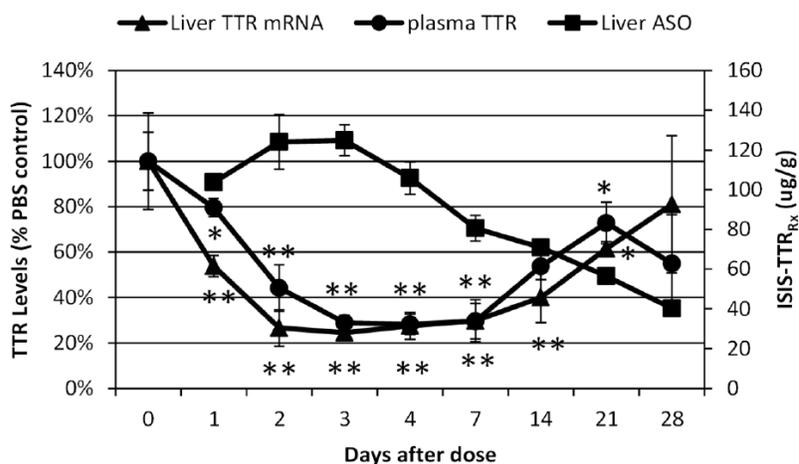
As shown in Figure 1 below, after 4 weeks of treatment, dose-dependent reductions in liver hTTR mRNA levels correlated with similar reductions in plasma hTTR protein levels and increases in liver ISIS 420915 concentrations, compared to PBS controls. Maximum reductions of 90% and 94% in liver hTTR mRNA and plasma hTTR protein, respectively, were achieved at the high dose of 100 mg/kg/week, which corresponded to a liver concentration of 631 µg/g ISIS 420915.



**Figure 1 Human TTR mRNA and Protein Reduction after ISIS 420915 Treatment in Transgenic Mice**

(page 10 of Study Report)

Following a single dose of 100 mg/kg ISIS 420915, maximum reductions of ~75% in liver hTTR mRNA and ~70% in plasma hTTR protein levels (compared to PBS controls) were observed on Days 2-3 and 3-4, respectively, correlating with maximal levels of ~124 µg/g ISIS 420915 concentrations in liver. The lag between reductions in liver hTTR mRNA and plasma hTTR protein was attributed to the 1- to 2-day half-life of pre-existing hTTR protein in plasma of the transgenic mice. Liver hTTR mRNA and plasma hTTR protein levels started to return toward baseline by Day 14 postdose but had not fully recovered by 28 days postdose.



**Figure 2 Duration of Action of ISIS 420915 Single-Dose Treatment in Transgenic Mice**

TTR I84S transgenic mice were treated with a single subcutaneous dose of ISIS 420915 (100 mg/kg). Levels of hepatic hTTR mRNA and plasma hTTR protein were measured, as well as ISIS 420915 concentrations in the liver. Results represent the mean  $\pm$  standard deviation (N = 4).

\* P < 0.05    \*\* P < 0.01 when compared to PBS control group

(page 10 of Study Report)

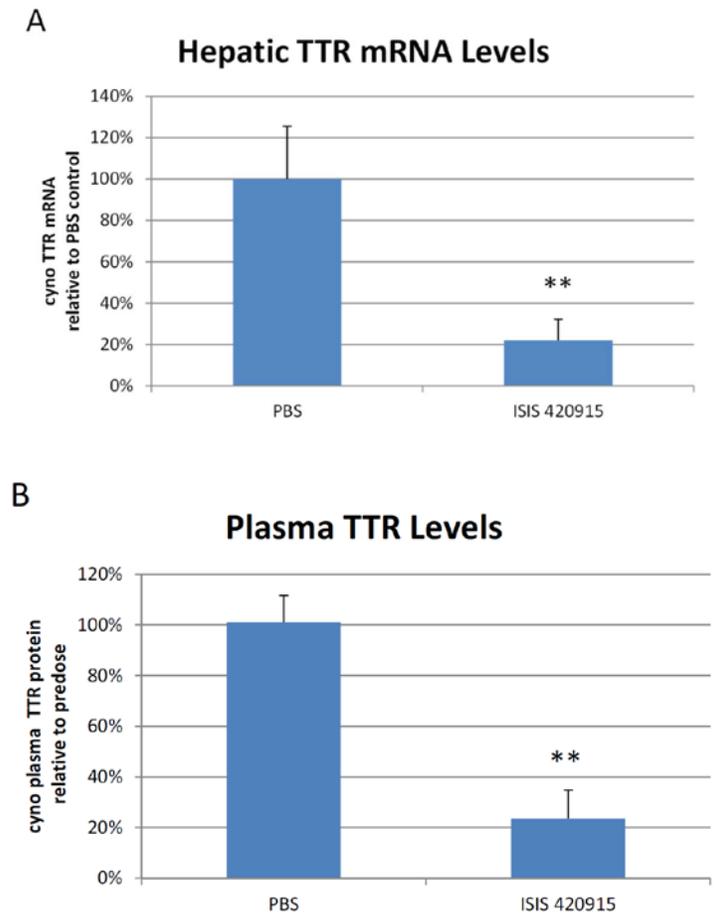
As noted for the pilot study, the questionable reliability of single data points due to the small number of animals per group is mitigated by the dose-dependence, time-dependence, small error bars, and consistency among the antisense effects on hTTR mRNA levels in liver and hTTR protein levels in plasma.

**A 12-Week Pharmacology Study of ISIS 420915 Administered by Subcutaneous Injection in Cynomolgus Monkeys**

(IONIS Study 420915-NP03; [REDACTED] (b) (4)  
[REDACTED] study initiated February 10, 2010; Final Report dated May 9, 2017; non-GLP; non-QA; ISIS 420915 Lot 420915-125-Q1199-118; 125 mg/mL in PBS)

Male Cynomolgus monkeys (N=4/group; 2-3 years old at initiation of dosing) were administered vehicle (PBS) or ISIS 420915 (25 mg/kg/dose) on Days 1, 3, 5, and 7, and twice weekly thereafter through Week 12 via SC injection. Following sacrifice at ~48 hours after the final dose, liver samples were collected for determination of hTTR mRNA by RT-PCR, normalizing to levels of cyclophilin mRNA. Blood samples were collected on Days -5, 9, 16, 23, 30, 44, 58, 72, and 86, ~48 hours after dosing for determination of plasma levels of hTTR protein and Retinol Binding Protein 4 (RBP4; using an ELISA). Serum levels of TSH, Total T4, Free T4, Total T3, and Free T3 were measured in blood samples collected on Days -5, 51, and 86 (methods not specified).

As shown in Figures 1 and 2 below, SC administration of ISIS 420915 for 4 weeks reduced liver hTTR mRNA and plasma hTTR protein levels ~80%, and plasma RBP4 levels ~60%, compared to controls. Reductions in plasma hTTR and RBP4 levels were time-dependent, as shown in Figures 2 and 3 below. No consistent drug-related effects were observed on serum TSH, T3, or T4 levels.



**Figure 1 TTR mRNA Levels in Liver and TTR Protein Levels in Plasma are Reduced in Monkeys Treated with ISIS 420915 for 12 Weeks**

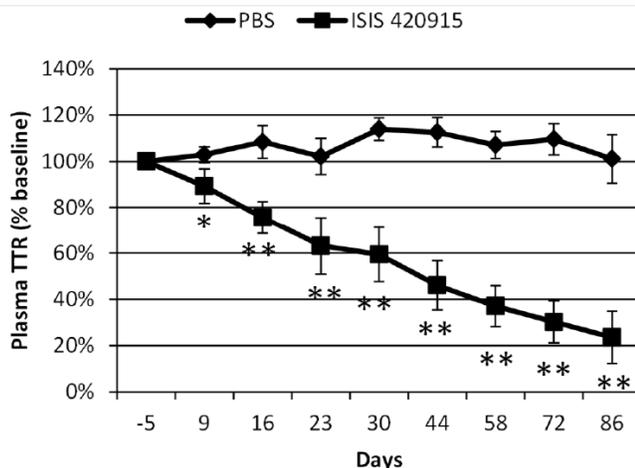
(A) TTR mRNA reduction in liver

(B) Plasma TTR protein reduction

Liver total RNA was purified and RT-PCR was carried out to determine the levels of TTR mRNA in the liver. The plasma protein levels were measured by immunoturbidimetry using an Olympus clinical analyzer. Results represent the mean ± standard deviation (N = 4).

\*\* P < 0.01 when compared to PBS group (A) or compared to pre-dose (B).

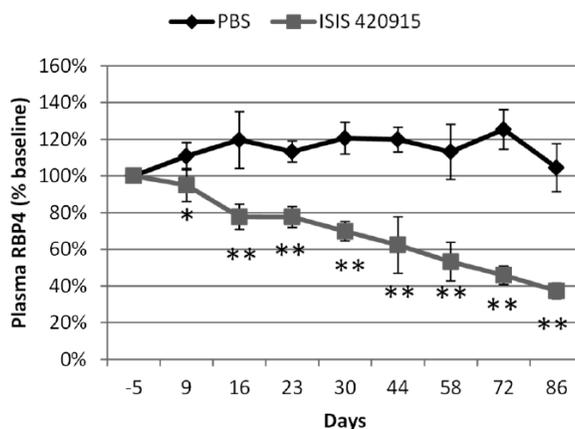
(page 11 of Study Report)



**Figure 2** ISIS 420915 Treatment Promotes a Time-Dependent Reduction of Plasma TTR Levels in Monkeys

ISIS 420915 was administered 25 mg/kg SC every other day in Week 1 and twice a week for Weeks 2 through 12. Plasma samples were obtained from each animal at Day -5, Days 9, 16, 23, 30, 44, 58, 72, and 86. TTR levels were determined by immunoturbidimetry using an Olympus clinical analyzer. Results represent the mean  $\pm$  standard deviation of relative levels to baseline (Day -5, N = 4).

\* P < 0.05      \*\* P < 0.01 when compared to PBS group



**Figure 3** ISIS 420915 Treatment Leads to Time-Dependent Reduction of Plasma RBP4 Levels in Monkeys

ISIS 420915 was administered 25 mg/kg SC every other day in Week 1 and twice a week for Weeks 2 through 12. Plasma samples were obtained from each animal at Day -5, Days 9, 16, 23, 30, 44, 58, 72, and 86. Plasma RBP4 levels were determined by an ELISA kit (Alpco cat. # 30-6110). Results represent the mean  $\pm$  standard deviation of relative levels to baseline (Day -5, N = 4).

\*\* : P < 0.01 when compared to PBS group.

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## 4.2 Secondary Pharmacology

No secondary pharmacology studies of inotersen were submitted.

### 4.3 Safety Pharmacology

#### Evaluation of the Effects of ISIS 420915 on Neurobehavior (Irwin's Test) and Body Temperature in Mice Following a Single Subcutaneous Administration

(ISIS Study 420915-AS03; [REDACTED] (b) (4) study initiated February 14, 2011; Final Report dated May 5, 2011; GLP and QA, except for analysis of drug in liver; ISIS 420915 Lot Numbers RP420915-003, -004, and -006-A)

CrljOri:CD1 (ICR) mice (N=10/sex/group; ~6 weeks old at initiation of dosing) were administered placebo (PBS) or ISIS 420915 (40, 100, or 300 mg/kg) via SC injection in the mid scapular region at 10 mL/kg. Evaluations were conducted prior to dosing and at 1, 2, 6, and 24 hours postdose, and included body temperature and neurobehavioral observations (Modified Irwin's Method: locomotor activity, tail elevation, tremors, convulsions, abdominal tone, catalepsy, traction, righting reflex, pinna reflex, piloerection, skin coloration, respiration rate, eyelid, exophthalmos, lacrimation, salivation, diarrhea, death, and startle reflex). Animals were sacrificed after the final observation, and liver samples were isolated from the first 4 males per dose group for determination of drug concentration (method not specified).

No consistent drug-related effects were observed on body temperature or neurobehavioral parameters. Dose-dependent increases were observed in ISIS 420915 concentrations in liver of male mice: 57, 111, and 175 µg/g, in LDM, MDM, and HDM, respectively.

#### A Cardiovascular and Respiratory Safety Pharmacology Study of ISIS 420915 by Subcutaneous Administration in Telemetered Cynomolgus Monkeys

(ISIS Study 420915-AS06; [REDACTED] (b) (4) study initiated October 11, 2010; Final Report dated July 18, 2011; ISIS 420915 Lot Numbers RP420915-006 and RP420915-007; GLP and QA, except for analysis of plasma drug levels)

Telemetered male Cynomolgus monkeys (N=4/group; 6-7 years old at initiation of dosing) were administered Placebo (PBS) on Day 1 and ISIS 420915 (12 or 40 mg/kg) on Day 3 via SC injection at 0.4 mL/kg. Parameters evaluated at one hour predose and at 2, 4, and 24 hours postdose included systolic BP, diastolic BP, mean arterial pressure, heart rate, respiratory rate, body temperature, and ECG. Clinical observations were recorded twice daily throughout the study and body weights were recorded on Day -1 only. Arterial blood gases were measured on Days 1 and 3 at 6 hours postdose, and venous blood was collected on Day 3 at ~6 hours postdose for analysis of plasma drug concentrations using a hybridization ELISA. Animals were returned to the stock colony on Day 5.

No drug-related effects were observed. Mean plasma drug levels at 6 hours after dosing with 12 and 40 mg/kg ISIS 420915 were 29.55 (±8.98) and 92.24 µg/mL (±11.67), respectively. The NOAEL for effects of ISIS 420915 on cardiovascular and respiratory parameters in male monkeys was the HD of 40 mg/kg SC.

**Effect of ISIS 420915 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells**

(ISIS Study 420915-IS01; [REDACTED] (b) (4)  
[REDACTED] study initiated October 29, 2010; Final Report dated April 26, 2011; GLP and QA, except for characterization of positive control and stability analysis of ISIS 420915; ISIS 420915 Lot Number RP420915-008)

The magnitude of the hERG rapid delayed rectifier potassium current was measured in HEK293 cells stably transfected with hERG mRNA (N=3 cells/concentration) using standard patch clamp techniques in the presence of vehicles (HEPES-buffered saline + 1% sterile water for drug, + 0.3% DMSO for positive control), ISIS 420915 (100 or 300  $\mu$ M), or terfenadine (60 nM; positive control).

Mean reductions in hERG current during incubation with ISIS 420915 at 100  $\mu$ M ( $0.4 \pm 0.7\%$ ) and 300  $\mu$ M ( $0.6 \pm 0.7\%$ ) were not significantly different from that observed with the vehicle control ( $0.3 \pm 1.0\%$ ). Incubation with 60 nM terfenadine reduced the hERG current 76.1% compared to baseline, as expected.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

#### ISIS 420915: Pharmacokinetic Study of ISIS 420915 in Mice

(ISIS Study 420915-APK01; (b) (4) study initiated July 2, 2014; Final Report dated January 28, 2016; GLP; QA)

Male CrljOri:CD1(ICR) mice (N=6/time point) were administered ISIS 420915 (10 or 40 mg/kg/dose) via SC injection on Days 1, 3, 5, 7, 14, 21, 28, 35, and 42. Blood samples were collected via the tail vein predose and at 0.5, 1, 3, 4, 6, 10, 24, and 48 hours after dosing on Days 1 and 42 for determination of ISIS 420915 concentrations in plasma using a validated hybridization ELISA method. Liver and kidney samples were collected from 3 animals/group following sacrifice on Day 44 for determination of ISIS 420915 concentrations in tissues using a validated HPLC-UV method. Urine samples were collected from 3 animals per group on Days 43 and 44 and assayed for ISIS 420915 concentrations using an LC/MS method.

No drug-related effects were observed on mortality, clinical signs, or body weight. As shown in the table below, plasma exposures ( $C_{max}$  and  $AUC_{0-48\text{ hr}}$ ) generally increased proportionally with dose and little to no accumulation was observed with repeated once weekly dosing following the loading doses in the first week. Distribution to kidney and liver was extensive, exceeding plasma  $C_{max}$  values by ~5-fold and ~8-fold, respectively, in HDM after 6 weeks of dosing. Excretion of ISIS 420915 in urine was low (~2-8% of dose administered).

Table 7. Summary of Selected Plasma Pharmacokinetic Parameters in Mice following Single and Multiple Subcutaneous Administrations of ISIS 420915

Group	Dose Level (mg/kg)	Study Day (No. of Dose)	Gender	$T_{max}$ (hr)	$C_{max}$ ( $\mu\text{g/mL}$ )	$AUC_{0-48\text{hr}}$ (hr* $\mu\text{g/mL}$ )	$MRT_{0-48\text{hr}}$ (hr)
1	10	1 (1 <sup>st</sup> )	Male	0.5	12.1	21.3	2.1
1	10	42 (9 <sup>th</sup> )	Male	0.5	9.3	20.8	3.2
2	40	1 (1 <sup>st</sup> )	Male	0.5	48.7	91.7	2.6
2	40	42 (9 <sup>th</sup> )	Male	0.5	42.5	115.8	4.0

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

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Table 8. ISIS 420915 Tissue Concentrations in Mice after Multiple (ninth dose) Subcutaneous Administrations of ISIS 420915

<b>Matrix</b>	<b>Group</b>	<b>Dose Level</b> <b>(mg/kg)</b>	<b>Study Day</b> <b>(No. of Dose)</b>	<b>Gender</b>	<b>N</b>	<b>Mean ± SD</b> <b>(µg/g)</b>
Kidney	1	10	42 (9 <sup>th</sup> )	Male	3	100.5 ± 50.5
Kidney	2	40	42 (9 <sup>th</sup> )	Male	3	203.5 ± 20.7
Liver	1	10	42 (9 <sup>th</sup> )	Male	3	85.6 ± 17.5
Liver	2	40	42 (9 <sup>th</sup> )	Male	3	359.1 ± 50.2

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Table 9. Percent ISIS 420915 Excreted in Urine Collected up to 48 Hours After Multiple (ninth dose) Subcutaneous Administrations of ISIS 420915 in Mice

<b>Group</b>	<b>Dose Level</b> <b>(mg/kg)</b>	<b>Study Day</b>	<b>Gender</b>	<b>% Excreted</b> <b>(0-48 hr)</b>	<b>CL<sub>r(0-48hr)</sub></b> <b>(mL/hr)</b>
1	10	42	Male	1.56	0.30
2	40	42	Male	7.79	1.11

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### **[<sup>3</sup>H]-ISIS 420915: Pharmacokinetics, Distribution, Metabolism, Excretion and Mass Balance of Radioactivity in Rats Following a Single Subcutaneous Bolus Injection**

(ISIS Study 420915-APK02; (b) (4))

study initiated June 16, 2016; Final Report dated July 10, 2017; GLP, except for lack of dosing formulation analyses; QA)

Sprague Dawley rats (M: 7-9 weeks old; F: 10-12 weeks old) were administered single doses of control (0.9% saline for infusion) or [<sup>3</sup>H]-ISIS 420915 (5 or 25 mg/kg; 2.5 mL/kg in PBS) via SC injection as described in the table below.

Study Phase	Number and sex of animals	Dose level (mg/kg)	Radioactivity level (μCi/kg)	Study type	Animal numbers
A	1M	0	0	Control (samples used as backgrounds and blank matrix for Metabolite Profiling)	1M
B	15M	5	100	Pharmacokinetics, and Tissue Distribution (and provision of samples for Metabolite ID and Profiling)	2-16M
C	3M	5	100	Mass Balance and Tissue Distribution (and provision of samples for Metabolite ID and Profiling)	17-19M
D	4M, 4F	25	500	Quantitative whole-body autoradiography	20M, 21M, 22M, 23M, 24F, 25F, 26F, 27F
E	1M, 1F	25	500	Provision of samples for Metabolite ID and Profiling	28M, 29F

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Peak concentrations of radioactivity (6.38 μg Eq/mL in whole blood; 9.04 μg Eq/mL in plasma) were observed at 1 hour postdose, followed by rapid and broad distribution into tissues and slow clearance from tissues ( $t_{1/2}$  elimination  $\geq$  12 days). Tissue concentrations generally peaked at 24-48 hours postdose and were highest in kidneys, followed by liver, mesenteric lymph nodes, bone marrow, thyroid, spleen, bone, and pancreas. Brain and spinal cord showed relatively low concentrations of radioactivity. Similar tissue distribution was observed using Quantitative Whole Body Autoradiography after administration of 25 mg/kg [<sup>3</sup>H]-ISIS 420915, with the highest levels observed in the kidney cortex and undetectable levels in brain and spinal cord.

Excretion of radioactivity from M following administration of 5 mg/kg [<sup>3</sup>H]-ISIS 420915 was slow, with ~13% of the administered dose eliminated within the first 24 hours, primarily via urine, and then < 2% per day throughout the 56-day observation period. Overall, ~80% of the administered dose was eliminated from 0 to 1344 hours postdose, with 45.2%, 11.5%, 1.41%, and 21% accounted for by urine, feces, cage washings, and carcass, respectively.

Unchanged ISIS 420915 accounted for ~73% of radioactivity in plasma at 2 hours postdose and >69% of radioactivity in kidney and liver at 24 hours postdose. Various 3'-

deletion metabolites (8-mers and longer) were present, each at < 10% of total sample radioactivity. Non-radiolabeled 5'-deletion metabolites were detected in kidney samples by LC-MS but were not quantified. Samples of urine and feces contained primarily 5- to 8-mer 3'-deletion oligomers, with very little parent drug present. No substantial differences were noted in the pattern of metabolites after administration of 5 vs. 25 mg/kg [<sup>3</sup>H]-ISIS 420915.

The sponsor's summary tables are presented below:

**Table 1 Non-compartmental pharmacokinetic parameters of radioactivity in whole blood and plasma following a single subcutaneous administration of [<sup>3</sup>H]-ISIS 420915 (5 mg/kg) to male Sprague Dawley rats (Phases B and C)**

Matrix	C <sub>max</sub> (µg eq/mL)	T <sub>max</sub> (h)	AUC <sub>0-24h</sub> (µg eq.h/mL)	AUC <sub>0-48h</sub> (µg eq.h/mL)	AUC <sub>last</sub> (µg eq.h/mL)	AUC <sub>0-∞</sub> (µg eq.h/mL)	% Extrapolated AUC <sub>0-∞</sub>	λ <sub>z</sub> (h <sup>-1</sup> )	t <sub>1/2 λz</sub> (h)	CL/F (mL/h/kg)	V <sub>z</sub> /F (mL/kg)
Plasma	9.04	1	36.6	39.0	67.0	(78.7) <sup>a</sup>	14.9	(0.00200) <sup>a</sup>	(347) <sup>a</sup>	63.5	31800
Blood	6.38	1	27.0	29.2	40.5	(46.4) <sup>a</sup>	12.7	(0.00369) <sup>a</sup>	(188) <sup>a</sup>	108	29200
Volatile <sup>b</sup>	1.67	2	-	-	8.24	(11.7) <sup>a</sup>	29.7	(0.00588) <sup>a</sup>	(118) <sup>a</sup>	426	72400

<sup>a</sup> Reported half-lives were outside acceptance criteria (see [Data Processing](#))

<sup>b</sup> Volatile radioactivity in plasma

Concentration units are expressed as µg equivalents of ISIS 420915/mL

Note: Results were acquired after sparse sampling where n=3 at each timepoint (see [Section 3.4.2](#))

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**Table 7 Pharmacokinetic parameters derived from total radioactivity concentrations in tissues/organs following a single subcutaneous administration of [<sup>3</sup>H]-ISIS 420915 (5 mg/kg) to male Sprague Dawley rats (Phases B and C)**

Tissue	C <sub>max</sub> (µg eq/g)	T <sub>max</sub> (h)	AUC <sub>last</sub> (µg eq.h/g)	AUC <sub>0-∞</sub> (µg eq.h/g)	% Extrapolated AUC <sub>0-∞</sub>	λ <sub>z</sub> (h <sup>-1</sup> )	t <sub>1/2λz</sub> (d)
Adrenal glands	3.14	168	1830	(2930) <sup>a</sup>	37.5 <sup>a</sup>	(0.000652) <sup>a</sup>	(44.2) <sup>a</sup>
Bone (femur)	3.69	48	2040	(2590) <sup>a</sup>	21.0 <sup>a</sup>	(0.00116) <sup>a</sup>	(24.9) <sup>a</sup>
Bone marrow	9.37	24	7710	(13800) <sup>a</sup>	44.2 <sup>a</sup>	(0.000616) <sup>a</sup>	(47.1) <sup>a</sup>
Brain	0.0740	24	43.5	(66.2) <sup>a</sup>	34.4 <sup>a</sup>	(0.000788) <sup>a</sup>	(36.7) <sup>a</sup>
Eyes	0.810	24	682	(1030) <sup>a</sup>	33.9 <sup>a</sup>	(0.000798) <sup>a</sup>	(36.2) <sup>a</sup>
Fat (perirenal)	1.38	24	673	779	13.5	0.00149	19.4
Fat (subcutaneous)	0.478	48	238	273	12.9	0.00151	19.2
Heart	0.894	48	746	(1070) <sup>a</sup>	30.6 <sup>a</sup>	(0.000871) <sup>a</sup>	(33.2) <sup>a</sup>
Kidney	122	168	100000	(157000) <sup>a</sup>	36.1 <sup>a</sup>	(0.000732) <sup>a</sup>	(39.5) <sup>a</sup>
Liver	12.7	48	7220	7530	4.12	0.00242	12.0
Lungs	0.997	168	833	(1030) <sup>a</sup>	19.3 <sup>a</sup>	(0.00126) <sup>a</sup>	(22.8) <sup>a</sup>
Lymph nodes (mesenteric)	10.6	24	7440	(11200) <sup>a</sup>	33.9 <sup>a</sup>	(0.000849) <sup>a</sup>	(34.0) <sup>a</sup>
Muscle (skeletal)	0.349	168	266	(347) <sup>a</sup>	23.3 <sup>a</sup>	(0.00109) <sup>a</sup>	(26.4) <sup>a</sup>
Pancreas	4.01	48	2830	3350	15.6	0.00139	20.8
Pituitary gland	1.21	48	903	(1690) <sup>a</sup>	46.4 <sup>a</sup>	(0.000556) <sup>a</sup>	(52.1) <sup>a</sup>
Prostate	0.971	168	709	(879) <sup>a</sup>	19.3 <sup>a</sup>	(0.00122) <sup>a</sup>	(23.7) <sup>a</sup>
Skin	1.13	48	685	841	18.5	0.00126	23.0
Spinal cord	0.460	24	174	(1060) <sup>a</sup>	83.6 <sup>a</sup>	(0.000131) <sup>a</sup>	(221) <sup>a</sup>
Spleen	4.63	168	4760	(22000) <sup>a</sup>	78.4 <sup>a</sup>	(0.000180) <sup>a</sup>	(160) <sup>a</sup>
Testes	1.21	168	1300	(3240) <sup>a</sup>	60.0 <sup>a</sup>	(0.000383) <sup>a</sup>	(75.4) <sup>a</sup>
Thymus	0.795	336	862	-	-	<sup>b</sup>	-
Thyroid	4.64	24	2830	3110	9.13	0.00174	16.6

<sup>a</sup> Reported λ<sub>z</sub> were outside acceptance criteria (see [Section 3.10.3](#)). Associated parameters (AUC<sub>0-∞</sub>, % extrapolated AUC<sub>0-∞</sub>, t<sub>1/2λz</sub>) should be interpreted with caution

<sup>b</sup> Could not be estimated

Concentration units are expressed as µg equivalents of ISIS 420915/g

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***In Vitro* Evaluation of the Extent of Plasma Protein Binding of ISIS 420915 in Human and Monkey Plasma by Ultrafiltration**

(ISIS Study 420915-IS04; ISIS Pharmaceuticals, Inc., Carlsbad, CA; Final Report dated July 2, 2012; non-GLP; non-QA)

Binding of ISIS 420915 to proteins in plasma isolated from human and Cynomolgus monkey (purchased from (b) (4)) was evaluated at 5 and 150 µg/mL using ultrafiltration followed by a non-validated nuclease-dependent hybridization ELISA method.

As shown in Table 1 below, plasma protein binding was high in both species at both concentrations tested.

**Table 1. In Vitro Plasma Protein Binding (%Bound) of ISIS 420915 in Whole Plasma in Both Species. % Bound values are presented as mean ± SD.**

ISIS 420915 Nominal Plasma Concentration (µg/mL)	Extent of Plasma Protein Binding (%)	
	Human	Monkey
5	97.84 ± 0.34	99.35 ± 0.05
150	94.41 ± 0.35	97.80 ± 0.22

SD = standard deviation

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**Identification and Profiling of ISIS 420915 and its Associated Metabolites by Ion-Pair HPLC Electrospray/Mass Spectrometry (IP-HPLC-ES/MS) in Selected Available Plasma, Tissue and Urine Samples from Mice, Monkeys, and Humans**

(ISIS Study 420915-IS09; Ionis Pharmaceuticals, Carlsbad, CA; Final Report dated May 25, 2017; non-GLP; non-QA)

Samples of plasma and/or urine were collected from mice (Study 420915-APK01), monkeys (Study 420915-AS02), and humans (Study ISIS (b) (4)-CS1), and analyzed using IP-HPLC-ES/MS to characterize parent drug and metabolites present following repeated SC dosing for 6, 13, or 3 weeks, respectively. No sex-related differences were observed, so data presented represent averages of males and females combined.

As shown in Tables 4, 5, and 6 below, parent drug (ISIS 420915, a 20-mer with five 2'-MOE-nucleotides on the 3' and 5' ends, flanking ten successive 2'-deoxy-nucleotides) was the most abundant drug-related oligonucleotide present in plasma, accounting for ~97%, ~95%, and ~74% of the total in mouse, monkey, and human, respectively. Shorter oligonucleotides, primarily 6-mer to 12-mers, each accounted for  $\leq$  ~1%,  $\leq$  ~2%, and  $\leq$  ~7% of total plasma oligonucleotide in mouse, monkey, and human, respectively.

IP-HPLC-ES/MS analyses of liver and kidney samples collected 48 hours after the final dose in a 13-week mouse (Study 420915-AS01; 100 mg/kg/week) and 13-week monkey (Study 420915-AS02; 40 mg/kg/week) studies confirmed the identities of ISIS 420915 and shorter oligonucleotide metabolites present in the tissues. HPLC-UV analyses included in the original study reports showed that parent drug accounted for 80-92% (mouse) or 82-95% (monkey) of total peak area in liver and kidney tissue samples, while shorter oligomers (19-mers and 7- to 14-mers in mouse; 19-mers and 8- to 15-mers in monkey) each accounted for < 5%.

As shown in Tables 10 and 11 below, analyses of urine samples collected 0-24 hours after the final dose revealed that ISIS 420915 accounted for 69% and 7.8% of total oligonucleotide present in mouse and human, respectively, while shorter oligomer metabolites (18- and 19-mers and 5- to 14-mers in mouse; 5- to 12-mers in human) each accounted for 0.2-8.4% and 0.7-17%, respectively. As shown in Table 12, urinary excretion of full-length ISIS 420915 within 24 hours after the final dose of 300 mg was only 0.78% of the dose administered, while parent + metabolites together accounted for 13.5%.

These data are consistent with rapid distribution of ISIS 420915 from plasma into tissues (especially in humans, at the doses tested), followed by slow metabolism in tissues by endonuclease and then exonuclease digestion of the ten inner, more vulnerable 2'-deoxy-nucleotides, down to the protected 5-mer "wings" consisting of the five 2'-MOE-nucleotides on either end. The shorter 5- to 12-mers are then slowly and steadily excreted in the urine as they bind less strongly than full-length ISIS 420915 to tissue and plasma proteins.

**Table 4 Relative Abundance (%) of ISIS 420915 and Its Associated Metabolites in Mouse Plasma (Ionis Study 420915-APK01)**

Oligonucleotide	Day 44, 48 hours Post-dose			Day 42, 0.5 hours Post-dose		
	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions <sup>b</sup>	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions <sup>b</sup>
ISIS 420915	NA	NA	0	NA	NA	97.02 ± 1.25
19-mer	0	0	0	0	0	0.91 ± 0.18 <sup>a</sup>
18-mer	0	0	0	0	0	0
17-mer	0	0	0	0	0	0
16-mer	0	0	0	0	0	0
15-mer	0	0	0	0	0	0
14-mer	0	0	0	0	0	0
13-mer	0	0	0	0	0	0
12-mer	0	0	0	0	0	0.16 ± 0.29 <sup>a</sup>
11-mer	0	0	0	0.28 ± 0.10	0	0.28 ± 0.10
10-mer	0	0	0	0	0	0.77 ± 0.46 <sup>a</sup>
9-mer	0	0	0	0.37 ± 0.29	0.24 ± 0.22	0.61 ± 0.15
8-mer	0	0	0	0	0	0.12 ± 0.22 <sup>a</sup>
7-mer	0	0	0	0	0	0
6-mer	0	0	0	0.09 ± 0.15	0	0.09 ± 0.15
5-mer	0	0	0	0	0	0

Note: Data presented are mean ± standard deviation (n=3).

NA= not applicable

<sup>a</sup> Due to similar masses, 3'- and 5'-deletion metabolites are indistinguishable

<sup>b</sup> Relative abundance listed as "0" when no full-length or shortmer oligonucleotides were detected  
(page 21 of Study Report)

**Table 5 Relative Abundance (%) of ISIS 420915 and Its Associated Metabolites in Monkey Plasma (Ionis Study 420915-AS02)**

Oligonucleotide	Day 91, Pre-dose			Day 91, 2 hours Post-dose		
	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions <sup>b</sup>	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions <sup>b</sup>
ISIS 420915	NA	NA	0	NA	NA	94.58 ± 2.02
19-mer	0	0	0	0	0	0
18-mer	0	0	0	0	0	0
17-mer	0	0	0	0	0	0
16-mer	0	0	0	0	0	0
15-mer	0	0	0	0	0	0
14-mer	0	0	0	0	0	0
13-mer	0	0	0	0	0	0
12-mer	0	0	0	0	0	0.64 ± 0.44 <sup>a</sup>
11-mer	0	0	0	1.51 ± 0.20	0	1.51 ± 0.20
10-mer	0	0	0	0	0	0.69 ± 0.85 <sup>a</sup>
9-mer	0	0	0	1.47 ± 0.64	0.19 ± 0.38	1.67 ± 0.83
8-mer	0	0	0	0	0	0.88 ± 0.64 <sup>a</sup>
7-mer	0	0	0	0	0	0
6-mer	0	0	0	0	0	0
5-mer	0	0	0	0	0	0

Note: Data presented are mean ± standard deviation (n=3).

NA= not applicable

<sup>a</sup> Due to similar masses, 3'- and 5'-deletion metabolites are indistinguishable

<sup>b</sup> Relative abundance listed as "0" when no full-length or shortmer oligonucleotides were detected  
(page 22 of Study Report)

**Table 6** Relative Abundance (%) of ISIS 420915 and Its Associated Metabolites in Human Plasma (Study **ISIS 420915-CS1**)

Oligonucleotide	Day 22, Pre-dose			Day 22, 4 hours Post-dose		
	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions <sup>b</sup>	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions <sup>b</sup>
ISIS 420915	NA	NA	0	NA	NA	74.23 ± 5.64
19-mer	0	0	0	0	0	0
18-mer	0	0	0	0	0	0
17-mer	0	0	0	0	0	0
16-mer	0	0	0	0	0	0
15-mer	0	0	0	0	0	0
14-mer	0	0	0	0	0	0
13-mer	0	0	0	0	0	0
12-mer	0	0	0	0	0	0.40 ± 0.81 <sup>a</sup>
11-mer	0	0	0	3.78 ± 0.38	0	3.78 ± 0.38
10-mer	0	0	0	0	0	6.19 ± 1.34 <sup>a</sup>
9-mer	0	0	0	2.17 ± 1.48	4.83 ± 1.14	7.01 ± 1.42
8-mer	0	0	0	0	0	2.97 ± 2.08 <sup>a</sup>
7-mer	0	0	0	2.07 ± 1.51	1.55 ± 1.79	3.62 ± 2.67
6-mer	0	0	0	1.25 ± 1.49	0.50 ± 1.00	1.76 ± 2.08
5-mer	0	0	0	0	0	0

Note: Data presented are mean ± standard deviation (n=3).

NA= not applicable

<sup>a</sup> Due to similar masses, 3'- and 5'-deletion metabolites are indistinguishable

<sup>b</sup> Relative abundance listed as "0" when no full-length or shortmer oligonucleotides were detected  
(page 23 of Study Report)

**Table 10** Relative Abundance (%) of ISIS 420915 and Its Associated Metabolites in Mouse Urine, Group 2, 40 mg/kg (Ionis Study 420915-APK01)

Oligonucleotide	Day 42, 0-24 hours Post-dose			Day 42, 24-48 hours Post-dose		
	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions	3'-Deletion	5'-Deletion	Sum of 3'- and 5'- Deletions
ISIS 420915	NA	NA	68.61	NA	NA	46.19
19-mer	0	0	1.00 <sup>a</sup>	0	0	1.34 <sup>a</sup>
18-mer	0	0	0.21 <sup>a</sup>	0	0	0
17-mer	0	0	0	0	0	0
16-mer	0	0	0	0	0	0
15-mer	0	0	0	0	0	0
14-mer	0.33	0.39	0.73	0	0	0
13-mer	1.08	0.40	1.49	4.6	0	4.60
12-mer	0	0	1.53 <sup>a</sup>	0	0	3.08 <sup>a</sup>
11-mer	4.26	0.34	4.60	8.63	0	8.63
10-mer	0	0	8.40 <sup>a</sup>	0	0	8.81 <sup>a</sup>
9-mer	2.93	1.73	4.66	6.21	0	6.21
8-mer	0	0	1.39 <sup>a</sup>	0	0	2.50 <sup>a</sup>
7-mer	1.79	0.66	2.46	2.98	0.52	3.50
6-mer	1.51	0.74	2.25	2.20	1.92	4.13
5-mer	0.26	2.33	2.60	0.67	10.29	10.96

Note: Data presented are pooled samples (n=1).

NA= not applicable

<sup>a</sup> Due to similar masses, 3'- and 5'-deletion metabolites are indistinguishable

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**Table 11 Relative Abundance (%) of ISIS 420915 and Its Associated Metabolites in Human Urine (Study ISIS 420915-CS1)**

Oligonucleotide	Day 22, 0-24 hours Post-dose		
	3'-Deletion	5'-Deletion	Sum of 3'- and 5'-Deletions
ISIS 420915	NA	NA	7.80 ± 2.33
19-mer	0	0	0
18-mer	0	0	0
17-mer	0	0	0
16-mer	0	0	0
15-mer	0	0	0
14-mer	0	0	0
13-mer	0	0	0
12-mer	0	0	0.71 ± 0.79 <sup>a</sup>
11-mer	3.16 ± 0.52	0	3.16 ± 0.52
10-mer	0	0	9.94 ± 1.10 <sup>a</sup>
9-mer	4.46 ± 0.59	16.79 ± 1.23	21.25 ± 1.76
8-mer	0	0	10.76 ± 0.68 <sup>a</sup>
7-mer	6.72 ± 0.84	8.14 ± 0.82	14.87 ± 1.57
6-mer	10.05 ± 0.40	10.17 ± 1.30	20.22 ± 1.58
5-mer	10.08 ± 0.88	1.17 ± 0.26	11.26 ± 1.01

Note: Data presented are mean ± standard deviation (n=5).

NA= not applicable

<sup>a</sup> Due to similar masses, 3'- and 5'-deletion metabolites are indistinguishable  
(page 30 of Study Report)

**Table 12 Urinary Excretion (%) of ISIS 420915, Its Associated Metabolites, and Total Measured Oligonucleotide in Human Urine Samples Collected over 0-24 hours following the Last Dose (Day 22) in Study **ISIS 420915-CS1****

Dose (mg)	Dose (μM)	Time Point	Study Day	Subject No.	Sum of Oligonucleotide (μM)	%Dose Excreted	Mean	SD	%Dose Excreted Parent
300	39469	0-24 hr	22	(b)(6)	3857.8	9.77	13.5%	5.0%	0.78
					5442.9	13.79			0.96
					7208.5	18.26			0.99
					3450.2	8.74			0.62
					8157.4	20.67			1.01
					3892.1	9.86			0.31

SD = Standard Deviation

<sup>a</sup> Dose Level (μmole) = [Dose Level (mg)\*1000]/MW, where MW is the molecular weight of ISIS 420915 (7600<sup>(b)</sup><sub>(4)</sub> Daltons).

<sup>b</sup> Values presented as ISIS 420915-equivalent. See [Section 3.3](#) for the calculation of %Dose Excreted as ISIS 420915-equivalent.

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**Assessment of ISIS 420915 As an Inhibitor or a Substrate of Human BCRP, P-gp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3 and BSEP Mediated Transport**

(ISIS Study 420915-IS06; [REDACTED] (b) (4)  
[REDACTED] Final Report dated June 1, 2016; non-GLP; non-QA)

The ability of ISIS 420915 to act as a substrate or inhibitor of human BCRP, P-gp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, and BSEP transporters was evaluated using standard methods and reagents. The results demonstrated that ISIS 420915 did not act as a transport inhibitor at 100  $\mu\text{M}$  or as a transporter substrate at 10  $\mu\text{M}$  under the conditions tested. Results with positive controls met criteria for a valid study.

***In Vitro* Assessment of Cytochrome P450 Induction Potential of ISIS 420915 in Plated Cryopreserved Human Primary Hepatocytes**

(ISIS Study 420915-IS07; [REDACTED] (b) (4)  
[REDACTED] Final Report dated June 2, 2016; non-GLP; non-QA)

No consistent increases were observed in enzyme activities or mRNA levels for CYP1A2, CYP2B6, and CYP3A4 measured in cryopreserved human primary hepatocytes incubated with up to 100  $\mu\text{M}$  ISIS 420915 for 72 hours, compared with vehicle controls. Positive controls resulted in induction of 5- to 77-fold in enzyme activity and 5- to 50-fold in mRNA levels, as expected. Uptake of ISIS 420915 into hepatocytes was confirmed using LCMS in a separate experiment, reaching a maximum concentration of 105.57  $\mu\text{M}$  after 72 hours. ISIS 420915 did not act as an inducer of CYPs 1A2, 2B6, or 3A4 under the conditions tested.

***In Vitro* Assessment of Cytochrome P450 Inhibition Potential of ISIS 420915 in Cultured Cryopreserved Human Primary Hepatocytes**

(ISIS Study 420915-IS08; [REDACTED] (b) (4)  
Amended Final Report dated June 9, 2017; non-GLP; non-QA)

The ability of ISIS 420915 at concentrations up to 100  $\mu\text{M}$  to inhibit CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 was evaluated using standard in vitro methods and reagents.  $\text{IC}_{50}$  values for ISIS 420915 were all > 100  $\mu\text{M}$ , whereas those for positive controls ranged from 0.00108 to 1.545  $\mu\text{M}$ . Concentrations of ISIS 420915 measured in hepatocytes after incubation with 1, 10, and 100  $\mu\text{M}$ , were 5.66, 25.18, and 52.18  $\mu\text{M}$  respectively, after 30 minutes; and 12.01, 42.06, and 105.57  $\mu\text{M}$ , respectively, after 120 minutes. ISIS 420915 did not act as an inhibitor of CYPs under the conditions tested.

**5.2 Toxicokinetics**

Toxicokinetic data for inotersen were reviewed with the toxicity studies in mouse, rat, rabbit, and monkey.

### 5.3 Methods of Analysis

The analytical method validation studies listed in the table below were reviewed and found to adequately support the conclusions reported in the pivotal toxicity studies of inotersen in mouse, rat, rabbit, and monkey. In addition, evaluations of the levels of anti-drug antibodies in serum samples from monkey and mouse were supported by method validation Study 420915-MV08 and qualification Study 420915-MV09, respectively.

**Table 2 List of Analytical Methods Used for Quantitation of Inotersen in Biological Matrices**

Species	Method	Sample Matrix Type	Quantitation Range (LLOQ to ULOQ)	Study No.	GLP
Mouse	HELISA	Plasma	0.001 to 0.15 µg/mL	420915-MV12	Yes
Mouse	HPLC-UV	Tissue Homogenate	10 to 1500 µg/g	420915-MV01	Yes
Rat	HELISA	Plasma	0.001 to 0.15 µg/mL	420915-MV14	Yes
Rat	HPLC-UV	Tissue Homogenate	10 to 1500 µg/g	420915-MV15	Yes
Rabbit	HPLC-UV	Tissue Homogenate	10 to 1500 µg/g	420915-MV06	Yes
Monkey	HELISA	Plasma	0.002 to 0.15 µg/mL	420915-MV02	Yes
Monkey	HELISA	Plasma	0.002 to 0.15 µg/mL	420915-MV10	Yes
Monkey	HPLC-UV	Tissue Homogenate	10 to 1500 µg/g	420915-MV03	Yes
Mouse, Monkey, Human	IP-HPLC-ES/MS	Plasma and Urine	Various	420915-IS09	No

Abbreviations: HELISA = hybridization enzyme-linked immunosorbent assay; HPLC = high performance liquid chromatography; IP-HPLC-ES/MS = ion-pair HPLC-electrospray/mass spectrometry; LC-MS = liquid chromatography-mass spectrometry; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation

*(pages 13-14 of sponsor's Pharmacokinetics Written Summary)*

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

No single-dose toxicity studies were submitted.

### 6.2 Repeat-Dose Toxicity

#### A 13-Week Repeated Subcutaneous Dose Toxicity Study of ISIS 420915 and ISIS 401724 in Mice with a 4-Week Interim Necropsy and a 13-Week Recovery

(ISIS Study 420915-AS01; (b) (4) initiated October 20, 2010; GLP [with acceptable exceptions]; QA)

CD-1 mice were administered placebo, ISIS 420915, or ISIS 401724 (a mouse TTR-specific homolog ASO) via SC injection once weekly (following every other day loading doses during Week 1) through Week 4 or 13 as described in the table below:

#### Study design

Group	Test Substance	Dose Level (mg/kg/wk) <sup>a</sup>	Number of Animals (Male/Female)	Necropsy (Male/Female)		
				Interim (Day 30)	Terminal (Day 93)	Recovery (Day 182)
1	Placebo	0	22/22	6/6	10/10	6/6
2	ISIS 420915	4	16/16	6/6	10/10	-
3	ISIS 420915	12	22/22	6/6	10/10	6/6
4	ISIS 420915	40	16/16	6/6	10/10	-
5	ISIS 420915	100	22/22	6/6	10/10	6/6
6	ISIS 401724	40	16/16	6/6	10/10	-

<sup>a</sup> Animals received 4 doses at these dose levels on Week 1 (Days 1, 3, 5, and 7), and one dose per week for the remainder of the dosing phase of the study. Throughout this report, doses will be referenced as mg/kg/week; however, it should be noted that the actual weekly amount administered during Week 1 is 4X that of the weekly doses administered for the remainder of the study.

No drug-related effects were observed on mortality, body weight, food consumption, ophthalmology, or gross pathology. Drug-related effects included reduced red cell mass (~10% in M at 100 mg/kg/week; RBC count, hematocrit, and/or hemoglobin; all time points); increased total cholesterol (1.5x in M at 100 mg/kg/week at the interim necropsy); and increased ALT (1.9x in M at 100 mg/kg/week at the terminal necropsy), compared to controls. Relative weights of spleen and thymus were increased and decreased, respectively, in animals administered  $\geq 40$  mg/kg/week ISIS 420915, compared to controls. Dose-dependent increases were observed in the incidence and severity of cytoplasmic basophilic granules in lymph node histiocytes, liver Kupffer cells, and kidney proximal tubular epithelium at the terminal necropsy. Additional effects included increased incidence of minimal mononuclear infiltrates in liver and injection sites, minimal to moderate Kupffer cell hypertrophy in liver, minimal to moderate

histiocyte hypertrophy in lymph nodes, and increased incidence of slight thymic involution. Recovery groups showed effects similar to those observed at the terminal necropsy but at lower incidence and severity, except for the effects on thymic weight and involution, which were maintained.

Toxicokinetic analyses showed extensive distribution of ISIS 420915 into kidney and liver tissues (884 and 810  $\mu\text{g/mL}$ , respectively, at 100 mg/kg/week on Day 93), reaching near steady-state levels by 30 days, except in the kidney of animals administered 100 mg/kg/week. Estimated tissue half-life values were 15.8 and 16.9 days in kidney and liver, respectively, at 100 mg/kg/week. Short oligonucleotide metabolites of ISIS 420915 observed in tissues were consistent with endonuclease cleavage as the primary route of metabolism. Parent drug accounted for 80-92% of total oligonucleotide present in tissues.

Animals administered 40 mg/kg/week ISIS 401724 showed similar toxicity to those given 40 mg/kg/day ISIS 420915, along with reductions of ~70% in liver TTR mRNA compared to controls. No unique toxicities were observed. The NOAEL was the high dose of 100 mg/kg/week ISIS 420915.

**A 13-Week Repeat Dose Toxicity Study of ISIS 420915 and ISIS 401724 in CByB6F1-Tg(HRAS)2Jic Wild Type and Hemizygous Transgenic Mice**

(ISIS Study 420915-AS12P; (b) (4) initiated April 7, 2015; GLP; QA; Final Report dated June 24, 2016; draft report reviewed by David B. Hawver, Ph.D., under IND 113968, February 11, 2016; no important changes were made between submission of the draft report and the final report)

CByB6F1-Tg(HRAS)2Jic wild type (WT) and hemizygous transgenic (HT) mice were administered placebo, ISIS 420915, or ISIS 401724 (a mouse TTR-specific homolog ASO) via SC injection once weekly (following every other day loading doses during Week 1) through Week 13 as described in the table below:

<b>Table B: Group Assignments</b>			
Group Number	Dose Level (mg/kg/week) <sup>a, b</sup>	Treatment	Number of Animals
			Male/Female <sup>b</sup>
<b>Main Study</b>			
1	0	Saline (Control) <sup>c</sup>	6/6
2	12	ISIS 420915 <sup>c</sup>	10/10
3	40	ISIS 420915 <sup>c</sup>	10/10
4	100	ISIS 420915 <sup>c</sup>	10/10
5	100	ISIS 420915 <sup>d</sup>	10/10
6	40	ISIS 401724 <sup>c, e</sup>	10/10
<p><sup>a</sup>The control and test articles were administered on Days 1, 3, 5, and 7 and then weekly (Days 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91) via subcutaneous injection. The loading dose during the first week is expressed as mg/kg/dose, while the maintenance doses on Weeks 2 through 13 are expressed as mg/kg/week.</p> <p><sup>b</sup>The control and test articles were administered via subcutaneous injection, with a scheduled necropsy occurring on Day 93.</p> <p><sup>c</sup>Wild type (Groups 1, 2, 3, 4, and 6)</p> <p><sup>d</sup>Hemizygous (Group 5 only)</p> <p><sup>e</sup>mouse-specific TTR oligonucleotide</p>			

(page 15 of Study Report)

One HDF WT mouse was found dead of uncertain cause on Day 72, with no prior clinical signs. No clear drug-related effects were observed in clinical signs, body weight, food consumption, or gross pathology. Drug-related effects on hematology parameters included decreases in red cell mass (RBC count, hematocrit, and hemoglobin; 7-13%), reticulocytes (30%), and platelets (31%) in HD groups compared to controls.

Drug-related microscopic changes were generally related to accumulation of oligonucleotide in tissues and minimal to mild inflammatory responses (basophilic granules in renal tubules and liver; vacuolated/granular macrophages in liver, spleen, lymph nodes, and injection sites; granulomatous inflammation in liver; and lymphocytic depletion in thymus). Minimal to mild individual cell necrosis was observed in liver in all drug-dosed groups (except LDM and MDF), and was correlated with slight (1.4-2x) increases in ALT, AST, and ALP in HD WT and HT groups. Microscopic changes observed only in HT mice included mild cystitis in urinary bladder (2/10 F) and minimal to mild degeneration/necrosis of skeletal muscle (9/10 M, 8/10 F); in the absence of control HT mice, it is unclear if these effects were drug-related. No important differences in toxicity were observed between animals administered 40 mg/kg/week ISIS 420915 and those given 40 mg/kg/week ISIS 401724. Liver TTR mRNA levels were reduced ~80% in animals administered 40 mg/kg/week ISIS 401724 for 13 weeks, compared to controls.

## 26-Week Repeat-Dose Subcutaneous Toxicity Study of ISIS 420915 in CD-1 Mice with a 13-Week Recovery

Study no.:	ISIS Study 420915-AS07 (b) (4)
Study report location:	edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 8, 2011
GLP compliance:	Yes, except for the cytokine/chemokine analysis
QA statement:	Yes
Drug, lot #, and % purity:	Inotersen Lot # CA420915-001, 93.8% Inotersen for Injection Lots RP420915-009, -010, -011, and -012

### Key Study Findings

- The NOAEL was the high dose of 80 mg/kg/week.

### Methods

Doses:	0, 3, 10, 40, and 80 mg/kg/week
Frequency of dosing:	Days 1, 3, 5, 7, then once weekly to Day 182
Route of administration:	Subcutaneous injection, alternating among 4 sites: left and right shoulders and flanks
Dose volume:	10 mL/kg
Formulation/Vehicle:	Sterile solution of inotersen sodium in water for injection, pH 7.5-8.5 Placebo Control: Phosphate buffered saline
Species/Strain:	Crl:CD1 <sup>®</sup> (ICR) mice
Number/Sex/Group:	12 Main Study; 6 at 0, 40, and 80 mg/kg/week 13-week recovery period
Age:	7.5 weeks old after acclimatization period
Weight:	28.2-33.5 g M; 22.5-28.0 g F
Satellite groups:	None
Unique study design:	Anti-drug antibody (ADA) analysis
Deviation from study protocol:	No deviations reported had an impact on the outcome or integrity of the study

### Observations and Results

#### Dosing Solution Analysis

Preformulated vials of each inotersen dosing solution were analyzed for inotersen concentration on Feb 14, 2012, and on March 27, 2012, using a validated ion-pair HPLC-MS method. Saline placebo control solutions were analyzed similarly on the latter date.

All preformulated inotersen dosing solutions were within acceptable ranges of nominal concentrations when analyzed on Feb 14, 2012 (102.4-102.8%) and on March 27, 2012 (101.3-102.3%). No inotersen was detected in the placebo control samples.

### **Mortality**

Mortality was assessed twice daily.

No drug-related effects were observed. One female (Animal 4516) administered 40 mg/kg/week was found dead on Day 248 due to lymphoma.

### **Clinical Signs**

Detailed clinical examinations were conducted twice daily (predose and 2-4 hours postdose on dosing days). The appearance of injection sites used was assessed prior to dosing on each dosing day.

No drug-related effects were observed.

### **Body Weights**

Body weight was assessed once weekly.

No drug-related effects were observed.

### **Food Consumption**

Food consumption was assessed once weekly.

No drug-related effects were observed.

### **Ophthalmoscopy**

Ophthalmoscopic examinations were conducted prior to dosing initiation and prior to the terminal and recovery necropsies.

### **Hematology**

Blood was collected via cardiac puncture from the first 6/sex/group prior to the terminal necropsy (Day 184) and from the first 3/sex/group prior to the recovery necropsy (Day 273). The following parameters were analyzed: erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, mean platelet volume, red cell distribution width (RDW), white blood cell count, differential leukocyte absolute count, and reticulocyte count.

As shown in the table below, slight and moderate reductions in red cell mass (erythrocyte counts, hemoglobin, and hematocrit) were observed in males administered 40 and 80 mg/kg/week inotersen, respectively. Moderate reductions in platelet counts and dose-dependent increases in RDW were also seen in males at these doses. Similar increases in RDW were observed in females. Slight reductions in red cell mass were still observed in the HDM group following the 13-week recovery period, demonstrating partial reversibility; effects on RDW and platelets showed full reversibility.

#### Effects of Inotersen on Hematology Parameters in Male Mice

	0 mg/kg/week		40 mg/kg/week		80 mg/kg/week	
	Terminal	Recovery	Terminal	Recovery	Terminal	Recovery
Erythrocytes (10 <sup>6</sup> /μL)	9.210	9.393	9.148 (-0.7%)	9.420 (0.3%)	7.966 (-13.5%)	8.923 (-5.0%)
Hemoglobin (g/dL)	14.20	14.57	13.76 (-3.1%)	14.30 (-1.9%)	11.76 (-17%)	13.57 (-6.9%)
Hematocrit (%)	46.56	48.33	44.42 (-4.6%)	47.37 (-2.0%)	38.74 (-16.8%)	43.97 (-9.0%)
RDW (%)	12.80	13.67	13.66 (6.7%)	13.87 (1.5%)	14.96 (17%)	13.50 (-1.2%)
Platelets (10 <sup>3</sup> /μL)	1408.6	1087.7	903.4 (-36%)	1139.0 (4.7%)	827.6 (-41%)	1267.7 (17%)

*(Reviewer's table based on data on pages 370-379 of Study Report; values in parentheses are percentage change from control values)*

#### Clinical Chemistry

Blood was collected via cardiac puncture from the last 6/sex/group prior to the terminal necropsy (Day 184) and from the last 3/sex/group prior to the recovery necropsy (Day 273). The following parameters were analyzed: BUN, total protein, albumin, globulin, albumin:globulin ratio, ALT, ALP, AST, calcium, chloride, cholesterol, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, triglyceride, and creatine kinase.

As shown in the table below, drug-related effects observed in animals administered 40 and 80 mg/kg/week included mild reductions in albumin and total protein and mild increases in AST and ALT, compared to controls. The increases in AST and ALT were consistent with slight hepatotoxicity, though no necrosis was observed in liver upon histopathological examination. Recovery groups showed mild reductions in albumin and total protein compared to controls, especially in the HDM group.

**Effects of Inotersen on Clinical Chemistry Parameters**

	0 mg/kg/week		40 mg/kg/week		80 mg/kg/week	
	M	F	M	F	M	F
Albumin (g/dL)	2.85	3.12	2.47 (-13%)	2.82 (-9.6%)	2.48 (-13%)	2.70 (-13%)
Total Protein (g/dL)	5.33	5.60	5.03 (-5.6%)	5.13 (-8.4%)	4.97 (-6.8%)	4.93 (-12%)
AST (U/L)	42.5	71.8	74.5 (75%)	62.0 (-14%)	73.0 (72%)	84.7 (18%)
ALT (U/L)	23.2	27.2	69.8 (201%)	28.0 (2.9%)	67.3 (190%)	41.2 (51%)

*(Reviewer's table based on data on pages 386-393 of Study Report; values in parentheses are percentage change from control values)*

**Cytokine/Chemokine Analysis**

Blood was collected via cardiac puncture from the first 6/sex/group prior to the terminal necropsy (Day 184). Serum levels of IL-10, IL-1 $\beta$ , IL-6, and MCP-1 were measured by ELISA.

No drug-related effects were observed.

**Serum Immunoglobulin (IgG and IgM) Analysis**

Blood was collected via cardiac puncture from the first 6/sex/group prior to the terminal necropsy (Day 184). Serum levels of total IgG and IgM were measured a qualified ELISA method.

No drug-related effects were observed.

**Urinalysis**

Urinalysis was not conducted.

**Gross Pathology**

All animals surviving to scheduled termination were necropsied at the terminal sacrifice (Day 182) or at the end of the recovery period (Day 456), following euthanization by carbon dioxide inhalation followed by exsanguination via the abdominal vena cava. Evaluations included palpable masses, subcutaneous masses, body cavities (abdominal, thoracic, and cranial), and organs, as they were removed, weighed, and placed in fixative.

No drug-related effects were observed.

## Organ Weights

Weights were recorded for the following organs: adrenal glands, brain, heart, kidneys, liver, lung (with bronchi), ovaries (with oviducts), pituitary gland, prostate/seminal vesicles, salivary glands (mandibular/sublingual), spleen, testes, thymus, thyroid (with parathyroid), and uterus (body and cervix).

As shown in the sponsor's tables below, increases were observed in the relative weights of liver and spleen, and reductions were observed in the relative weights of thymus and heart in males, compared to controls. Effects on the liver and heart were maintained in the HD group following the 13-week recovery period.

<b>Test Article-related Organ Weight Changes - Terminal Male and Female (Percent change relative to control)</b>				
<b>Dose level: mg/kg/week</b>	40		80	
<b>Sex (Male, Female)</b>	M	F	M	F
<b>Liver (g)</b>	NSD	NSD	↑16.36 <sup>b</sup>	NSD
<b>Liver/BWt%</b>	NSD	NSD	↑12.90 <sup>b</sup>	↑13.58 <sup>a</sup>
<b>Spleen (g)</b>	NSD	NSD	↑27.08 <sup>b</sup>	NSD
<b>Spleen/BWt%</b>	NSD	NSD	↑23.78 <sup>a</sup>	NSD
<b>Thymus (g)</b>	↓41.94 <sup>b</sup>	NSD	NSD	NSD
<b>Thymus/BWt%</b>	↓42.77 <sup>b</sup>	NSD	NSD	NSD
<b>Heart (g)</b>	NSD	NSD	NSD	NSD
<b>Heart/BWt%</b>	NSD	NSD	↓14.08 <sup>a</sup>	NSD
<sup>a</sup> Significantly different from control; (p<0.05) <sup>b</sup> Significantly different from control; (p<0.01) BWt - Body Weight ↑ - Increased, ↓ - Decreased, NSD- No Significant Difference M - Male, F - Female, Number examined: 12/sex/group				

<b>Test Article-related Organ Weight Changes - Recovery Male and Female (Percent change relative to control)</b>		
<b>Dose level: mg/kg/week</b>	80	
<b>Sex (Male, Female)</b>	M	F
<b>Liver (g)</b>	↑23.50 <sup>a</sup>	↑34.03 <sup>a</sup>
<b>Liver/BWt%</b>	NSD	↑19.57 <sup>a</sup>
<b>Heart (g)</b>	NSD	NSD
<b>Heart/BWt%</b>	↓17.48 <sup>a</sup>	NSD
<sup>a</sup> Significantly different from control; (p<0.05) BWt - Body Weight Number examined: 6/sex/group (5/sex for 40 mg/kg/week female)		
↑ - Increased, ↓ - Decreased M - Male, F - Female NSD- No Significant Difference		

(pages 498-499 of Study Report)

## Histopathology

The following tissues from all animals were examined microscopically: adrenal glands, aorta, bone and bone marrow (femurs, sternum), brain, cecum, colon, duodenum, epididymides, esophagus, eyes (with optic nerves), gallbladder, GALT, Harderian gland, heart, ileum, injection sites, jejunum, joint (tibiofemoral), kidneys, lacrimal gland, larynx, liver, lung (with bronchi), lymph node (mandibular, mesenteric, inguinal), mammary gland (F), ovaries (with oviducts), pancreas, pituitary gland, prostate, rectum, salivary glands (submandibular/sublingual, parotid), sciatic nerves, seminal vesicles, skeletal muscles (biceps femoris), skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach (glandular and nonglandular), testes, thymus, thyroid (with parathyroid), tongue, trachea, ureter, urinary bladder, uterus (body and cervix), vagina, and gross lesions (if any).

The battery of tissues examined was adequate. No Peer Review was conducted. A signed pathology report was provided.

### Histological Findings

As shown in the table below, drug-related effects included accumulation of basophilic granules (inotersen) in kidney tubular epithelial cells ( $\pm$  vacuolation) and Kupffer cells in liver; increased incidence and severity of lymphoid depletion in thymus cortex; and increased incidence of extramedullary hematopoiesis in spleen. The effects on Kupffer cells were often seen together with slight hypertrophy and/or cytoplasmic vacuolation. Minimal to moderate accumulation of basophilic granules was also observed in macrophages in multiple organs throughout the body, particularly in lymph nodes, spleen, and the skin of the injection sites.

**Histopathological Effects of Inotersen, Terminal Sacrifice**

Dose (mg/kg/week)	0		3		10		40		80	
	M	F	M	F	M	F	M	F	M	F
Kidney tubular cell basophilic granules	0	0	0	0	5 min 1 mild	11 min 1 mild	9 min 2 mild	12 min	9 min 1 mild 2 mod	10 min 2 mild
Kidney tubular cell vacuolation	0	0	0	0	1 mild	0	0	0	3 min 0 mild 1 mod	0
Liver Kupffer cell basophilic granules	0	0	0	0	11 min	12 min	7 min 5 mild	6 min 6 mild	0 min 12 mild	0 min 12 mild
Thymus cortex lymphoid depletion	12 min	11 min 1 mild	11 min 1 mild	10 min 1 mild 1 mod	12 min	10 min	12 min	11 min 1 mild	4 min 6 mild 1 mod	9 min 3 mild
Spleen extramedullary hematopoiesis	0	0	0	0	1 min	0	0	4 min	3 min	6 min

(Reviewer's table based on data on pages 578-627 of Study Report; N=12/sex/group; min = minimal; mod = moderate)

As shown in the table below, the incidence and severity of the drug-related effects were partially or completely reversed following the 13-week recovery period, except for the thymic cortical lymphoid depletion. However, the study pathologist reported that thymic medullary hyperplasia in these animals made it difficult to assess the cortical lymphoid depletion; therefore, it is not clear if the increased severity of these effects was related to drug.

### Histopathological Effects of Inotersen, Recovery Sacrifice

Dose (mg/kg/week)	0		40		80	
	M	F	M	F	M	F
Kidney tubular cell basophilic granules	0	0	1 min 1 mild	1 min	1 min 0 mild 1 mod	1 min
Kidney tubular cell vacuolation	0	0	0 min 0 mild 1 mod	0	0 min 0 mild 1 mod	0
Liver Kupffer cell basophilic granules	0	0	6 min	2 min	6 min	5 min
Thymus cortex lymphoid depletion	6 min	4 min 2 mild	4 min 1 mild 1 mod	0 min 3 mild 2 mod	3 min 2 mild	1 min 3 mild 2 mod
Spleen extramedullary hematopoiesis	0	0	1 min	0	0 min 1 mild	1 min

(Reviewer's table based on data on pages 635-652 of Study Report; N=6/sex/group)

### Toxicokinetics

Samples of liver and kidney were collected from the first 6/sex/group at the terminal necropsy (Day 184) and from all surviving animals at the recovery necropsy (Day 274) and analyzed for levels of inotersen using a validated HPLC assay. No blood samples were collected for TK analysis.

Concentrations of inotersen were greater in kidney than in liver in the two lower dose groups and increased in both organs less than proportionally with dose from 10 to 80 mg/kg/week. No consistent sex-related differences were observed. The estimated half-life of elimination from these tissues was 15.5 to 19.3 days.

Dose (mg/kg/wk):	0 <sup>b</sup>	3	10	40	80
<b>Tissue Concentrations (Mean ± SD)</b>					
Liver (µg/g)					
Day 184	NA	25.2 ± 6.23	90.1 ± 12.6	296 ± 55.9	528 ± 103
Day 273	NA	NA	NA	8.11 ± 10.2	21.5 ± 19.4
Kidney (µg/g)					
Day 184	NA	53.4 ± 17.5	239 ± 264	307 ± 104	535 ± 250
Day 273	NA	NA	NA	5.70 ± 13.3	13.3 ± 18.7

(page 12 of sponsor's Toxicology Tabulated Summary)

**Anti-Inotersen Antibody Analysis**

Blood was collected via cardiac puncture from the first 6/sex/group prior to the terminal necropsy (Day 184). Serum levels of anti-Inotersen antibodies were measured by ELISA.

Only 2 of 60 samples assayed were positive for anti-Inotersen antibodies: Control Animal 1503 (titer = 1); and 3 mg/kg/week Animal 2504 (titer = 2).

**A 26-Week Repeat Dose Toxicity Study of ISIS 420915 and ISIS 594799 in Rats**

(ISIS Study 420915-AS11; (b) (4) initiated January 5, 2015; GLP; QA; Final Report dated October 27, 2016; draft report reviewed by David B. Hawver, Ph.D., under IND 113968, February 11, 2016; no important changes were made between submission of the draft report and the final report)

Sprague-Dawley rats were administered placebo, ISIS 420915, or ISIS 594799 (a rat TTR-specific homolog ASO) via SC injection once weekly (following twice-weekly loading doses during the first 2 weeks) through Week 26 as described in the table below:

<b>Table B: Study Design</b>			
Group Number	Dose Level (mg/kg/week) <sup>a, b</sup>	Treatment	Number of Animals
			Male/Female <sup>b</sup>
<b>Main Study</b>			
1	0	Saline (Control)	10/10
2	5	ISIS 420915	10/10
3	15	ISIS 420915	10/10
4	40	ISIS 420915	10/10
5	15	ISIS 594799 <sup>c</sup>	10/10
<b>Toxicokinetics (TK)<sup>d</sup></b>			
6	15	ISIS 420915	24 males
7	40	ISIS 420915	24 males

<sup>a</sup>The control and test articles were administered on Days 1, 5, 10, and 14 and then weekly (Days 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, 119, 126, 133, 140, 147, 154, 161, 168, 175, 181) via subcutaneous injection. The loading dose during the first two weeks is expressed as mg/kg/dose, while the maintenance doses on Weeks 3 through 26 are expressed as mg/kg/week. Dosing for TK animals was the same regimen, but through Day 91 only.

<sup>b</sup>The control and test articles were administered via subcutaneous injection, with the scheduled necropsy occurring on Day 183 for main study animals.

<sup>c</sup>Rat-specific TTR oligonucleotide

<sup>d</sup>TK groups; Plasma was collected from animals in Groups 6 and 7 at various time points (cohorts of 6 rats/group/time point) following dose administration through Day 91 (last dose on Day 91). Tissues were collected from animals in Groups 6 and 7 (12 rats/group) at necropsy on Day 93 (48 hours post the Day 91 dose).

(page 16 of Study Report)

One HDM was euthanized in extremis on Day 161; the cause of morbidity and relationship to drug was unclear. Body weight gain was reduced 3%, 16%, 39%, and 40% in M; and 13%, 18%, 42%, and 49% in F; in LD, MD, HD, and ISIS 594799 groups, respectively, compared to controls. Final body weight was reduced 2%, 11%, 26%, and 27% in M; 6%, 9%, 20%, and 24% in F; in LD, MD, HD, and ISIS 594799 groups, respectively, compared to controls. Effects on body weight were related to reduced food consumption. Drug-dependent effects on hematology parameters included decreases in hemoglobin (up to 28-22%), neutrophils (up to 69%), eosinophils (up to 85%), and platelets (up to 31%); and increases in reticulocytes (up to 44%), lymphocytes (up to 52%), and monocytes (up to 232%). Serum TNF $\alpha$  levels increased dose-dependently (2x to 8x) compared to controls.

Increased urine protein/creatinine ratio (up to 10.7x, compared to controls) and albumin/creatinine ratio (up to 163x) correlated with microscopic observations in kidney in MD, HD, and ISIS 594799 groups (basophilic granules and cytoplasmic vacuolation in tubular epithelial cells, vacuolated/granular macrophages, interstitial mononuclear cell infiltration, and increased glomerular cellularity and matrix). Macrophages containing cytoplasmic basophilic granules (likely representing oligonucleotide) and mononuclear cell infiltration were observed throughout various tissues. Drug-related effects were also observed in liver (bile duct hyperplasia, minimal individual hepatocyte necrosis), lymph nodes (hyperplasia), spleen (hyperplasia, increased hematopoietic cellularity), thymus (lymphoid depletion), bone marrow (increased hematopoietic cellularity, vacuolated cells), bone (increased trabecular bone), ovaries (interstitial cell hypertrophy/hyperplasia), uterus (increased endometrial thickness), and injection sites (macrophages, infiltration, fibrosis, edema).

Results of toxicokinetic analyses of plasma, kidney, and liver are shown in the tables below:

**Table 2. Summary of Plasma Toxicokinetic Parameters in Male Rats Following Single or Multiple Subcutaneous Administrations of ISIS 420915**

Group	Dose Level (mg/kg)	Number of Doses	Study Day	T <sub>max</sub> (hr)	C <sub>max</sub> (μg/mL)	AUC <sub>0-24hr</sub> (μg•hr/mL)	AUC <sub>0-48hr</sub> (μg•hr/mL)	CL/F <sub>0-48hr</sub> (mL/hr/kg)	MRT <sub>0-48hr</sub> (hr)
6	15	1	1	1.0	32.3	138	139	108	3.23
		15	91	1.0	61.3	408	421	35.6	4.95
7	40	1	1	2.0	93.7	465	471	85.0	3.57
		15	91	0.50	147	1000	1120	35.8	8.01

(page 811 of Study Report)

**Table 3. Summary of ISIS 420915 Tissue Concentrations in Rats (Gender combined) Following Multiple Subcutaneous Administrations of ISIS 420915**

Matrix	Group	Dose Level (mg/kg)	Number of Doses	Study Day	Time After Last Dose (hr)	N	ISIS 420915 Mean $\pm$ SD ( $\mu$ g/g)
Kidney	2	5	28	183	48	12	865 $\pm$ 279
	3	15	28	183	48	12	1270 $\pm$ 434
	4	40	28	183	48	12	2180 $\pm$ 341
	6	15	15	93	48	12	1090 $\pm$ 266
	7	40	15	93	48	12	1950 $\pm$ 473
Liver	2	5	28	183	48	12	165 $\pm$ 28.2
	3	15	28	183	48	12	415 $\pm$ 158
	4	40	28	183	48	12	772 $\pm$ 281
	6	15	15	93	48	12	383 $\pm$ 254
	7	40	15	93	48	12	476 $\pm$ 210

*(page 811 of Study Report)*

Liver TTR mRNA levels were reduced 34% in M and 18% in F animals administered 15 mg/kg/week ISIS 594799 for 26 weeks, compared to controls.

Based on adverse effects on the kidney (increased cellularity of glomeruli and increased glomerular matrix, correlated with marked increases in urine protein/creatinine and albumin/creatinine ratios and body weight gain reductions of  $\geq 16$ -18%) observed at  $\geq 15$  mg/kg/week, the NOAEL was the LD of 5 mg/kg/dose ISIS 420915. No important differences in toxicity were observed between animals administered 15 mg/kg/week ISIS 420915 and those given 15 mg/kg/dose ISIS 594799.

**A 13-Week Repeated Subcutaneous Dose Toxicity Study of ISIS 420915 in Cynomolgus Monkeys with a 4-Week Interim Necropsy and a 13-Week Recovery** (ISIS Study 420915-AS02; (b) (4) initiated October 12, 2010; GLP [with acceptable exceptions]; QA)

Cynomolgus monkeys were administered placebo or ISIS 420915 via SC injection once weekly (following every other day loading doses during Week 1) through Week 1, 4, or 13 as described in the table below:

Group	Dose Level (mg/kg/week)*	Number of Animals (Male/Female)	Necropsy (Male/Female)		
			Interim (Day 30)	Terminal (Day 93)	Recovery (Day 182)
1	0	7/7	2/2	3/3	2/2
2	4	5/5	2/2	3/3	-
3	8	5/5	2/2	3/3	-
4	12	7/7	2/2	3/3	2/2
5	40	7/7	2/2	3/3	2/2
6 (TK)	8	7 <sup>a</sup> /7 <sup>a</sup>	-	-	-

<sup>a</sup> Dose Days 1, 3, 5, 7, with 1 animal/sex necropsy on Days 3 (this animal was not dosed prior to necropsy), 9, 16, 23, 30, 44, and 58

\* During Week 1, this dose was the amount administered on Days 1, 3, 5, and 7. Throughout this report, doses will be referenced as mg/kg/week; however, it should be noted that the actual weekly amount administered during Week 1 is 4X that of the weekly doses administered for the remainder of the study.

- : No animals in this category

*(page 19 of Study Report)*

No drug-related effects were observed on mortality, clinical signs, body weight, food consumption, ECG, or ophthalmology parameters. Plasma levels of complement split product Bb were transiently increased within 4 hours of dosing at 12 and 40 mg/kg/week (2.4x and 11.6x, respectively, on Day 1; and 1.4x and 6.2x, respectively, on Day 91), compared to pre-dose values. Reductions in serum albumin (15%) and albumin/globulin ratios (30%) were seen in HD animals compared to controls on Day 93.

Dose-dependent accumulation of basophilic granules was observed in kidney proximal tubular epithelial cells (associated with cytoplasmic vacuolation), liver Kupffer cells (associated with hypertrophy), and lymph node histiocytes (associated with hypertrophy). The effects observed in kidney were considered adverse in the HD group, as they also included minimal to mild multifocal tubular epithelial degeneration/regeneration, tubular dilatation, erythrocytic tubular casts, multifocal interstitial mononuclear cell infiltration, fibroconnective tissue proliferation, and hemorrhage. No adverse effects were observed following the 13-week recovery period. The NOAEL was 12 mg/kg/dose.

As shown in the tables below, ISIS 420915 was rapidly and extensively distributed into tissues following SC administration in monkey, reaching near steady-state concentrations in kidney and liver by Day 30.

**Table A. Summary of Selected ISIS 420915 Plasma Toxicokinetic Parameters Calculated in Cynomolgus Monkeys. Values presented as Mean  $\pm$  SD.**

Group	Dose Level (mg/kg)	Day	Number of Doses	Route	C <sub>max</sub> (µg/mL)	AUC <sub>0-48hr</sub> (hr*µg/mL)	MRT <sub>last</sub> <sup>a</sup> (hr)	t <sub>1/2λz</sub> (days)
2	4	28	7	SC	11.6±3.51	53.0±12.1	4.56±1.01	NA
		91	16	SC	7.45±2.80	49.5±13.5	6.83±0.990	NA
3	8	28	7	SC	20.8±3.27	121±15.9	4.94±0.814	NA
		91	16	SC	24.3±7.61	145±19.5	5.33±1.49	NA
4	12	28	7	SC	35.6±6.57	220±28.9	5.01±0.766	NA
		91	16	SC	38.5±5.96	254±36.5	5.59±0.482	29.2±4.93
5	40	28	7	SC	85.9±21.2	935±104	8.37±0.885	NA
		91	16	SC	118±15.7	1290±130	8.60±0.681	27.5±6.75
6	8	1	1	SC	30.0±4.41	148±26.6	4.22±0.844	NA
		7	4	SC	24.2±4.04	135±26.0	4.67±0.656	17.0

NA = Not Applicable

<sup>a</sup> MRT<sub>last</sub> was calculated from plasma concentrations ranging from time 0 to 48 hours.

(page 1985 of Study Report)

**Table B. Summary of ISIS 420915 Kidney Cortex and Liver Concentrations after Dosing and Estimated Elimination Half-Life Values in Monkeys. Values presented as Mean  $\pm$  SD.**

Matrix	Group	Dose Level (mg/kg)	Route	Study Day	Number of Doses	N	ISIS 420915 Conc. ( $\mu$ g/g)	$t_{1/2\lambda z}$ (days)
Kidney Cortex	2	4	SC	30	7	4	449 $\pm$ 115	NA
				93	16	6	462 $\pm$ 149	
	3	8	SC	30	7	4	848 $\pm$ 348	NA
				93	16	6	717 $\pm$ 175	
	4	12	SC	30	7	4	1030 $\pm$ 223	NA
				93	16	6	823 $\pm$ 170	
				182	16	4	37.4 $\pm$ 14.2	
	5	40	SC	30	7	4	2110 $\pm$ 299	NA
				93	16	6	2090 $\pm$ 272	
				182	16	4	223 $\pm$ 149	
	6	8	SC	3	1	2	507 $\pm$ 106	13.5 <sup>a</sup>
				9	4	2	771 $\pm$ 484	
				16	4	2	290 $\pm$ 21.7	
				23	4	2	389 $\pm$ 281	
				30	4	2	179 $\pm$ 31.2	
				44	4	2	80.2 $\pm$ 41.3	
				58	4	2	60.4 $\pm$ 11.7	
	Liver	2	4	SC	30	7	4	369 $\pm$ 52.5
93					16	6	374 $\pm$ 55.3	
3		8	SC	30	7	4	531 $\pm$ 44.0	NA
				93	16	6	479 $\pm$ 80.8	
4		12	SC	30	7	4	677 $\pm$ 67.6	NA
				93	16	6	772 $\pm$ 62.0	
				182	16	4	14.1 $\pm$ 11.1	
5		40	SC	30	7	4	1510 $\pm$ 429	NA
				93	16	6	1440 $\pm$ 189	
				182	16	4	163 $\pm$ 35.7	
6		8	SC	3	1	2	126 $\pm$ 17.8	18.8 <sup>a</sup>
				9	4	2	381 $\pm$ 66.4	
				16	4	2	417 $\pm$ 63.0	
				23	4	2	291 $\pm$ 55.6	
				30	4	2	222 $\pm$ 43.4	
				44	4	2	89.8 $\pm$ 56.6	
				58	4	2	101 $\pm$ 21.0	

NR = Not Reported

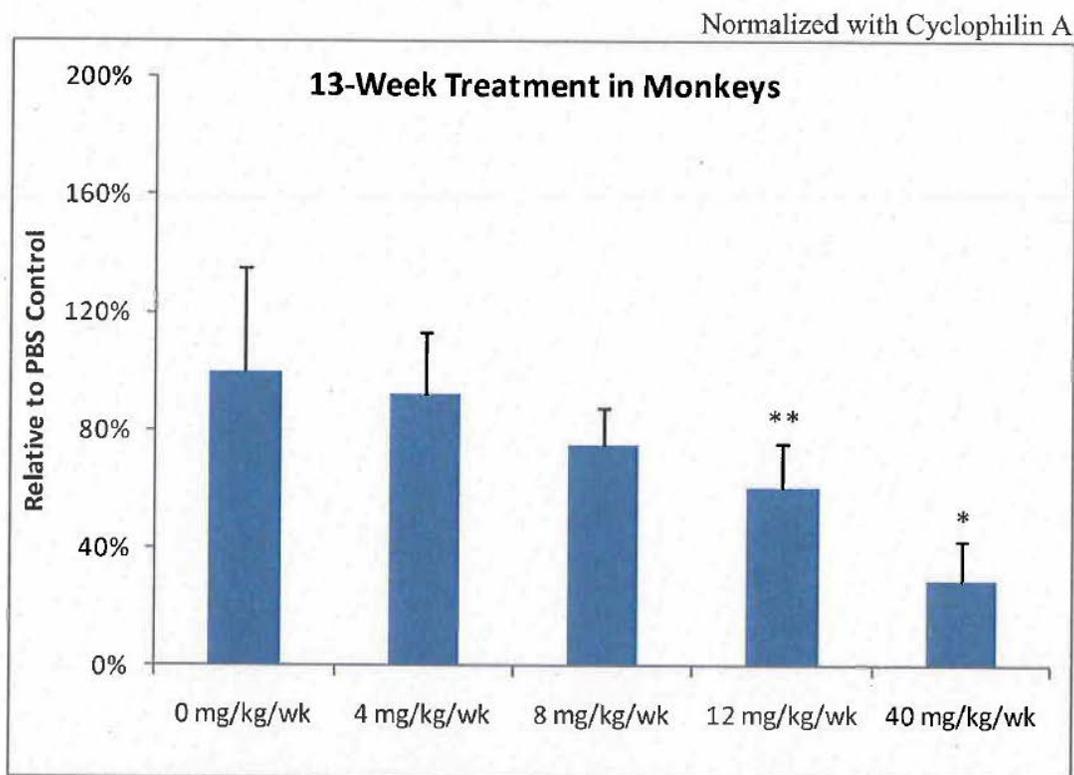
<sup>a</sup>  $t_{1/2\lambda z}$  values reported for 8 mg/kg on Day 7 (after 4 doses)

(page 1987 of Study Report)

Evaluation of metabolites showed that parent drug was the major species present in liver and kidney cortex (~83-95% of total), with nuclease-mediated cleavage products present at low levels ( $\leq$  5%).

As shown in the figure below, TTR liver mRNA levels were dose-dependently reduced up to 70% following 13 weeks of SC administration of ISIS 420915; TTR mRNA levels remained partially reduced (~35%) after 13 weeks of recovery, compared to controls.

**Figure 2. Dose-Dependent Reductions of Liver TTR mRNA Levels after 13-Week Treatment**



\*: Statistically significant from control; ( $p < 0.01$ )

\*\* : Statistically significant from control; ( $p < 0.05$ )

(page 2101 of Study Report)

### A 39-Week Repeat Subcutaneous Dose Toxicity Study of ISIS 20915 in Cynomolgus Monkeys with a 26-Week Interim Necropsy and a 26-Week Recovery

Study no.: Sponsor Study 420915-AS08  
 (b) (4)

Study report location: edr

Conducting laboratory and location: (b) (4)

Date of dosing initiation: April 26, 2011

GLP compliance: Yes, with the following exceptions:  
 analyses of complement split product Bb and C3, cytokine/chemokines, target liver TTR mRNA, plasma TTR and RBP4, diagnostic blood and fecal swab cultures, and blood smears for Plasmodium.

QA statement: Yes

Drug, lot #, and % purity: Inotersen Lot # CA420915-001, 93.8%  
 Inotersen for Injection Lots RP420915-012, -013, -014, and -015

### Key Study Findings

- Marked thrombocytopenia with anemia necessitated early euthanasia of 1/14 monkeys at 10 mg/kg/week (Study Specific Animal Number [SSAN] 48; Day 74) and 1/18 at 20 mg/kg/week (SSAN 68; Day 151). Observations included petechial hemorrhages on the face and petechiae or bruising on the chest and/or abdomen prior to euthanasia, and hemorrhages in multiple organs upon necropsy and microscopic examination. Platelet counts in SSAN 48 and 68 were reduced from  $349 \times 10^3/\mu\text{L}$  and  $521 \times 10^3/\mu\text{L}$ , respectively, during acclimation, to  $3 \times 10^3/\mu\text{L}$  and  $2 \times 10^3/\mu\text{L}$ , respectively, at euthanasia. Platelet counts in SSAN 68 rebounded from  $14 \times 10^3/\mu\text{L}$  on Day 94 to  $227 \times 10^3/\mu\text{L}$  on Day 122 following a 4-week dosing holiday, but then decreased dramatically during the subsequent 3 weeks as weekly dosing was resumed.
- Platelet counts were reduced  $\geq 49\%$  from baseline in 6/60 animals at some point during dosing with ISIS 420915 (1-2 animals each at doses of 3, 6, 10, and 20 mg/kg/week, respectively), compared to  $\leq \sim 30\%$  in 18 controls.
- Drug-related microscopic findings were observed in all drug-treated groups, and included minimal to moderate accumulation of basophilic granules (consistent with drug-related material) associated with slight vacuolation in the proximal tubular epithelium of the kidney, Kupffer cells in the liver, and histiocytes in the mandibular and mesenteric lymph nodes; minimal to moderate infiltration of mononuclear or mixed inflammatory cells, edema, and/or hemorrhage at the injection sites; and minimal to slight perivascular mixed cell infiltration in multiple organs.
- Widespread perivascular infiltration observed in 5 individual animals across the three lowest dose groups was associated with increases in ADA, CRP, IL-6, MIP-1 $\beta$ , TNF $\alpha$ , and total IgG and IgM; and (in 4/5 animals) with moderate reductions in platelet counts compared to baseline (49-70%).

- The NOAEL was 6 mg/kg/week, based on the severe thrombocytopenia observed at 10 and 20 mg/kg/week.

## Methods

Doses:	0, 3, 6, 10, and 20 mg/kg/week
Frequency of dosing:	Once weekly for 26 (Interim) or 39 weeks (Main)
Route of administration:	Subcutaneous injection, rotating among four interscapular sites
Dose volume:	0.4, 0.38, 0.4, 0.4, and 0.32 mL/kg at doses of 0, 3, 6, 10, and 20 mg/kg/week, respectively
Formulation/Vehicle:	Sterile solution of inotersen sodium in water for injection, pH 7.5-8.5 Placebo Control: Phosphate buffered saline
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group Interim (26 weeks) 4/sex/group Main Study (39 weeks) 2/sex Con & HD 26-week Recovery
Age at study initiation:	3.02-4.64 years old (M); 3.63-6.05 years old (F)
Weight at study initiation:	2.53-4.06 kg (M); 2.41-4.03 kg (F)
Satellite groups:	None
Unique study design:	Analyses of complement split product Bb and C3, cytokines/chemokines, D-dimer, plasmodium and Streptococcus DNA, TTR, RBP4, ADA, anti-platelet Ab, anti-PF4 Ab, and liver TTR mRNA
Deviation from study protocol:	No deviations reported had an impact on the outcome or integrity of the study

## Observations and Results

### Dosing Solution Analysis

Preformulated vials of each inotersen dosing solution were analyzed for inotersen concentration on Feb 14, 2012, and on March 27, 2012, using a validated ion-pair HPLC-MS method. Saline placebo control solutions were analyzed similarly on the latter date.

All preformulated inotersen dosing solutions were within acceptable ranges of nominal concentrations when analyzed on Feb 14, 2012 (102.4-104.7%) and on March 27, 2012 (102.0-104.1%). No inotersen was detected in the placebo control samples.

### Mortality

Mortality was assessed twice daily in all surviving animals.

Two animals were euthanized early due to marked thrombocytopenia with anemia: 1 of 7 males (SSAN 48) given 10 mg/kg/week, on Day 74; and 1 of 9 males (SSAN 68) given 20 mg/kg/week, on Day 151.

SSAN 48 showed petechial hemorrhages on the face on Day 68; more extensive bruising of the chest and abdomen was observed on Day 72, along with lethargy. A diagnostic blood sample on Day 74 revealed reduced red cell mass, increased reticulocytes (indicative of bone marrow regeneration), and a very low ( $3 \times 10^3 /\mu\text{L}$ ) platelet count compared to baseline ( $349 \times 10^3 /\mu\text{L}$ ). Associated hemorrhages (dark, red, discolorations) were observed upon necropsy on Day 74 over 40% of the abdomen and thorax, large areas of the hind legs, and on the tongue, stomach, colon, epididymides, and urinary bladder. Histopathology evaluation showed mild to moderate hemorrhages in the epididymides, gallbladder, injection site, colon, and skin. Other findings included moderate, perivascular, multifocal, mononuclear cell and mild, dermal, focal, mixed cell infiltrates in the injection site; minimal, perivascular, multifocal, mixed cell infiltrate in liver; mild, focal, mononuclear cell infiltrate in kidneys; mild, multifocal, mononuclear cell infiltrate in salivary glands; moderate, chronic, focal, pleural inflammation and mild, multifocal, alveolar macrophages in lung; minimal to mild basophilic granules in liver and lymph nodes; and minimal to mild hypertrophy of Kupffer cells in liver and sinusoidal histiocytes in lymph nodes.

SSAN 68 showed petechiae on the chest starting on Day 94 and near the eyebrows on Day 99. The platelet count for this animal was  $521 \times 10^3 /\mu\text{L}$  prior to initiation of dosing,  $14 \times 10^3 /\mu\text{L}$  on Day 94,  $227 \times 10^3 /\mu\text{L}$  on Day 122 (following dosing holidays on Days 99, 106, 113, and 120),  $6 \times 10^3 /\mu\text{L}$  on Day 150 (following resumption of dosing on Days 127, 134, and 141, but not on Day 148, due to observations of faint petechial hemorrhages on the face on Day 147), and  $4 \times 10^3 /\mu\text{L}$  on Day 151, the day of euthanasia. Associated hemorrhages were observed in the stomach upon necropsy. Histopathology evaluation showed mild hemorrhages in the duodenum, stomach, and thymus; minimal to mild mononuclear cell infiltrates in the esophagus, salivary gland, and stomach; minimal mixed cell infiltrate in the dermis of the injection site; mild basophilic granules in the kidneys, liver, and lymph nodes; mild to moderate hypertrophy of Kupffer cells in liver and sinusoidal histiocytes in lymph nodes; and minimal multifocal regeneration of proximal tubules in the kidneys.

No effects were observed in bone marrow in SSAN 48 or 68, suggesting that the severe thrombocytopenia was not caused by decreased production of platelets.

### **Clinical Signs**

Clinical signs were assessed twice daily in all surviving animals. The appearance of the injection site used the previous week was assessed each week prior to dosing.

No drug-related clinical signs were observed other than those described under Mortality above and minor irritation at the injection site noted for 8 weeks in one animal (M SSAN 40, 6 mg/kg/week) and once each in two additional animals (F SSAN 29, 3 mg/kg/week; and M SSAN 48, 10 mg/kg/week). The animal most affected (SSAN 40) also showed signs of inflammation (increased CRP, IgG, and cytokines/chemokines) and the highest anti-drug antibody titer (ADA), consistent with a more pronounced response to inotersen compared to the other animals.

**Body Weights**

Body weight was assessed once weekly in all surviving animals.

No drug-related effects were observed.

**Food Consumption**

Food consumption was assessed once weekly in all surviving animals.

No drug-related effects were observed.

**Ophthalmoscopy**

Ophthalmoscopy was conducted on all surviving animals during acclimation, Week 26 [Interim only], Week 39, and the last week of recovery.

No drug-related effects were observed.

**ECG**

ECG was conducted on all surviving animals during acclimation, Week 26 [Interim only], Week 39, and the last week of recovery.

No drug-related effects were observed.

**Hematology**

Blood was collected from a peripheral vein from each surviving animal on Day -13, Day -7, and 48 hours after dosing on Days 92, 120, 148, 183, 211, 239, and 274; during Recovery on Days 304, 395, and 456; and at the time of unscheduled euthanasia or assessment of health concerns. The following parameters were analyzed: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, mean platelet volume, red cell distribution width, white blood cell count, differential leukocyte absolute count, and reticulocyte count and percentage.

No drug-related effects were observed in group mean hematology parameters. However, as shown in the sponsor's table below, reductions in platelet counts of 49% or greater (compared to baseline) were observed in 6 animals given various doses of inotersen. No controls showed reductions greater than ~30% throughout the dosing period. No reductions in platelet counts were observed during the recovery period. The partial recovery of platelet counts in animal 68 during the dosing holidays, followed by a dramatic reduction upon re-challenge with inotersen, provides additional evidence that this effect is drug-related. The cause of this apparently idiosyncratic drug-related reduction in platelet counts in 6 of 60 monkeys was not determined.

**In-text Table 14: Dosing phase platelet count changes**

Dose (mg/kg/week)	SSAN	Necropsy Day	Sex	Time Point	Platelet Count	Notes
3	32	Day 276 (terminal)	M	Acclimation <sup>a</sup>	367 x 10 <sup>3</sup> /μL	NA
				Day 185	187 x 10 <sup>3</sup> /μL	
6	40	Day 276 (terminal)	M	Acclimation <sup>a</sup>	474 x 10 <sup>3</sup> /μL	Animal was not dosed on Days 176, 218, and 225
				Day 122	251 x 10 <sup>3</sup> /μL	
				Day 150	159 x 10 <sup>3</sup> /μL	
				Day 185	242 x 10 <sup>3</sup> /μL	
				Day 213	124 x 10 <sup>3</sup> /μL	
				Day 241	187 x 10 <sup>3</sup> /μL	
6	45	Day 185 (interim)	F	Acclimation <sup>a</sup>	392 x 10 <sup>3</sup> /μL	NA
				Day 94	221 x 10 <sup>3</sup> /μL	
				Day 122	179 x 10 <sup>3</sup> /μL	
				Day 150	170 x 10 <sup>3</sup> /μL	
				Day 185	141 x 10 <sup>3</sup> /μL	
10	48	Day 74 (unscheduled)	M	Acclimation <sup>a</sup>	349 x 10 <sup>3</sup> /μL	Animal euthanized on Day 74 due to thrombocytopenia
				Day 74	3 x 10 <sup>3</sup> /μL	
10	47	Day 185 (interim)	F	Acclimation <sup>a</sup>	527 x 10 <sup>3</sup> /μL	NA
				Day 150	227 x 10 <sup>3</sup> /μL	
				Day 185	326 x 10 <sup>3</sup> /μL	
20	68	Day 151 (unscheduled)	M	Acclimation <sup>a</sup>	521 x 10 <sup>3</sup> /μL	Animal was not dosed on Days 99, 106, 113, 120, and 148 Animal euthanized on Day 151 due to thrombocytopenia
				Day 94	14 x 10 <sup>3</sup> /μL	
				Day 122	227 x 10 <sup>3</sup> /μL	
				Day 150	6 x 10 <sup>3</sup> /μL	
				Day 151	4 x 10 <sup>3</sup> /μL	

<sup>a</sup> Acclimation values presented are the mean calculated from measurements taken on Days -13 and -7.

(pages 60-61 of Study Report)

### Coagulation

Blood was collected from a peripheral vein from each surviving animal on Day -7, and 48 hours after dosing on Days 92, 183, 274, and 456, or at the time of unscheduled euthanasia. The following parameters were analyzed: fibrinogen, prothrombin time (PT), and activated partial thromboplastin time (APTT).

Reductions were observed in mean PT (5-9%) and APTT (3-4% M; 12-15% F) in the HD group compared to controls on Days 94, 183, and 276. No significant differences were observed in these parameters in Recovery groups.

### **Clinical Chemistry**

Blood was collected from a peripheral vein from each surviving animal on Day -13, Day -7, and 48 hours after dosing on Days 92, 120, 148, 183, 211, 239, and 274; during Recovery on Days 304, 395, and 456; and at the time of unscheduled euthanasia or assessment of health concerns. The following parameters were analyzed: BUN, total protein, albumin, globulin, albumin:globulin ratio, ALT, ALP, AST, GGT, LDH, CRP, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, triglyceride, creatine kinase, bicarbonate, IgG, and IgM.

Albumin was decreased 8-19% in the HD group from Day 122 to Day 276, compared to baseline and controls. Greater decreases in albumin (23-45%), along with increases in globulin and decreases in A/G ratio, were observed in individual animals at 6 mg/kg/week (SSAN 45) and 20 mg/kg/week (SSAN 73 and 77).

Mean IgM levels were increased up to 1.5- to 1.7-fold in the HD group during the dosing period compared to baseline and controls. Males given 10 mg/kg/week also showed mean increases in IgM of up to 1.5-fold.

Increases observed in mean ALT levels of 2- to 3.3-fold compared to baseline were primarily driven by large increases in a few animals: one F at 10 mg/kg/week (SSAN 83, 10-fold); two HDM (SSAN 66, 8.1-fold; SSAN 74, 4.3-fold); and two HDF (SSAN 65, 27-fold; SSAN 85, 10-fold). No histopathological correlates were noted.

Sporadic increases in CRP were observed in individual animals in all groups including controls. These changes were associated with infection or parasitic infestation in two controls and in one animal at 6 mg/kg/week (SSAN 35). The sponsor also noted that CRP was increased (from below the level of detection to 5.8 mg/dL) in SSAN 45 (6 mg/kg/week), which was found to have a small parasite under the skin at necropsy; however, the increased CRP in this animal seems more likely to be drug-related, as it was correlated with increased cytokines and perivascular mixed cell infiltrate in multiple organs, as were the CRP increases up to 4.3-9.5 mg/dL in SSAN 32, 40, 43, and 47.

No drug-related differences in clinical chemistry parameters were observed between control and HD 26-week recovery groups.

### **Urinalysis**

Urine was collected in pans under the cage of each surviving animal during overnight fasting on Days -7, 94, 185, 276, 304, and 456. Parameters evaluated included: clarity, color, volume, specific gravity, pH, protein, creatinine, total protein (micro protein), glucose, ketones, bilirubin, urobilinogen, occult blood, leukocytes, nitrites, protein/creatinine ratio, and microscopic examination of sediment.

No drug-related effects were observed.

### **Gross Pathology**

All animals surviving to scheduled termination were necropsied on Days 185 (Interim), 276 (Main study), or 456 (Recovery), following sedation with ketamine, anesthetization with pentobarbital and phenytoin, and exsanguination. Animals SSAN 48 and SSAN 68 were euthanized moribund and necropsied on Days 74 and 151, respectively.

No drug-related effects were observed.

### **Organ Weights**

Weights were recorded for the following organs: adrenal glands, brain, epididymides, heart, kidneys, liver (with gallbladder), lung (with bronchi), lymph node (mesenteric), ovaries (with oviducts), pituitary gland, prostate/seminal vesicles, spleen, testes, thymus, thyroid (with parathyroid), and uterus (body and cervix).

After 6 months of treatment, the HD group showed increases in the weights (relative to body weight) of liver (1.6-fold) and spleen (3- to 4-fold) compared to controls; relative spleen weight was increased 2- to 3-fold in the group given 10 mg/kg/week. SSAN 45 (a F given 6 mg/kg/week, with multiple markers of inflammation, and evidence of a parasitic infection) showed ~8-fold and ~2-fold increases in the relative weights of the spleen and kidneys, respectively. SSAN 47 (a F given 10 mg/kg/week, with multiple markers of inflammation) showed an increase of ~2-fold in the relative weight of the kidneys, compared to controls.

Following 9 months of treatment, increases were observed in the relative weight of liver (1.2- to 1.4-fold in M at 10 and 20 mg/kg/week) and spleen (2.6-fold in HDM; 1.6-fold in HDF). Individual animals in all dose groups showed increases in relative spleen weights of 2.5- to 11-fold that were not correlated with histopathological findings. Three individual animals (SSAN 43, 40, and 32) showing increases in relative spleen weight of > 4-fold correlated with multiple signs of inflammation (increased CRP, chemokines/cytokines, and/or IgG and IgM). SSAN 40 also showed increases in relative liver (> 2-fold) and kidney weights (> 1.5-fold), relative to controls.

No consistent drug-related effects on organ weights were observed following the 26-week recovery period.

### **Histopathology**

The following tissues from all animals were examined microscopically: adrenal glands, aorta, bone (femurs) with joints (knee), bone and bone marrow (sternum), brain, cecum, colon, duodenum, epididymides, esophagus, eyes (with optic nerves), gallbladder, GALT, heart, ileum, injection sites, jejunum, kidneys, lacrimal gland, liver, lung (with bronchi), lymph node (mandibular, mesenteric), mammary gland, ovaries (with oviducts), pancreas, pituitary gland, prostate, rectum, salivary glands (submandibular), sciatic nerves, seminal vesicles, skeletal muscles (quadriceps femoris), skin

(mammary), spinal cord (thoracic), spleen, stomach, testes, thymus, thyroid (with parathyroid), tongue, trachea, urinary bladder, uterus (body and cervix), vagina, and gross lesions (if any).

The battery of tissues examined was adequate. No Peer Review was conducted. A signed pathology report was provided.

#### Histological Findings

Drug-related effects were observed in the kidneys, liver, lymph nodes, injection sites, and perivascular areas in multiple organs. Findings were similar in animals sacrificed after 6 (Interim) or 9 months (Main Study) of treatment.

Dose-dependent, minimal to moderate accumulation of basophilic granules (consistent with drug-related material) associated with slight vacuolation was observed in the proximal tubular epithelium of the kidney, Kupffer cells in the liver, and histiocytes in the mandibular and mesenteric lymph nodes. The changes in Kupffer cells and histiocytes were associated with hypertrophy.

Minimal to moderate infiltration of mononuclear or mixed inflammatory cells, edema, and/or hemorrhage was observed at the injection sites in all dose groups, including controls. Incidence and severity of these effects were increased in drug-treated groups compared to controls but did not appear to be dose-dependent.

Minimal to slight perivascular mixed cell (neutrophils, macrophages, and lymphocytes) infiltrates were observed in multiple organs in individual animals in all drug-treated groups but were usually only one or two cell layers thick in the vessel wall. SSANs 45 (6 mg/kg/week) and 47 (10 mg/kg/week), however, showed perivascular lesions of up to 20 or more cell layers in thickness in multiple organs (e.g., pancreas, gallbladder, GI tract, kidneys, lung, and/or reproductive organs), characterized by “infiltration of minimal to moderate numbers of the mixed inflammatory cell population into the tunica adventitia and outer segments of the tunica media” (*page 1468 of Study Report*) at the interim sacrifice on Day 185. Similar findings were observed at the terminal sacrifice on Day 276 in SSANs 43 and 40 (6 mg/kg/week) and 32 (3 mg/kg/week). The perivascular infiltration observed in  $\geq 8$  organs (in addition to the injection site) in these five animals was associated with increases in CRP and cytokines/chemokines. These lesions were not associated with hemorrhages or necrosis in the tunica media or tunica intima, and the endothelium appeared to be normal.

In the 6-month recovery group, no drug-related effects were observed in F at 20 mg/kg/week, and fewer basophilic granules were observed in kidneys and lymph nodes in M at 20 mg/kg/week, compared to observations at the terminal necropsy.

#### **Toxicokinetics**

Blood was collected from a peripheral vein from each surviving animal on Day 1 at 0.5, 1, 2, 4, 8, 24, and 48 hours postdose; predose on Days 8, 15, 43, 99, 148, 183, 218, and 274; on Days 183 and 274 at 0.5, 1, 2, 4, 8, 24, and 48 hours postdose; and during recovery on Days 281, 295, 330, 421, and 456. TK samples were not taken on Day 99

from animals SSAN 68 and SSAN 25 due to concerns about low platelets and electrolyte imbalance, respectively. Plasma samples were analyzed for inotersen concentration using a validated Hybridization-ELISA method.

Tissue samples were collected from up to 4/sex/group at each necropsy date (liver and kidney cortex from all dose groups; brain, heart, kidney medulla, lung, mesenteric lymph node, pituitary, spleen, testis, uterus, and ovary from control and HD groups only). Liver and kidney cortex samples were analyzed for inotersen concentration using a validated HPLC photodiode array detector method; other tissues were not analyzed.

Control plasma and tissue samples were collected but were not analyzed for inotersen concentrations. Values for males and females were combined because no consistent sex-related differences were observed.

As shown in the sponsor's table below, inotersen plasma exposure increased slightly greater than dose-proportionately after a single dose, and there was significant accumulation with repeated once weekly dosing between Days 1 and 183, except at the highest dose. Plasma inotersen levels peaked within 2-4 hours after subcutaneous injection and then declined rapidly during an initial tissue distribution phase, and then more slowly during a prolonged elimination phase. The terminal half-life of elimination for the HD group was estimated to be 27.6 days.

Group	Dose Level (mg/kg/wk)	No. of doses	Profile Day	N	C <sub>max</sub> (µg/mL)		T <sub>max</sub> (hr)	AUC <sub>0-48hr</sub> (hr*µg/mL)	
					Mean ± SD	Median (Range)	Median (Range)	Mean ± SD	Median (Range)
2	3	1	1	14	9.55 ± 2.76	8.79 (6.72 - 14.9)	2 (1 - 2)	36.3 ± 6.42	33.7 (28.6 - 48.6)
		27	183	6	5.74 ± 1.92	5.12 (3.99 - 8.29)	4 (2 - 4)	56.4 ± 21.5	52.2 (32.7 - 84.1)
		40	274	7	7.29 ± 4.23	5.75 (2.94 - 16.5)	2 (2 - 4)	44.8 ± 12.8	44.1 (31.4 - 61.6)
3	6	1	1	14	20.6 ± 5.38	20.0 (12.3 - 29.5)	2 (1 - 2)	98.8 ± 22.8	92.0 (65.6 - 140)
		27	183	4	19.5 ± 4.71	17.7 (15.6 - 26.9)	4 (2 - 4)	162 ± 30.4	150 (139 - 206)
		40	274	5	22.9 ± 6.42	22.4 (13.1 - 34.8)	2 (1 - 4)	168 ± 43.3	169 (105 - 220)
4	10	1	1	14	35.7 ± 5.89	36.2 (27.7 - 44.0)	2 (1 - 4)	207 ± 30.9	199 (173 - 272)
		27	183	5	35.0 ± 10.4	33.1 (23.4 - 49.0)	4 (2 - 4)	477 ± 295	443 (136 - 935)
		40	274	8	33.8 ± 5.61	32.6 (26.3 - 45.3)	2 (2 - 4)	377 ± 92.7	391 (264 - 504)
5	20	1	1	18	81.6 ± 13.2	80.2 (53.2 - 111)	2 (1 - 2)	529 ± 84.5	530 (376 - 736)
		27	183	5	73.6 ± 25.7	62.9 (50.1 - 114)	2 (2 - 4)	599 ± 163	615 (411 - 770)
		40	274	12	50.8 ± 12.1	53.5 (27.7 - 66.2)	4 (2 - 4)	556 ± 114	534 (415 - 706)

SD = Standard Deviation

Group	Dose Level (mg/kg/wk)	No. of doses	Profile Day	N	MRT <sub>0-48hr</sub> (hr)		CL <sub>p</sub> /F <sub>0-48hr</sub> <sup>a</sup> (mL/hr/kg)	
					Mean ± SD	Median (Range)	Mean ± SD	Median (Range)
2	3	1	1	14	3.74 ± 0.603	3.67 (2.97 - 5.22)	84.9 ± 13.4	89.2 (61.8 - 105)
		27	183	6	9.42 ± 3.23	8.67 (6.94 - 15.7)	60.3 ± 22.8	59.7 (35.7 - 91.9)
		40	274	7	8.93 ± 3.42	8.55 (5.58 - 15.6)	71.9 ± 20.1	68.0 (48.7 - 95.5)
3	6	1	1	14	3.82 ± 0.473	3.74 (3.02 - 4.55)	63.8 ± 14.8	65.2 (42.7 - 91.5)
		27	183	4	9.49 ± 4.44	7.90 (5.58 - 17.1)	38.0 ± 6.18	39.9 (29.1 - 43.1)
		40	274	5	9.02 ± 3.44	8.76 (4.30 - 14.5)	38.0 ± 11.7	35.5 (27.3 - 57.4)
4	10	1	1	14	4.32 ± 0.665	4.18 (3.46 - 5.93)	49.2 ± 6.64	50.3 (36.7 - 57.9)
		27	183	5	8.80 ± 3.07	7.04 (6.19 - 13.5)	30.9 ± 24.7	22.6 (10.7 - 73.6)
		40	274	8	7.78 ± 1.42	7.61 (6.01 - 10.1)	28.0 ± 7.21	25.6 (19.8 - 37.9)
5	20	1	1	18	4.86 ± 0.455	4.80 (4.15 - 6.06)	38.7 ± 6.30	37.7 (27.2 - 53.2)
		27	183	5	7.52 ± 1.12	7.87 (6.36 - 9.00)	35.6 ± 10.2	32.5 (26.0 - 48.7)
		40	274	12	7.26 ± 0.773	7.40 (6.03 - 8.50)	37.4 ± 7.67	37.5 (28.3 - 48.2)

SD = Standard Deviation

(page 2786 of Study Report)

As shown in the sponsor's table below, inotersen concentrations in liver and kidney tissue increased less than proportionally with dose and generally appeared to have reached steady state or near steady state levels within 26 weeks of dosing. Mean Day 276 inotersen concentrations were 25-54 and 12 to 29 times greater in kidney cortex and liver, respectively, compared to mean Day 274 plasma C<sub>max</sub>. Clearance from tissues was complete (liver) or nearly complete (kidney cortex) by the end of the 26-week recovery period; the mean half-life of elimination from kidney cortex was estimated to be 27.3 days.

**Table 9 Summary of ISIS 420915 Tissue Concentrations in Monkeys (Genders Combined) Collected during Multiple Subcutaneous Administration of ISIS 420915 and after the Recovery Period (Day 456)**

Matrix	Group	Dose Level (mg/kg/wk)	Number of Doses	Day of Collection	N	ISIS 420915 Concentration (µg/g)	
						Mean ± SD	Median (Range)
Kidney Cortex	2	3	27	185	6	408 ± 114	401 (255 - 586)
			40 <sup>a</sup>	276	8	395 ± 77.8	404 (257 - 536)
	3	6	27	185	6	519 ± 206	594 (161 - 729)
			40 <sup>b</sup>	276	8	607 ± 272	605 (53.8 - 985)
	4	10	27	185	5 <sup>c</sup>	757 ± 302	782 (271 - 1024)
			40	276	8	849 ± 84.0	846 (750 - 1003)
	5	20	27	185	5 <sup>d</sup>	1403 ± 178	1338 (1267 - 1695)
			40	276	8	1414 ± 454	1381 (845 - 1997)
			40	456	4	14.6 ± 11.0	17.3 (0 - 23.7)
	Liver	2	3	27	185	6	241 ± 75.5
40 <sup>a</sup>				276	8	208 ± 117	219 (38.9 - 340)
3		6	27	185	6	358 ± 159	396 (95.2 - 564)
			40 <sup>b</sup>	276	8	279 ± 180	311 (58.8 - 509)
4		10	27	185	5 <sup>c</sup>	596 ± 85.7	616 (464 - 698)
			40	276	8	518 ± 158	500 (318 - 779)
5		20	27	185	5 <sup>d</sup>	680 ± 161	743 (448 - 840)
			40	276	8	902 ± 137	895 (696 - 1135)
			40	456	4	0 ± 0 <sup>e</sup>	0 (0 - 0) <sup>e</sup>

SD = Standard Deviation

<sup>a</sup> SSAN 25 received 2 fewer doses than the other animals in Group 2 (see Table 1)

<sup>b</sup> SSAN 40 received 3 fewer doses than the other animals in Group 3 (see Table 1)

<sup>c</sup> SSAN 48 sacrificed early on Day 74, see Section 7.1.

<sup>d</sup> SSAN 68 sacrificed early on Day 151, see Section 7.1.

<sup>e</sup> Values below the limit of quantification (<10.0 µg/g) were treated as "0" for summary statistic calculations

(page 2272 of Study Report)

### Anti-Inotersen Antibodies

Blood was collected from a peripheral vein from each surviving animal on Day -13, 48 hours after dosing on Days 92, 183, and 274, and during recovery on Day 456. Serum levels of anti-Inotersen antibodies were assessed using a validated ELISA method.

Anti-drug antibody (ADA) was not detected in any samples from the 18 control animals, or in any baseline (Day -13) samples from any group.

ADA was detected in samples from 4 of 14 animals administered 3 mg/kg/day, 7 of 14 animals administered 6 mg/kg/day, 6 of 13 animals administered 10 mg/kg/week, and 8 of 18 animals administered 20 mg/kg/day. Titers observed in samples that tested positive are shown in the table below:

**Anti-Inotersen Antibody Titers Observed during Dosing and Recovery**

<b>Animal Number</b>	<b>Dose (mg/kg/week)</b>	<b>Titer Day 94</b>	<b>Titer Day 185</b>	<b>Titer Day 276</b>	<b>Titer Day 456</b>
SSAN 23	3	32	32	NA	NA
SSAN 24	3	2	Negative	NA	NA
SSAN 30	3	Negative	2	1	NA
SSAN 32	3	Negative	128	256	NA
SSAN 34	6	8	16	NA	NA
SSAN 35	6	Negative	1	NA	NA
SSAN 38	6	Negative	8	NA	NA
SSAN 39	6	Negative	Negative	4	NA
SSAN 40	6	8	256	512	NA
SSAN 43	6	Negative	128	256	NA
SSAN 45	6	Negative	128	NA	NA
SSAN 47	10	32	256	NA	NA
SSAN 52	10	Negative	4	NA	NA
SSAN 57	10	Negative	2	Negative	NA
SSAN 58	10	4	4	16	NA
SSAN 59	10	Negative	Negative	2	NA
SSAN 83	10	Negative	1	NA	NA
SSAN 61	20	8	32	NA	NA
SSAN 63	20	Negative	8	NA	NA
SSAN 65	20	Negative	1	2	NA
SSAN 73	20	Negative	Negative	1	8
SSAN 75	20	4	Negative	Negative	Negative
SSAN 76	20	8	8	16	2
SSAN 77	20	Negative	1	4	NA
SSAN 82	20	4	16	NA	NA

*(Reviewer's table based on data in Table 5 on pages 432-446 of Study Report)*

All five animals that showed ADA titers of  $\geq 125$  also showed widespread perivascular infiltration, increased levels of CRP, cytokines/chemokines, and (perhaps related to the ADA) total IgG and IgM. The presence of ADA had no consistent effect on inotersen plasma PK parameters, tissue levels, or pharmacodynamic effects.

**Anti-Platelet Antibodies**

Concentrations of IgG and IgM anti-platelet antibodies were measured in serum samples from selected monkeys (4 controls, 4 drug-treated animals that showed reduction in platelets, and 4 drug-treated animals that did not show reductions in platelets) following incubation with isolated human platelets (pre-stimulated with prostaglandin E1) in the presence and absence of inotersen using a flow cytometry method that was not validated for use in testing non-human primate serum samples.

Substantial increases in anti-platelet IgG levels were observed in 5 animals during the dosing period: one control (SSAN 11); 3 of the 4 drug-treated animals that showed reduced platelet levels (SSAN 40, 48, and 68; but not 45); and one drug-treated animal that maintained normal platelet levels (SSAN 73).

Substantial increases in anti-platelet IgM levels were observed in 5 animals during the dosing period: 2 of the 4 drug-treated animals that showed reduced platelet levels (SSAN 48 and 68; but not 40 or 45); and 3 drug-treated animals that maintained normal platelet levels (SSAN 65, 69, and 73). These data suggest that increases in anti-platelet antibodies may be neither necessary nor sufficient to cause substantial reductions in platelets in cynomolgus monkeys. However, the small number of animals examined and the lack of validation of the assays warrant caution in the interpretation of the data.

### **Anti-Platelet Factor 4 (PF4) Antibodies**

Concentrations of IgG and IgM anti-PF4 antibodies were measured in serum samples from 12 selected monkeys using an ELISA that was not validated for use in testing non-human primate serum samples.

Substantial increases in anti-PF4 IgG were observed in two of the four monkeys that showed reduced platelet levels during dosing (SSAN 40 and 45), but not in the two that showed the most severe reductions in platelets (SSAN 48 and 68). Changes occurring in anti-PF4 IgM were not clearly interpretable, partly due to widely varying baselines among different individuals.

All four animals that showed reductions in platelets during the dosing period showed substantial increases in anti-platelet IgG and/or IgM and/or anti-PF4 IgG. Therefore, it is possible that such increases might contribute to the thrombocytopenia observed. However, other factors must also be involved as similar increases in anti-platelet IgG were observed in one control (SSAN 11) and three drug-treated animals (SSAN 65, 69, and 73) that did not show reduced platelets during the dosing period. The small number of animals examined and the lack of validation of the assay warrant caution in the interpretation of the data.

### **Complement Split Product Bb**

Blood was collected from a peripheral vein from each surviving animal on Day 1 at 1, 4, 8, and 24 hours postdose; and on Days 92, 183, and 274 predose and at 4 hours postdose. Plasma Bb levels were measured by ELISA.

As shown in the sponsor's table below, plasma levels of complement split product Bb increased (compared to baseline) in all groups, including controls, after each injection. The greatest effects were observed 4-8 hours after the first dose, with increases of ~3-fold observed at 10 mg/kg and ~6-fold at 20 mg/kg; levels had returned toward baseline by 24 hours postdose.

Group Averages as Percent Predose Day 1						
Study Day	Time Point	Group 1	Group 2	Group 3	Group 4	Group 5
		PBS	3 mg/kg/wk	6 mg/kg/wk	10 mg/kg/wk	20 mg/kg/wk
1	pre	100	100	100	100	100
	1hr	139	143	140	137	177
	4hr	210	267	230	316	610
	8hr	223	291	249	303	463
	24hr	129	194	140	143	156
92	pre	194	266	193	139	157
	4hr	241	340	256	203	352
183	pre	201	289	273	191	226
	4hr	261	405	597	295	382
274	pre	167	255	266	134	206
	4hr	241	458	702	256	422

(page 1824 of Study Report)

### Complement C3

Blood was collected from a peripheral vein from each surviving animal on Day -7; predose and 24 hours postdose on Days 1, 92, 183, and 274; and during recovery on Days 281, 289, 304, 395, and 456. Serum C3 levels were measured using a non-GLP turbidimetric assay.

No toxicologically important effects were observed on mean serum complement C3 levels, though a statistically significant reduction of ~20% was noted in HD animals compared to controls by the end of the dosing period.

### Flow Cytometry Immunophenotyping

Blood was collected from a peripheral vein from each surviving animal given 0, 10, or 20 mg/kg/week, on Day -13, Day -7, and 48 hours after dosing on Day 274. Flow cytometric immunophenotyping was conducted on isolated leukocytes using antibodies to CD3, CD4, CD8, CD14, CD159a, and CD20.

No drug-related effects were observed.

### Cytokine/Chemokine Analysis

Blood was collected from a peripheral vein from each surviving animal on Day -7, 48 hours after dosing on Days 183 and 274, and during recovery on Day 456. Serum levels of IL-1 $\beta$ , IL-6, IL-8, IFN- $\gamma$ , TNF- $\alpha$ , MCP-1, MIP-1 $\alpha$ , and MIP-1 $\beta$  were measured by ELISA.

No consistent dose-dependent effects on cytokine/chemokine levels in serum were observed. However, increases in IL-6, IL-8, TNF- $\alpha$ , MCP-1, and/or MIP-1 $\beta$  were observed in individual animals (SSAN 32 at 3 mg/kg/week; SSAN 40, 43, and 45 at 6 mg/kg/week; SSAN 47, 56, and 83 at 10 mg/kg/week; and SSAN 65, 73, and 82 at 20 mg/kg/week) on Day 185 and/or 276 compared to baseline and control values. As shown in the table below, the five animals that showed the most widespread microscopic findings of perivascular mixed cell infiltration also showed increases in MIP-1 $\beta$ , IL-6, TNF- $\alpha$ , and ADA, and reductions in platelet counts (in 4/5). These five animals also had increases in total serum IgG (1.9-3.4x) and IgM (1.4-5.2x), compared to baseline.

SSAN	# of organs w/ perivascular infiltration	MIP-1 $\beta$ (vs. Con)	IL-6 (vs. Con)	TNF- $\alpha$ (vs. Con)	CRP* (mg/dL)	Anti-Drug Antibody <sup>^</sup>	Platelet Count Reduction <sup>#</sup>
32	8	3.4x	10x	21x	9.5	256	49%
40	8	4.2x	239x	22x	8.6	512	70%
43	11	4.8x	31x	11.5x	4.3	256	26% <sup>\$</sup>
45	7	1.9x	82x	86x	5.8	128	64%
47	9	3.4x	986x	9.5x	6.4	256	57%

\*Below detectable levels at baseline; max value; <sup>^</sup> titer; <sup>#</sup>maximum reduction from baseline (mean of Days -7 and -13); <sup>\$</sup>within the range of reductions observed in controls

The two animals euthanized early due to severe thrombocytopenia (SSAN 48 and 68) did not show perivascular mixed cell infiltration in multiple organs or substantial increases in serum cytokine/chemokine, IgG, or IgM levels, except for increases of 2.9x in TNF- $\alpha$  observed in SSAN 68 at necropsy on Day 151, compared to baseline; CRP and ADA were not increased in SSAN 68 and were not available for SSAN 45. Among those showing an apparent drug-related (but not dose-related) inflammatory response, the three animals with the greatest increases in IL-6 also showed the greatest reductions in platelet counts (SSANs 40, 45, and 47). Bone marrow cellularity was slightly increased in SSANs 32 (myeloid), 40 (myeloid), 43 (granulocyte and megakaryocyte), and 45 (myeloid and megakaryocyte), but no changes in bone marrow were observed in SSANs 47, 48, or 68.

No substantial increases in cytokines/chemokines were observed in the four HD animals evaluated on Day 456, compared to controls, following 26 weeks of recovery.

**D-dimer Analysis**

Blood was collected from a peripheral vein from each surviving animal 48 hours after dosing on Days 120, 183, and 274. Plasma levels of D-dimer were assessed using a two-site enzyme immunoassay.

Sporadic increases in plasma levels of D-dimer were observed in all groups, including controls. Mean levels were increased 1.7- to 3.4-fold in the HD group compared to controls during the dosing period.

**Plasmodium and Streptococcus Analysis**

Blood was collected from a peripheral vein from each surviving animal on Day 185 (Interim study only; *Plasmodium* and *Streptococcus pyogenes*) or on Day 276 (Main study only; *Streptococcus pyogenes*); or prior to unscheduled euthanasia (SSAN 48: *Plasmodium* and *Streptococcus pneumoniae*; SSAN 68: *Plasmodium*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*). The presence of *Plasmodium* and *Streptococcus* species was assessed using PCR.

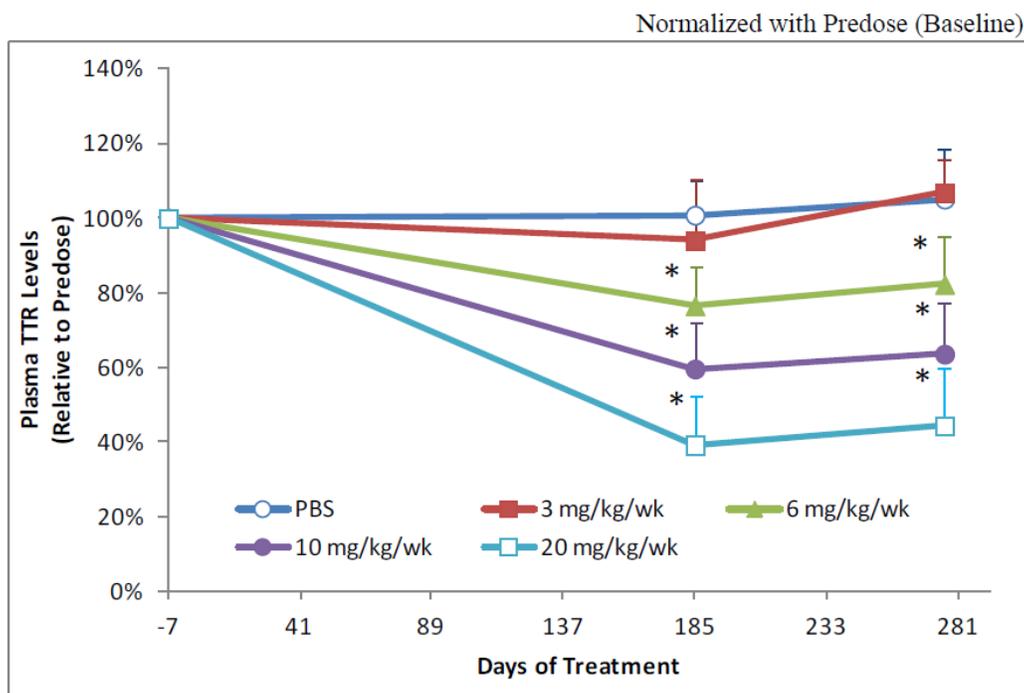
All samples were negative for all species tested.

**Transthyretin (TTR) and Retinol Binding Protein (RBP4)**

Blood was collected from a peripheral vein from each surviving animal on Day -7 and 48 hours after dosing on Days 183 and 274. Plasma levels of TTR and RBP4 were assessed using an Olympic Clinical Analyzer AU480 and an ELISA, respectively.

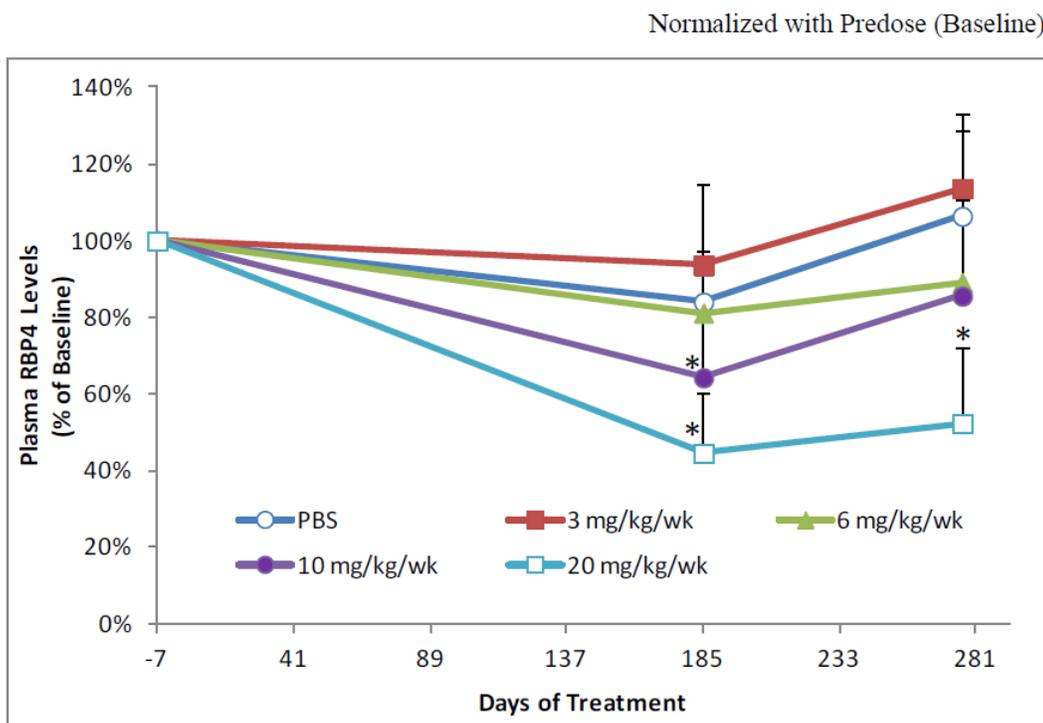
As shown in the sponsor's figures below, treatment with SC inotersen once weekly for 26 or 39 weeks resulted in dose-dependent reductions in plasma levels of TTR and RBP4 of up to ~60%, compared to baseline levels.

**Figure 2. Plasma TTR Protein Levels After 6- and 9-month treatment**



\*: Statistically significant from control; ( $p < 0.01$ ); Student's t-test

**Figure 3. Plasma RBP4 Protein Levels After 6- and 9-month Treatment**



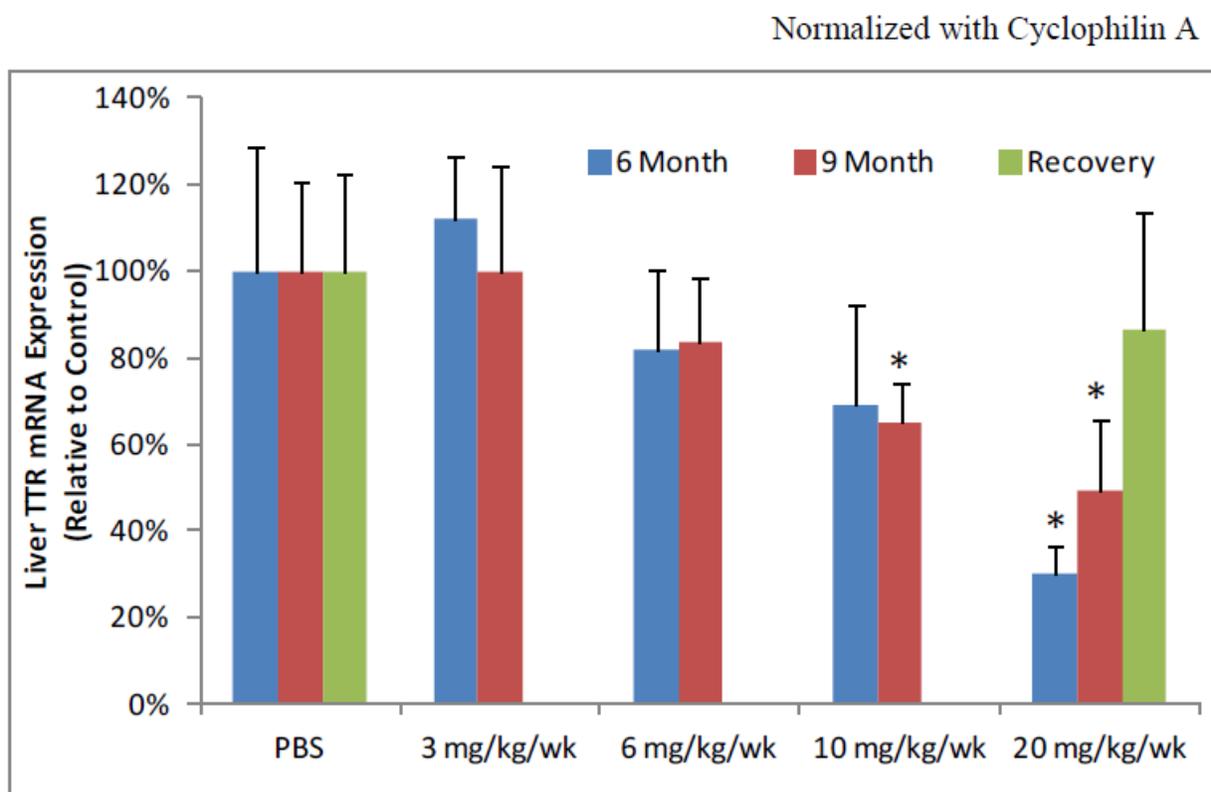
\*: Statistically significant from control; ( $p < 0.01$ ); Student's t-test  
(pages 2352-2353 of Study Report)

### Liver TTR mRNA Expression

Liver samples were collected from all animals within 10 minutes of sacrifice, flash frozen in liquid nitrogen, and stored at  $\leq -60$  °C prior to analysis of TTR mRNA by RT-PCR.

As shown in the sponsor's figure below, once weekly SC injection of inotersen to cynomolgus monkeys for 26 or 39 weeks resulted in dose-dependent reduction of TTR mRNA. The HD group showed reductions of 78.8% and 62.3% after 6 and 9 months of dosing, respectively, compared to controls. No significant reduction was observed following the 26-week recovery period.

**Figure 1. Dose-Dependent Reductions of Liver TTR mRNA Levels After 6- and 9-Month of Treatment**



\*: Statistically significant from control; ( $p < 0.01$ ); Student's t-test

(page 2352 of Study Report)

## 7 Genetic Toxicology

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

#### Evaluation of ISIS 420915 in the Bacterial Reverse Mutation Assay

Study number: ISIS Study 420915-IS02  
 Study report location: edr  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: March 28, 2012  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: ISIS 420915, 200 mg/mL, Lot RP420915-008, 103.2%  
 Drug Substance Lot CA420915-001, 91.9%

#### Key Study Findings

In the *in vitro* bacterial reverse mutation assay with the plate incorporation method, ISIS 420915 was negative for mutagenicity in all strains tested, in the presence and absence of Aroclor-induced rat liver S9 metabolic activation.

#### Methods

Strains: *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537;  
*E. coli* tester strain WP2 *uvrA*

Concentrations in definitive study: 50, 150, 500, 1500, and 5000 µg/plate ± S9;  
 retest of TA1537 -S9 used 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate

Basis of concentration selection: Absence of mutagenicity, precipitates, or bacterial lawn toxicity in initial assay at ISIS 420915 concentrations up to 5000 µg/plate

Negative control: Sterile water for injection

Positive control: +S9: 2-aminoanthracene (all tester strains)  
 -S9: 2-nitrofluorene (TA98)  
 sodium azide (TA100, TA1535)  
 9-aminoacridine (TA1537)  
 Methyl methanesulfonate (WP2 *uvrA*)

Formulation/Vehicle: Sterile water for injection

Incubation & sampling time: 48 to 72 hours at 37±2°C  
 Initial plate incorporation (duplicate)  
 Confirmatory plate incorporation (triplicate)  
 Metabolic activation: liver S9 from Aroclor 1254-induced male Sprague-Dawley rats

## Study Validity

Selection of bacterial tester strains was adequate based upon current guidelines. Positive and negative controls produced expected responses. Dose selection was adequate based upon use of the limit concentration of 5000 µg/plate. The S9 concentration (10%) was within acceptable limits. 2-aminoanthracene was used as the positive control for all tester strains in the presence of metabolic activation, and the activity of the S9 preparation was adequately characterized by the sponsor, as recommended by OECD guidelines. Analysis of samples of the dosing formulations confirmed that concentrations of the test article were within ±15% of nominal concentrations, except for the low dose formulations in the initial assay, which were slightly outside of this acceptable range (82.7-104.9%). This deviation did not affect the validity of the study conclusion.

In the confirmatory study without S9, reductions in revertant colonies were observed at 50-1500 µg/plate ISIS 420915 for tester strain TA 1537 (mean = 4-5 revertants/plate), compared to the negative control (mean = 12 revertants/plate). Therefore, this strain was retested at 1.5, 5.0, 15, 150, 500, 1500, and 5000 µg/plate ISIS 420915 -S9. Results of the retest were acceptable and negative, with all values in the range of 5-7 revertants/plate, including the negative control.

## Results

Criteria for a positive response were provided in the study report. Positive findings had to show increases in the mean number of revertants per plate that were dose-related over at least two increasing concentrations. For tester strains TA98, TA100, and WP2uvrA, the test article was considered positive if it produced at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. For tester strains TA1535 and TA1537, the test article was considered positive if it produced at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control.

No positive mutagenic responses were observed with any of the tester strains in the presence or absence of metabolic activation in the initial or confirmatory plate incorporation study; or in the retest of strain TA 1537 -S9. No cytotoxicity or precipitation was observed, except for the apparent reduction in mean revertants per plate for TA 1537 -S9 at 50-1500 µg/plate described above under Study Validity.

## Conclusion

All criteria for a valid study were met. ISIS 420915 was negative for mutagenicity in the in vitro bacterial mutation assay in the presence and absence of metabolic activation.

## 7.2 *In Vitro* Assays in Mammalian Cells

### The Effect of ISIS 420915 on the *In Vitro* Induction of Chromosome Aberrations in Chinese Hamster Lung (CHL) Cells

Study no.: ISIS Study 420915-IS03  
 (b) (4)

Study report location: edr

Conducting laboratory and location: (b) (4),  
 (b) (4)

Date of study initiation: March 21, 2012

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: ISIS 420915, 200 mg/mL, Lot RP420915-008, 103.1%  
 Drug Substance Lot CA420915-001, 91.9%

### Key Study Findings

In an in vitro chromosome aberration assay using CHL cells, ISIS 420915 was negative for clastogenicity in the presence and absence of rat liver S9 metabolic activation.

### Methods

Cell line: Chinese Hamster Lung (CHL) cells

Concentrations in definitive study: 625, 1250, 2500, and 5000 µg/mL

Basis of concentration selection: preliminary cytotoxicity assessment

Negative control: phosphate buffered saline (PBS)

Positive control: cyclophosphamide monohydrate (+S9)  
 ethyl methanesulfonate (-S9)

Formulation/Vehicle: PBS

Incubation time: -S9: 6 and 22 hours; +S9: 6 hours

Sampling time: 24 hours after start of incubation

Metabolic activation system: liver S9 from Aroclor 1254-induced male Sprague-Dawley rats

### Study Validity

The following criteria for a valid study were listed in the study report, and were met: 1) "There needed to be a minimum of 3 analyzable concentrations;" 2) "The number of metaphases with structural aberrations (excluding gaps) in the vehicle group was within or close to the range(s) of (b) (4) historical control data;" and 3) "The positive control groups induced statistically significant increases in the number of metaphases with structural aberrations (excluding gaps) compared to the concurrent vehicle groups." Dose selection was adequate as the highest concentration used (5000 µg/mL) exceeded the maximum recommended concentration of 0.5 mg/mL specified for in vitro mammalian cell assays (*Guidance for Industry S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, CDER, CBER, ICH, June 2012*). The S9 concentration used (3% final) was within acceptable limits. The study

was performed using standard procedures. Analysis of the ISIS 420915 dosing formulations confirmed that concentrations of ISIS 420915 were within 4% of nominal concentrations.

## Results

A positive response was defined in the study report as one in which there was “a concentration-related and/or reproducible increase in the number of cells with chromosome aberrations (excluding gaps), at one or more concentrations.” A negative response was defined as one in which “all test concentrations show frequencies of metaphases with structural aberrations (excluding gaps) that are not increased over the concurrent vehicle groups and fall within the range of <sup>(b) (4)</sup> historical control data (both replicates).”

No statistically significant increases in the number of metaphases with structural or numerical aberrations (relative to vehicle control) were observed in CHL cells incubated with ISIS 420915 at up to 5000 µg/mL in the presence or absence of rat liver S9 metabolic activation. No precipitate or excessive toxicity was observed up to the highest concentration tested, 5000 µg/mL, which was associated with reductions in relative cell counts of 22%, 32%, and 7% in the 4-hour assay without S9, the 20-hour assay without S9, and the 4-hour assay with S9, respectively.

## Conclusion

All criteria for a valid study were met. ISIS 420915 was negative for clastogenicity in the in vitro chromosomal aberration assay in CHL cells in the presence and absence of metabolic activation.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

#### **In Vivo Mammalian Micronucleus Assessment in Bone Marrow Erythrocytes Following Treatment with ISIS 420915 in ICR Mice**

Study no: ISIS Study 420915-AS05  
 (b) (4)

Study report location: edr

Conducting laboratory and location: (b) (4)

Date of study initiation: November 30, 2010

GLP compliance: Yes, except for TK analyses

QA statement: Yes

Drug, lot #, and % purity: RP 420915-007-A, 100 mg/mL, 104.2%  
 RP 420915-008, 200 mg/mL, 103.1%  
 Drug Substance Lot CA420915-001,  
 91.9%

#### **Key Study Findings**

ISIS 420915 was negative for clastogenicity in the *in vivo* mouse bone marrow micronucleus assay.

#### **Methods**

Doses in definitive study: 0, 500, 1000, 2000 mg/kg

Frequency of dosing: single dose

Route of administration: SC injection in the interscapular region

Dose volume: 5 mL/kg for LD; 10 mL/kg for MD & HD

Formulation/Vehicle: sterile water for injection

Species/Strain: CrljOri:CD1(ICR) mice

Number/Sex/Group: 6/sex/time point (24 hrs and 48 hrs post-dose)

Satellite groups: none

Basis of dose selection: DRF study in mice at 500, 1000, 2000 mg/kg

Negative control: PBS

Positive control: CPA, 70 mg/kg i.p., 10 mL/kg

#### **Study Validity**

The study was deemed valid for the following reasons: 1) all animals had PCE/(PCE+NCE) ratios > 0.1; 2) mean micronucleated polychromatic erythrocyte (MNPCE) incidence was ≤ 0.5% in the negative control group; 3) mean MNPCE incidence in the negative control group was within the historical control range; and 4) mean MNPCE incidence in the positive control group was significantly greater than that in the negative control group.

**Results**

A positive response was defined in the study report as one in which “there was a statistically significant and dose-related increase or a reproducible increase in the frequency of MNPCEs at least at one dose level.”

No drug-related effects were observed other than swelling at the injection site observed in most animals at all doses. Single doses of up to 2000 mg/kg SC ISIS 420915 did not induce significant increases (compared to negative control) in the incidence of MNPCEs in bone marrow of male or female ICR mice. No consistent differences were observed in the ratios of PCEs/(PCEs+NCEs). Analyses of dosing formulations confirmed that ISIS 402915 concentrations were within 4% of nominal concentrations. Analyses of ISIS 420915 concentrations in liver of male mice confirmed substantial exposure to tissues within 24 to 48 hours of dosing: 183-216 µg/g, 304-332 µg/g, and 377-386 µg/g at 500, 1000, and 2000 mg/kg, respectively.

**Conclusions**

All criteria for a valid study were met. ISIS 420915 was negative for clastogenicity in the in vivo mouse bone marrow micronucleus assay.

## 8 Carcinogenicity

### A 26-Week Subcutaneous Carcinogenicity Study of ISIS 420915 and ISIS 401724 in CByB6F1-Tg(HRAS)<sup>2</sup>Jic Hemizygous Transgenic Mice

Study no.:	Ionis Study 420915-AS12 (b) (4)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 30, 2015
GLP compliance:	Yes, except for target mRNA analysis and NMU content in positive control solutions
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 420915 Lot CA420915-006, 95.0% ISIS 401724 Lot 401724-6, purity not provided
CAC concurrence:	“The Committee recommended doses of 0 (saline), 10, 30, and 100 mg/kg/wk SC ISIS 420915, and 30 mg/kg/wk SC ISIS 401724 for M and F, with the high dose based on observations of moderate proinflammatory effects in the liver of mice treated for 13 weeks with 100 mg/kg/wk ISIS 420915.” The sponsor chose to use a HD of 80 mg/kg/wk.

#### Key Study Findings

- The NOAEL for neoplastic and non-neoplastic effects was the HD of 80 mg/kg/week ISIS 420915.

#### Adequacy of Carcinogenicity Study

The HD of 80 mg/kg/week was lower than the dose of 100 mg/kg/week recommended by the FDA Executive Carcinogenicity Committee (see Meeting Minutes of December 1, 2015) and did not result in adverse effects. However, toxicokinetic analyses of the liver and kidney demonstrated substantial tissue exposure following administration at this dose for 26 weeks. The study was adequately conducted.

#### Appropriateness of Test Models

The Tg.rash2 mouse model is appropriate for assessing the carcinogenic potential of this drug.

#### Evaluation of Tumor Findings

No drug-related increases in tumors were observed.

## Methods

Doses: 0 (Saline), 10, 30, or 80 mg/kg/week SC ISIS 420915, or 30 mg/kg/week SC ISIS 401724 (mouse homolog); or 75 mg/kg IP N-Nitroso-N-methylurea (NMU) positive control on Day 1

Frequency of dosing: Once weekly, except positive control

Dose volume: 14.29, 14.29, 10.34, and 9.30 mL/kg at 0, 10, 30, and 80 mg/kg/week SC ISIS 420915; 10.34 mL/kg at 30 mg/kg/wk SC ISIS 401724; 10 mL/kg at 75 mg/kg NMU

Route of administration: SC injection for ISIS 420915 and 401724, alternating sites between interscapular and proximal tail regions on the dorsal surface; IP injection for NMU

Formulation/Vehicle: 0.9% Sodium Chloride for Injection (USP) for ISIS 420915 and 401724; citrate buffer, pH 4.5±0.2, diluted 10-fold in 0.9% Sodium Chloride for Injection (USP) for NMU

Basis of dose selection: "...findings of mortality, reduced body weight gain, and extent of proinflammatory effects and associated secondary changes (e.g. splenomegaly, increased ALT, decreased RBC mass) observed at 100 mg/kg/week in a 13-week Tg. rasH2 mouse study (Study No. 420915-AS12P)." The mortality observed at 100 mg/kg/wk was of uncertain relationship to the drug.

Species/Strain: CByB6F1-Tg(HRAS)2Jic Hemizygous [RasH2] mice (Tg.rasH2 mice)

Number/Sex/Group: 10 for positive controls; 25 for all other groups

Age: 6-7 weeks old at initiation of dosing

Weight: 19.1-23.4 g (M); 15.7-19.3 g (F); at randomization

Animal housing: Male animals were housed individually; female animals were housed 2-3/cage

Dual control employed: None

Satellite groups: None

Deviation from study protocol: No important deviations were reported

## Observations and Results

### Mortality (twice daily)

No statistically significant drug-related differences in mortality were observed. The mortality rate was higher in positive control animals compared to saline controls, as expected.

<b>Table E. Survival Rate</b>			
The number of animals surviving to the scheduled terminal necropsy (Day 184)*			
Dose Level (mg/kg/week)	Male	Female	Overall (M+F)
0 (Saline Control)	24 (96%)	25 (100%)	49 (98%)
10	23 (92%)	25 (100%)	48 (96%)
30	24 (96%)	25 (100%)	49 (98%)
80	23 (92%)	24 (96%)	47 (94%)
30 (ISIS 401724)	24 (96%)	24 (96%)	48 (96%)
75 mg/kg (Positive Control)	7 (70%)	7 (80%)	14 (70%)

\*Respective survival percentage calculations are included in parentheses [one female at 75 mg/kg (Positive Control) was found dead on the scheduled day of necropsy (Day 184)].

<b>Table F. Unscheduled Euthanasia and/or Deaths During the Course of the Study - Males</b>				
Animal Number	Dose Level (mg/kg/week)	Fate/Animal Disposition	Fate Day	Cause of Death/Euthanasia
1021	0 (Saline Control)	FD	172	undetermined
2016	10 (ISIS 420915)	FD	179	testes; hemangiosarcoma; unilateral
2024	10 (ISIS 420915)	EE	138	urinary bladder; mesothelioma
3007	30 (ISIS 420915)	EE	91	lymphoid tumor
4013	80 (ISIS 420915)	EE	20	undetermined
4016	80 (ISIS 420915)	FD	97	undetermined
5007	30 (ISIS 401724)	EE	174	hemangiosarcoma/hemangioma
6005	75 (Positive Control)	EE	173	lymphoid tumor
6009	75 (Positive Control)	FD	138	glandular stomach; squamous cell carcinoma
6010	75 (Positive Control)	EE	144	glandular stomach; squamous cell carcinoma

EE-Euthanized *in extremis*  
FD-Found Dead

Table G. Unscheduled Euthanasia and/or Deaths During the Course of the Study - <u>Females</u>				
Animal Number	Dose Level (mg/kg/week)	Fate/Animal Disposition	Fate Day	Cause of Death/Euthanasia
4512	80 (ISIS 420915)	FD	121	undetermined
5513	30 (ISIS 401724)	FD	152	undetermined
6501	75 (Positive Control)	FD	141	lymphoid tumor
6502	75 (Positive Control)	FD	184	lung; bronchiolar alveolar carcinoma
6508	75 (Positive Control)	EE	129	lymphoid tumor

EE-Euthanized *in extremis*  
 FD-Found Dead

(pages 24-25 of Study Report)

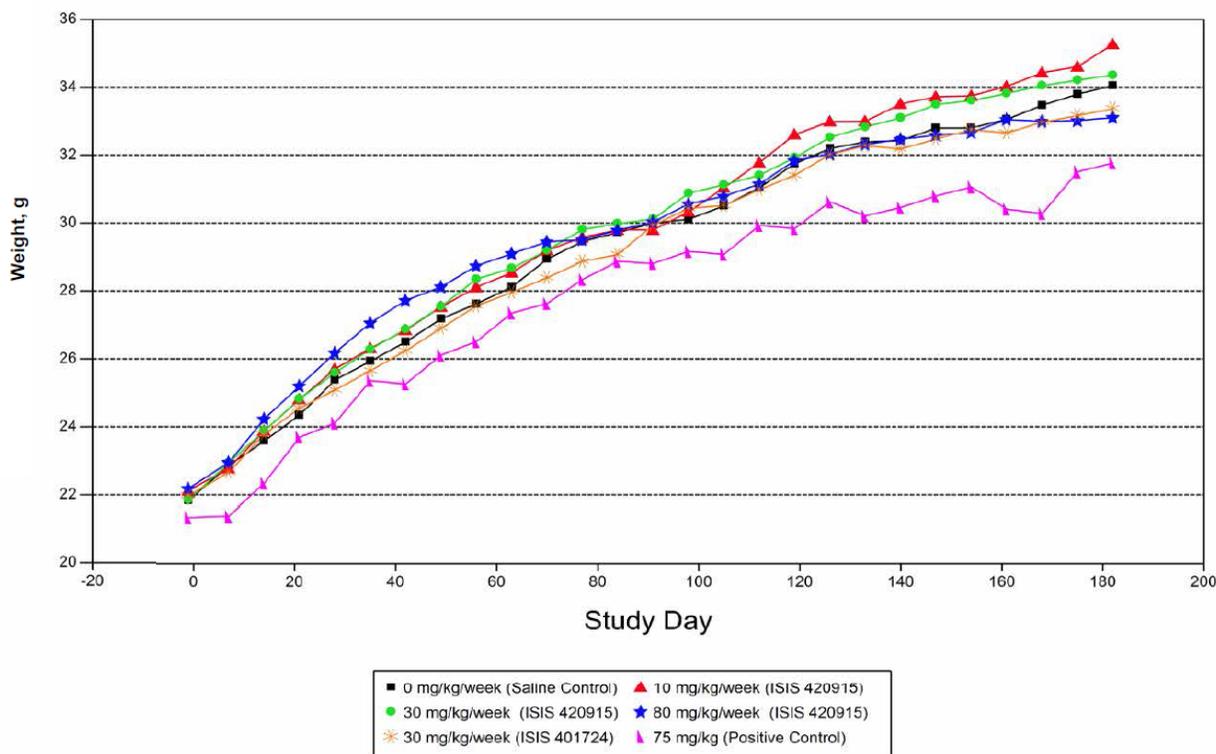
**Clinical Signs** (weekly; predose and at 3 ± 1 hour postdose)

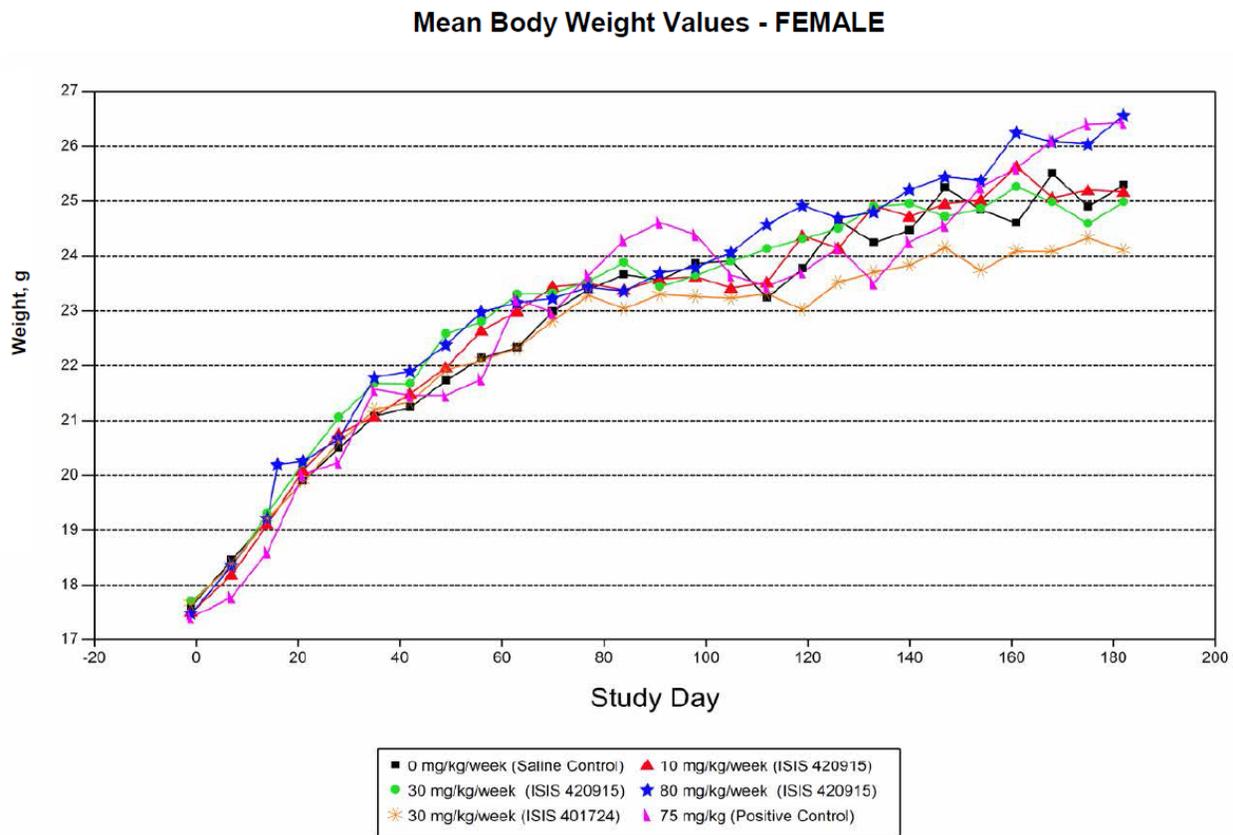
No drug-related effects on clinical signs were observed in animals administered ISIS 420915 or ISIS 401724. The incidence of nodules was increased in the positive control group (8/10 M; 4/10 F) compared to the saline control group (0/25 M; 1/25 F).

**Body Weights** (weekly)

As shown in the figures below, no consistent drug-related effects on body weight were observed.

**Mean Body Weight Values - MALE**





### Food Consumption (weekly for 14 weeks, then every two weeks)

No consistent drug-related effects on food consumption were observed.

### Clinical Chemistry

(standard parameters were measured in blood collected from the vena cava after carbon dioxide inhalation at necropsy)

Drug-related effects observed at Week 26 included increases in ALP (M:30%), ALT (M:33%; F:63%), and AST (F:43%) at 80 mg/kg/week ISIS 420915; increases in urea nitrogen in males at 30 and 80 mg/kg/week ISIS 420915 (15% and 22%, respectively); and decreases at 30 mg/kg/week ISIS 420915, 80 mg/kg/week ISIS 420915, 30 mg/kg/week ISIS 401729, and 75 mg/kg NMU in total protein (5% [M], 5-7%, 4% [M], and 5-12%, respectively) and albumin (3-7%, 8-9%, 5% [M], and 6-12%, respectively).

**Gross Pathology**

(all animals euthanized in extremis, found dead, or surviving to scheduled necropsy on Day 184; parameters evaluated included palpable masses, abnormalities on external surface and in abdominal, thoracic, and cranial cavities; and protocol-specified organs)

No drug-related macroscopic findings were reported in groups administered ISIS 420915, ISIS 401729, or vehicle control.

Macroscopic findings attributed to the administration of the positive control included enlarged thymus, spleen, lymph node, and/or liver; discolored spleen; and mass and/or raised area of the stomach, lung, pancreas, duodenum, diaphragm, spleen, adipose tissue, and/or skin/subcutis.

**Histopathology**

(all protocol-specified tissues from all animals were fixed directly in neutral buffered formalin, except eyes and testes, which were first fixed in modified Davidson's fixative; hematoxylin and eosin-stained paraffin sections were examined microscopically by a board-certified veterinary pathologist)

Adequate Battery: Yes

Peer Review: No

Signed Pathology Report: Yes

**Neoplastic Findings**

No drug-related neoplasms were observed in animals administered ISIS 420915 or ISIS 401729. NMU-related neoplasms observed included malignant lymphoma, squamous cell papilloma, squamous cell carcinoma, and hemangiosarcoma.

Table C: Summary of Neoplastic Lesions												
Group	1		2		3		4		5		6	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
<b>Clitoral glands</b>												
sarcoma, stromal, malignant	-	-	-	-	-	-	-	1/25	-	-	-	-
<b>Epididymides</b>												
sarcoma, stromal, malignant	-	-	-	-	-	-	-	-	1/25	-	-	-
<b>Harderian glands</b>												
adenocarcinoma, malignant	-	-	-	-	-	-	-	-	-	1/25	-	-
adenoma, benign	-	-	-	2/25	-	3/25	-	-	-	2/25	-	1/10
<b>Heart</b>												
mesothelioma, malignant	-	-	-	-	-	-	-	-	-	1/25	-	-
<b>Injection site, interscapular</b>												
papilloma, squamous cell, benign	-	-	-	-	-	-	-	-	-	-	1/10	-
<b>Injection site, proximal to tail</b>												
papilloma, squamous cell, benign	-	-	-	-	-	-	-	-	-	-	1/10	-
<b>Lung</b>												
adenoma, bronchiolar	3/25	2/25	2/25	3/25	4/25	4/25	1/25	-	3/25	1/25	-	1/10
alveolar, benign												
carcinoma, bronchiolar	-	-	-	-	-	-	-	-	-	-	1/10	1/10
alveolar, malignant												
<b>Multicentric neoplasm</b>												
hemangioma, benign	-	-	-	-	-	-	-	1/25	-	-	-	-
hemangiosarcoma, malignant	1/25	-	3/25	2/25	-	1/25	2/25	2/25	2/25	1/25	1/10	2/10
lymphoma, malignant	-	-	-	-	1/25	-	-	-	-	-	1/10	2/10
<b>Skin/skin, subcutis</b>												
carcinoma, squamous cell, malignant	1/25	-	-	-	-	-	-	-	-	-	-	-
papilloma, squamous cell, benign	-	-	-	-	-	-	-	-	-	1/24	7/10	3/10
<b>Stomach, glandular</b>												
carcinoma, squamous cell, malignant	-	-	-	-	-	-	-	-	-	-	2/10	-
<b>Stomach, nonglandular</b>												
papilloma, squamous cell, benign	-	-	-	-	-	-	-	-	-	-	8/10	9/10
<b>Thymus</b>												
thymoma, malignant	-	-	-	2/24	-	-	1/21	-	1/25	-	-	-
<b>Thyroid gland</b>												
adenoma, follicular cell, benign	-	-	-	-	-	-	-	1/24	-	-	-	-
<b>Urinary Bladder</b>												
mesothelioma, malignant	-	-	1/25	-	-	-	-	-	-	-	-	-
M-Male; F-Female					Group 3: 30 mg/kg/week (ISIS 420915)							
Number of tumor-bearing animals/number of animals examined at site					Group 4: 80 mg/kg/week (ISIS 420915)							
Group 1: 0 mg/kg/week (Saline Control)					Group 5: 30 mg/kg/week (ISIS 401724)							
Group 2: 10 mg/kg/week (ISIS 420915)					Group 6: 75 mg/kg (Positive Control)							

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Table D: Tumor Incidence and Historical Control Data										
Group	Male					Female				
	1	2	3	4	5	1	2	3	4	5
<b>Harderian Glands</b>										
Adenocarcinoma, malignant <sup>1</sup> (Incidence %)	0	0	0	0	0	0	0	0	0	1/25 (4%)
Historical Data %	0-4%					0-5%				
Adenoma, benign (Incidence %)	0	0	0	0	0	0	2/25 (8%)	3/25 (12%)	0	2/25 (8%)
Historical Data %	0-5.7%					0-10%				
<b>Lung</b>										
Adenoma, bronchiolar alveolar, benign (Incidence %)	3/25 (12%)	2/25 (8%)	4/25 (16%)	1/25 (4%)	3/25 (12%)	2/25 (8%)	3/25 (12%)	4/25 (16%)	0	1/25 (4%)
Historical Data %	0-25%					0-12%				
<b>Multicentric neoplasm</b>										
Hemangioma, benign (Incidence %)	0	0	0	0	0	0	0	0	1/25 (4%)	0
Historical Data %	0%					0-4%				
Hemangiosarcoma, malignant (Incidence %)	1/25 (4%)	3/25 (12%)	0	2/25 (8%)	2/25 (8%)	0	2/25 (8%)	1/25 (4%)	2/25 (8%)	1/25 (4%)
Historical Data %	0-12%					0-16%				
Lymphoma, malignant (Incidence %)	0	0	1/25 (4%)	0	0	0	0	0	0	0
Historical Data %	0-5%					0-5%				
<b>Thymus</b>										
Thymoma, malignant (Incidence %)	0	0	0	1/21 (5%)	1/25 (4%)	0	2/24 (8%)	0	0	0
Historical Data %	0-4%					0-12%				
Group 1: 0 mg/kg/week (Saline Control)					Group 4: 80 mg/kg/week (ISIS 420915)					
Group 2: 10 mg/kg/week (ISIS 420915)					Group 5: 30 mg/kg/week (ISIS 401724)					
Group 3: 30 mg/kg/week (ISIS 420915)										
Number of tumor-bearing animals/number of animals examined at site										
MPI Research Historical Control, Neoplastic Data, rash2 Transgenic Mouse, 26 Week Studies, 10/1/05 to 1/1/17										

(page 487 of Study Report)

Non-neoplastic Findings

As shown in the table below, minimal to moderate drug-related microscopic changes were observed in the kidney, liver, mesenteric lymph node, spleen, thymus, and injection sites. These findings are typical of those observed with chronic administration of oligonucleotides and are not adverse at the level of severity seen in this study.

<b>Microscopic Findings in 26-Week Tg.rasH2 Mouse Study</b>										
<b>Treatment</b>	<b>Saline</b>		<b>ISIS 420915</b>		<b>ISIS 420915</b>		<b>ISIS 420915</b>		<b>ISIS 401729</b>	
Dosage (mg/kg/week)	0		10		30		80		30	
Sex	M	F	M	F	M	F	M	F	M	F
kidney, tubular cell basophilic granules	0	0	1 min	1 min	23 min 1 mild	25 min	4 min 18 mild 2 mod	0 min 3 mild 22 mod	13 min	24 min
liver, Kupffer cell basophilic granules	0	0	0	1 min	5 min	25 min	23 min 2 mild	2 min 22 mild 1 mod	25 min	20 min 4 mild
liver, subacute inflammation	5 min	19 min 1 mild	4 min	16 min	12 min	22 min 1 mild	15 min 3 mild	9 min 14 mild 2 mod	8 min	21 min 1 mild 1 mod
mesenteric lymph node, vacuolation of macrophages	0	0	0	0	3 min	0	15 min 8 mild	16 min 5 mild	17 min 8 mild	13 min 10 mild
mandibular lymph node, vacuolation of macrophages	0	0	0	0	0	0	6 min	4 min	8 min	13 min
spleen, lymphoid depletion	0 min 1 mild 1 mod	0	0	2 min 2 mild	1 min 1 mild	1 min 1 mild	3 min 6 mild 1 mod	5 min 3 mild 1 mod	2 min	2 min
spleen, intrasinusoidal cell vacuolation	0	0	0	21 min 1 mild	23 min	24 min 1 mild	17 min 5 mild 1 mod	16 min 7 mild	17 min	18 min 5 mild
thymus, lymphoid depletion	0 min 0 mild 2 mod	0 min 2 mild	0 min 0 mild 1 mod	1 min	1 min 1 mild	1 min 3 mild	5 min 3 mild 2 mod	0 min 6 mild 1 mod	0	0 min 3 mild
injection site, interscapular, mixed cell inflammation	2 min	8 min 1 mild	1 min	1 min 1 mild	2 min	5 min	1 min 0 mild 1 mod	5 min	0	5 min
injection site, near tail, mixed cell inflammation	7 min 2 mild	14 min 4 mild	4 min	16 min 4 mild	4 min	13 min 1 mild	2 min	13 min 1 mild	3 min 1 mild	15 min 2 mild

(Reviewer's Table; min = minimal; mod = moderate; N=25/sex/group)

### Toxicokinetics

(concentrations of ISIS 420915 were measured in the left kidney and a portion of the left lateral lobe of the liver in the first 10/sex/group treated with ISIS 420915; and in the left lateral lobe of the liver in saline controls; using a validated method)

As shown in the table below, ISIS 420915 tissue concentrations increased slightly less than dose-proportionately in kidney and substantially less than dose-proportionally in liver. Mean tissue concentrations were greater in kidney in females and in liver in males.

<b>Table E. ISIS 420915 Concentration in Tissue (µg/g)</b>								
Matrix	Group	Dose Level (mg/kg/week)	Study Day	Time Point	N	Female	N	Male
Kidney	2	10	184	48 hr	10	274 ± 58.2	10	134 ± 17.5
	3	30	184	48 hr	10	642 ± 105	10	320 ± 23.7
	4	80	184	48 hr	9*	1444 ± 189	10	820 ± 199
Liver	1	0	184	48 hr	10	BLQ	10	BLQ
	2	10	184	48 hr	10	109 ± 15.2	10	125 ± 17
	3	30	184	48 hr	10	238 ± 51.5	10	323 ± 44.3
	4	80	184	48 hr	9*	409 ± 51.4	10	613 ± 86

Data presented are mean ± standard deviation (N= 9 or 10).  
 BLQ: Below the lower limit of quantitation (LLOQ= 10 µg/g).  
 \*Animal 4501 (Group 4) was excluded due to the possible swap of kidney and liver samples.

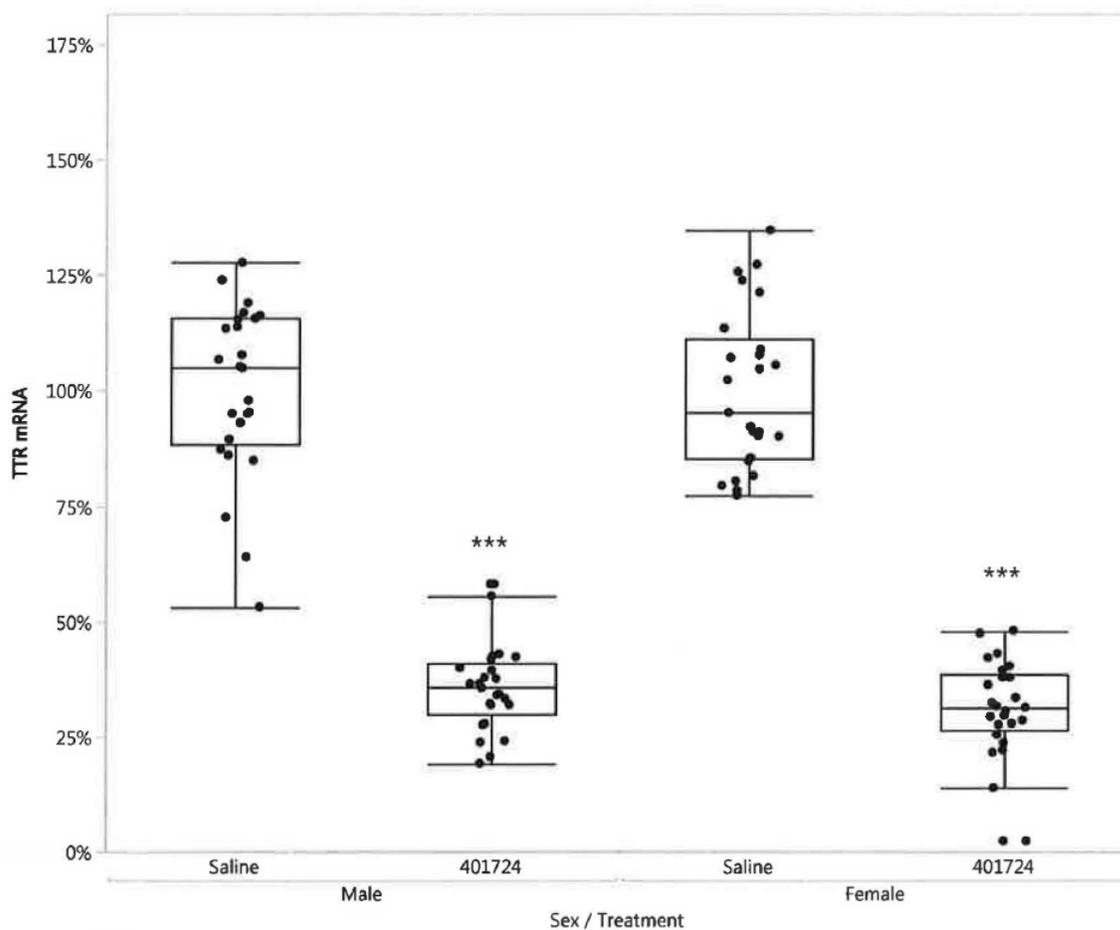
(page 30 of Study Report)

### mRNA Analysis

(levels of TTR mRNA were measured in portions of the right lobe of the liver from all animals administered saline or ISIS 401724, using RT-PCR; liver samples were flash frozen within 5 minutes of sacrifice)

As shown in the figure below, mean TTR mRNA levels in liver (normalized to total RNA levels) were reduced 65-70% after 26 weeks of administration of ISIS 401724 at 30 mg/kg/week SC, compared to saline controls.

**Figure 1. Statistically Significant Reduction in Mouse Liver TTR mRNA 26-Weeks of Treatment**



\*\*\*One-way ANOVA with post-hoc Dunnett's Method ( $p < 0.0001$ )

(page 1716 of Study Report)

### Dosing Solution Analysis

Dosing solutions analyzed were within acceptable limits: 100.6-101.3% of nominal concentrations pre-study, and 99.6-101.6% at the end of the dosing phase.

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

#### Study of Fertility and Embryo-Fetal Development in Mice with ISIS 420915 and ISIS 401724 Administered Subcutaneously

Study no.:	ISIS Study 420915-AS09 (b) (4)
Study report location:	edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 22, 2011
GLP compliance:	Yes, except for liver TTR mRNA, placenta, and TK analyses
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 420915, using Lot CA420915-001: RP420915-009, 0.3 mg/mL, 102.7% RP420915-011, 4 mg/mL, 102.8% ISIS 401724 using Lot 401724-6: RP401724-002, 1.5 mg/mL, 106.4%

#### Key Study Findings

- Male mice administered the HD of 87.5 mg/kg/week ISIS 420915 SC showed increases in body weight, body weight gain, and weights of spleen and liver; and reductions in hematocrit, hemoglobin, albumin, and A/G ratio, compared to controls. Male mice administered 52.5 mg/kg/week ISIS 401724 showed similar effects, except for the albumin and A/G changes.
- The NOAEL for reproductive performance, fertility, and developmental toxicity was the HD of 87.5 mg/kg/week ISIS 420915.
- Administration of 52.5 mg/kg/week ISIS 401724 SC every other day from prior to mating through GD 15 resulted in a 60% reduction in liver TTR mRNA, compared to controls; no adverse effects due to TTR inhibition were observed.

## Methods

Doses:	0, 10.5, 52.5, 87.5 mg/kg/week ISIS 420915, (0, 3, 15, 25 mg/kg/dose) 52.5 mg/kg/week ISIS 401724 (15 mg/kg/dose)
Frequency of dosing:	Every other day for ~10 weeks in M, starting 28 days prior to pairing; through GD 15 in F, starting 14 days prior to pairing
Dose volume:	10, 4, or 6 mL/kg at 3, 15, and 25 mg/kg/dose ISIS 420915; 10 mL/kg at 15 mg/kg/dose ISIS 401724
Route of administration:	SC injection, rotating among 4 sites on the dorsal surface of the back
Formulation/Vehicle:	PBS
Species/Strain:	Crl:CD1 <sup>®</sup> (ICR) mice
Number/Sex/Group:	25 M and 25 F per group
Satellite groups:	TK satellite groups at 0, 3, 15, and 25 mg/kg/dose ISIS 420915; 6/sex/group
Study design:	every other day dosing 28 (M) or 14 (F) days prior to mating, through GD 15; necropsy/Caesarean GD 17
Deviation from study protocol:	No important deviations were reported

## Observations and Results

### Mortality

All animals were observed for mortality twice daily throughout the study.

No drug-related effects on mortality were observed.

### Clinical Signs

Cageside observations were conducted twice daily. Detailed clinical observations were conducted once daily, at 4 hours postdose on dosing days.

No drug-related effects on clinical signs were observed.

### Body Weight

Body weight was recorded twice weekly for M through Day 71 and for F through Day 15 and then on GD 0, 3, 6, 9, 12, 15, 16, and 17.

No drug-related effects on body weight were observed in the LD and MD ISIS 420915 groups. Final body weight and body weight gain (Days 1-71) were increased 6.2% and 25%, respectively, in HDM compared to controls. Body weights in HDF were increased 4.2% and 5.0% on GD 0 and GD 3, respectively, compared to controls. Final body weight and body weight gain (Days 1-71) were increased 7% and 38%, respectively, in M administered 52.5 mg/kg/week ISIS 401724 compared to controls.

### **Food Consumption**

Food consumption was measured at the time of weighing, with the exception that no measurements were recorded for M during the first 14 days of the mating period.

No drug-related effects on food consumption were observed.

### **Hematology**

Blood samples were collected via the vena cava following carbon dioxide inhalation from the first 10 surviving main study animals/sex/group at termination, ~48 hours after the final dose. Animals were not fasted prior to blood collection. The following parameters were analyzed: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, red cell distribution width, white blood cell count, differential leukocyte absolute count, and reticulocyte count.

Hemoglobin and hematocrit were reduced up to 9-10% in MDM, HDM, and M administered 52.5 mg/kg/week ISIS 401724, compared to controls.

### **Clinical Chemistry**

Blood samples were collected via the vena cava following carbon dioxide inhalation from the second 10 surviving main study animals/sex/group at termination, ~48 hours after the final dose. Animals were not fasted prior to blood collection. The following parameters were analyzed: BUN, total protein, albumin, globulin, albumin:globulin ratio, ALT, AST, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, triglyceride.

Albumin and albumin/globulin ratio were reduced 12% and 16%, respectively, in HDM compared to controls.

### **Toxicokinetics**

Samples of liver and kidney were collected from all TK animals following euthanasia by carbon dioxide inhalation and exsanguination ~48 hours following the final dose. Fetuses from TK animals were euthanized by decapitation and samples of liver, kidney, and placenta were collected from each. Levels of ISIS 420915 were analyzed in fetal and maternal samples using a validated HPLC/Photodiode Array Detector method. Kidney samples were not analyzed.

As shown in the sponsor's summary table below, dose-dependent increases were observed in concentrations of ISIS 420915 in maternal liver and placenta, but the drug was undetectable in fetal liver.

Mean Tissue Concentrations of ISIS 420915 in Mice				
Dose Level	Day	Organ		
		Fetal Liver	Liver	Placenta
		Mean ± SD	Mean ± SD	Mean ± SD
10.5 mg/kg/week	71 (Males)	NA	123.32 ± 26.63	NA
	GD 17	0.00	52.52 ± 17.86	3.78 ± 5.86
52.5 mg/kg/week	71 (Males)	NA	631.17 ± 134.62	NA
	GD 17	0.00	243.83 ± 36.81	5.47 ± 5.99
87.5 mg/kg/week	71 (Males)	NA	826.33 ± 162.58	NA
	GD 17	0.00	384.33 ± 58.67	14.77 ± 1.82

NA – Not applicable

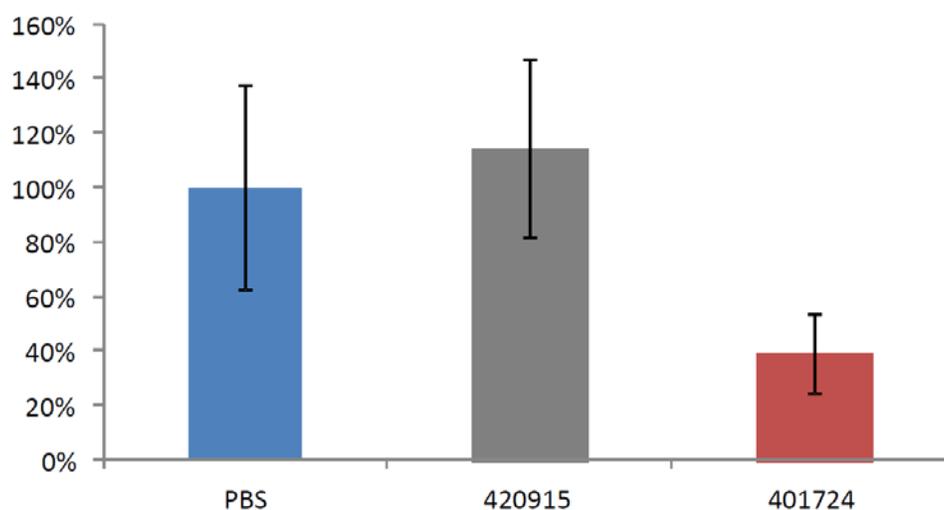
(page 30 of Study Report;  $\mu\text{g}$  ISIS 420915 per gram of tissue)

### Liver TTR mRNA Analysis

Levels of TTR mRNA were measured in samples of liver collected from 6/sex/group TK M, main study F administered ISIS 401724 at 52.5 mg/kg/week, and TK F administered placebo or 52.5 mg/kg/week ISIS 420915.

As shown in the sponsor's figure below, liver TTR mRNA levels were reduced ~60% in mice administered 52.5 mg/kg/week ISIS 401724, compared to controls. As expected, no significant reduction was observed in mice administered the same dose of ISIS 420915 because this ASO (which is complementary to human TTR mRNA) does not bind effectively to the mouse TTR mRNA sequence.

### Liver TTR mRNA Levels in CD-1 Mice



(page 36 of Study Report; M and F combined)

### **Dosing Solution Analysis**

Unopened vials of placebo control, ISIS 420915, and ISIS 401724 dosing formulations were analyzed by ion-pair high performance liquid chromatography-mass spectrometry following completion of the dosing period.

All samples of dosing solutions were within 6% of the nominal concentrations. No ISIS 420915 was detected in the placebo control dosing solutions.

### **Necropsy**

All main study animals were euthanized by carbon dioxide inhalation and exsanguination, and necropsied ~48 hours after the last dose. Organs weighed included testes, epididymides, seminal vesicles (with coagulating gland), prostate, liver, spleen, kidneys, gravid uterus, ovaries (with oviduct). Sperm analysis conducted on all main study males included sperm motility, caudal epididymal sperm concentrations per gram of tissue, morphological examination (% abnormal sperm). Fertility parameters evaluated included corpora lutea and uterine implantation; fetal number, survival, body weight, sex ratio; and fetal external, visceral, and skeletal malformations and variations.

No drug-related effects were observed on mating, reproductive, or fertility parameters; corpora lutea, uterine implantation, or gravid uterine weight; fetal sex ratio or body weight; or on the incidence of fetal external, visceral, skeletal, or overall malformations or variations, compared to controls.

No consistent drug-related effects were observed on sperm motility, concentration, or morphology.

No drug-related macroscopic findings were observed.

Increases in absolute and relative weights of spleen (1.3-1.4x) and liver (1.1-1.2x) were observed in MDM, HDM, and M administered ISIS 401724, compared to controls.

## 9.2 Embryonic and Fetal Development

### Preliminary Embryo-Fetal Development Study of ISIS 420915 by Subcutaneous Administration in Rabbits

(ISIS Study 420915-AD10; [REDACTED] (b) (4)  
[REDACTED] initiated November 7, 2011; Final Report dated March 7, 2013; non-GLP; non-QA)

Pregnant female New Zealand White rabbits (6/group) were administered placebo (PBS) or ISIS 420915 once every other day (5, 15, or 30 mg/kg/dose; 17.5, 52.5, or 105 mg/kg/week) from GDs 6 to 18 via SC injection. Evaluations included mortality, clinical signs, body weight, food consumption, necropsy (Day 28), hematology, clinical chemistry, organ weights (gravid uterus, placenta, liver, kidney, and spleen), corpora lutea, implantation sites; and fetal number, weight, sex, and external variations and malformations.

Drug-related effects included premature delivery (1/6 MD, GD 28); decreases in body weight and food consumption (MD & HD; GD 22-28; body weight loss, compared to GD 17 weights); increases in WBC count (HD, 1.4x); decreases in platelets (HD, 31%), monocyte % (all, 65-72%), and basophil % (all, 26-63%); increases in BUN (HD, 1.3x) and creatinine (MD, 1.3x; HD, 1.6x); decreases in total protein and albumin (MD & HD, 19-23%) and calcium (MD & HD, ~14%); and decreases in mean weight of male fetuses (LD, 25.3%; MD, 21.7%; HD, 21.4%) and female fetuses (HD, 26.5%), compared to controls.

Based on the adverse effects observed on maternal and fetal body weights, doses of 0, 8.75, 17.5, and 52.5 mg/kg/week were selected for the definitive embryofetal development study in rabbit.

## Embryo-Fetal Development Study of ISIS 420915 by Subcutaneous Administration in Rabbits

Study no.: ISIS Study 420915-AS10  
 (b) (4)

Study report location: edr

Conducting laboratory and location: (b) (4),  
 (b) (4)

Date of study initiation: December 23, 2011

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: ISIS 420915, using Lot CA420915-001:  
 RP420915-017, 10 mg/mL, 103.1%  
 RP420915-018, 20 mg/mL, 103.0%  
 RP420915-019, 60 mg/mL, 103.9%

### Key Study Findings

- The NOAEL for maternal and developmental toxicity was the MD of 17.5 mg/kg/week, based on findings of body weight loss in dams, premature delivery, and reduced fetal body weight at the HD of 52.5 mg/kg/week. No teratogenicity was observed.
- Increases in white blood cells, neutrophils, and lymphocytes observed in HDF compared to controls were consistent with proinflammatory effects commonly seen with administration of oligonucleotides.
- Concentrations of ISIS 420915 increased dose-dependently in maternal liver but were undetectable in fetal liver.

### Methods

Doses: 0, 8.75, 17.5, and 52.5 mg/kg/week ISIS 420915  
 (0, 2.5, 5, and 15 mg/kg/dose, respectively)

Frequency of dosing: every other day from GDs 6 to 18

Dose volume: 0.25 mL/kg

Route of administration: SC injection, rotating among 4 sites on the back

Formulation/Vehicle: controls received PBS;  
 ISIS 420915 was administered as received, in sterile water for injection

Species/Strain: New Zealand White rabbits, *Yac:NZW(KBL)*,  
 timed pregnant

Number/Sex/Group: 23 F/group main study

Satellite groups: 4 F/group TK

Study design: every other day dosing GD 6-18,  
 necropsy/cesarean GD 28

Deviation from study protocol: Minor deviations were reported that did not  
 affect study interpretations

## Observations and Results

### Mortality

All animals were observed for mortality twice daily throughout the study.

No drug-related effects on mortality were observed. HDFs #70 and 74 were euthanized following premature delivery on GD 27 and GD 28, respectively, exhibiting signs of maternal toxicity (body weight loss of 11% and 19%, respectively, associated with reduced food consumption).

### Clinical Signs

Clinical observations were recorded twice daily (~30-60 minutes pre-dose and ~2 hours postdose) on dosing days and once daily on non-dosing days.

No drug-related effects were observed on clinical signs.

### Body Weight

Body weights were recorded on GD 0, 4, 6, 9, 12, 15, 18, 20, 23, 26, and 28.

Mean body weight loss of 49.2 g was observed in HDF during the interval GD 15 to 23, while control F showed a gain of 22.8 g.

### Food Consumption

Food consumption was recorded once daily from GD 5 to GD 28.

HDF showed reductions in food consumption of 11-27% during the intervals from GD 18 to GD 28 and a 2-fold increase in the incidence of unconsumed feed, compared to controls.

### Hematology

Blood samples were collected from all (non-fasted) animals via the auricular artery on GD 28 prior to sacrifice. The following parameters were analyzed: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count (total, absolute, and percent differential), red cell distribution width, and reticulocyte count (absolute and percent).

HDF showed increases in white blood cells (1.5x), neutrophils (1.7x), and lymphocytes (1.5x); and decreases in hemoglobin (5.8%), hematocrit (5.5%), platelets (19.4%), % monocytes (67.3%), absolute monocytes (52.0%), and % basophils (37.5%), compared to controls.

## Clinical Chemistry

Blood samples were collected from all (non-fasted) animals via the auricular artery on GD 28 prior to sacrifice. The following parameters were analyzed: BUN, total protein, albumin, globulin, A/G ratio, ALT, AST, ALP, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, triglyceride.

No drug-related effects were observed on clinical chemistry parameters.

## Toxicokinetics

Maternal and fetal liver and kidney, as well as placenta, samples were collected from all TK animals following sacrifice on GD 20 (~48 hours after the final dose) and from main study LD, MD, and HD groups following sacrifice on GD 28 (10 days after the final dose). Placenta and maternal and fetal liver samples were analyzed for concentrations of ISIS 420915 using a qualified HPLC method. Placental samples were analyzed under non-GLP conditions.

As shown in the sponsor's summary table below, dose-dependent increases in ISIS 420915 concentration were observed in maternal liver, but levels in fetal liver were below the lower limit of quantification (LLOQ, 10 µg/g). Concentrations of ISIS 420915 were measurable in placenta from all 3 HDF evaluated 48 hours after the final dose but were below the LLOQ in all 5 HDF evaluated 10 days after the final dose.

Text Table 4. Mean Tissue Concentrations of ISIS 420915 in Rabbits

Group 2	Dose Level	Number of Doses	Study Day	N	Organ Concentration (µg/g)		
					Fetal Liver	Liver	Placenta
2	2.5 mg/kg	7	GD28	5	0 ± 0	0 ± 0	0 ± 0
3	5 mg/kg	7	GD28	5	0 ± 0	18.4 ± 8.75	0 ± 0
4	15 mg/kg	7	GD28	5	0 ± 0	140 ± 73.7	0 ± 0
5	2.5 mg/kg	7	GD20	3	0 ± 0	30.7 ± 11.5	0 ± 0
6	5 mg/kg	7	GD20	3	0 ± 0	92.6 ± 4.71 <sup>a</sup>	0 ± 0
7	15 mg/kg	7	GD20	3	0 ± 0	345 ± 52.8	17.9 ± 3.42

<sup>a</sup> One value in this group (1759 µg/g) was excluded from the calculation as an outlier. Median (range) of group with all data included is 95.9 µg/g (89.2 to 1759 µg/g).

GD = Gestation Day

All values below the lower limit of quantification (BLQ) were treated as zero (0) for the statistical summary analyses

SD = Standard Deviation

(page 27 of Study Report)

## Dosing Solution Analysis

Unopened vials of placebo control and ISIS 420915 dosing formulations were analyzed by ion-pair high performance liquid chromatography-mass spectrometry following completion of the dosing period.

All dosing solutions were within 4% of the nominal concentrations. No ISIS 420915 was detected in the placebo control dosing solution.

### **Necropsy**

Main study animals were euthanized using thiopental sodium and necropsied on GD 28 (except for HD #73, which delivered prematurely on GD 27 and was sacrificed immediately following observation of delivered pups, and HD #74, which delivered prematurely on GD 28 and was sacrificed following blood collection). Liver, kidney, spleen, and gravid uterus were weighed for all pregnant animals.

No drug-related effects were observed on macroscopic findings or gravid uterine weights. Absolute and relative weights of spleen were increased ~40% in HDF compared to controls.

### **Cesarean Section Data**

Corpora lutea, implantations sites, fetal number/weight/survival/sex, resorptions, and placenta weight were recorded for each pregnant female.

No drug-related effects on these parameters were observed, other than a reduction of 12.9% in fetal weight in the HD group, compared to controls.

### **Offspring**

No drug-related effects were observed on the incidence of fetal external, visceral, or skeletal malformations or variations, compared to controls.

### 9.3 Prenatal and Postnatal Development

#### Pre and Postnatal Development of ISIS 420915 and ISIS 401724 via Subcutaneous Administration Including Maternal Function in CD-1 Mice

Study number:	ISIS Study 420915-AS14 (b) (4)
Study report location:	edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 17, 2015
GLP compliance:	Yes, except for analyses of liver TTR mRNA and milk ISIS 420915
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 420915, using Lot CA420915-001: RP420915-033, 0.57 mg/mL, 102.1% RP420915-034, 2.29 mg/mL, 102.2% RP420915-035, 4.57 mg/mL, 105.3% ISIS 401724, using Lot 401724-6: RP401724-005, 2.29 mg/mL, 96.5%

#### Key Study Findings

- SC administration of ISIS 420915 (2.9, 11.4, and 22.9 mg/kg/dose every other day during GDs 6-16, then approximately once weekly at 10, 40, and 80 mg/kg/dose through LD 20) to pregnant/lactating female mice resulted in slight reductions in food consumption (HD) and increases in relative weights of liver (HD) and spleen (MD and HD) compared to controls.
- The NOAEL for maternal and developmental toxicity was the HD of 80 mg/kg/week ISIS 420915.
- Toxicokinetic analysis showed dose-dependent increases in ISIS 420915 concentrations in liver of F<sub>0</sub> pregnant/lactating female mice.
- Dose-dependent increases in ISIS 420915 concentration were also observed in milk collected from F<sub>0</sub> females on LD 13, but levels were ~700-7000x lower than those measured in liver.
- SC administration of ISIS 401724 (~40 mg/kg/week), a mouse TTR-specific ASO, to F<sub>0</sub> pregnant/lactating female mice resulted in a ~60% reduction in liver TTR mRNA compared to controls. No adverse maternal or developmental effects were associated with the reductions in TTR mRNA.

## Methods

Group Number	ISIS 420915 Dose Level (mg/kg/dose) <sup>a</sup>	ISIS 420915 Dose Level (mg/kg/week) <sup>a</sup>	Number of Animals		
			P Females	Selected F <sub>1</sub>	
				Male	Female
1	0 (Control)	0 (Control)	46	46	46
2	2.9	10	30	26	26
3	11.4	40	30	26	26
4	22.9	80	30	26	26
5	11.4 (ISIS 401724)	40 (ISIS 401724)	26	26	26

<sup>a</sup> During gestation, doses were administered once every other day at a dose volume of 5 mL/kg; therefore, the weekly dose was equivalent to 3.5 times each dose. During lactation, doses were administered approximately once a week at a dose volume of 17.5 mL/kg to provide approximately the same weekly dose administration.

(page 11 of Study Report)

Route of administration: SC injection, alternating between 2 sites: interscapular and proximal to the tail

Formulation/Vehicle: controls received 0.9% Sodium Chloride for Injection, USP; ISIS 420915 and ISIS 401724 were administered as received, in sterile water for injection

Species/Strain: Crl:CD1<sup>®</sup> (ICR) mice

Study design: dams were dosed at 2.9, 11.4, and 22.9 mg/kg/dose on GD 6, 8, 10, 12, 14, and 16; and at ~10, 40, and 80 mg/kg/dose on LD 0, 6, 12, and 20; necropsies were performed on F<sub>0</sub> F on PND 22, and on F<sub>1</sub> M and F on GD 13 (following 1:1 mating starting at PND ≥85)

Deviation from study protocol: minor deviations were reported that did not affect study interpretations

## Observations and Results

### Dosing Solution Analysis

Samples of ISIS 420915 and ISIS 401724 dosing formulations were analyzed by ion-pair HPLC-UV-MS following completion of the dosing period.

All dosing solutions were within 6% of the nominal concentrations.

**Pregnant Females (Fo)****Mortality**

All animals were observed for mortality twice daily throughout the study.

No drug-related effects on mortality were observed. One LDF was euthanized in extremis on LD 14 with swelling and impairment of a hind limb; the cause of moribundity was not clear. Two LDF and one HDF were found dead on LD 13, following completion of milk collection—these deaths were attributed to the stress of the procedure.

**Clinical Observations**

Details clinical examinations were conducted on each animal once daily.

No drug-related effects on clinical signs were observed.

**Body Weight**

Body weights were recorded on GD 0, 4, 6, 7, 8, 9, 12, 15, and 17; and on LD 0, 4, 7, 10, 14, 17, 19, and 21.

No drug-related effects on body weight were observed.

**Food Consumption**

Food consumption was recorded on days animals were weighed.

Food consumption was reduced in HDF during LD 4-7 (9%), LD 17-21 (11%), and LD 0-21 (8%), compared to controls.

**Necropsy**

Necropsy examinations were performed on pregnant females found dead, euthanized in extremis, or euthanized at scheduled termination on PND 22.

No drug-related effects on macroscopic findings were observed.

**Organ Weights**

Organs weighed included kidneys, liver, and spleen.

Increases were observed in relative weights of liver (HDF, 8%) and spleen (MDF, 19%; HDF, 42%) compared to controls.

**F<sub>1</sub> Litter Data****F<sub>0</sub> Parturition Data**

Parturition parameters evaluated included gestation length, number of pups, number of stillborn pups, gestation index, stillborn index, uterine implantation scar counts, pup weights, number of each sex, and gross abnormalities of pups for each litter at birth.

No drug-related effects were observed on parturition parameters.

**F<sub>1</sub> Pup Survival**

F<sub>1</sub> pup survival was evaluated daily over LD 0 to 4 (pre-cull) and LD 4 to 21 (post-cull).

No drug-related effects were observed on F<sub>1</sub> pup survival.

**F<sub>1</sub> Pup Sex Ratio**

F<sub>1</sub> pup sex ratio (% males per litter) was recorded throughout lactation.

No drug-related effects were observed on F<sub>1</sub> pup sex ratio.

**F<sub>1</sub> Pup Clinical Observations**

Eight pups (4 M, 4 F) were selected from each litter for ongoing evaluation beyond LD 4. Pups were observed daily, and individual external examinations were conducted on LD 0, 4, 7, 14, and 21.

No drug-related effects were observed on F<sub>1</sub> pup clinical signs.

**F<sub>1</sub> Pup Body Weight**

F<sub>1</sub> pup body weights were recorded on LD 0, 4, 7, 14, and 21.

No drug-related effects were observed on F<sub>1</sub> pup body weights.

**F<sub>1</sub> Pup Necropsy**

Necropsies were conducted on F<sub>1</sub> pups that were stillborn, culled at LD 4, found dead during lactation, or not selected on PND 28 for further evaluations.

No drug-related effects were observed on F<sub>1</sub> pup macroscopic findings.

**F<sub>1</sub> Pup Behavioral, Sensory, and Developmental Evaluations**

Evaluations included static righting reflex (LD 2 onward), pinna detachment (LD 2 onward), eye opening (LD 13 onward), air drop righting reflex (LD 16 onward), neuropharmacological evaluation (LD 22, comparable to Irwin test), and auditory response (PND 23).

No drug-related effects were observed on F<sub>1</sub> pup preweaning assessments for physical development, sensory response, or reflex performance.

### **F<sub>1</sub> Pup Sexual Maturation**

Pups were examined for vaginal opening (PND 21 onward) or preputial separation (PND 28 onward).

No drug-related effects were observed on F<sub>1</sub> pup sexual maturation endpoints.

### **F<sub>1</sub> Pup Motor Activity**

Pups selected on PND 28 for evaluation were assessed for motor activity on PND 35 using an automated motion detector for four 5-minute test intervals per animal to record basic movements, fine movements, rearing counts, and total distance.

No drug-related effects were observed on F<sub>1</sub> pup motor activity.

### **F<sub>1</sub> Pup Learning and Memory Assessments**

Pups selected on PND 28 for evaluation were assessed for learning and memory using a fully automated step-through passive avoidance test, initiated between PND 70 and 85, and consisting of a maximum of five 3-minute trials during a single day of testing.

No drug-related effects were observed on F<sub>1</sub> pup learning and memory performance.

### **F<sub>1</sub> In-Life Examinations**

#### **F<sub>1</sub> Mortality**

All animals (at least one per sex per litter) selected on PND 28 for evaluation were observed for mortality twice daily throughout the study.

No drug-related effects were observed on F<sub>1</sub> animal mortality.

#### **F<sub>1</sub> Clinical Observations**

Detailed clinical examinations were conducted once weekly in animals selected on PND 28 for evaluation.

No drug-related effects were observed on F<sub>1</sub> animal clinical signs.

#### **F<sub>1</sub> Body Weight**

Body weight was recorded once weekly from PND 28 through termination for M; and through evidence of positive copulation in F, then on GDs 0, 3, 6, 10, and 13.

No drug-related effects were observed on F<sub>1</sub> animal body weight.

**F<sub>1</sub> Food Consumption**

Food consumption was recorded once weekly from PND 28 through termination for M; and through pairing for F, then on GDs 0, 3, 6, 10, and 13.

No drug-related effects were observed on F<sub>1</sub> animal food consumption.

**F<sub>1</sub> Reproductive Performance**

Following completion of the passive avoidance testing, M and F of the same treatment group were paired 1:1 for mating. Measures of reproductive performance evaluated in F<sub>1</sub> animals included mating index, fecundity index, fertility index, and copulatory interval.

No drug-related effects were observed on F<sub>1</sub> animal reproductive performance.

**F<sub>1</sub> Uterine/Ovarian Examinations**

Following euthanization of F<sub>1</sub> F on GD 13, uterine and ovarian examinations were conducted to determine the mean number of corpora lutea, uterine implantation sites, viable embryos, and resorption sites per animal, as well as mean pre- and post-implantation loss indices.

No drug-related effects were observed on F<sub>1</sub> animal uterine or ovarian parameters.

**F<sub>1</sub> Organ Weights**

Organs weighed in M and F on GD 13 included epididymides, kidneys, liver, ovaries, prostate, seminal vesicles (with coagulating glands), spleen, testes, and uterus (both horns) with cervix.

No drug-related effects were observed on F<sub>1</sub> animal organ weights.

**Milk Analysis**

Mouse milk samples were collected from pregnant F<sub>0</sub> females (4/group) on LD 13, following isoflurane anesthesia, for determination of ISIS 420915 concentrations using a non-GLP ELISA method.

As shown in the sponsor's summary table below, ISIS 420915 concentrations in milk increased dose-dependently.

Group	Dose Level (mg/kg/dose)	Weekly Dose (mg/kg/week)	N	Mean ISIS 420915 Concentration in Milk <sup>a</sup> (µg/mL) ± SD
2	2.9	10	4	0.01242 ± 0.0229
3	11.4	40	4	0.1370 ± 0.0767
4	22.9	80	4	0.7008 ± 1.092

Note: The lower limit of quantitation (LLOQ) for the assay was 0.005 µg/mL. Values are presented as mean ± standard deviation (SD)

<sup>a</sup>All values below the lower limit of quantitation (BLQ) were treated as zero for the statistical summary analysis

N = Number

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### **Toxicokinetics**

Samples of kidney and liver were collected from the first 10 surviving F<sub>0</sub> animals/group and the first 10 surviving F<sub>1</sub> animals/sex/group following scheduled necropsies on PND 22 and GD 13, respectively. Liver samples from female F<sub>0</sub> mice were analyzed for ISIS 420915 concentrations using a validated HPLC-UV method. Liver samples from F<sub>1</sub> mice and kidney samples from F<sub>0</sub> and F<sub>1</sub> mice were not analyzed.

As shown in the sponsor's summary table below, dose-dependent increases were observed in the concentration of ISIS 420915 in maternal liver.

Group	Dose Level (mg/kg/week) <sup>a</sup>	Day	Gender	Number of Doses	N	Liver Concentration (µg/g)
1	0	PND 22	Female	10	10	BLQ
2	10	PND 22	Female	10	9 <sup>b</sup>	88.4 (±9.04) <sup>b</sup>
3	40	PND 22	Female	10	10	275 (±36.2)
4	80	PND 22	Female	10	10	468 (±67.0)

Note: The lower limit of quantitation (LLOQ) for the assay was 10 µg/g. 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).

<sup>a</sup>For all dose groups, doses were administered on GD 6, 8, 10, 12, 14, and 16 and on LD 0, 6, 12, and 20.

<sup>b</sup>Animal number 355 was excluded from the mean and standard deviation due to the fact that it was identified as an outlier (liver concentration for animal number 355 was 446.4 µg/g). The mean (±SD) liver concentration would be 124 (±114) if animal number 355 was not excluded.

BLQ = Below the lower limit of quantitation (10 µg/g); PND = Postnatal Day

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### **Liver TTR mRNA Analysis**

Samples of liver were collected on from all F<sub>0</sub> females administered control or 40 mg/kg/week ISIS 401724 surviving to necropsy on PND 22. Levels of liver TTR mRNA were measured using RT-PCR, normalized to total RNA.

Administration of 40 mg/kg/week SC ISIS 401724 to pregnant/lactating mice from GD 6 through LD 20 (9 doses) resulted in a ~60% reduction in liver TTR mRNA compared to controls. No adverse maternal or developmental effects were associated with the reductions in TTR mRNA.

## **10 Special Toxicology Studies**

### **Evaluation of ISIS 420915 and ISIS 401724 in the Mouse Influenza Host Resistance Model: Viral Clearance, TDAR, and Biological Mediators**

(ISIS Study 420915-AS16; [REDACTED] (b) (4)  
[REDACTED] initiated May 31, 2016; Amended Final Report dated June 28, 2017; GLP; QA)

Female Balb/c mice (N=50/group) were administered ISIS 420915 (10, 40, or 80 mg/kg/week; Groups 3, 4, and 5, respectively), ISIS 401724 (40 mg/kg/week; Group 6), or placebo (0.9% Sodium Chloride for Injection, USP; Group 2) via SC injection once weekly for 8 weeks (Weeks -8 to -1) prior to infection with influenza virus (~4 x 10<sup>4</sup> plaque forming units [PFU], intranasally), on the day of infection (Day 0), and for 3 weeks after infection (Days 7, 14, and 21). A positive control group (N=50; Group 7) was administered dexamethasone (20 mg/kg/day, PO) once daily from Day -3 to Day 21. Parallel groups (N=10/group) received placebo (Group 8) or ISIS 420915 (0, 10, 40, or 80 mg/kg/week; Groups 9, 10, and 11, respectively) from Week -8 to Day 0, but were not infected with influenza. A naïve group (N=10; Group 1) was left untreated. Groups 1-7 were sacrificed on Days 2, 6, 8, 10, and 21 (N=10/group/time point) for determination of terminal body, lung, and spleen weight; viral clearance (from lung); and serum influenza-specific IgG levels. Groups 8-11 were sacrificed on Days 1 and 3 (N=5/group/time point) for determination of terminal body, spleen, and liver weight; and serum cytokine (MCP-1), total IgG/M, anti-dsDNA Ab, and serum amyloid A (SAA) levels.

No drug-related effects were observed on viral clearance, influenza-specific IgG levels (TDAR), total IgG/M, SAA, anti-dsDNA Ab, body weight, or organ weights. MCP-1 was slightly increased (61%) in the HD group compared to controls. Under the conditions tested, SC administration of ISIS 420915 or ISIS 401724 to F Balb/c mice did not result in immunosuppression or immunotoxicity in this viral host resistance model. As expected, administration of the positive control, dexamethasone, resulted in suppressed viral clearance from lung and decreased anti-influenza IgG compared to placebo controls.

**Assessment of ISIS 420915 to Directly Activate Human Platelets**

(IONIS Study 420915-IS10; IONIS Pharmaceuticals, Carlsbad, CA; Final Report dated June 14, 2017; non-GLP; non-QA)

Platelet rich plasma (PRP) was isolated from whole blood samples collected from healthy volunteers (Donors 2, 6, and 60) for use in these in vitro assays of platelet activation, as measured by increases in activation markers (% CD41<sup>+</sup>/CD62P<sup>+</sup> and % CD41<sup>+</sup>/PAC-1<sup>+</sup> cells) observed with flow cytometry following incubation with the test compounds. ADP was used as a positive control for platelet activation, and ISIS 141923 was used as a non-CpG MOE-modified ASO comparator that does not match any human mRNA sequence.

In Part A, ADP activated platelets from Donors 6 and 60 dose-dependently at 2.5, 5, and 10  $\mu\text{M}$ , as expected. In contrast, no significant platelet activation was observed with ISIS 141923 (0.0064-100  $\mu\text{M}$ ) or ISIS 420915 (0.4-4  $\mu\text{M}$ ; within the range of clinically relevant concentrations).

In Part B, no activation of platelets from Donor 2 was observed following incubation with 16  $\mu\text{M}$  normal TTR (from 2 patients) or 3 different TTR mutations (from 6 different patients). Positive (5  $\mu\text{M}$  ADP) and negative (100 mg/mL albumin) controls performed as expected.

In Part C, no activation of platelets was observed following incubation with normal TTR (0.5, 1, or 6  $\mu\text{M}$ ) in the presence or absence of ISIS 141923 or ISIS 420915 (2  $\mu\text{M}$ ). Positive (5  $\mu\text{M}$  ADP  $\pm$  normal TTR) and negative (albumin; 0.5, 1, or 6  $\mu\text{M}$ ) controls performed as expected, except that platelet activation by ADP was decreased in the presence of TTR.

### A 13-Week Repeat Dose Toxicity and Impurity Qualification Study of GSK2998728 (ISIS 420915) in CD-1 Mice

(ISIS Study 420915-AS15   (b) (4) initiated October 7, 2016; final report dated July 28, 2017; GLP and QA, except for analysis of drug levels in liver)

CrI:CD1® (ICR) mice (N=6/sex/group) were administered vehicle (PBS) or ISIS 420915 ± IM 1 or IM 2 (mixtures of ISIS 420915 impurities) via SC injection once weekly for 13 weeks, alternating between two dosing sites (interscapular and proximal to the tail), as described in Table A below:

<b>Table A: Group Assignments</b>			
Group Number	Dose Level (mg/kg/week) <sup>a</sup>	Treatment	Number of Animals Male/Female
<b>Main Study</b>			
1	0	Saline (Control)	6/6
2	10	ISIS 420915	6/6
3	50	ISIS 420915	6/6
4	10	ISIS 420915 IM 1	6/6
5	50	ISIS 420915 IM 1	6/6
6	10	ISIS 420915 IM 2	6/6
7	50	ISIS 420915 IM 2	6/6
<sup>a</sup> The control and test articles were administered weekly via subcutaneous injection with a scheduled necropsy occurring on Day 93. IM – Impurity Mixture			

*(page 15 of Study Report)*

Evaluations included mortality, clinical observations, body weight, food consumption, ophthalmic examinations, hematology, clinical chemistry, gross pathology, organ weights, and histopathology.

Drug-related effects were limited to clinical chemistry, organ weights, and histopathology findings. Administration of all 3 HD formulations resulted in similar increases in ALT (up to 2.0x) and AST (up to 1.8x), compared to controls. Absolute and relative organ weights were increased for spleen (up to 1.3x in LDF groups and up to 1.6x in HD M and F groups) and decreased for thymus (up to 1.4x in HD groups), compared to controls. Dose-dependent increases were observed in the incidence and severity of accumulation of basophilic granules in tubular epithelium of the kidneys;

accumulation of vacuolated/granular macrophages in liver, lymph nodes, injection sites, and many other tissues; and increased incidence of extramedullary hematopoiesis in spleen. These microscopic findings were generally minimal to mild. There were no apparent additional toxicities related to the administration of the impurities present in IM-1 or IM-2 formulations. Levels of ISIS 420915 in liver were similar among the three formulations tested: ~60-80 µg/g in LD groups and ~320-370 µg/g in HD groups.

The sponsor's tables below list the types of drug substance impurities included in IM-1 and IM-2:

**Summary of Control Methods for Product-Related Impurities**

Product-Related Impurities Group	Control Method
(b) (4) impurities	(b) (4)
(b) (4) impurities	
Any unspecified impurity	
Total impurities	
(b) (4) impurities	
(b) (4) impurities	
(b) (4) impurities	

(page 5 of sponsor's Justification of Specification, Section 3.2.S.4.5 of NDA 211172)

**Table 2 Levels of Impurities Dosed in Toxicological Safety Study 420915-AS15**

Product-Related Impurities	(b) (4) Impurities Content (%w/w)	Inotersen Sodium Specification Limit (%w/w)
(b) (4) impurities	(b) (4)	(b) (4)
(b) (4) impurities		
(b) (4) impurities		
(b) (4) impurities		

(page 6 of sponsor's Justification of Specification, Section 3.2.S.4.5 of NDA 211172)

The study report states that (b) (4)

[Redacted text block]



(b) (4)

The number of animals in each dose group (N=6/sex/group) was lower than the minimum (10/sex/group) expected for a pivotal toxicity study in rodent. Therefore, this study, on its own, does not provide adequate qualification of the impurities tested.

## 11 Integrated Summary and Safety Evaluation

Inotersen is a 20-base 2'-methoxyethyl-modified phosphorothioate antisense oligonucleotide designed to bind specifically to a sequence within the 3'-untranslated region of the human transthyretin (hTTR) mRNA, resulting in degradation of the mRNA by RNase H1 and reduced synthesis of TTR protein in liver cells. The proposed indication is "for the treatment of adult patients with hereditary TTR amyloidosis with polyneuropathy (hATTR-PN) (b) (4)

Patients with hATTR-PN have mutations in the hTTR gene that destabilize the protein's normal tetrameric conformation and allow the TTR protein to misfold into amyloidogenic conformations that accumulate in the peripheral nervous system (and multiple other organs), causing damage. The proposed therapeutic dosing regimen is subcutaneous injection of 284 mg inotersen (300 mg inotersen sodium salt) (b) (4) once weekly (b) (4)

In vitro transfection studies demonstrated that inotersen concentration-dependently reduced hTTR mRNA levels in HepG2 cells, isolated hepatocytes from a transgenic mouse expressing hTTR, and isolated hepatocytes from a cynomolgus monkey ( $IC_{50} = 0.2, 0.1, \text{ and } 0.8 \mu\text{M}$ , respectively), compared to controls. The specificity of inotersen for the intended target, TTR mRNA, was demonstrated by the absence of in vitro antisense activity toward potential off-target mRNAs identified via bioinformatics analyses. Additional bioinformatics analyses showed that the frequency of spontaneously occurring SNP-related mismatches in the target TTR mRNA sequence is very low ( $<0.00005$ ), suggesting that reduced effectiveness of inotersen based on such mismatches would be rare.

In vivo studies using transgenic mice expressing human TTR I84S demonstrated statistically significant, dose-dependent reductions of up to 90-94% in liver TTR mRNA and plasma hTTR protein levels following twice-weekly administration of 20, 50, or 100 mg/kg SC inotersen for 4 weeks, compared to PBS controls. A time-course study showed that maximal reduction of hTTR mRNA in liver and hTTR protein in plasma occurred within 3 days of administration of a single dose of 100 mg/kg SC, correlating with the maximum level of inotersen ASO observed in liver. Similarly, in a 12-week study in cynomolgus monkey, administration of 25 mg/kg inotersen twice weekly (following 3x/week in Week 1) resulted in reductions of ~80% in hTTR mRNA in liver (compared to controls) and ~80% and ~60% in plasma levels of hTTR and RBP4 protein, respectively (compared to baseline); no effects were observed on serum TSH, T3, or T4 levels. In the 39-week chronic toxicity study in monkey, the HD of 20 mg/kg/week resulted in reductions of ~60% in hTTR mRNA in liver (compared to controls) and hTTR and RBP4 protein in plasma (compared to baseline).

No consistent drug-related effects were observed in standard safety pharmacology studies conducted with inotersen (an Irwin's test in CD-1 mice, a cardiovascular and respiratory study in telemetered cynomolgus monkeys, and an in vitro hERG assay HEK293 cells).

Absorption, distribution, metabolism, and excretion studies were conducted in mouse, rat, monkey, and human. In a 6-week SC study in male CD-1 mice, inotersen distributed

extensively to liver and kidney and ~2-8% was excreted intact in urine within 48 hours of the final dose. In SD rats administered [<sup>3</sup>H]-ISIS 420915 via SC injection, radioactivity levels peaked in plasma at 1 hour postdose, then distributed rapidly and broadly into tissues, primarily kidneys, liver, mesenteric lymph nodes, bone marrow, thyroid, spleen, bone, and pancreas. Excretion of radioactivity from rat tissues was slow, at ~2% per day after ~13% in the first 24 hours postdose, primarily in urine (~45%) and feces (~12%). Metabolism of inotersen to shorter oligonucleotides via endonuclease and exonuclease digestion apparently occurred over time, as unchanged parent drug accounted for ~70% of radioactivity in plasma, kidney, and liver, but very little was present in urine. Similarly, analysis of samples collected after repeated dosing in mouse, monkey, and human showed that parent drug accounted for ~74-95% of total oligonucleotide present in plasma, while shorter metabolites (mostly 6- to 12-mers), accounted for ≤ ~7%; in mouse and monkey, parent drug accounted for ~80-95% of total oligonucleotide present in liver and kidney samples, while shorter oligomers each accounted for < 5%. In urine collected over 24 hours after a single dose of 300 mg inotersen, parent drug accounted for 0.78% of the total dose administered, while metabolites and parent together accounted for 13.5%.

In vitro studies demonstrated that plasma protein binding of inotersen was high (>94%) in human and monkey, and that inotersen did not act as a substrate or inhibitor of any of the transporters tested (human BCRP, P-gp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, or BSEP) or as an inducer of CYPs 1A2, 2B6, or 3A4.

The general toxicology of inotersen injected subcutaneously was assessed in repeated-dose studies in mouse (13- and 26-week), rat (26-week), and monkey (13- and 39-week). The primary effects observed across species were related to accumulation of basophilic granules (consistent with drug-related material) in Kupffer cells in liver, tubular epithelial cells in kidney, histocytes in lymph nodes, and macrophages at injection sites and in other organs, along with associated inflammatory responses typically seen with administration of ASOs. In addition, severe thrombocytopenia or perivascular mixed cell infiltration in multiple organs associated with increased cytokines/chemokines occurred in individual animals across dose groups in the 39-week monkey study, and kidney toxicity was observed in the two highest dose groups in the 26-week rat study.

In a 13-week study in CD-1 mice, SC administration of inotersen (4, 12, 40, or 100 mg/kg on Days 1, 3, 5; then once weekly from Day 7 onward) resulted in the following changes, compared to vehicle controls: modest non-adverse effects including reduced red cell mass (~10%); increased ALT (~2x, M); increased relative weight of spleen decreased relative weight of thymus; dose-dependent increases in the incidence and severity of basophilic granules in kidney proximal tubular epithelial cells and (with hypertrophy) in liver Kupffer cells and lymph node histiocytes; mononuclear infiltrates in liver and injection sites; and thymic involution. All changes showed at least partial reversibility during the 13-week recovery period except for those in thymus. No additional toxicities were observed in a parallel group administered 40 mg/kg/dose SC ISIS 401724, a mouse-specific ASO that reduced liver TTR mRNA ~70% compared to controls.

In a 13-week study in CByB6F1 mice, SC administration of inotersen (12, 40, or 100 mg/kg on Days 1, 3, 5; then once weekly from Day 7 onward) resulted in similar observations of reduced red cell mass, accumulation of oligonucleotide in tissues, and related inflammatory responses, in addition to the following changes, compared to vehicle controls: reductions in reticulocytes and platelets; individual cell necrosis in liver (associated with slight increases in ALT, AST, and ALP); and thymic lymphocytic depletion. As in the previous study, no additional toxicities were observed after administration of the mouse-specific ASO, ISIS 401724, which reduced liver TTR mRNA ~80% compared to controls.

In a 26-week study in CD-1 mice, SC administration of inotersen (3, 10, 40, or 80 mg/kg on Days 1, 3, 5; then once weekly from Day 7 onward) resulted in the following changes, compared to vehicle controls: reductions in RBC mass, platelets, albumin, and total protein; increased relative weights of liver and spleen; decreased relative weights of thymus and heart; basophilic granules/vacuolation in renal cortical tubular epithelium; basophilic granules/vacuolation/hypertrophy of Kupffer cells in liver; infiltration of macrophages with basophilic granules in lymph nodes, spleen, injection sites, and other organs; thymic cortical lymphoid depletion; and increased splenic extramedullary hematopoiesis. All effects were at least partially reversed during the 13-week recovery period except for the changes in relative weights of liver and heart and the thymic lymphoid depletion. The NOAEL was the high dose of 80 mg/kg/dose.

In a 26-week study in Sprague-Dawley rats, SC administration of inotersen (5, 15, or 40 mg/kg on Days 1, 5, 10; then once weekly from Day 14 onward) resulted in the following changes, compared to vehicle controls: reduced food consumption, body weight, and body weight gain; decreased hemoglobin, neutrophils, eosinophils, and platelets; increased reticulocytes, lymphocytes, monocytes, and serum TNF levels; increased urine protein/creatinine and albumin/creatinine ratio, correlated with histopathological findings in kidneys (basophilic granules and cytoplasmic vacuolation in tubular epithelial cells, vacuolated/granular macrophages, interstitial mononuclear cell infiltration, and increased glomerular cellularity and matrix); basophilic granules in macrophages and mononuclear cell infiltration in multiple organs; bile duct hyperplasia and individual hepatocyte necrosis in liver; hyperplasia in spleen and lymph nodes; thymic lymphoid depletion; increased hematopoietic cellularity in spleen and bone marrow; increased trabecular bone; interstitial cell hypertrophy/hyperplasia in ovaries; increased endometrial cell thickness in uterus; and macrophages, infiltration, fibrosis, and edema at injection sites. Based on the adverse effects on the structure and function of the kidneys and substantial reductions in body weight gain observed at  $\geq 15$  mg/kg/week, the NOAEL in this study was the low dose of 5 mg/kg/dose. No additional toxicities were observed in a parallel group administered 15 mg/kg/dose SC ISIS 594799, a rat-specific ASO that reduced liver TTR mRNA 34% in M and 18% in F, compared to controls.

In a 13-week study in cynomolgus monkeys, SC administration of inotersen (4, 8, 12, or 40 mg/kg on Days 1, 3, 5; then once weekly from Day 7 onward) resulted in the following changes, compared to vehicle controls: transiently increased complement split product Bb in plasma; reduced serum albumin and albumin/globulin ratio; basophilic granules, vacuolation, degeneration/regeneration in proximal tubular epithelial cells, along with tubular dilatation, erythrocytic tubular casts, fibroconnective tissue

proliferation, and hemorrhage in kidneys; and basophilic granules and hypertrophy in liver Kupffer cells and lymph node histiocytes. No adverse effects were observed following a 13-week recovery period. The NOAEL in this study was 12 mg/kg/dose, based on the adverse kidney findings observed at 40 mg/kg/dose.

In a 39-week study in cynomolgus monkey (with a 26-week interim sacrifice), SC administration of inotersen (3, 6, 10, or 20 mg/kg/dose once weekly) resulted in marked drug-related thrombocytopenia in one animal in each of the two highest dose groups, with observations of petechiae and/or bruising starting on Days 68 and 94 and necessitating early euthanasia on Days 74 and 151, respectively. Platelet counts in both animals were extremely low at euthanasia ( $2-3 \times 10^3/\mu\text{L}$ ) and postmortem examinations revealed hemorrhages in multiple organs. Drug-related microscopic findings included basophilic granules and vacuolation in the proximal tubular epithelium of the kidney, Kupffer cells in the liver, and histiocytes in the lymph nodes; infiltration of mononuclear and mixed cells, edema, and/or hemorrhage at the injection sites; and mixed cell perivascular infiltration in multiple organs. The occurrence of the most widespread perivascular infiltration in five individual animals across the three lowest dose groups was correlated with increases in the levels of anti-drug antibody (only these 5 had titers  $\geq 128$ ), CRP, IL-6, MIP-1 $\beta$ , TNF $\alpha$ , and total IgG and IgM; and (in 4/5 animals) with moderate reductions in platelet counts (49-70%, compared to baseline). The basis for the susceptibility of individual animals across dose groups to an exaggerated inflammatory response in multiple organs (correlated with increases in ADA, CRP, cytokines/chemokines, and total IgG and IgM, and moderate reductions in platelets) or to a severe potentially fatal thrombocytopenia (which was not correlated with ADA or an exaggerated inflammatory response) was not explained. No effects were observed in bone marrow in the two animals euthanized early due to severe thrombocytopenia; these animals showed increased levels of anti-platelet IgG and IgM compared to baseline, but other animals showed similar increases that were not correlated with changes in platelet levels. Anti-platelet factor 4 antibody levels were not increased in the two animals with the most severe thrombocytopenia. The NOAEL in this study was 6 mg/kg/week, based on the severe thrombocytopenia observed at 10 and 20 mg/kg/week. While exaggerated inflammatory responses were observed in one animal at 3 mg/kg/week and 3 animals at 6 mg/kg/week, these effects were not clearly correlated with adverse clinical signs or body weight loss.

Severe thrombocytopenia was observed in three patients during clinical trials of inotersen, and a reduction in platelet count of >30% from baseline was observed in ~70% of patients given inotersen compared to ~5% of controls; both severe and moderate thrombocytopenia are known potential adverse effects of oligonucleotide therapies, and may occur via different mechanisms (see Chi, Gatti, and Papoian, 2017, *Drug Discovery Today* 22(5):823-833).

Inotersen was negative in a standard battery of valid genotoxicity studies (in vitro bacterial reverse mutation and CHL cell chromosome aberration assays and an in vivo mouse bone marrow micronucleus assay).

In a 6-month carcinogenicity study in Tg.rasH2 mice, administration of inotersen (10, 30, or 80 mg/kg/week SC) or ISIS 401729 (30 mg/kg/week SC) resulted in no drug-related

increases in neoplasms or non-neoplastic adverse effects, compared to vehicle controls. The CDER Executive Carcinogenicity Assessment Committee concluded that “the study was adequately conducted and negative for drug-related neoplasms in males and females” (ECAC Meeting Minutes dated February 28, 2018). Non-adverse drug-related effects observed included increased serum ALP, ALT, and AST; increased urea nitrogen in males; decreased total protein and albumin; basophilic granules in kidney tubular cells and liver Kupffer cells; subacute inflammation in liver; vacuolation in macrophages in lymph nodes and in intrasinusoidal cells in spleen; lymphoid depletion in spleen and thymus; and mixed cell inflammation at the injection sites. Administration of 30 mg/kg/week SC ISIS 401724, the mouse-specific ASO, resulted in mean reduction of 65-70% in TTR mRNA levels in liver, compared to controls.

The reproductive and developmental toxicity of inotersen was assessed in a fertility and embryofetal development study in mouse, an embryofetal development study in rabbit, and a pre- and postnatal development study in mouse. In the fertility and embryofetal development study, SC administration of inotersen (3, 15, or 25 mg/kg/dose once every other day from prior to mating through GD 15) to CD-1 mice resulted in the following effects in males at 25 mg/kg/dose (87.5 mg/kg/week): increases in body weight, body weight change, and weights of spleen and liver; and reductions in hematocrit, hemoglobin, albumin, and A/G ratio, compared to controls. The NOAEL for reproductive performance, fertility, and developmental toxicity was the HD of 87.5 mg/kg/week ISIS 420915. A parallel group administered 15 mg/kg/dose SC ISIS 401724, the mouse-specific ASO, showed reduction of ~60% in liver TTR mRNA, compared to controls; no adverse effects were observed.

In the GLP embryofetal development study, SC administration of inotersen (2.5, 5, and 15 mg/kg/dose once every other day from GD 6 to 18) to New Zealand White rabbits resulted in the following effects at 15 mg/kg/dose (52.5 mg/kg/week): premature delivery, body weight loss, and increased white blood cells, neutrophils, and lymphocytes in dams; and reduced mean fetal body weight, compared to controls. No teratogenicity was observed. The NOAEL for maternal and fetal developmental toxicity was 5 mg/kg/dose (17.5 mg/kg/week), based on the premature delivery and reduced maternal and fetal body weights. Inotersen concentrations in fetal liver were below detectable levels; mean concentrations in placenta were ~20-fold lower than those in maternal liver on GD 20, and were undetectable on GD 28.

In the pre- and postnatal development study, SC administration of inotersen to CD-1 mouse dams (2.9, 11.4, or 22.9 mg/kg/dose once every other day from GD 6 to GD 16, then 10, 40, or 80 mg/kg once weekly through LD 20) resulted in reduced food consumption and increased relative weights of liver and spleen in dams at 22.9 mg/kg/dose, compared to controls. The NOAEL for maternal and developmental toxicity was the high dose of 80 mg/kg/week. Concentrations of inotersen measured in milk on LD 13 were 700 to 7000 times lower than concentrations in maternal liver. A parallel group administered 40 mg/kg/week SC ISIS 401724, the mouse-specific ASO, showed reduction of ~60% in liver TTR mRNA, compared to controls; no adverse effects were observed.

Special toxicology studies of inotersen included an assessment of immunosuppression and immunotoxicity in a mouse influenza host resistance model, an in vitro platelet activation assay, and an assessment of the potential toxicity of process-related and starting material impurities in a 13-week SC toxicity study in mouse. In the host resistance assay, female Balb/c mice administered inotersen (10, 40, or 80 mg/kg/week SC) for 8 weeks prior to infection with influenza virus resulted in a 61% increase in MCP-1 in the high dose group, compared to controls, but no evidence of immunosuppression or immunotoxicity was observed; the positive control, dexamethasone, suppressed viral clearance from lung and decreased anti-influenza IgG compared to placebo controls. No drug-related effects were observed in a parallel group administered 40 mg/kg/week SC ISIS 401729, the mouse-specific ASO. Inotersen had no effect on the activation of isolated human platelets at up to 5  $\mu$ M alone, or at 2  $\mu$ M in the presence of normal TTR protein; the positive control, ADP, activated platelets as expected, though the extent of activation was reduced in the presence of TTR protein.

In the assessment of the potential toxicity of impurities, SC administration of inotersen (10 or 50 mg/kg/week once weekly for 13 weeks) in the presence or absence of

(b) (4)

impurities) to CD-1 mice resulted in similar toxicities: increases in ALT, AST, and relative spleen weight; decreases in relative thymus weight; accumulation of basophilic granules in tubular epithelium of the kidneys; accumulation of vacuolated/granular macrophages in liver, lymph nodes, injection sites, and many other tissues; and increased incidence of extramedullary hematopoiesis in spleen. However, the number of animals per group (N=6/sex/group) was too low for this study, on its own, to provide adequate qualification of these impurities. Based on discussion of potential safety risks of these types of impurities with FDA experts, the proposed product-related impurity specifications are acceptable even without this qualification study.

### Summary of Key Toxicities and Plasma Exposures in Pivotal Chronic Studies

Toxicity	Duration & Species	NOAEL	Mean AUC <sub>0-24 hr</sub> (µg•hr/mL)	Safety Margin Based on AUC*
↓platelets and red cell mass (M)	26-week mouse	80 mg/kg/wk	NA	NA
↓BW & BWG ↑cellularity of glomeruli ↑glomerular matrix ↑urine protein & albumin/creatinine ratios ↓hemoglobin	26-week rat	5 mg/kg/wk	~130 <sup>#</sup>	~1.6x
Severe thrombocytopenia associated w/hemorrhages, necessitating early euthanasia	39-week monkey	6 mg/kg/wk	~168 <sup>&amp;</sup>	~2.1x

\*Mean Day 449 AUC<sub>0-24 hr</sub> in human = 80.4 µg•hr/mL at 300 mg/week SC inotersen (see Appendix)

<sup>#</sup>Estimated based on mean values of 408 and 1000 µg•hr/mL at 15 and 40 mg/kg/week, respectively, observed in M

<sup>&</sup>This mean Day 274 AUC<sub>0-48 hr</sub> at 6 mg/kg/week is reasonably close to the AUC<sub>0-24 hr</sub> value, as the mean inotersen plasma level was ~87% lower than the C<sub>max</sub> by 24 hours postdose  
NA: Not available

As illustrated in the table above, the safety margins for severe, life-threatening thrombocytopenia in monkeys and adverse kidney function in rats were quite low; therefore, adequate monitoring for these toxicities in patients is warranted.

### Recommendations

The nonclinical data submitted adequately support the approval of inotersen for the treatment of adult patients with hereditary transthyretin amyloidosis with polyneuropathy. Per prior agreement, a two-year carcinogenicity study of inotersen in rat should be conducted as a post-marketing requirement.

## 12 Appendix

**Table 11 Summary of Key Plasma Pharmacokinetic Parameters for Inotersen by Study following SC Administration(s) of 300 mg Inotersen**

Study/Day	Dose (mg)	N	C <sub>max</sub> (µg/mL)	T <sub>max</sub> (h)	AUC <sub>0-24h</sub> (µg*h/mL)	CL <sub>0-24h/F</sub> (L/h)	t <sub>1/2λz</sub> (day)
ISIS 420915-CS1							
Day 1	300	8	6.58 (43.6)	3.00 (3.00-8.00)	74.4 (41.4)	4.03 (41.4)	NA
Day 22	300	7	7.09 (42.3)	3.00 (1.50-4.00)	70.8 (31.5)	4.24 (31.5)	17.6 (29.1)
ISIS 420915-CS2							
Day 1	300	10	10.1 (84.2)	3.18 (0.470, 8.03)	90.6 (48.5)	3.31 (48.5)	NA
Day 240	300	7	6.26 (38.8)	3 (2.00, 4.08)	74.3 (28.7)	4.04 (28.7)	NA
Day 449	300	8	7.83 (39.5)	3.77 (2.95, 6.00)	80.4 (68.8)	3.73 (68.8)	25.5 (45.1) <sup>a</sup>

Note: Data are presented as geometric mean (geometric mean coefficient of variation CV%), with the exception of T<sub>max</sub>, which is presented as median (min-max)

a The terminal elimination half-life (t<sub>1/2λz</sub>) was determined from subjects not rolling over to OLE study CS3 (N = 10) and are not from subjects in the PK subgroup

Source: [CTD Section 5.3.4.1 CS1 CSR Table 14.2.1.2](#) and [Table 14.2.1.3](#)  
[CTD Section 5.3.5.1 CS2 CSR Table 3.03](#) and [3.04](#)

*(page 45 of sponsor's Summary of Clinical Pharmacology Studies)*

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/s/  
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09/19/2018

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09/19/2018