CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203568Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 203568
Agency receipt date: March 29, 2012
Drug: Kynamro (mipomersen sodium) Injection
Sponsor: Genzyme Corp.
Indication: Treatment of Homozygous Familial Hypercholesterolemia

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of mipomersen for the indication listed above.

The recommended pharmacologic class for mipomersen is oligonucleotide inhibitor of apolipoprotein B-100 synthesis.

Mipomersen was not teratogenic in mice or rabbits at doses providing modest margins of exposure (2 to 5 times) compared to humans. Some effects were noted in pups from female rats treated with mipomersen from gestation day 6 through day 20 of lactation. Decreases in pup body weights, impaired reflexes and grip strength were observed at approximately 2-times the anticipated human dose and decreased rat pup survival was observed at approximately 3-times the anticipated human dose.

Two year carcinogenicity studies of mipomersen were conducted in rats and mice. The Executive Carcinogenicity Assessment Committee found the studies adequate. The Committee concluded that hepatocellular adenomas and combined hepatocellular adenomas or carcinomas were drug-related in female mice administered 60 mg/kg/week mipomersen. In addition, hepatocellular adenomas or carcinomas, combined, in both sexes were drug-related in mice administered 60 mg/kg/week of a mouse surrogate oligonucleotide.
Fibrosarcoma of the skin/subcutis in males and hemangiosarcomas in females administered 60 mg/kg/week were also considered drug-related. Lower doses did not exhibit increases in drug-related neoplasms.
Fibrous histiocytoma of the skin/subcutis in male and female rats at ≥ 10 mg/kg/week were considered to be drug-related. In addition, fibrosarcoma of the skin/subcutis in females at ≥ 10 mg/kg/week and combined fibroma/fibrosarcoma/fibrous histiocytoma of the skin/subcutis in females at ≥ 10 mg/kg/week were considered to be drug-related.

Conclusions:
I agree with the division pharm/tox conclusion that mipomersen can be approved from the pharm/tox perspective for the indication of homozygous familial hypercholesterolemia. Some of the nonclinical findings occurred at doses or

exposures that are relatively close to clinical doses/exposures. However, some of the findings are likely to be of low relevance to humans or offer a risk that may be acceptably balanced if adequate clinical benefit has been shown for this particular indication. I reviewed draft labeling and provided comments to the division separately.

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/s/

PAUL C BROWN
01/23/2013

DEPARTMENT OF HEALTH & HUMAN SERVICES
Food and Drug Administration

Memorandum

Date: December 3, 2012
From: Karen Davis-Bruno PhD; Pharmacology Supervisor; DMEP
Subject: Supervisory Pharmacology/Toxicology Memo
To: NDA 203-568

Reference is made to the primary Pharmacology/Toxicology Review which recommends approval. I agree with this recommendation.

Mipomersen (Kynamro) is a drug product with a novel mechanism of action for treatment of an orphan dyslipidemia indication of homozygous familial hypercholesterolemia (hoFH). It is a phosphorothioate anti-sense oligonucleotide (ASO) that inhibits the synthesis of human apoB100. Pharmacology studies in multiple species have established that apoB100 synthesis reduces the circulating levels of apoB containing lipoproteins (i.e. VLDL, LDL and lipoprotein a). Although rodent models do not imitate the atherosclerotic plaque rupture and ensuing myocardial infarction seen in the human disease, the mouse models of atherosclerosis (e.g. LDLr-KO mouse, human apoB transgenic/ LDLr-KO mouse and apoE-KO mouse) do show a reduction in aortic plaque burden that correlates with ASO mediated decreases in apoB100 expression.

In contrast, phosphorothioate ASOs are anticipated to have proinflammatory effects as a result of stimulation of the innate immune system, in particular macrophages and dendritic cells leading to release of cytokines and chemokines. Additionally, ASOs activate complement via the alternative pathway by inhibition of factor H, a negative regulator of the alternative pathway. The proinflammatory signals seen in all animal species tested with mipomersen are dose-related and include:

- Evidence of acute phase response
- Changes in lymphoid organ weights
- Infiltration of histiocytes ± lymphocytes into multiple tissues
- Histiocyte activation (i.e. hypertrophy/ hyperplasia)
- Occasional infiltrates of leukocytes (e.g. neutrophils or eosinophils) into multiple tissues
- Lymphoid hyperplasia
- Changes in leukocyte levels
- Increased cytokine and chemokines
- Injection site inflammation

Complement activation was investigated in monkeys (not rodents) in the

mipomersen development program. This is observed at a 20 mg/kg SC dose of mipomersen (Cmax=47 mcg/ml) in the monkey (~6-times the anticipated systemic exposure at the maximum clinical dose, MRHD= 200 mg qw). *In vitro* assessments indicate an absence of direct mast cell activation in the mouse. An influenza host resistance assay in mice did not find perturbations in the ability to resolve a viral infection, although a slight to moderate decrease in the magnitude of an anti-influenza IgG response at all doses (20-100 mg/kg/week) of mipomersen or the mouse surrogate tested was observed. This indicates that it is unlikely that mipomersen induces immunosuppression directly. It may however, potentiate immunosuppression at high drug exposures by an indirect effect through for example depletion of C3 complement levels.

The immunologic effects of mipomersen by complement activation, may be implicated in the observed inflammatory effects observed in chronic toxicity studies in multiple tissues (vascular, cardiac, renal, liver) in multiple species.

General Toxicology: Chronic studies included a 6-month mouse and 12-month monkey toxicity study with mipomersen treatment weekly. The mouse study also included dosing with a mouse specific surrogate (ISIS 147764) at a single dose of 75 mg/kg/wk.

Mouse Toxicity:

Findings in a 6-month SC mouse study

Dose (mg/kg/wk)	Human AUC Multiple	Findings
2	<1	Lymphohistiocytic infiltrate injection site Kupffer cell basophilic granules
10	<1	Lymphohistiocytic infiltrate multiple tissues ↓ RBC ↑ MCP-1
25	1 X	↑platelets ↓ albumin Extramedullary hematopoiesis (spleen) ↑ weight: liver, spleen, heart (up to 1.5X)
75	4 X	↑ ALT, AST, ALKP (2-3X) ↓ Protein, A/G Bone marrow granulocyte hyperplasia Thymus lymphoid depletion ↓ Liver apoB mRNA (30-50%) w/ ISIS 147764 mouse surrogate

The sponsor has performed cytokine/chemokine analysis on a variety of proinflammatory factors. MCP-1 is the only factor that demonstrates a dose-dependent increase at mipomersen doses ≥25 mg/kg/wk. MCP-1 (monocyte chemotactic protein) recruits monocytes, dendritic and memory T cells to sites of injury and infection. It is produced by macrophages and endothelial cells.

Minimal- mild lymphohistiocytic infiltrates (macrophages containing basophilic granular material) in multiple tissues and minimal –mild lymph node sinus histiocytosis reflect proinflammatory effects and cellular uptake of the drug. Minimal-mild basophilic granules in the kidney proximal tubule and liver Kupffer cells (minimal-moderate) represents tissue accumulation of mipomersen (or surrogate). The liver being the intended target has a greater severity of this finding than kidney. The mouse specific surrogate (ISIS 147764) was associated with minimal-mild hepatic karyomegaly in female mice.

Lymphoid depletion of the thymus (mild-severe) is considered a proinflammatory effect of ASOs. The increased granulocytic hyperplasia of the bone marrow is likely a secondary response to the proinflammatory effects of the drug class. There was no evidence of steatosis in mice treated with either mipomersen or the mouse surrogate.

Minimal to mild "foreign material" is seen in the skin of males given ≥ 10 mg/kg/day. It is unclear if this bears any relationship to the increased incidence of fibrosarcomas in male mice treated throughout their lifetime at 60 mg/kg/day (3X human AUC @MRHD).

Monkey Toxicity:

Table 3 Findings in the 12-month s.c. monkey study

Dose (mg/kg/wk)	Human AUC multiple	Findings
1	0.3x	Injection site inflammation Accumulation of basophilic granules (lymph nodes, Kupffer cells, kidney, urinary bladder, injection site) Lymph node lymphoid hyperplasia, sinus histiocytosis and histiocyte hypertrophy
3	1x	Lymph node enlargement and weight increase Thrombocytopenia (mild)
10	5x	Renal tubule cell epithelial vacuolation (Peri)vasculitis and intimal thickening (heart, cecum, colon)
30	15x	APR (CRP increase); IgG increase; Complement product Bb increase; C3 decrease (trend); IL-1b, MCP-1, MIP-1b increase; Activated platelets increase Serum and/or urine B-2-microglobulin increase Neutrophil decrease Thrombocytopenia (severe) Megakaryocytic hyperplasia Liver and spleen weight increase Kupffer cell and spleen lymphoid hyperplasia/hypertrophy Renal tubule cell epithelial vacuolation/degeneration Vasculitis/perivasculitis and intimal hyperplasia in multiple tissues

Human AUC 200 mg = 31ug•h/mL (N=26; Clinical trials CS1, 3, 8)

In the 1-year monkey toxicity study, 2 monkeys given 30 mg/kg/wk (animal # 5007, 5603) had findings initially characterized as extensive vasculitis and /or perivasculitis with intimal hyperplasia and rarely reported medial hyperplasia. Subsequent peer review by a vascular pathology expert and macaque pathologist re-characterized these lesions as intimal thickening/hyperplasia with lymphocytic infiltration occasionally in combination with eosinophils. The endothelium in some arteries from these monkeys were reactive (rounded nuclei)

and were focally denuded. These damaged areas were sporadically associated with light eosinophilic material suggestive of early fibrin accumulation in the lumen. The etiology of these vascular lesions is unclear although complement activation has been implicated in the development.

A deficiency in complement C3 (consumed during complement activation) interferes with the ability of complement to clear immune complexes which is one of the basic functions of complement. The deficiency of C3 in conjunction with a pathogenic agent could result in high levels of antigen-antibody complexes, resulting in renal tubular deposition and inability to effectively clear immune complexes systemically. Both of these moribund sacrificed monkeys had bacterial infections while others in the dose group did not. The monkeys had decreases in plasma C3 and their clinical signs corresponded with transient increase in C-reactive protein (CRP) or IL-1β. Animal #5007 had a very low C3 level compared to the rest of the cohort and both monkeys had the greatest reductions in platelets and up-regulation of platelet activation markers (CD62P). Complement activation can result in activation of platelets, increased consumption and removal via the spleen. Animal #5506 exhibited the most profound decrease in platelets. In addition to thrombocytopenia, severe anemia and schistocytic RBCs were noted prior to sacrifice, consistent with microangiopathic hemolytic anemia. Necropsy indicated widespread multifocal hemorrhage with thrombocytopenia being contributory with multifocal necrosis-considered secondary to tissue anoxia associated anemia and altered vascular perfusion.

Common inflammatory signs with mipo in rodents and monkeys was a dose dependent increase in lymphoid organ weight, lymphoid hyperplasia, and multi tissue lymphohistiocytic cell infiltrates. In rodents these effects correlate with increases in chemokines as MCP-1. Fibrosis and morphologic tissues changes are not observed. Multi tissue lymphocytic infiltrates were observed in the monkey primarily at the SC injection site. Instead basophilic granules in histiocytes in lymphoid tissue of multiple organs is seen in monkey with all doses of mipo. Monkeys additionally had sporadic increases in CRP and IL-1β coincidental with signs of infection. Repeated complement activation and subsequent impairment of innate immunity may have contributed to the sum total effects observed. One monkey each given 10 mg/kg/wk and 30 mg/kg/wk had arterial cellular infiltrates without intimal thickening in the GI tract. Arterial perivascular/intimal mononuclear or mixed cell infiltrates with and without intimal thickening in other tissues and accompanied by CRP and IL-1β and chronic complement activation that were considered treatment related. The plasma IgG levels in these two monkeys were the highest sustained levels compared to others in the HD group. The vascular pathology noted in these 2 monkeys is secondary and attributable to chronic repeated complement activation as significant decreases in plasma C3 in these two were considered sufficient to impair the innate immune surveillance and clearance of immune complex by the complement pathways leading to secondary effects on blood vessels.

Dose related proinflammatory response evident by detection of biomarkers of the acute phase response, expansion of lymphoid tissues, infiltration of lymphocytes into multiple tissues, establishment of lymphoid follicles in multiple tissues and hypertrophy of histiocytes-which contained basophilic granules possibly containing mipomersen are consistent with the experience with distribution of other ASOs. Impaired clearance of the immune complex might also contribute to some of the additional inflammatory pathology observed in the liver and kidney.

Liver & Kidney Effects
The liver Kupffer cell (less in the endothelial and hepatocytes) and renal proximal tubular epithelium receive the highest distribution of mipomersen. In addition to pro-inflammatory signals described above, the liver had increased incidence of minimal cytoplasmic vacuolation at high exposures. Kupffer cell hypertrophy/hyperplasia is considered an adaptive response to the liver uptake of the ASO. In the kidney in addition to the pro-inflammatory findings found in other tissues there was minimal-moderate cytoplasmic vacuolation and minimal-slight degeneration of the tubular epithelium in monkeys given ≥10 mg/kg/wk (3-times MRHD).

Effects on coagulation have been observed in all species. Mechanistic studies with other ASOs suggest that the interaction of the ASO with a component of the intrinsic tenase complex and to a lesser degree, thrombin inhibits the intrinsic clotting cascade resulting in increased aPTT

Renal adverse effects were seen in monkeys given 12 mg/kg IV where Cmax was higher compared to the 20 mg/kg/wk SC dose. The systemic AUC or renal tissue levels did not correlate with the adverse renal effect which further suggests that the renal toxicity is perhaps secondary to complement activation. Similarly, no steatosis was observed in any animal species including a hyper cholesterolemic mouse treated for 22 weeks with a mouse surrogate (ISIS 147764) on a high fat diet. Hepatic findings including hepatocytes necrosis, increased transaminases, Kupffer cell hypertrophy/hyperplasia and decreased serum albumin were observed at exposures >3-times exposures at the MRHD. Fortunately, complement activation occurs at >3-times the maximum anticipated clinical exposure and can be monitored.

Carcinogenicity: ECAC agreed that both the mouse and rat 2-year bioassays were adequate, noting prior ECAC concurrence with the mouse doses as well as the early termination of dosing and sacrifice of the high dose of both sexes and mid-dose females. The primary cause of morbidity/mortality in the mice were either unknown causes or cardiac thrombosis. Despite the absence of prior FDA concurrence on the rat doses and a high mortality rate in the drug treated groups, ECAC accepted the rat study as valid. The primary cause of morbidity/mortality in the rat carc study was kidney failure. Polyarteritis was seen in multiple tissues in the 2-year rat carcinogenicity study with a safety margin up to 3-times

maximum clinical systemic exposure. These vascular inflammatory findings were considered secondary to the marked exacerbation of spontaneous chronic progressive nephropathy with uremia in this species treated with mipomersen. The 2-year mouse carcinogenicity study suggests that mipomersen and the mouse surrogate increased in the incidence of cardiac thrombus formation. This finding appears confounded by the exacerbation of a spontaneous finding in aged mice of this strain. Carciac thrombus formation has been observed with a prior, similar structure ASO (ISIS 2302) in a 2-year mouse study. Slight-moderate atrial/ventricular dilatation occurred in ~3% of female mice given ≥20 mg/kg/wk (~6-times MRHD) which correlated with a slight worsening of severity of cardiomyopathy. These findings were also observed with the mouse surrogate (ISIS 147764).

The following mouse neoplasms were drug related:

- Hepatocellular adenomas and combined hepatocellular adenomas or carcinomas in females given 60 mg/kg/w (1.5X MRHD) mipomersen. The mouse surrogate (ISIS 147764), increased hepatocellular adenomas or carcinomas, combined, in both sexes administered 60 mg/kg/w.

- Fibrosarcomas of the skin/subcutis in males given 60 mg/kg/w (1.5X MRHD)

- Hemangiosarcomas in females given 60 mg/kg/w (1.5X MRHD)

The following rat skin/subcutis neoplasms were drug related and not strictly associated with injection site:

- Fibrous histiocytoma in males and females given ≥10 mg/kg/w (2X MRHD)

- Fibrosarcoma in females given ≥10 mg/kg/w (2X MRHD)

- Combined fibroma/fibrosarcoma/fibrous histiocytoma in females given ≥10 mg/kg/w (2X MRHD)

The fibrosarcomas are seen in the skin/subcutis of male mice and female rats at 2X clinical exposure. Soft tissue tumors consisting of fibromas/fibrosarcomas/fibrous histiocytomas were observed in male and female rats at 2X clinical exposure. Together this suggests drug related soft tissue tumors in multiple species and genders at clinically relevant exposures that are not specific to the injection site. Another phosphorothioate antisense oligo was also associated with fibrohistiocytic sarcoma, histiocytoma and hemangiosarcomas in mice suggesting that these soft tissue and vascular tumor in mice may be a class effect. Hemangiosarcomas were seen in female mice at 2X clinical exposure.

Based on the intended liver target and hepatic histopathology observed with mipomersen, the presence of hepatocellular adenomas and carcinomas with both mipomersen and the mouse surrogate support the drug related nature of these hepatic tumors.

The potential clinical risk from these drug-related rodent tumors can be adequately conveyed in product labeling.

Impurities: Studies designed to qualify impurities at various tested concentrations were performed which included testing two different manufacturing (b) (4) for synthesis of mipomersen were evaluated in 5 week mouse toxicity studies. These studies adequately qualify the impurities specifications sought by the sponsor according to ONDQA and Pharm/Tox assessments.

Juvenile Rat/Pediatric Support:: A 10-week juvenile rat (PND 22 at initiation) was performed with a 4-week recovery period. There were decreases in food consumption and body weight gain which did not reverse in the recovery period. Findings seen in the juvenile rat but not in the adult included a reduction in fat tissue in the bone marrow (secondary to decreased food and not reversible) and thymic atrophy (reversible). No meaningful effects on long bone growth, FOB or sexual maturation were observed. Decreases in RBC mass and reticulocyte count, increases in spleen, kidney and liver weight, accumulation of foamy macrophages in these tissues and significant immune stimulation e.g. lymphoid hyperplasia in the spleen, were similar to adults. Injection site reactions were apparent in juveniles and these effects were partially reversible following recovery. Females had minimal-slight tubular vacuolation which was not reversible. The NOAEL in this study approximates systemic exposures at the MRHD. A pediatric development plan has not been proposed to date.

Labeling Recommendations:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B

There are no adequate and well-controlled studies in pregnant women. Reproduction and embryofetal development studies performed in mice at doses up to 87.5 mg/kg/wk given by subcutaneous administration from mating through organogenesis and in pregnant rabbits given 52.5 mg/kg/wk, show no evidence of impaired fertility or harm to the fetus at 2 (mice) to 5 (rabbits) times clinical exposure at a 200 mg/wk therapeutic dose. Because animal reproduction studies are not always predictive of the human response, this drug should be used during pregnancy only if clearly needed.

Pregnant rats given subcutaneous doses of 7, 35, 70 mg/kg/wk mipomersen sodium from gestation day 6 through weaning on lactation day 20, resulted in decreased rat pup survival at 70 mg/kg/wk, 3-times clinical exposure at a 200 mg/wk therapeutic dose based on body surface area comparisons across species. Dose related decreases in

pup body weights, impaired reflexes and grip strength were observed at 35 mg/kg/wk (2-times the anticipated human dose. Levels of mipomersen in rat milk were very low (≤0.92 µg/mL at subcutaneous doses up to 70 mg/kg/wk). Due to the poor oral bioavailability of mipomersen sodium, it was considered unlikely that these low milk exposure levels adversely affected the pups during lactation.

8.3 Nursing Mothers

It is not known whether KYNAMRO is excreted in human milk. Because many drugs are excreted in human milk a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother. Levels of mipomersen present in rat milk were low (≤0.92 µg/mL) given subcutaneous doses up to 70 mg/kg/wk. Oral bioavailability is expected to be less than 10%. However a risk to newborns/infants cannot be excluded, therefore caution should be used when KYNAMRO is administered to a nursing woman.

Lactating rats administered mipomersen sodium at doses up to 70 mg/kg/wk (3-times the anticipated systemic exposure from a 200 mg/wk dose, based on body surface area comparison) consumed less food while nursing. This correlated with reduced weight gain in the rat pups, and decreased pup survival from litters given 70 mg/kg/wk.

8.4 Females of Reproductive Potential

KYNAMRO may cause fetal harm [see Use in Specific Populations (8.1)]. Females who become pregnant during KYNAMRO therapy should notify their healthcare provider.

(b) (4)

Contraception

Females of reproductive potential should use effective contraception during KYNAMRO therapy.

8.5 Pediatric Use

The safety and effectiveness of KYNAMRO in pediatric patients has not been established. (b) (4)

A juvenile toxicity study was conducted in rats at doses up to 50 mg/kg/wk (2-times the systemic exposure from a 200 mg/wk clinical dose based on body surface area comparisons). Doses > 10 mg/kg/wk were associated with reduced body weight gain in young rats, but had no effect on long bone growth or sexual development.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a subcutaneous carcinogenicity study in mice, mipomersen sodium was administered for up to 104 weeks at doses of 5, 20, 60 mg/kg/week. There were statistically significant increases in the incidences of hepatocellular adenoma and combined adenoma and carcinoma in female mice at 60 mg/kg/wk (2-times the systemic clinical exposure at 200 mg/wk, based on a body surface area comparison) for both mipomersen sodium and the mouse-specific analog. This dose also resulted in statistically significant increases in the incidence of hemangiosarcomas in female mice and fibrosarcomas of the skin/subcutis in male mice.

In a subcutaneous carcinogenicity study in rats, mipomersen sodium was administered for up to 104 weeks at doses of 3, 10, 20 mg/kg/wk. The incidence of fibrosarcomas of the skin/subcutis and the combination of fibroma, fibrosarcomas and malignant fibrous histiocytoma of the skin/subcutis was statistically significantly increased in female rats at 10 mg/kg/wk, at less than clinical exposure at the 200 mg/wk dose based on body surface area comparisons. Both sexes of rats also had statistically significant increases in the incidence of malignant fibrous histiocytoma of the skin/subcutis at 20 mg/kg/wk (at clinical exposure at the 200 mg/wk dose based on body surface area comparisons. Mipomersen sodium did not exhibit genotoxic potential in a battery of studies, including the *in vitro* Bacterial Reverse Mutation (Ames) assay, an *in vitro* cytogenetics assay using a mouse lymphoma cell line, and an *in vivo* micronucleus assay in mice. Mipomersen sodium had no effect on fertility in mice at doses up to 87.5 mg/kg/wk (2-times clinical exposure at the 200 mg/wk dose based on body surface area comparisons).

13.2 Animal Pharmacology and/or Toxicology

The principal target organs for mipomersen sodium pathology are the kidneys and liver. These organs represent the highest distribution of compound, and exhibit microscopic changes reflective of cellular uptake in macrophages. The most widespread toxicological effect of mipomersen sodium was a spectrum of inflammatory changes in numerous organs, including lymphohistiocytic cell infiltrates and increases in lymphoid organ weights, associated with increases in plasma cytokines, chemokines and total serum IgG. In a chronic monkey study, multi-focal intimal hyperplasia with mixed inflammatory infiltrates was evident in vascular beds in 2 of 6 monkeys treated for 12 months with 30 mg/kg/week with a no-observed-adverse-effect-level (NOAEL) of 10 mg/kg/week (clinical exposures anticipated from a 200 mg/wk dose based on body surface area comparisons across species).

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/s/

KAREN L DAVIS BRUNO
12/04/2012
Pharm/Tox Sup memo re:AP

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	203568
Supporting document/s:	0000
Applicant's letter date:	03/29/2012
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Product:	Kynamro (mipomersen sodium) Injection
Indication:	Treat Homozygous Familial Hypercholesterolemia
Applicant:	Genzyme Corp.
Review Division:	Metabolism and Endocrinology Products
Reviewer:	Ronald L. Wange, PhD
Supervisor/Team Leader:	Karen Davis-Bruno, PhD
Division Director:	Mary Park, MD
Project Manager:	Kati Johnson

Template Version: September 1, 2010

Disclaimer

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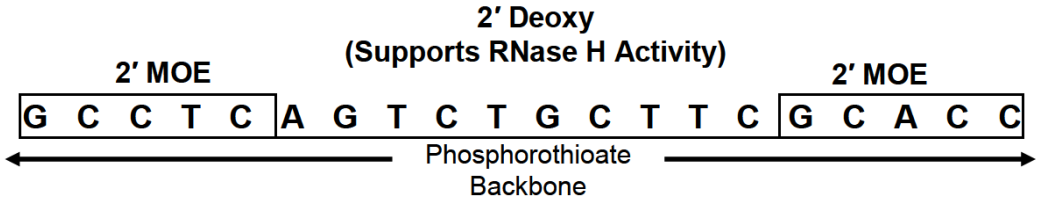
1 Executive Summary

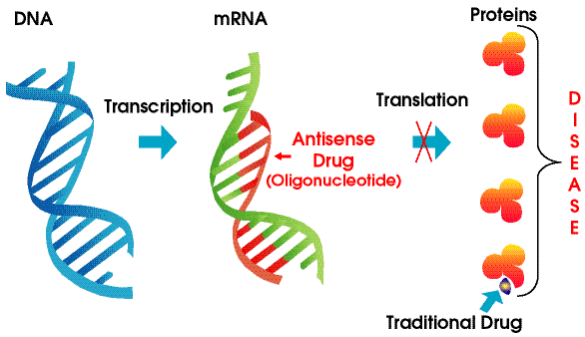
1.1 Introduction

Mipomersen is an antisense oligonucleotide (ASO) inhibitor targeted to human apolipoprotein B_{100} ($apoB_{100}$), the principal apolipoprotein of low density lipoprotein (LDL), its metabolic precursor, VLDL, and a component of Lp(a). Mipomersen is a second generation phosphorothioate (PS) oligonucleotide, which means that in addition to having phosphorothioate linkages in place of the natural phosphodiester linkage between bases, some of the bases of the 5' and 3' ends have a methoxyethyl (MOE) group on the 2' position of the ribose sugar. Both of these modifications serve to increase the stability of the oligonucleotide towards nucleases. The MOE modifications in particular make an oligonucleotide highly resistant to exonucleases. Mipomersen is a 5-10-5 MOE gapmer, meaning that the five bases at both the 5' and 3' ends are MOE-modified, while the central 10 bases are unmodified (2'-deoxy). Cytosine bases are also methylated to reduce the immunostimulatory potential of mipomersen.



Note: The sequence of mipomersen is shown (all cytodine residues are methylated at the 5-position)

The base sequence of mipomersen is complementary to a 20-nucleotide segment of the coding region of the messenger RNA (mRNA) for $apoB_{100}$, to which it binds by Watson-Crick base-pairing (Figure).



The hybridization (binding) of mipomersen to the cognate mRNA results in reduced translation of the mRNA to protein by multiple mechanisms including activation of RNase H-mediated degradation of the cognate mRNA. This leads to decreased synthesis of apo-B_{100} in the liver (the primary site of apo-B_{100} generation), a reduction in transport of apo-B_{100}-containing lipoprotein out of the liver and a reduction in circulating LDL-cholesterol (LDL-C). Based on the Sponsor's analysis, mipomersen is not predicted to productively bind to any human or animal mRNA other than apoB.

Mipomersen is a first-in-class compound for LDL-C reduction via $apoB_{100}$ inhibition. Unlike the statins, mipomersen is not dependent on LDL receptor (LDLr) upregulation, nor on LDLr function for its pharmacodynamic effects. Mipomersen is also the first systemically administered phosphorothioate antisense oligonucleotide (PS ASO) for which a marketing application has been received.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology

Species-specific differences in the nucleotide sequence of apoB mRNA caused a reduction (monkey) or complete loss (mouse, rat, dog and rabbit) of the intended pharmacological activity of mipomersen in these species. For this reason, most pharmacology studies have been conducted with surrogate molecules that optimize base pairing between the ASO and the species-specific apoB target mRNA. Pivotal toxicology studies in rodents have also included an arm with the species-specific surrogate. Nonclinical pharmacology studies with these apoB antisense inhibitors have shown rapid reduction of hepatic apoB mRNA and protein, and reductions in serum apoB, LDL-C and total-C in mouse, hamster, rabbit and monkey models. In mouse models of atherosclerosis, antisense apoB inhibition was shown to be anti-atherogenic (reduced aortic plaque volume or formation). These studies provide proof-of-concept support for the utility of targeting $apoB_{100}$ using antisense molecules. While none of the pharmacology studies revealed a clear potential for apoB antisense inhibitors to cause or exacerbate hepatic steatosis, it is notable that studies in the models that have the greatest degree of hypercholesterolemia (the LDLr- Deficient Mouse, the Human ApoB transgenic/LDLr-Deficient Mouse, and the ApoE-Deficient Mouse) failed to assess hepatic lipid levels.

Cardiovascular, pulmonary, and neurobehavioral safety pharmacology studies did not identify any safety concerns. Mipomersen also had no effect on $ApoB_{48}$ function in the mouse. $ApoB_{48}$ shares sequence identity to $ApoB_{100}$ in the region targeted by mipomersen, is expressed in the intestines, and is the essential apoB lipoprotein in chylomicron formation and absorption of dietary fat. The chronic toxicity studies also were consistent with there being no effect of mipomersen on absorption of dietary fat or fat soluble vitamins.

Pharmacokinetics

The pharmacokinetic profile of mipomersen is similar in all evaluated species (mice, rats, dogs & monkeys). Mipomersen is rapidly systemically absorbed following subcutaneous (SC) injection, with high bioavailability (> 80%). In circulation, mipomersen, like other PS ASOs, is highly protein bound (>85%), binding primarily to albumin at non-specific, low-affinity hydrophilic sites. PS ASOs do not displace drugs that are hydrophobically bound to plasma proteins. Upon absorption, mipomersen is rapidly cleared from the plasma by distribution to tissues. Across species T_{max} is typically between 0.5 to 2 hours. Tissue concentrations show accumulation due to slow tissue clearance, reaching steady state after ~3 months in rodents and ~6 months in monkeys. Consistent with slow tissue clearance, terminal plasma half-life was 16 to 30 days across species.

Notably, organ uptake is highly heterogeneous and cell type specific, and exhibits saturability, especially in the mouse kidney. Cellular entry of PS ASOs is by endocytosis or phagocytosis, and some of the differences in oligo uptake likely arise from intrinsic differences in the rates of endocytosis/phagocytosis in different cell types. Kidney uptake is primarily to the proximal tubule endothelium, where ASOs are taken up by pinocytosis in a manner similar to that normally involved in reabsorption of filtered proteins. The organs showing the highest tissue levels in all species are kidney, liver, spleen, lymph nodes and bone marrow, with the kidney and liver showing the highest concentrations. Little to no drug distributes to skeletal muscle, eye or the brain. Based on a comparison of interspecies tissue levels of mipomersen in the liver and kidneys at steady state, it is apparent that extrapolation of administered dose between species on a mg/m^2 basis provides a good prediction of tissue levels, while mg/kg extrapolations poorly predict tissue levels

Mipomersen is metabolized in tissues by intracellular nucleases to produce chain-shortened oligonucleotide metabolites, which are subsequently eliminated by renal clearance. The urinary metabolites of mipomersen are shorter oligonucleotides typically in the range of 5 to 15 bases long, consistent with a model of endonuclease cleavage within the central ten 2'-deoxynucleotides, followed by sequential exonuclease cleavage primarily of the residual 2'-deoxynucleosides. Notably mipomersen is neither a substrate, an inhibitor or an inducer of the major drug-metabolizing cytochrome P450 enzymes, nor a substrate or inhibitor of P-glycoprotein.

Toxicity

Mipomersen toxicity has been assessed primarily in the mouse, rat and monkey, with studies of up to 2-years duration (carcinogenicity studies) available for evaluation in the rodents and up to 1-year for the monkey. The effects of mipomersen on reproductive function, embryofetal development, pre- and postnatal development and development in juvenile animals has also been assessed. In general the toxicities seen are consistent with the expected toxicities of a PS ASO[1].

Immunological Effects

Proinflammatory effects were generally dose-related, and were seen in all nonclinical species, and included 1) evidence of an acute phase response, 2) changes in lymphoid organ weight (↑ spleen weight, ↓ thymus weight), 3) infiltration/expansion of histiocytes ± lymphocytes into myriad tissues, 4) activation of histiocytes (hypertrophy, and/or hyperplasia), 5) occasional infiltration of other leukocytes (*e.g.,* neutrophils or eosinophils) into multiple tissues, 6) lymphoid hyperplasia, 7) changes in circulating levels of leukocytes, 8) elevation of certain cytokines/chemokines, 9) increased immunoglobulin levels, 10) decreased platelet count and platelet activation, and 11) injection site inflammation. Not all findings were seen in all species, and not all findings

[1] Henry, S.P., *et al.,* Toxicologic Properties of 2'-*O*-Methoxyethyl Chimeric Antisense Inhibitors in Animals and Man, in *Antisense Drug Technology,* Crooke, S.T., ed., CRC Press, Boca Raton, 2008.

were seen at all doses. At lower doses the effects were generally confined to the injection site and secondary lymphoid tissues (*e.g.,* lymph nodes and spleen).

Complement activation was apparent in monkeys at SC doses of ≥ 20 mg/kg (~7x clinical exposure) and at intravenous (IV) doses as low as 12 mg/kg, as evidenced by transient rises in the level of the complement factor B split product, Bb, and the depletion of complement factor C3 with repeated dosing. The observation that IV dosing is more potent in activating complement is consistent with literature evidence indicating that activation of complement by PS ASOs is a C_{max}-driven response. Per the scientific literature, primates are considered to be more sensitive to PS ASO-mediated complement activation, and the ability of mipomersen to activate complement was only assessed in monkeys. Acutely, complement activation produces anaphylactic split products (*i.e.*, C3a and C5a), and secondarily affects hemodynamic and inflammatory responses. Persistent activation of the complement pathway can result in consumption of some complement factors at a rate faster than they are synthesized, resulting, for example, in depletion of complement C3, which can alter innate immune surveillance and clearance of immune complex.

An *in vitro* assessment revealed no potential for direct activation of murine Mast cells by mipomersen; however, this study does not address the potential for indirect activation of Mast cells, for example via complement activation. An influenza host resistance assay in mice found no clinically meaningful effect on the ability to control and clear the infection, although there was a slight to moderate decrease in the magnitude of anti-influenza IgG response at all doses (20-100 mg/kg/week [7x-33x clinical exposure]) of mipomersen and the mouse surrogate. While these data indicate that it is unlikely that mipomersen has high potential for inducing immunosuppression by itself, they also suggest that mipomersen has the potential for augmenting immunosuppression, albeit at relatively high exposures.

Cardiovascular Effects

Mipomersen was associated with various vascular effects in the long-term studies in rodents (2-year) and monkeys (1-year). In rats, polyarteritis was seen in multiple tissues in males at doses ≥ 10 mg/kg/week (< 1x clinical exposure) and in females at 20 mg/kg/week (highest dose [~1x clinical exposure]); however, these findings may be of limited clinical relevance, as it seems likely that these changes were secondary to a marked exacerbation of spontaneous chronic progressive nephropathy (with uremia) that was elicited by mipomersen only in rats.

In the mouse 2-year (lifetime) study, mipomersen at doses ≥ 20 mg/kg/week (< 1x clinical exposure) (and the mouse surrogate at 60 mg/kg/week) increased the incidence of cardiac thrombus. However clear attribution to the test items is confounded by the fact that this is a common finding in aged mice of this strain, and the highest incidence in each sex lies just outside of the highest incidence in the historical background database. It is notable though that a different PS ASO also exhibited an apparent increase in the incidence of cardiac thrombus in a long-term mouse study. Given the absence of a mipomersen-related increase in incidence of thrombotic events in other

tissues, or an increase in thrombotic events in other species, these data may be interpreted as indicating a propensity to worsen or accelerate formation of spontaneous lesions in the mouse. The significance of this finding to humans with pre-existing vascular lesions is unclear.

Another cardiovascular finding in the mouse apparently related to lifetime PS ASO exposure is slight-moderate atrial/ventricular dilatation in ~3% of females at doses $\geq$ 20 mg/kg/week, which was correlated with a slight worsening of severity and incidence of cardiomyopathy in these dose groups. Comparable findings were observed with the mouse surrogate (ISIS 147764).

In the 1-year monkey study, 2 monkeys (animals 5007 and 5603) treated with mipomersen at 30 mg/kg/week (highest dose [~10x clinical exposure]) had findings that were initially characterized as extensive vasculitis and/or perivasculitis, with intimal hyperplasia (and rare medial hyperplasia). Upon subsequent peer review by a vascular pathology expert (Dr. Kerns) and an expert in the pathology of macaques (Dr. Palate), these lesions were re-characterized as intimal thickening/hyperplasia with lymphocytic infiltration, occasionally in combination with eosinophils. Dr. Palate also noted that the endothelium in some arteries from these animals appeared reactive (with rounded nuclei) and appeared focally denuded. Moreover, these areas of damaged endothelial cells were sporadically associated with light eosinophilic material suggestive of early fibrin accumulation in the lumen.

The precise etiology of these lesions is unclear. Sponsor posits that a deficiency in complement C3 (which is consumed during complement activation) interferes with the ability of complement to clear immune complexes from the vasculature, which is one of the recognized functions of basal complement activity. This deficiency, especially in the face of an immune response to a pathogenic agent (resulting in high levels of antigen-antibody complexes), could result in vascular lesions. In support of such an etiology, it is notable that both monkeys #5007 and 5603 were diagnosed with bacterial infections (Campylobacter and Shigella, respectively) around study day 310. Indeed, animal #5007 had very low C3 levels compared to the rest of the high-dose monkeys; however, animal #5603 had C3 levels that were comparable to the rest of the dose cohort. It is also notable that these two animals had among the greatest reductions in platelets, and also the greatest percentage of platelets with upregulated activation markers (CD62P). Complement activation can cause consumption of platelets by inducing platelet activation and subsequent removal in the spleen.

It is also notable that the HD monkey (#5506) that exhibited the most profound decrease in platelets was ultimately sacrificed moribund. In addition to thrombocytopenia, this animal also had severe anemia (RBCs ~13% of baseline), and schistocytic RBCs 2 days prior to sacrifice, consistent with microangiopathic hemolytic anemia (although microangiopathy was not noted in the histopathology report). The postmortem was notable for widespread multifocal hemorrhage (with thrombocytopenia presumably being contributory), and multifocal necrosis (possibly secondary to local tissue anoxia associated with anemia and altered tissue perfusion). The etiology of the

vascular findings in this animal, and its relevance to humans is unknown. A contribution of complement activation is possible.

In ascribing significance to the CV findings noted above, it should be borne in mind that the animals studied in the toxicology program have normal LDL-C levels, and therefore can derive little or no (presumed) CV benefit from the LDL-C lowering effect of inhibition of $apoB_{100}$ synthesis. Based on the anti-atherogenic effect of apoB synthesis inhibition in mouse models of atherosclerosis, it is plausible that inhibition of apoB synthesis would provide a protective CV effect on a background of elevated LDL-C.

Renal Effects

The kidneys are generally the site of highest tissue exposures, especially in the renal cortex proximal tubules, where PS ASOs concentrate. Following 5 to 6 months with a comparable dose of 25-30 mg/kg/week, the highest kidney concentration of mipomersen was seen in the monkey (~2000 μg/g), the lowest was seen in mice (182 μg/g), and the rat was intermediate (~1350 μg/g). All species show a dose-related finding of the presence of basophilic granules in the cytoplasm of kidney tubule epithelial cells. These granules represent oligonucleotide (mipomersen or surrogate) taken up by these cells.

In mice there was no indication of mipomersen-related renal toxicity. But, as noted above, mice had the lowest levels of mipomersen in the kidney of any of the assessed species (~10-fold lower than the monkey). Mipomersen loading of the kidney appears to saturate at relatively low doses in mice, compared to monkeys and rats, and mice are not likely to be a good model for renal toxicity.

In rats, doses ≥ 10 mg/kg/week (<1x clinical exposure) were associated with a profound worsening of chronic progressive nephropathy (CPN) in males and a worsening and increase in incidence of CPN in females. This was associated with proteinuria, increased blood urea nitrogen, and increased deaths due to CPN/uremia in both sexes in the 2-year rat study. Given the apparent etiology of the toxicity as an exacerbation of an underlying condition, the clinical significance of this finding is unclear, but does suggest caution in administering to patients with underlying kidney disease.

In monkeys, SC doses ≥ 10 mg/kg/week (~1x clinical exposure) were associated with minimal to moderate multifocal cytoplasmic vacuolation and minimal to slight degeneration of the tubular epithelium. Sponsor considers that the vacuoles are an artifact of fixation, arising from fluid influx into oligonucleotide-laden (hydroscopic) phagolysosomes, resulting in washing out of the drug and swelling of the phagolysosomes, and notes that basophilic material (presumably residual oligonucleotide) was visible in some of the vacuoles. While plausible, this etiology has not been rigorously established. Intravenous dosing at 12 mg/kg q4d (21 mg/kg/week) for 3 months was additionally associated with minimal-moderate tubular epithelial cell regeneration. This IV dose was also associated with minimal-moderate intratubular hemorrhage in 4/6 monkeys, accompanied by hematuria. This finding was not seen in the concurrent 20 mg/kg q4d (35 mg/kg/week) SC dose group, following 3 months of

dosing, despite a higher plasma and tissue AUC. This suggests that it is the higher C_{max} associated with the 12 mg/kg q4d IV dose that was instrumental in the higher degree of renal toxicity; possibly implicating complement activation, which was much greater in the monkeys treated with 12 mg/kg q4d IV than in the monkeys treated with the 20 mg/kg q4d SC dose. Minimal multifocal tubular hemorrhage (positive for occult blood) was also seen in the 12-month study in 1/6 monkeys dosed at 30 mg/kg/week, a dose that was also associated with marked complement activation. This monkey (#5007) also had elevated urine β2-microglobulin beginning Week 27 and proteinuria beginning Week 39. This animal also had high levels of serum β2-microglobulin during Week 39 (4.6-fold the mean control value and 2.7-fold the mean of the rest of 30 mg/kg/week dose cohort).

On the basis of tubular epithelial cell degeneration (mild) with tubular vacuolation (moderate) in 1/6 monkeys after 1 year of treatment with 10 mg/kg/week of mipomersen by SC injection, the NOAEL is considered to be 3 mg/kg/week SC (<1x clinical exposure) for kidney toxicity.

Liver Effects

The liver is generally the organ with the second highest tissue concentration (except for mice where liver concentrations typically exceed kidney concentrations). Liver concentrations of mipomersen follow the same general pattern as the kidney, with the lowest level in mice (~280 μg/g), the highest in monkeys (~1090 μg/g) and an intermediate level in rats (~750 μg/g) following a comparable exposure of 25-30 mg/kg/week for 5-6 months. Unlike in the kidney (where oligonucleotide remains largely confined to the proximal tubule), oligonucleotide in the liver is distributed to all cell types. Nonetheless, the highest levels of mipomersen are seen in Kupffer cells, which concentrate the oligo in the lysosomes, giving rise to basophilic granules.

Liver findings in mice following 3 to 6 month of mipomersen treatment included accumulation of basophilic granules in Kupffer cells at all doses and increased liver weight at doses ≥ 25 mg/kg/week (<1x clinical exposure). Higher doses (≥ 44 mg/kg/week [~1x clinical exposure]) were also associated with elevations in ALT, AST & ALP and decreases in albumin. Treatment with the mouse surrogate, ISIS 147764, at a dose of 75 mg/kg/week for 6 months was associated with hepatocyte Karyomegaly, and occasionally with single cell necrosis.

After 2 years of dosing, the findings in mice also include an increase in the incidence of basophilic foci of cellular alteration in males at all doses ≥ 5 mg/kg/week and eosinophilic foci of cellular alteration in both sexes at doses of 60 mg/kg/week. Foci of cellular alteration often occur as a precursor to neoplastic changes. There was also an increase in the incidence and severity of minimal-moderate single cell necrosis at all doses of mipomersen (≥ 5 mg/kg/week) in both sexes. An increased incidence and/or severity of extramedullary hematopoiesis was seen at 60 mg/kg/week in both sexes. Counterintuitively, given the proinflammatory action of PS ASOs, the incidence of mononuclear cell infiltration was decreased in females at doses ≥ 5 mg/kg/week, and in males at 60 mg/kg/week of mipomersen, but were unaffected by 60 mg/kg/week of ISIS

147764 (mouse surrogate). The mouse surrogate was however associated with the foci of cellular alteration (both basophilic and eosinophilic) and increases in single cell necrosis noted above as well as a > 3-fold increases in ALT and > 2-fold increase in AST in both sexes. Whether this apparent increase in hepatotoxicity (elevated transaminases) over that seen with mipomersen is a consequence of antisense inhibition of apoB synthesis is unknown.

In the rat, treatment with mipomersen for 5 months at doses ≥ 10 mg/kg/week (<1x clinical exposure) was associated with increased liver weight in both sexes, and all doses were associated with accumulation of basophilic granules in the Kupffer cells. There was no increase in inflammatory infiltrates noted, and, as in mice, the incidence of monocyte cell infiltration was actually decreased at mipomersen ≥ 10 mg/kg/week in females and ≥ 30 mg/kg/week (~2x clinical exposure) in males. There were no changes in serum transaminases in males, but females had elevated AST and ALT at doses ≥ 30 mg/kg/week. Cholesterol (total, HDL & LDL) was increased at 50 mg/kg/week (~3x clinical exposure). VLDL and triglyceride were decreased at mipomersen ≥ 3 mg/kg/week in males and ≥ 30 mg/kg/week in females.

In the 2-year rat study, liver weights were increased at doses ≥ 10 mg/kg/week, and accumulation of basophilic granules in Kupffer cells was seen at all doses. As for the 5-month study, there was no increase in inflammatory infiltrates, and the incidence of monocyte cell infiltration was decreased by mipomersen at all doses (≥ 3 mg/kg/week). Doses ≥ 10 mg/kg/week (mipomersen or rat surrogate) were associated with an increased incidence of centrilobular vacuolation and necrosis. Both sexes saw decreases in AST and albumin (but no change in ALT) at ≥ 10 mg/kg/week mipomersen (but not the rat surrogate). There were marked increases in triglycerides and cholesterol (total, HDL, LDL and VLDL) at 20 mg/kg/week, but not with the surrogate.

Mipomersen treatment in monkeys for 1 year was likewise associated with accumulation of basophilic granules in Kupffer cells at all doses and increases in liver weight at 30 mg/kg/week. Doses ≥ 3 mg/kg/week (<1x clinical exposure) were also associated with diffuse hypertrophy/hyperplasia of Kupffer cells (a finding that was not seen in rodents). Unlike rodents there were no changes in serum transaminases and no effect on serum lipid parameters. There was a reversible decline in albumin levels at 30 mg/kg/week, possibly related to an acute phase response.

Steatosis was not apparent with mipomersen or species-specific surrogates in any species in any study, including a 22-week study in mice rendered hypercholesterolemic by consumption of a high fat diet and treated with a mouse surrogate (ISIS 147764) or a 5-week study in monkeys rendered hypercholesterolemic by feeding a high fat diet and treated with a monkey surrogate (ISIS 326358).

Based on the foregoing, the NOAEL for liver toxicity is considered to be < 5 mg/kg/week (<1x clinical exposure) in mice on the basis of the increased incidence/severity of single hepatocyte necrosis at all doses examined in the 2-year study; 3 mg/kg/week (<1x clinical exposure) in the rat on the basis of increased incidence/severity of centrilobular

vacuolation and necrosis in the 2-year study; and 30 mg/kg/week (>3x clinical exposure) in the monkey (the highest dose tested in the 1-year study).

Carcinogenicity

2-year carcinogenicity studies were conducted in both the mouse and the rat with mipomersen and the relevant species-specific surrogate. Mipomersen treatment was associated with multiple tumors in both species: 1) Hepatocellular adenomas and combined hepatocellular adenomas or carcinomas in female mice administered 60 mg/kg/week mipomersen (2x clinical exposure); 2) Hepatocellular adenomas or carcinomas, combined, in both sexes of mice administered 60 mg/kg/week ISIS 147764 (mouse surrogate); 3) Fibrosarcoma of the skin/subcutis in male mice administered 60 mg/kg/week mipomersen; 4) Hemangiosarcomas in female mice given 60 mg/kg/week mipomersen; 5) Fibrous histiocytoma (malignant) of the skin/subcutis in male and female rats at ≥ 10 mg/kg/week (<1x clinical exposure); 6) Fibrosarcoma of the skin/subcutis in female rats at ≥ 10 mg/kg/week; 7) Combined fibroma/fibrosarcoma/fibrous histiocytoma of the skin/subcutis in female rats at ≥ 10 mg/kg/week.

The hepatocellular adenomas are of particular note as they are occurring at a clinically relevant exposure in the target tissue of intended pharmacology. It is also notable that the pharmacologically active surrogate oligonucleotide caused ~2x the tumor incidence of mipomersen. On the other hand, this is not a malignant neoplasm, and conversion of hepatocellular adenoma to hepatocellular carcinoma in humans is reportedly rare. The clinical significance of the remaining tumors is doubtful. Mice are reportedly susceptible to formation of hemangiosarcoma under conditions of hypoxia and macrophage activation, both of which were apparent in the 2-year study. With regard to the observed fibrohistiocytic tumors (fibrosarcomas, malignant fibrous histiocytoma) of the skin/subcutis, rodents are reportedly highly susceptible to these tumors with chronic irritation/inflammation of the subcutis, which was observed in the 2-years studies. Overall, it is judged that the possible tumor risk attending mipomersen treatment in humans can be adequately addressed in the label.

Reproductive & Developmental Toxicity

Reproduction and embryofetal development studies performed in mice at doses up to 87.5 mg/kg/wk mipomersen given by subcutaneous administration from mating through organogenesis and in pregnant rabbits given 52.5 mg/kg/wk, show no evidence of impaired fertility or harm to the fetus at 2 (mice) to 5 (rabbits) times clinical exposure at a 200 mg/wk therapeutic dose.

Pregnant rats given subcutaneous doses of 7, 35, 70 mg/kg/wk mipomersen from gestation day 6 through weaning on lactation day 20, resulted in decreased rat pup survival at 70 mg/kg/wk, 3-times clinical exposure at a 200 mg/wk therapeutic dose based on body surface area comparisons across species. Dose related decreases in pup body weights, impaired reflexes (visual placing) and grip strength were observed at 35 mg/kg/wk (2-times the anticipated human dose). Levels of mipomersen in rat milk were very low (≤0.92 µg/mL at subcutaneous doses up to 70 mg/kg/wk). Due to the

poor oral bioavailability of mipomersen sodium, it was considered unlikely that these low milk exposure levels adversely affected the pups during lactation.

A juvenile toxicity study was conducted in rats at doses up to 50 mg/kg/wk (2-times the systemic exposure for a 200 mg/wk clinical dose based on body surface area comparisons). Doses ≥ 10 mg/kg/wk were associated with reduced body weight gain in young rats, but had no effect on long bone growth or sexual development. Body weights and food consumption remained lower throughout a 4-week treatment-free recovery period.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data submitted in this application support the marketing approval of mipomersen for the treatment of HoFH.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B

There are no adequate and well-controlled studies in pregnant women. Reproduction and embryofetal development studies performed in mice at doses up to 87.5 mg/kg/wk given by subcutaneous administration from mating through organogenesis and in pregnant rabbits given 52.5 mg/kg/wk, show no evidence of impaired fertility or harm to the fetus at 2 (mice) to 5 (rabbits) times clinical exposure at a 200 mg/wk therapeutic dose. Because animal reproduction studies are not always predictive of the human response, this drug should be used during pregnancy only if clearly needed.
Pregnant rats given subcutaneous doses of 7, 35, 70 mg/kg/wk mipomersen sodium from gestation day 6 through weaning on lactation day 20, resulted in decreased rat pup survival at 70 mg/kg/wk, 3-times clinical exposure at a 200 mg/wk therapeutic dose based on body surface area comparisons across species. Dose related decreases in pup body weights, impaired reflexes and grip strength were observed at 35 mg/kg/wk (2-times the anticipated human dose. Levels of mipomersen in rat milk were very low (≤0.92 µg/mL at subcutaneous doses up to 70 mg/kg/wk). Due to the poor oral bioavailability of mipomersen sodium, it was considered unlikely that these low milk exposure levels adversely affected the pups during lactation.

8.3 Nursing Mothers

It is not known whether KYNAMRO is excreted in human milk. Because many drugs are excreted in human milk a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

Levels of mipomersen present in rat milk were low (≤0.92 µg/mL) given subcutaneous doses up to 70 mg/kg/wk. Oral bioavailability is expected to be less than 10%. However a risk to newborns/infants cannot be excluded, therefore caution should be used when KYNAMRO is administered to a nursing woman.

Lactating rats administered mipomersen sodium at doses up to 70 mg/kg/wk (3-times the anticipated systemic exposure from a 200 mg/wk dose, based on body surface area comparison) consumed less food while nursing. This correlated with reduced weight gain in the rat pups, and decreased pup survival from litters given 70 mg/kg/wk.

8.4 Females of Reproductive Potential

KYNAMRO may cause fetal harm [see Use in Specific Populations (8.1)]. Females who become pregnant during KYNAMRO therapy should notify their healthcare provider.

(b) (4)

Contraception

Females of reproductive potential should use effective contraception during KYNAMRO therapy.

8.5 Pediatric Use

The safety and effectiveness of KYNAMRO in pediatric patients has not been established. (b) (4)

A juvenile toxicity study was conducted in rats at doses up to 50 mg/kg/wk (2-times the systemic exposure from a 200 mg/wk clinical dose based on body surface area comparisons). Doses ≥ 10 mg/kg/wk were associated with reduced body weight gain in young rats, but had no effect on long bone growth or sexual development.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In a subcutaneous carcinogenicity study in mice, mipomersen sodium was administered for up to 104 weeks at doses of 5, 20, 60 mg/kg/week. There were statistically significant increases in the incidences of hepatocellular adenoma and combined adenoma and carcinoma in female mice at 60 mg/kg/wk (2-times the systemic clinical exposure at 200 mg/wk, based on a body surface area comparison) for both mipomersen sodium and the mouse-specific analog. This dose also resulted in

statistically significant increases in the incidence of hemangiosarcomas in female mice and fibrosarcomas of the skin/subcutis in male mice.
In a subcutaneous carcinogenicity study in rats, mipomersen sodium was administered for up to 104 weeks at doses of 3, 10, 20 mg/kg/wk. The incidence of fibrosarcomas of the skin/subcutis and the combination of fibroma, fibrosarcomas and malignant fibrous histiocytoma of the skin/subcutis was statistically significantly increased in female rats at 10 mg/kg/wk, at less than clinical exposure at the 200 mg/wk dose based on body surface area comparisons. Both sexes of rats also had statistically significant increases in the incidence of malignant fibrous histiocytoma of the skin/subcutis at 20 mg/kg/wk (at clinical exposure at the 200 mg/wk dose based on body surface area comparisons.
Mipomersen sodium did not exhibit genotoxic potential in a battery of studies, including the *in vitro* Bacterial Reverse Mutation (Ames) assay, an *in vitro* cytogenetics assay using a mouse lymphoma cell line, and an *in vivo* micronucleus assay in mice.
Mipomersen sodium had no effect on fertility in mice at doses up to 87.5 mg/kg/wk (2-times clinical exposure at the 200 mg/wk dose based on body surface area comparisons).

13.2 Animal Pharmacology and/or Toxicology

The principal target organs for mipomersen sodium pathology are the kidneys and liver. These organs represent the highest distribution of compound, and exhibit microscopic changes reflective of cellular uptake in macrophages. The most widespread toxicological effect of mipomersen sodium was a spectrum of inflammatory changes in numerous organs, including lymphohistiocytic cell infiltrates and increases in lymphoid organ weights, associated with increases in plasma cytokines, chemokines and total serum IgG. In a chronic monkey study, multi-focal intimal hyperplasia with mixed inflammatory infiltrates was evident in vascular beds in 2 of 6 monkeys treated for 12 months with 30 mg/kg/week with a no-observed-adverse-effect-level (NOAEL) of 10 mg/kg/week (clinical exposures anticipated from a 200 mg/wk dose based on body surface area comparisons across species).

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

629167-92-6

Generic Name

Mipomersen Sodium

Code Name

ISIS 301012

Chemical Name

2'-*O*-(2-methoxyethyl)-*P*-thioguanylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-deoxy-*P*-thioadenylyl-(3'→5')-2'-deoxy-*P*-thioguanylyl-(3'→5')*P*-thiothymidylyl-(3'→5')-2'-deoxy-5-methyl-*P*-thiocytidylyl-(3'→5')-*P*-thiothymidylyl-(3'→5')-2'-deoxy-*P*-thioguanylyl-(3'→5')-2'-deoxy-5-methyl-*P*-thiocytidylyl-(3'→5')-*P*-thiothymidylyl-(3'→5')-*P*-thiothymidylyl-(3'→5')-2'-deoxy-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*thioguanylyl-(
3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*-thioadenylyl(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5 methylcytidine nonadecasodium salt

Molecular Formula/Molecular Weight

$C_{230}H_{305}N_{67}Na_{19}O_{122}P_{19}S_{19}$/ 7595

Structure or Biochemical Description

5′-$\underline{G^{Me}C^{Me}C^{Me}U^{Me}C}$ AGT ^{Me}CTG ^{Me}CTT ^{Me}C $\underline{G^{Me}CA^{Me}C^{Me}C}$- 3′

The underlined residues are 2′-MOE nucleosides. It should be noted that 2′-*O*-(2-methoxyethyl)-5-methyluridine (2′-MOE ^{Me}U) nucleosides are also sometimes designated as 2′-*O*-(2-methoxyethyl)ribothymidine (2′-MOE T).

Pharmacologic Class

Antisense Oligonucleotide Apolipoprotein B Synthesis Inhibitor
-this would be a novel pharmacologic class

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 70,969 (ISIS 301012, IND supporting this NDA, Isis→Genzyme)

(b) (4)

2.3 Drug Formulation

Sterile solution in pH adjusted (7.5 to 8.5) water, for injection.

Table 1: Composition of Mipomersen Sodium Injection, 200 mg/mL, in Vials

Component	Quality Standard	Function	Quantity per Vial[a]
Mipomersen sodium	In-house	Active Ingredient	200 mg
Water for injection	PhEur[b] USP-NF[c]	[redacted]	(b) (4)
Sodium hydroxide (b) (4)	PhEur USP-NF	pH adjustment	Sufficient to reach target pH
Hydrochloric acid (b) (4)	PhEur USP-NF	pH adjustment	Sufficient to reach target pH

[a] These values are calculated for the label claim of 1.0 mL. (b) (4)
[b] European Pharmacopoeia
[c] United States Pharmacopoeia-National Formulary

2.4 Comments on Novel Excipients

None present

2.5 Comments on Impurities/Degradants of Concern

From the P/T perspective, Sponsor has adequately addressed the potential toxicological significance of impurities and degradants (see reviews of study #s 301012-AS18 & GT-348-TX-7).

2.6 Proposed Clinical Population and Dosing Regimen

Patients with familial homozygous hypercholesterolemia, subcutaneous administration of 200 mg once per week.

2.7 Regulatory Background

IND 70,969 was opened on 8 June 2005 when a pre-IND meeting request was received by DMEP from then Sponsor, Isis Pharmaceuticals regarding their planned phase 3 program (early clinical development was ex-USA). This meeting request was initially granted on 20 June 2005 as a type B meeting to be held on 1 September 2005. However, during an internal meeting on 25 August 2005, it was determined that there was insufficient information available in the absence of a complete IND submission to adequately address Sponsor's questions. Preliminary comments were provided (internal meeting minutes issued in an IR letter dated 1 September 2005) to aid Sponsor in preparation of their IND submission. The IND was submitted on 17 November 2005.

Fast Track designation was requested 11 April 2006, and denied on 30 May 2006 on the basis that the clinical development program was not designed to address whether mipomersen would affect a serious aspect of HoFH (*e.g.,* cardiovascular morbidity or mortality).

An End-of-Phase 2 meeting request was received on 7 May 2007 and accepted 4 June 2007 as a type B to be held on 11 September 2007. This meeting was held as scheduled; however, the focus was changed to a discussion of the vascular findings

observed in the 1-year monkey study (Study ISIS 301012-AS15) and their implications for the continued clinical development of mipomersen.

On 29 January 2008 clinical development was placed on a partial clinical hold, which prohibited administration of mipomersen to patients who were not at high risk of cardiovascular disease. The basis of the partial clinical hold was the adverse vascular findings in the 1-year monkey study. These vascular findings and their significance to further clinical development were also discussed at an internal Regulatory Briefing on 15 February 2008.

Sponsorship of the IND was transferred from Isis Pharmaceuticals to Genzyme on 23 July 2008.

On 19 December 2008 FDA concurred with Sponsor's plan to discontinue dosing in a given dose group in the ongoing mouse carcinogenicity study when the number of surviving animals in a dose group falls to 20 and early sacrifice of the dose group when the number of survivors in a dose group drops to 15.

On 24 September 2010 Sponsor requested a pre-NDA meeting with the Agency, which was granted on 29 September 2010, and scheduled for 13 December 2010.

NDA was submitted on 29 March 2012 and accepted for filing.

This application was discussed by the Endocrinologic and Metabolic Drugs Advisory Committee on 18 October 2012.

3 Studies Submitted

3.1 Studies Reviewed

With the exception of studies noted in section 3.2, all submitted studies (see tables, below) were reviewed.

Pharmacology

Genzyme (ISIS) Study Number	Study Title
GT-348-EF-8 (EX/2401-170)	Concentration-Response Effect of Multiple Apo B Antisense Oligonucleotides on the Levels of Apo B mRNA after 24 Hr in HepG2 Cells
GT-348-EF-9 (EX/2359-75)	Effect of Concentration on the Inhibition of Apo B mRNA after 24 and 48-Hour Treatment with ISIS 301012 in Hep3B and HepG2 Cells
GT-348-EF-33	SNP Discovery for Antisense Oligonucleotide Target Regions
GT-348-EF-34	BLAST and Complimentary Gene Expression Analysis for ISIS 301012
GT-348-EF-1 (EX/2551-31)	Effect of a Murine Apo B ASO (ISIS 147764) Upon Intestinal [14C]-Oleic Acid Absorption in C57BL/6 Mice
GT-348-EF-12 (EX/2293-06)	Onset of Action of Apo B ASO Treatment in a High Fat Fed Mouse Model
GT-348-EF-6 (EX/1848-13)	Evaluation of Murine Apo B Antisense Oligonucleotide in Lean C57BL/6 Mice
GT-348-EF-7	Evaluation of Apo B ASO Treatment in a Lean Mouse Model

(EX/2551-03)	
GT-348-EF-10 (EX/2016-03)	Dose Response Evaluation of Apo B ASO Treatment in a High Fat Mouse Model
GT-348-EF-4 (EX/2293-12)	Dose Response Evaluation of Apo B ASOs in High Fat Fed C57BL/6 Mice
GT-348-EF-11 (EX/2016-22)	Evaluation of Apo B ASO Effects in Combination with Atorvastatin in a High Fat Mouse Model
GT-348-EF-3 (EX/2293-07)	Duration of Action Evaluation of Apo B ASO Treatment in High Fat Fed C57BL/6 Mice
GT-348-EF-19 (MA7)	Global Gene Expression Profiling in Mice Treated with Atorvastatin and Apo B ASO
GT-348-EF-18 (MA11)	Kinetics of Global Gene Expression Changes in Liver RNA of Mice Treated with Apo B ASO (ISIS 147764) Over 4 Weeks as Determined by Affymetrix Gene Chips
GT-348-EF-24 (EX/3309-059)	Analysis of Lipoprotein Secretion and Liver Lipid Concentrations in High- Fat Fed Mice Administered Apo B ASO (ISIS 147764) or MTP ASO (ISIS 144477) for Six Weeks
GT-348-EF-25 (EX/3309-172)	Analysis of Fatty Acid Synthesis and Other Metabolic Parameters in DIO C57BL/6 Mice Treated with Either Apo B or MTP ASOs
GT-348-EF-22 (EX/3315-49)	Metabolomic Analysis of DIO C57BL/6 Mice Treated with a Murine Specific Apo B Antisense Oligonucleotide
GT-348-EF-30 (EX/2551-32)	Effect of a Murine Apo B ASO on Apo B-48 Levels in High Fat Fed C57BL/6 Mice
GT-348-EF-5 (EX/2293-20)	Long Term Evaluation of Apo B Treatment in High Fat Fed C57BL/6 Mice
GT-348-EF-20 (EX/3261-01)	A 12-Week Onset of Activity Study in High Fat Fed C57BL/6 Mice: Metabolomic and Transcriptional Comparison of an Apo B ASO, MTP ASO and a Small Molecule MTP Inhibitor
GT-348-EF-20a (EX/3261-01a)	Effect of Murine ApoB and MTP ASO Treatment on Hepatic Endoplasmic Reticulum Stress and Heat Shock Proteins
GT-348-EF-21 (EX/3310-29)	Analysis of Fatty Acid Synthesis and Other Metabolic Parameters in DIO C57BL/6 Mice Treated with Apo B Antisense Oligonucleotides
GT-348-EF-14 (EX/2293-10)	Evaluation of Apo B ASO Dose-Dependent Effects in LDLr-/-Deficient Male and Female Mice
GT-348-EF-27 (EX/3309-244)	Analysis of Lipoprotein Particles in Western Diet-Fed LDLr-/- Mice Administered Apo B and MTP Antisense Oligonucleotides
GT-348-EF-26 (EX/3309-152)	Apo B vs. MTP ASO Comparison in LDLr-/- Mice Fed a High Fat, High Cholesterol Diet
GT-348-EF-17 (EX/3381-47)	Effect of ISIS 147764 on Atherosclerosis in LDLr-Deficient Mice
GT-348-EF-16 (EX/2947-01)	Effect of ISIS 301012 on Atherosclerosis in Human Apo B-100, LDLr -/- Transgenic Mice
GT-348-EF-15 (EX/2551-41)	Effect of ISIS 147764 on Atherosclerosis in ApoE-Deficient Mice
GT-348-EF-2 (EX/2016-06)	Evaluation of Increasing Doses of a Murine Apo B ASO in ApoE-Deficient Mice
GT-348-EF-13 (EX/2016-19)	Kinetic Evaluation of a Murine Apo B-ASO in ApoE-Deficient Mice
GT-348-EF-29 (EX/2653-12)	Effect of Species-Specific Apo B ASOs in Lean Hamsters After 3 Weeks of Treatment
GT-348-EF-28 (GL/1521-101)	An Exploratory Study to Evaluate the Potential Effects of Four Rabbit Apo B Antisense Oligonucleotides on Hepatic mRNA Levels in Male New Zealand White Rabbits
GT-348-EF-31 (301012-AS10)	Investigation of Effects of ISIS 301012 and ISIS 326358 on Apo B and Cholesterol in Cynomolgus Monkeys Fed a High Fat Diet
GT-348-EF-35	In Vitro Pharmacology: High-Throughput Profile Study of Compound 301012

301012-AS13	Study on the Effects of ISIS 301012 on Respiration Rate, Tidal Volume and Minute Volume in ICR Mice Following a Single Subcutaneous Administration
301012-AS16	Effect of ISIS 301012 on Neurobehavior (Irwin's Test) and Body Temperature in Mice After a Single Subcutaneous Administration
301012-IS03	Effects of ISIS 301012 on cloned hERG Channels Expressed in Mammalian Cells
301012-AS02	13-Week Toxicity Study and Pharmacokinetic Study of ISIS 301012 Administered by Intravenous Infusion or Subcutaneous Injection to Cynomolgus Monkeys, with a 13-Week Recovery Period[a]

[a] toxicology study with cardiac endpoints

Pharmacokinetics

Genzyme (ISIS) Study Number	Study Title
301012-MV01	ELISA Analysis of ISIS 301012 in Mouse EDTA Plasma
301012-MV02	Validation of a Method for the Extraction of ISIS 301012 from Monkey Tissues with Analysis by Capillary Electrophoresis
301012-MV03	Partial Validation for the Extraction of ISIS 301012 From Mouse Tissues with Analysis by Capillary Electrophoresis
301012-MV04	ELISA Analysis of ISIS 301012 in Monkey EDTA Plasma
301012-MV05	Validation of a Method for the Determination of ISIS 301012 in Monkey, and Cross-Validation to Mouse Urine, Using 96- Well Plate Extraction with Analysis by Capillary Gel Electrophoresis
301012-MV08	ELISA Analysis of ISIS 301012 in Beagle EDTA Plasma
301012-MV09	Partial Validation for the Extraction of ISIS 301012 From Dog Tissues with Analysis by Capillary Electrophoresis
301012-MV10	Partial Validation for the Extraction of ISIS 301012 from Fetal Mouse Tissues with Analysis by Capillary Electrophoresis
301012-MV13	Partial Validation for the Extraction of ISIS 301012 From Fetal and Adult Rabbit Tissues with Analysis by Capillary Electrophoresis
301012-MV18	ELISA Analysis of ISIS 301012 in Rat K3 EDTA Plasma
301012-MV19	Partial Validation for the Extraction of ISIS 301012 From Rat Tissues with Analysis by Capillary Gel Electrophoresis
301012-APK01M	Characterization of ISIS 301012 Concentrations in Selected Plasma, Tissue and Urine Samples from Study 301012-APK01 Using ISIS 301012-Specific Non-Radiolabel Analytical Methods
301012-AS06	A pharmacokinetic Study with ISIS 301012 Administered Orally and Subcutaneously to Dogs
301012-APK01	Pharmacokinetics, Excretion and Mass Balance of Radioactivity in Rat and Tissue Distribution in Rat and Mouse Following a Single Intravenous Bolus Injection of 3H-ISIS 301012
DMPK10-R024	Assessment of Human Cytochrome P450 Involvement in the Metabolism of ISIS 301012
301012-IS07	Mipomersen (ISIS 301012) Metabolites Identified by Ion-Pair HPLC Electrospray/Mass Spectrometry (IPHPLC-ES/MS) in Liver and Kidney from Mice, Rats, and Monkeys and Urine from Rats, Monkeys, and Humans
301012-IS05	Determination of the Inhibitory Potential of ISIS 301012 on the Activities of CYPlA2, CYP2C9, CYP2Cl9, CYP2D6, and CYP3A4 in Cryopreserved Human Hepatocytes
DMPK10-R015	In Vitro Assessment of CYP450 Induction Potential in Human Hepatocytes
DMPK09-R037	In Vitro Evaluation of the P-Glycoprotein Substrate and Inhibition of ISIS 301012
301012-IS04	ISIS 301012: In Vitro Plasma Protein Binding Studies

Toxicology

Genzyme (ISIS) Study Number	Study Title
301012-AS01	3-Month Repeat-Dose Subcutaneous Toxicity Study of ISIS 301012 in CD-1 Mice With 3-Month Recovery
301012-AS14	6-Month Repeat-Dose Subcutaneous Toxicity Study of ISIS 301012 and ISIS 147764 in CD-1 Mice with 3-Month Recovery
301012-AS21	5-Month Repeat-Dose Subcutaneous Toxicity Study of ISIS 301012 in Sprague-Dawley Rats with a 3-Month Recovery
301012-AS02	A 13-Week Toxicity Study and Pharmacokinetic Study of ISIS 301012 Administered by Intravenous Infusion or Subcutaneous Injection to Cynomolgus Monkeys, with a 13-Week Recovery Period
301012-AS15	A One Year Toxicity Study of ISIS 301012 Administered by Subcutaneous Injection to Cynomolgus Monkeys
301012-IS01	Bacterial Reverse Mutation Study of ISIS 301012 in the Salmonella typhimurium/Escherichia coli Plate Incorporation Assay
301012-IS02	*In Vitro* L5178Y/TK+/- Mouse Lymphoma Cell Gene Mutation Assay of ISIS 301012
301012-AS12	Mammalian Erythrocyte Micronucleus Assay of ISIS 301012 in ICR Mice
GT-348-TX-1	2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 and ISIS 147764 in CD-1 Mice
GT-348-TX-2	2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats
301012-AS07	Combined Fertility and Development Toxicity Study of ISIS 301012 and ISIS 147764 Administered by Subcutaneous Injection in Mice
301012-AS08	Subcutaneous Developmental Toxicity Study of ISIS 301012 and ISIS 233183 in Rabbits
GT-348-TX-6	Study of Pre and Postnatal Development in the Rat
GT-348-TX-5	10-week toxicity study in the Juvenile Rat with a 4 week recovery Period
GT-348-TX-8	Effects of Three Oligonucleotides in a Mouse Mast Cell Degranulation Assay
301012-AS20	Evaluation of ISIS 301012 in the Mouse Influenza Host Resistance Model
301012-AS18	Repeat-Dose Subcutaneous Injection Toxicity Study with ISIS 301012 Manufactured by [redacted] (b) (4) with Process-Related Impurities in Mice
GT-348-TX-7	5 Week Subcutaneous (Impurities Qualification) Toxicity Study in the Mouse

3.2 Studies Not Reviewed

1. 301012-AS04: 13-Week Oral Dietary Admix Toxicity Study with ISIS 301012 and Penetration Enhancer in Mice with a 4-Week Interim Sacrifice
   -*Not the intended route of administration. Cursory review revealed no findings material to the review of this NDA.*
2. 301012-AS05: 13-Week Oral Tablet Toxicity and Toxicokinetic Study with ISIS 301012 in Dogs with a 4-Week Interim Sacrifice
   -*Not the intended route of administration. Cursory review revealed no findings material to the review of this NDA*.
3. 301012-AS09: A 5-Week Toxicity Study of ISIS 326358 Administered by Subcutaneous Injection Alone or in Combination with Atorvastatin Administered by Oral Gavage to Cynomolgus Monkeys, Followed by a 3-Month Recovery Period

-Not the clinical candidate. Cursory review revealed no findings uniquely material to the review of this NDA.

4. GT-348-TX-4: Subcutaneous Range-Finding Study of Pre- and Postnatal Development in the Rat.
   -Cursory review revealed no unique findings compared to the definitive study (GT-348-TX-6).
5. GT-348-TX-3: Subcutaneous Range-Finding Toxicity Study in the Juvenile Rat
   -Cursory review revealed no unique findings compared to the definitive study (GT-348-TX-5).
6. 301012-AS17: A 13-Week Dose-Range Finding Study of ISIS 301012 Administered by Weekly Subcutaneous Injection to Sprague-Dawley Rats
   -Cursory review revealed no unique findings compared to the definitive 5-month study (301012-AS21).

3.3 Previous Reviews Referenced

A. 13-JAN-2006, Initial safety review of IND 70,969 by Dr. Gemma Kuijpers
B. 15-MAY-2007, Review of 6-month interim data from 1-year monkey study by Dr. Gemma Kuijpers
C. 04-JUN-2007, Special protocol assessment (SPA) for mouse 2-year carcinogenicity study by Dr. Gemma Kuijpers

4 Pharmacology

4.1 Primary Pharmacology

Mipomersen exerts its intended pharmacology through an antisense mechanisms that relies upon its ability to selectively bind, in a base-pair sequence specific manner, to the messenger ribonucleic acid (mRNA) of apolipoprotein B-$_{100}$ (apoB$_{100}$) through Watson-Crick base-pairing. Once bound to the mRNA, mipomersen disrupts translation of the mRNA into apoB$_{100}$ protein via activation of RNAse H-dependent cleavage of the mipomersen-mRNA duplex. Decreased translation of apoB$_{100}$ mRNA into protein results in a reduction in apoB$_{100}$ protein level and therefore reduced apoB$_{100}$ activity.

Mipomersen is a second generation phosphorothioate oligonucleotide. The five bases on both the 5' and 3' ends are modified by attachment of a methoxyethyl (MOE) group on the 2' position of the ribose ring, giving rise to a chimeric RNA-DNA-RNA structure referred to as a MOE-gapmer. The "gap" refers to the ten 2'-deoxynucleosides in the middle that are necessary to support enzymatic cleavage of the mRNA. The 2' MOE modifications protect mipomersen from DNA exonuclease cleavage, and also decrease the degree of unintended immune stimulation. Additionally each of the nine cytosine bases carry a methyl group at the 5 position of the ribose ring to further reduce the proinflammatory activity of mipomersen.

Apolipoprotein B is a critical component of lipoproteins such as VLDL and LDL-C and provides structural stability to the lipoprotein. A lipoprotein consists of an apoprotein,

triglycerides, cholesteryl esters, and/or free cholesterol. ApoB also functions as a ligand in lipoprotein-receptor (*e.g.*, LDL receptor) interactions. ApoB is synthesized primarily in the liver. ApoB-VLDL containing particles are atherogenic.

Overproduction of ApoB has been observed in an autosomal dominant disease (familial hypercholesterolemia) and is associated with premature atherosclerosis. By contrast, in individuals with hypobetalipoproteinemia (low apoB levels due to mutation) the incidence of cardiovascular disease is low.

Mipomersen is 100% complementary to a 20 base-pair portion of the coding region of human apoB, and targets a region that appears to have a low frequency of SNPs (single nucleotide polymorphisms). No SNPs were observed in the complementarity region of ApoB from 213 donor sequences. This is significant since single base-pair mismatches can significantly reduce binding. BLAST analyses show that ApoB is the only human gene that is 100% complementary to the sequence of mipomersen. There were no human genes with a single base-pair mismatch. Six genes were identified that have a 2 base-pair mismatch. Two of these were only complementary to the negative strand (and therefore incapable of having an antisense effect). The expression levels of the other 4 genes (hypothetical protein FLJ32642, MDM2, EST similar to B cell growth factor, cGMP-dependent protein kinase-2) were not affected my mipomersen.

The mouse AboB sequence is only 81% homologous and the monkey sequence ~95% homologous to the mipomersen sequence. Because of the base-pair mismatches between mipomersen and ApoB mRNA in the animal models, species-specific surrogate ASOs with 100% complementarity to the corresponding ApoB mRNA were used for most pharmacology assessments.

The *in vitro* pharmacologic activity of mipomersen was characterized in human hepatoma cell lines (HepG2, Hep3B) and in human and cynomolgus monkey primary hepatocytes. In these experiments, mipomersen selectively reduced apo B mRNA, protein and secreted protein in a concentration- and time-dependent manner. The effects of mipomersen were shown to be highly sequence-specific. Mipomersen significantly reduced both apo B mRNA and protein levels after 24-hour treatment in human primary hepatocytes. The half maximal inhibitory concentration (IC_{50}) for mRNA reduction in these cells was <10 nM. When a series of mismatches were introduced into the mipomersen sequence and tested in HepG2 cells, a single mismatch abolished pharmacologic activity.

The pharmacology of inhibiting apo B with species-specific ASOs was examined in mouse, hamster, rabbit and monkey models. Studies were performed in animals with normal cholesterol levels, as well as several rodent models of hyperlipidemia and atherosclerosis. Mipomersen itself was also evaluated in mice containing the human apo B genomic transgene.

Results showed that apo B antisense inhibition reduced hepatic apo B mRNA and protein and serum apo B, low density lipoprotein (LDL-C) and total cholesterol in a

dose-, drug-, concentration- and time-dependent manner. In addition to effects on lipids, reduction of apo B resulted in the reduction of atherosclerosis (aortic sinus plaque volume) in 3 murine models.

Interfering with hepatic VLDL assembly and transport, as seen with microsomal triglyceride protein (MTP) inhibitors, can produce hepatic and intestinal steatosis (Cuchel, 2007, *N Engl J Med*; Chandler, 2003, *J Lipid Res*). Hepatic and intestinal steatosis has not been observed following chronic treatment with apo B ASOs in mice or monkeys. This may be accounted for by favorable secondary changes in the transcription of key hepatic genes involved in lipid synthesis and fatty acid oxidation (Crooke, 2005, *J Lipid Res*), which were demonstrated in mice using gene expression array, quantitative reverse transcription PCR and metabolic pathway analyses.

Apolipoprotein B exists in the plasma in 2 main isoforms, apo B-48 and apo B-100. In man, apo B-48 is synthesized exclusively by the gut while apo B-100 is synthesized in the liver (Powell, 1987, *Cell*). Apo B-48, which is essential for chylomicron formation, is derived from the same mRNA as apo B-100, which includes the mipomersen binding site. In preclinical pharmacology studies, it has been shown that while intestinal apo B-100 mRNA is reduced by approximately 50%, no reduction in apo B-48 protein is observed with apo B ASO treatment, and intestinal fat absorption is not affected. Enterocytes of the intestinal brush border have a half life of several days, mediate fat absorption and are the site of synthesis and export of chylomicrons. The lack of effect of apo B ASOs on chylomicron synthesis is likely due to several factors, including the low distribution of antisense drugs to the gastrointestinal tract and the rapid turnover of the intestinal brush border that together preclude accumulation of mipomersen to reach steady state concentrations.

Acute safety pharmacology studies to evaluate functional effects on cardiovascular, respiratory or central nervous system functions were conducted. No adverse effects were seen in any of these potential target organ systems.

4.2 Secondary Pharmacology

Potential secondary pharmacodynamic effects of mipomersen were evaluated in a high throughput *in vitro* screen with a broad collection of ~80 transmembrane and soluble receptors, ion channels and monamine transporters. The assay was performed at a single concentration of 10 µM. At this concentration, mipomersen inhibited ligand binding to various degrees to six receptors: Cholecystokinin A-R (↓59%), Cholecystokinin-B-R (↓53%), Galanin-R2 (↓75%), PDGF-R (↓72%), CXCR2 (↓70%) and Melanocortin-R4 (↓59%). These results likely reflect a non-specific interaction of mipomersen with these six specific receptors. The inhibitory activity of mipomersen at these receptors is unlikely to be clinically significant since: 1) the mean clinical C_{max} at the intended clinical dose of 200 mg is only 0.6 µM, and 2) these are all cell surface receptors, and mipomersen is rapidly internalized intracellularly.

4.3 Safety Pharmacology

Cardiovascular
Heart rate, mean arterial pressure and EKG were evaluated by telemetry in monkeys after single and repeat doses of 3 to 35 mg/kg/wk for 13 weeks (Study AS02). EKG's were also performed in all monkeys pre-study and prior to sacrifice using external leads. There were no effects on CV function.

hERG assay was performed in stably transfected human embryonic kidney cells (HEK 293) (Study ISIS 301012-IS03). No effect on ion current at concentrations up to 150 μM. Conclusion was mipomersen was negative in hERG assay.

Neurobehavioral
(AS16) Mice received a single SC dose of ISIS 301012 at 0, 50, 100 or 250 mg/kg. Body temperature and performance in a modified Irwin's test were measured at time zero (predose) and then 30, 60, 120, 360 min and 24 h after dosing. ISIS 301012 treatment was without effect on behavior at any dose. 250 mg/kg was associated with small s.s. ↓s in body temperature between 120 and 360 min after dosing; however the body temperatures remained within the range of control values.

Renal
Renal function was evaluated in the repeat dose studies in mice (AS01) and monkeys (AS02) after single or repeat dosing up to 13 weeks. There were no effects on renal function at doses up to 35 mg/kg/wk (monkey) or 88 mg/kg/wk (mouse), based on serum chemistry (BUN, creatinine) and urinalysis.

Pulmonary
A pulmonary safety pharmacology study was conducted to evaluate the effects of a single subcutaneous administration of ISIS 301012 on lung compliance, airway resistance, respiration rate, or tidal volume in ICR mice (AS13). Doses were selected at 25, 50, and 200 mg/kg ISIS 301012. Mice were positioned into a plethysmometer and pulmonary function was measured at 30, 60, 120, 360 minutes and 24 hours after administration. No changes on the respiratory rate, tidal volume and minute volume were observed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

METHODS
A variety of bioanalytical methods were used to quantitate mipomersen and its short-chain oligonucleotide metabolites. Several non-competitive hybridization nuclease-based enzyme-linked immunosorbent assay (ELISA) procedures were developed to quantitate mipomersen in plasma in nonclinical species. Capillary gel electrophoresis with UV detection (CGE-UV) was used to determine concentrations of mipomersen and its metabolites in urine and various tissues to support *in vivo* metabolism and excretion

studies. Liquid scintillation counting (LSC) was used to determine total radioactivity concentrations in plasma, urine, and tissue homogenates to support rat mass balance and tissue distribution study using [^{3}H]-mipomersen. Quantitative whole-body autoradiography (QWBA) was used to quantify the radioactivity concentration in tissues when [^{3}H]-mipomersen was administered to mouse and rat to study organ distributions. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays were developed to quantify mipomersen concentration in microsome and hepatocyte incubations used in *in vitro* metabolism studies. Analyses of plasma, tissue and urine concentrations of mipomersen and its related oligonucleotide metabolites were conducted with fully validated methods during development of mipomersen.

ABSORPTION

The plasma pharmacokinetic properties of mipomersen were consistent across multiple nonclinical species and man. Following SC administration, mipomersen was rapidly absorbed into the systemic circulation with a mean bioavailability close to 100% and Tmax values between 0.5 and 2 hours across the evaluated species. The disposition kinetics of mipomersen in plasma following SC administration was generally biphasic, with a rapid tissue distribution phase (half-life <5 hours) followed by a prolonged elimination phase (half-life >16 days). The mean apparent plasma terminal half-life of mipomersen was in the range of 16 to 30 days in the species evaluated.

Following a single SC dose in mouse, rat, and monkey, the mean plasma Cmax and AUC of mipomersen were shown to be dose-dependent across species, with Cmax increasing in a roughly dose proportional manner in mouse and monkey but not rat, and AUC increasing approximately 2- to 3-fold greater than that predicted based on dose proportionality.

Following repeated SC administration of mipomersen to mouse (5 to 25 mg/kg, once every fourth day [Q4D]), rat (3 to 30 mg/kg, once weekly [QW]), dog (2 mg/kg, once daily [QD]), and monkey (1 to 30 mg/kg, QW), the mean plasma Cmax and AUC of mipomersen were shown to be dose-dependent across species, and the Cmax and AUC increases were either dose-proportional or greater than dose-proportional, depending on the dose level.

Following repeated SC administration, mean trough plasma concentrations (C_{trough}) of mipomersen increased by 2- to 5-fold in mouse and monkey (once weekly dosing) and 10-fold in dog (QD dosing) compared to the plasma C_{trough} post loading doses, consistent with mipomersen accumulation in major tissues. Plasma C_{trough} reached steady-state in approximately three months in rodents and six months in monkey, consistent with the observed long terminal half-life.

Following IV dosing, the mean plasma clearance of mipomersen was 0.135 and 0.0616 L/h/kg in rat and monkey, respectively. The apparent volume of distribution was close to the total body water volume, indicating tissue distribution.

Table 3-A: Absorption after a Single Dose in Mouse (SC) and Rat (SC)

Report Number:	301012-AS01PK		301012-AS14PK		301012-AS21PK	
	Mean ± SD [a]					
PK Parameters	5 mg/kg (mouse)	25 mg/kg (mouse)	10 mg/kg (mouse)	75 mg/kg (mouse)	3 mg/kg (rat)	30 mg/kg (rat)
T_{max} (h):	0.5	1	0.50	0.25	0.5	0.5
C_{max} (µg/mL):	3.80 ± 0.57	29.8 ± 6.8	8.15 ± 2.31	47.1 ± 12.9	2.88 ± 0.571	45.6 ± 6.30
AUC_{0-t} (µg*h/mL):	N/D	N/D	14.6	118	10.1	234
0-t (h):	NA	NA	0-48	0-48	0-168	0-168
$AUC_{0-\infty}$ (µg*h/mL):	7.41	102	14.7	119	10.2	234
$t_{½}$ (h) [b]:	N/D	N/D	7.77	8.48	2.40 (days)	1.77 (days)
t_{last} (h):	48	48	48	48	168	168
CL/F (mL/h/kg):	674	245	679	630	N/D	N/D
V_d/F (mL/kg):	4715	2189	7612	7706	N/D	N/D
$t_{½\alpha}$ (h) [c]:	0.33	0.57	N/D	N/D	1.54	2.55
$t_{½\beta}$ (h) [b, c]:	5.6	3.9	N/D	N/D	0.41 (days)	2.59 (days)

N/D = Not determined, NA = Not applicable.
[a] Standard deviations are reported if determined.
[b] Half-life values considered underestimations due to limited duration of sampling.
[c] Parameters determined by compartmental analysis and reported in unit of hour unless specified in the table.

Table 3-B: Absorption after a Single Dose in Rat (SC, IV)

Report Number:	301012-AS23PK (GT-348-TX-5)						301012-APK01M	301012-APK01
	Mean ± SD [a]							
PK Parameters	Female (F)			Male (M)			Male (M)	Male (M)
	3 mg/kg	10 mg/kg	50 mg/kg	3 mg/kg	10 mg/kg	50 mg/kg	5 mg/kg	5 mg/kg
T_{max} (h) [b]:	0.25	0.5	0.5	0.5	0.5	0.5	0.0333	0
C_{max} (µg/mL) [b]:	4.14	17.9	79.4	4.49	19.3	85.5	75.9	85.1 [c]
AUC_{0-t} (µg*h/mL)	7.76	40.1	305	8.12	36.6	270	37.0	250 [c]
0-t (h)	0-24	0-24	0-24	0-24	0-24	0-24	0-336	0-672
$AUC_{0-\infty}$ (µg*h/mL):	N/D	N/D	N/D	N/D	N/D	N/D	37.1	368 [c, d]
$t_{½}$ (h):	N/D	N/D	N/D	N/D	N/D	N/D	126 [e]	457 [d]
t_{last} (h):	NA	NA	NA	NA	NA	NA	336	672
CL/F (mL/h/kg) [f]:	N/D	N/D	N/D	N/D	N/D	N/D	135	13.6 [d]
V_d/F (mL/kg) [f]:	N/D	N/D	N/D	N/D	N/D	N/D	604	7881 [d]
$t_{½\alpha}$ (h) [g]:	N/D	N/D	N/D	N/D	N/D	N/D	N/D	0.476
$t_{½\beta}$ (h) [g]:	N/D	N/D	N/D	N/D	N/D	N/D	N/D	391

N/D = Not determined, NA = Not applicable, TRA = total radioactivity.
[a] Standard deviations are reported if determined.
[b] Representing first time point and concentration values at first time point for bolus IV studies.
[c] Concentration is in unit of µg-eq for radioactivity measurements.
[d] Values calculated from $AUC_{0-\infty}$ that was extrapolated by more than 20% of the $AUC_{0-tlast}$ value.
[e] Value considered an underestimation due to limited duration of sampling.
[f] Representing CL and V_d for IV studies.
[g] Parameters determined by compartmental analysis and reported in unit of hour unless specified in the table.

Table 3-C: Absorption after a Single Dose in Dog (SC) and Monkey (IV)

Report Number:	301012-AS06PK	301012-AS15PK				301012-AS02PK
	Mean ± SD					
PK Parameters	n=6 (M&F Dog)	n=10 (M&F Monkey)			n=14 (M&F Monkey)	n=14 (M&F Monkey)
	2 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	4 mg/kg
T_{max} (h):	1.25 ± 0.27	2 (1-2) [a]	2 (2-2) [a]	2 (1-4) [a]	2 (1-2) [a]	1 ± 0
C_{max} (µg/mL):	1.80 ± 0.30	0.817 ± 0.230	3.82 ± 1.25	15.8 ± 3.9	46.6 ± 12.0	39.2 ± 5.6
AUC_{0-t} (µg*h/mL):	5.20 ± 1.24	5.16 ± 1.04	18.5 ± 2.9	112 ± 42	518 ± 119	47.4 ± 7.8
0-t (h):	0-24	0-48	0-48	0-48	0-48	0-24
$AUC_{0-\infty}$ (µg*h/mL):	N/D	5.25 ± 1.06	18.7 ± 2.9	113 ± 42	522 ± 118	67.5 ± 14.0
$t_{½}$ (h):	N/D	9.51 ± 1.82 [b]	9.35 ± 1.08 [b]	8.00 ± 2.88 [b]	6.60 ± 1.36 [b]	12.3 ± 4.1 [b]
t_{last} (h):	NA	48	48	48	48	48
CL/F (mL/h/kg) [c]:	N/D	N/D	N/D	N/D	N/D	61.6 ± 12.0
V_d/F (mL/kg) [c]:	N/D	N/D	N/D	N/D	N/D	112 ± 25 [d]
$t_{½\alpha}$ (h) [e]:	N/D	N/D	N/D	N/D	N/D	0.710 ± 0.061
$t_{½\beta}$ (h) [e]:	N/D	N/D	N/D	N/D	N/D	N/D

N/D = Not determined, NA = Not applicable.
[a] Median (Min-Max)
[b] Value considered an underestimation due to limited duration of sampling.
[c] Representing CL and V_d for IV studies.
[d] The value reported is volume of distribution at steady-state (V_{ss}) as opposed to V_d, which was not determined in the study 301012-AS02PK.
[e] Parameters determined by compartmental analysis

Table 4-A: Absorption after Repeated Doses in Mouse (SC), Rat (SC), and Dog (SC)

Report Number:	301012-AS01PK		301012-AS21PK		301012-AS23PK(GT-348-TX-5)						301012-AS06PK
	Mean ± SD [a]										
Sample Collection Day:	31		148		Postnatal Day (PND) 91						29
PK Parameters	5 mg/kg (mouse)	25 mg/kg (mouse)	3 mg/kg (rat)	30 mg/kg (rat)	Female Rat			Male Rat			2 mg/kg (dog)
					3 mg/kg	10 mg/kg	50 mg/kg	3 mg/kg	10 mg/kg	50 mg/kg	
T_{max}(h):	0.5	0.5	2	1	1	0.5	1	0.5	0.5	1	1.08 ± 0.49
C_{max} (µg/mL):	5.04 ± 0.56	19.9 ± 4.2	3.75 ± 0.513	49.1 ± 0.928	4.16	16.9	107	3.07	15.0	82.4	1.78 ± 0.42
AUC_{0-t} (µg*h/mL):	N/D	N/D	21.5 / 25.1	395 / 455	14.6	91.1	681	21.8	89.9	801	6.86 ± 1.94
0-t (h)	NA	NA	0-48 / 0-1344	0-48 / 0-1344	0-24	0-24	0-24	0-24	0-24	0-24	0-24
$AUC_{0-\infty}$ (µg*h/mL):	14.4	69.2	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
$t_{½}$ (day) [b]	N/D	N/D	17.5	22.4	N/D	N/D	N/D	N/D	N/D	N/D	N/D
t_{last} (h):	48	48	1344	1344	NA	NA	NA	NA	NA	NA	NA
$t_{½\alpha}$ (h) [c]:	0.58	1.51	2.31 ± 0.79	3.35 ± 0.42	N/D	N/D	N/D	N/D	N/D	N/D	N/D
$t_{½\beta}$ (h) [c]:	11.2h [d]	130h [d]	16.3 ± 3.6	21.5 ± 4.1	N/D	N/D	N/D	N/D	N/D	N/D	N/D

N/D = Not determined, NA = Not applicable.
[a] Standard deviations are reported if determined.
[b] Apparent terminal half-life determined from concentration-time curve after last dose of repeated dosing.
[c] Parameters determined by compartmental analysis and reported in the indicated units unless specified in the table.
[d] Value considered an underestimation due to limited duration of sampling.

Table 4-B: Absorption after Repeated Doses in Monkey (SC)

Report Number:	301012-AS15PK							
	Mean ± SD [a]							
Sample Collection Day:	183	358	183	358	183	358	183	358
PK Parameters	1 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
T_{max} (h):	1 (1-2) [b]	2 (1-4) [b]	2 (1-4) [b]	2 (1-2) [b]	2 (1-2) [b]	2 (1-4) [b]	2 (1-4) [b]	2 (1-4) [b]
C_{max} (µg/mL):	1.31 ± 0.45	1.05 ± 0.40	5.54 ± 1.42	3.24 ± 1.33	23.2 ± 4.5	17.4 ± 5.8	53.1 ± 10.0	33.9 ± 9.9
$AUC_{0\text{-}t}$ (µg*h/mL):	7.30 ± 1.62	8.88 ± 2.14	26.7 ± 6.6	19.3 ± 4.1	127 ± 33	156 ± 40	627 ± 180	448 ± 116
0-t (h):	0-48	0-48	0-48	0-48	0-48	0-48	0-48	0-48
$AUC_{0\text{-}\infty}$ (µg*h/mL) [c]:	8.02 ± 2.10	9.45 ± 2.22	28.4 ± 8.1	20.4 ± 3.8	128 ± 35	162 ± 45	638 ± 194	466 ± 121
$t_{½}$ (h) [d]:	14.6 ± 4.8	12.9 ± 1.5	14.9 ± 4.2	14.1 ± 3.6	10.0 ± 2.5	10.7 ± 2.4	7.46 ± 1.48	10.2 ± 2.4
t_{last} (h):	48	48	48	48	48	48	48	48

N/D = Not determined, NA = Not applicable.

[a] Standard deviations are reported for PK parameters, if determined.

[b] Data represented as median (range).

[c] $AUC_{0\text{-}\infty}$ =($AUC_{0\text{-}tlast}$+$AUC_{tlast\text{-}\infty}$), where the extrapolated $AUC_{tlast\text{-}\infty}$ is calculated based on the $t^{1}/_{2}$ estimated from the terminal phase of concentration-time course following the dose administered on the day specified in the table.

[d] Apparent terminal half-life following the dose administered on the day specified in the table; values reported in the table are considered being underestimated due to limited duration of sampling.

Table 4-C: Absorption after Repeated Doses in Monkey (SC, IV)

Report Number:	301012-AS02PK										
	Mean ± SD [a]										
Sample Collection Day:	7	87	7	87	7	7	87	7	87	7	87
PK Parameters	n=10 (M&F)	n=4 (M&F)	n=6 (M&F)		n=12 (M&F)	n=10 (M&F)	n=6 (M&F)	n=14 (M&F)	n=6 (M&F)	n=12 (M&F)	n=6 (M&F)
	IV 1-hour Infusion		SC		IV 1-hour Infusion			IV 1-hour Infusion		SC	
	2 mg/kg				4 mg/kg			12 mg/kg		20 mg/kg	
	QOD x 4 doses / Q4D x 12 weeks		QOD x 13 weeks		QOD x 4 doses / Q4D x 12 weeks			QOD x 4 doses / Q4D x 12 weeks		QOD x 4 doses / Q4D x 12 weeks	
T_{max} (h):	1.01± 0.03	1.08 ± 0.09	1.33 ± 0.52	1.33 ± 0.52	0.96 ± 0.14	1 ± 0	1.08 ± 0.09	1.07 ± 0.27	1.07 ± 0.06	2.17 ± 0.58	2.5 ± 1.22
C_{max} (µg/mL):	14.8 ± 4.3	10.6 ± 5.0	4.6 ± 1.9	2.6 ± 0.6	38.5 ± 5.6	39.8 ± 6.7	32.0 ± 5.3	123 ± 24.6	97.3 ± 25.8	42.4 ± 10.1	47.2 ± 16.7
$AUC_{0\text{-}t}$ (µg*h/mL):	17.5 ± 5.0	12.2 ± 5.8	6.4 ± 2.6	3.5 ± 0.6	50.5 ± 10.5	50.5 ± 10.5	39.9 ± 7.0	148 ± 30.7	119 ± 30	54.0 ± 13.7	58.8 ± 15.7
0-t (h)	0-2	0-2	0-2	0-2	0-2	0-48	0-48	0-2	0-2	0-2	0-2
$AUC_{0\text{-}\infty}$ (µg*h/mL) [b]:	26.2 ± 6.8	19.6 ± 11.6	20.6 ± 7.6	21.9 ± 5.9	80.9 ± 9.6	82.0 ± 19.1	76.1 ± 20.9	310 ± 67.1	253 ± 74	428 ± 119	557 ± 130
t½ (h) [c]	15.2 ± 5.0 [d]	21.4 ± 4.2 [d]	8.8± 1.1 [d]	12.9 ± 7.7 [d]	229 ± 169 [d]	11.6 ± 3.0 [d]	28.3 ± 12.8 [d]	10.1 ± 2.0 [d]	15.1 ± 2.1 [d]	6.9 ± 0.8 [d]	7.9 ± 1.1 [d]
t_{last} (h):	48	48	48	48	48	48	48	48	48	48	48
$t_{½\,\alpha}$ (h) [e]:	0.567 ± 0.152	0.601± 0.185	1.41 ± 1.297	1.86 ± 3.28	0.829 ± 0.098	0.676 ± 0.194	0.815 ± 0.236	1.19 ± 0.328	1.53 ± 0.489	3.83 ± 9.83	4.54 ± 17.3
F (%) [f]	NA	NA	78.6	111	NA	NA	NA	NA	NA	82.9	132

DISTRIBUTION

- Mipomersen is highly bound to plasma protein in all tested species (>=85%)
  - Extent of binding is independent of mipomersen concentration over 2 logs (0.1 to 10μM)
  - Mipomersen does not displace statins from plasma protein, nor do statins displace mipomersen binding
  - Mipomersen binds to multiple different human plasma proteins
- Tissue distribution: highest in kidney (especially cortex), liver, spleen, lymph nodes, thyroid/parathyroid, stomach and bone marrow
- Minimal distribution to muscle or brain
- Mipomersen does not cross the placenta
- Tissue concentration is dose-dependent, and scales best between species on a mg/m^2 basis
- Mipomersen distribution to the mouse kidney saturates at relatively low drug levels compared to the rat and monkey
- Tissue half-life is 10-35 days

Table 6-A: Plasma Protein Binding Across Species

Report Number:	301012-IS04			
Location in CTD:	4.2.2.6			
Test Article:	[^{32}P]-ISIS 301012			
Type of Study:	*In vitro* plasma protein binding study			
Method:	Plasma protein binding determined by ultrafiltration and analysis by liquid scintillation spectrometry (number of replicates per species = 5)			
Species:	Mouse, rat, monkey, human			
	% Bound (Mean ± SD)			
ISIS 301012 Concentration (μM)	**Mouse**	**Rat**	**Monkey**	**Human**
0.1	N/D	N/D	N/D	95.87 ± 0.83
1	87.98 ± 1.22	91.26 ± 2.21	92.46 ± 1.76	95.82 ± 0.32
2	88.66 ± 2.62	93.15 ± 1.02	94.81 ± 0.67	95.53 ± 0.29
4	86.73 ± 1.30	92.32 ± 1.11	90.84 ± 2.40	95.14 ± 0.13
6	86.70 ± 1.03	90.82 ± 1.06	93.72 ± 2.04	93.96 ± 2.03
8	84.79 ± 0.78	91.33 ± 0.39	92.72 ± 0.45	93.82 ± 0.93
10	84.47 ± 1.07	92.03 ± 1.15	92.76 ± 1.15	94.35 ± 0.23
20	84.71 ± 0.66	90.05 ± 0.43	92.08 ± 0.34	92.65 ± 1.62
50	N/D	N/D	N/D	90.38 ± 1.64
100	N/D	N/D	N/D	88.54 ± 2.69
600	N/D	N/D	N/D	84.81 ± 0.70

N/D = Not determined.

Table 6-B: Binding to Individual Protein Constituents of Human Plasma

Report Number:	301012-IS04		
Location in CTD:	4.2.2.6		
Test Article:	[^{33}P]-ISIS 301012		
Type of Study:	*In vitro* binding to individual protein constituents of human plasma		
Method:	Binding of mipomersen to individual protein constituents of human plasma determined by ultrafiltration and analysis by liquid scintillation spectrometry		
Protein Constituents:	Albumin, α-2 Macroglobulin, α-1 Acid glycoprotein		
	% Bound [a] (Mean ± SD) [b]		
ISIS 301012 Concentration (μM)	**Albumin**	**α-2 Macroglobulin**	**α-1 Acid glycoprotein**
0.1	97.28 ± 3.07	92.05 ± 0.55	94.7 ± 1.54
1	98.37 ± 1.24	91.16 ± 5.57	95.96 ± 0.22
10	99.07 ± 0.05	90.06 ± 0.29	86.81 ± 0.6
20	98.14 ± 1.25	87.45 ± 0.82	80.91 ± 3.57
40	95.14 ± 0.68	80.9 ± 2.7	52.38 ± 4.54
60	98.13 ± 1.14	75.93 ± 0.63	54.81 ± 5.85
80	99.25 ± 0.1	87.66 ± 0.69	63.98 ± 11.44
100	98.49 ± 0.61	87.74 ± 0.77	69.09 ± 2.51

[a] Physiological concentrations of albumin (600 μM, 39.6 mg/mL), α-2 macroglobulin (2 μM, 1.64 mg/mL), and α-1 acid glycoprotein (17 μM, 0.7 mg/mL) were used in the study.

[b] n=5

Figure 6: Tissue Concentrations of Radioactivity (μg-eq/g) in Male CD-1 Mouse at 48 hours after a Single 22.3 mg/kg IV Dose of [^{3}H]-Mipomersen (301012-APK01)

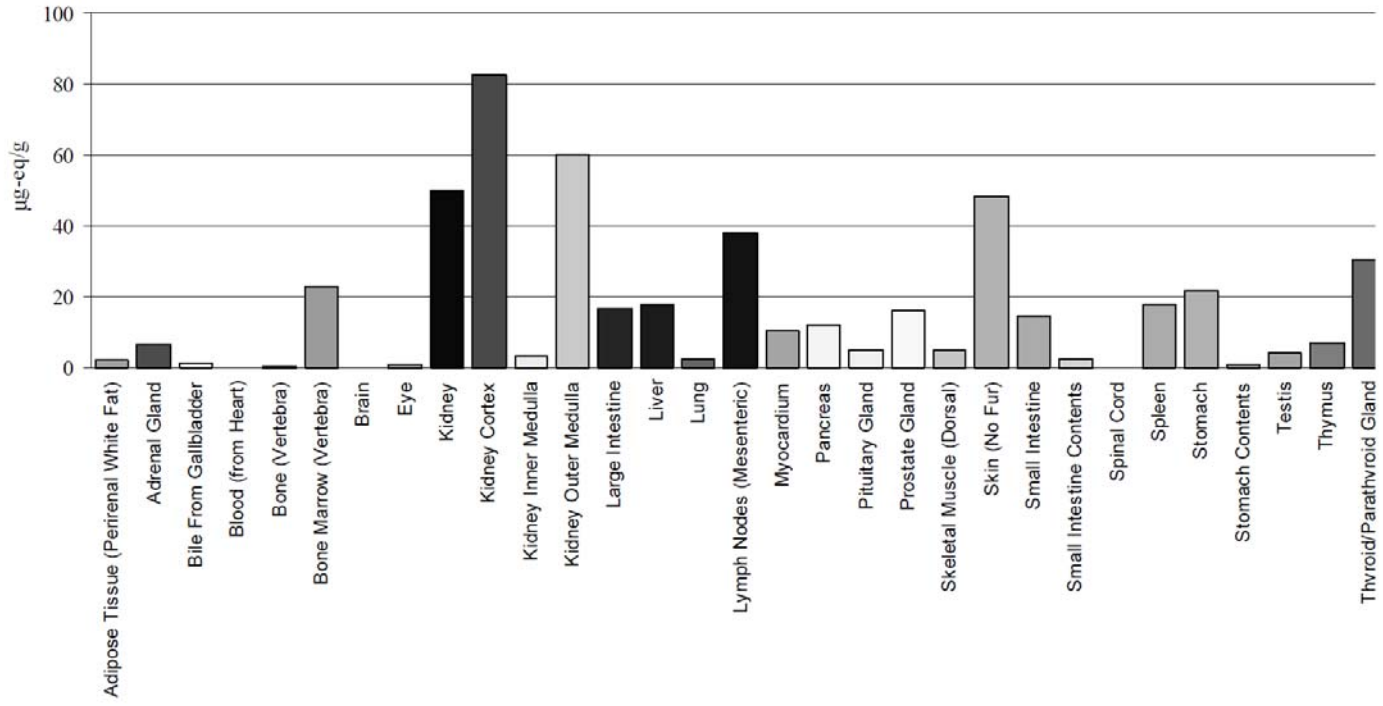


Figure 10: Tissue Concentrations of Radioactivity (µg-eq/g) at 48 hours Following a Single IV Administration of [^{3}H]-Mipomersen in Male (23.1 mg/kg) and Female (24.5 mg/kg) Sprague-Dawley Rats (n = 1 per gender) (301012-APK01)

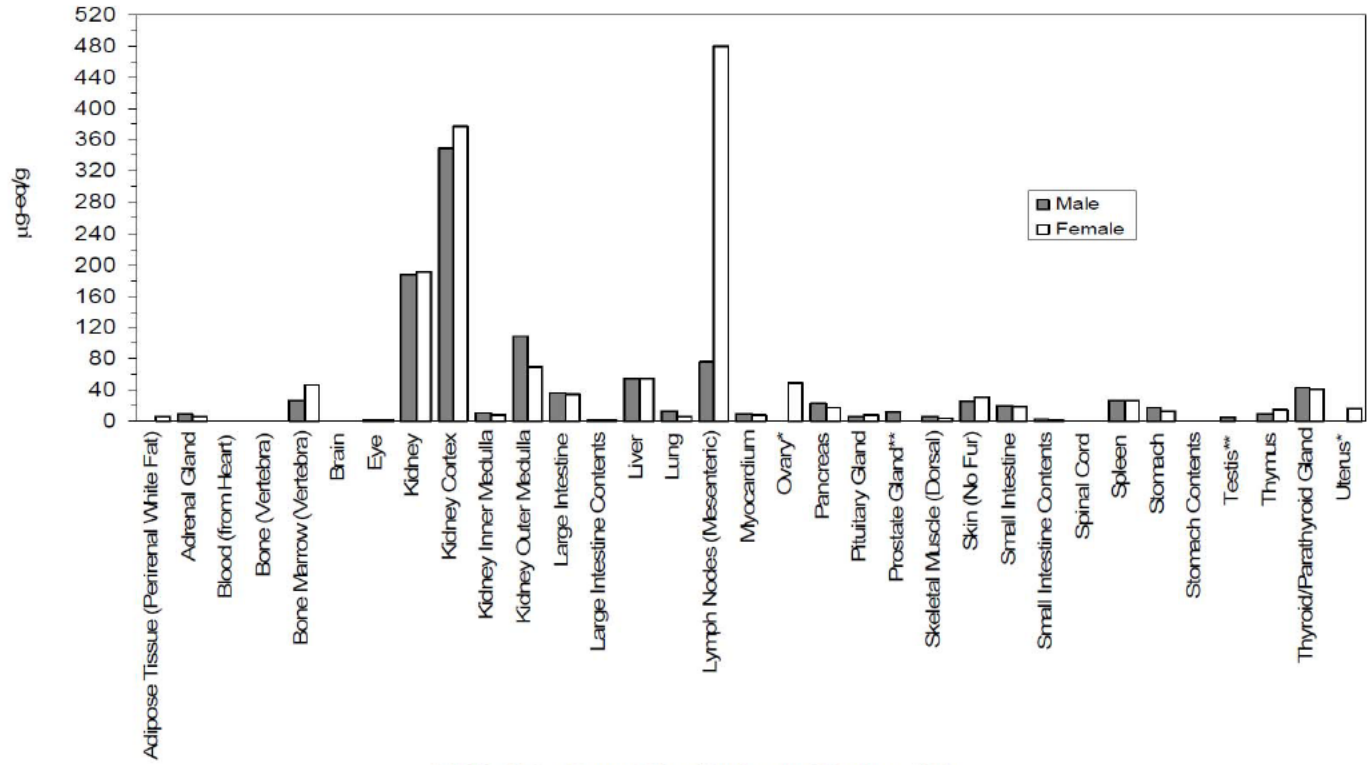


Table 5-S: Organ Distribution in Monkey (SC)

Report Number:	301012-AS02PK							
	Concentration (µg/g) (Mean ± SD) [a]							
Sample	**ISIS 301012**				**Total Oligonucleotides**			
	n=6 (M&F)	**n=4 (M&F)**	**n=6 (M&F)**	**n=2 (M&F)**	**n=6 (M&F)**	**n=4 (M&F)**	**n=6 (M&F)**	**n=2 (M&F)**
	2 mg/kg	**20 mg/kg**	**20 mg/kg**	**20 mg/kg**	**2 mg/kg**	**20 mg/kg**	**20 mg/kg**	**20 mg/kg**
	QOD	**QOD x 4 doses/Q4D**			**QOD**		**QOD x 4 doses/Q4D**	
	Day 89	**Day 33**	**Day 89**	**Day 181**	**Day 89**	**Day 33**	**Day 89**	**Day 181**
Plasma (µg/mL) [b]**:**	0.0830 ± 0.0376	N/D	0.887 ± 0.385	N/D	N/D	N/D	N/D	N/D
Bone marrow:	59.2 ± 27.1	69.1 ± 42.6	217 ± 48	47.1	78.1 ± 27.7	69.1 ± 42.6	241 ± 60	76.9
Brain:	N/D	BLQ	0.294 ± 0.719	BLQ	N/D	BLQ	0.294 ± 0.719	BLQ
Heart:	N/D	61.1 ± 7.6	93.2 ± 8.2	10.1	N/D	61.1 ± 7.6	98.5 ± 10.8	10.1
Kidney Cortex:	343 ± 112	1210 ± 592	1593 ± 518	245	347 ± 116	1502 ± 640	1700 ± 628	267
Kidney Medulla:	236 ± 125	647 ± 383	1221 ± 910	197	236 ± 125	917 ± 361	1316 ± 1099	218
Liver:	382 ± 130	840 ± 194	1129 ± 242	154	448 ±170	1395 ± 411	1142 ± 249	212
Lung:	N/D	46.9 ± 14.6	61.6 ± 12.2	9.48	N/D	46.9 ± 14.6	67.5 ± 13.9	9.48
Mesenteric lymph nodes:	N/D	324 ± 281	767 ± 255	107	N/D	324 ± 281	802 ± 273	107
Ovaries:	N/D	34.1	124 ± 32	41.3	N/D	34.1	128 ± 40	41.3
Spleen:	56.1 ± 19.5	294 ± 96	291 ± 125	31.1	83.5 ± 34.3	326 ± 51	305 ± 145	65.2
Testes:	N/D	27.3	207 ± 130	52.9	N/D	27.3	231 ± 162	52.9
Uterus:	N/D	100	112 ± 57	26.2	N/D	100	119 ± 62	26.2

Table 7-A: Study in Pregnant Mouse and Rabbit

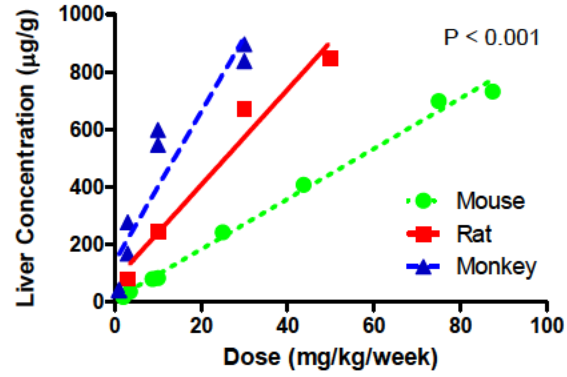
Report Number:	301012-AS07			301012-AS08					
	Concentration (Mean ± SD) (µg/g)								
Sample	**3 mg/kg (mouse)**	**10 mg/kg (mouse)**	**25 mg/kg (mouse)**	**2.5 mg/kg**	**5 mg/kg**	**15 mg/kg**	**2.5 mg/kg**	**5 mg/kg**	**15 mg/kg**
				n=3 (rabbit)			**n=5 (rabbit)**		
	Day 18			**Day 20**			**Day 29**		
Dam, Placenta:	2.75 ± 1.95	9.12 ± 1.47	22.9 ± 2.4	BLQ	4.28 ± 2.32	15.1 ± 4.5	BLQ [c]	BLQ [c]	6.93 ± 2.60
Dam, Kidney:	153 ± 47	393 ± 187	416 ± 127	767 ± 190	1239 ± 496	2336 ± 607	447 ± 189	756 ± 233	2152 ± 512
Dam, Liver:	47.2 ± 7.0	112 ± 37	252 ± 80	28.5 ± 13.2	99.2 ± 8.0	194 ± 19	22.4 ± 8.6	62.2 ± 15.1	178 ± 44
Fetus, Kidney:	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Fetus, Liver:	BLQ [a]	BLQ	BLQ [b]	BLQ	BLQ	BLQ	BLQ	BLQ [d]	BLQ

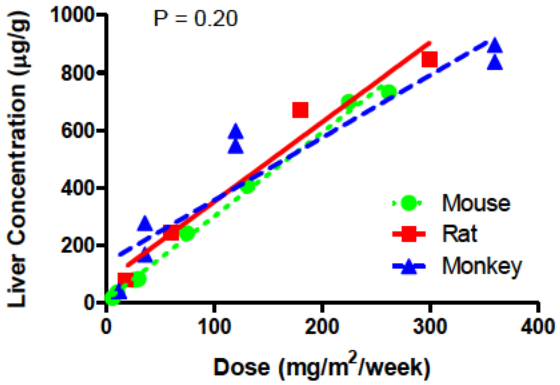
BLQ = Below the lower limit of quantitation.
[a] Two samples not quantified due to interference on electropherogram.
[b] One sample not quantified due to interference on electropherogram.
[c] Only one of the samples had measurable levels, others were BLQ.
[d] One sample not quantitated due to contamination at sample preparation.

Table 7-B: Study in Pre- and Postnatal Rats

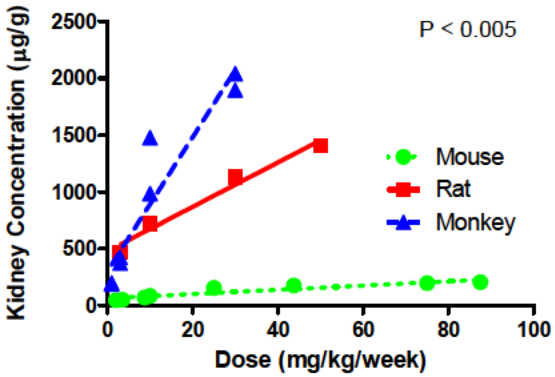
Report Number:	301012-AS24PK					
Location in CTD:	4.2.3.5.3					
Test Article:	ISIS 301012					
Species:	Rat					
Gender (M/F) / Strain / Number of Animals:	F/Sprague-Dawley/n=10 per group					
Feeding Condition:	Non-Fasted					
Vehicle/Formulation:	PBS / Solution					
Method of Administration:	SC					
Dose (mg/kg/dose):	2, 10, 20					
Dose Frequency:	QOD (Gestation Day 6 to 21 post partum)					
Sampling Time:	Day 22 post partum (24 hours post 19th dose)					
Analyte:	ISIS 301012, total oligonucleotides					
Assay:	CGE-UV					
LLOQ:	1.52 µg/g					
	Concentration (µg/g) (Mean ± SD)					
Sample	**ISIS 301012**			**Total Oligonucleotides**		
	2 mg/kg	**10 mg/kg**	**20 mg/kg**	**2 mg/kg**	**10 mg/kg**	**20 mg/kg**
Kidney	929 ± 109	2153 ± 384	2012 ± 246	1007 ± 109	2250 ± 497	2132 ± 422
Liver:	109 ± 20	511 ± 67	756 ± 183	121 ± 26	511 ± 67	821 ± 282
Spleen:	44 ± 11	215 ± 33	318 ± 39	46 ± 15	227 ± 35	327 ± 46

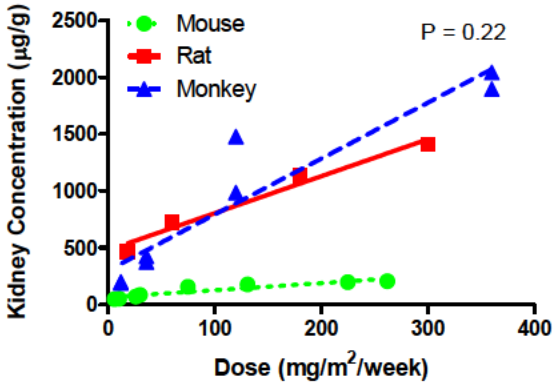
Relationship Between Liver Concentration of Mipomersen and Administered Dose Expressed Either as mg/kg (Bodyweight Scaling) or mg/m^2 (Body Surface Area Scaling)





Relationship Between Kidney Concentration of Mipomersen and Administered Dose Expressed Either as mg/kg (Bodyweight Scaling) or mg/m^2 (Body Surface Area Scaling)





METABOLISM

- Mipomersen is primarily metabolized by intracellular endonucleases and exonucleases to produce chain-shortened oligonucleotide metabolites.
- The rate limiting step in metabolism is likely to be endonuclease cleavage within the central 10-base deoxynucleotide (non-MOE modified) portion of the molecule.
- Metabolism is thought to occur in all tissues to which mipomersen distributes, since the requisite endo- and exonucleases are ubiquitously expressed in all tissues.
- Mipomersen is not a substrate for P450 metabolism.

- Chain-shortened metabolites (primarily 7-15 bases in length) were observed in tissues and urine. The metabolite profiles were similar across species. No unique metabolites were identified in human urine samples.
- Chain-shortened oligos are unlikely to exhibit pharmacologic activity due to their relatively low levels in tissues and the intrinsically reduced binding affinity towards RNA of shorter oligonucleotides.

Figure 22: Simple Scheme Depicting Mipomersen Metabolism and Elimination (301012-IS07)

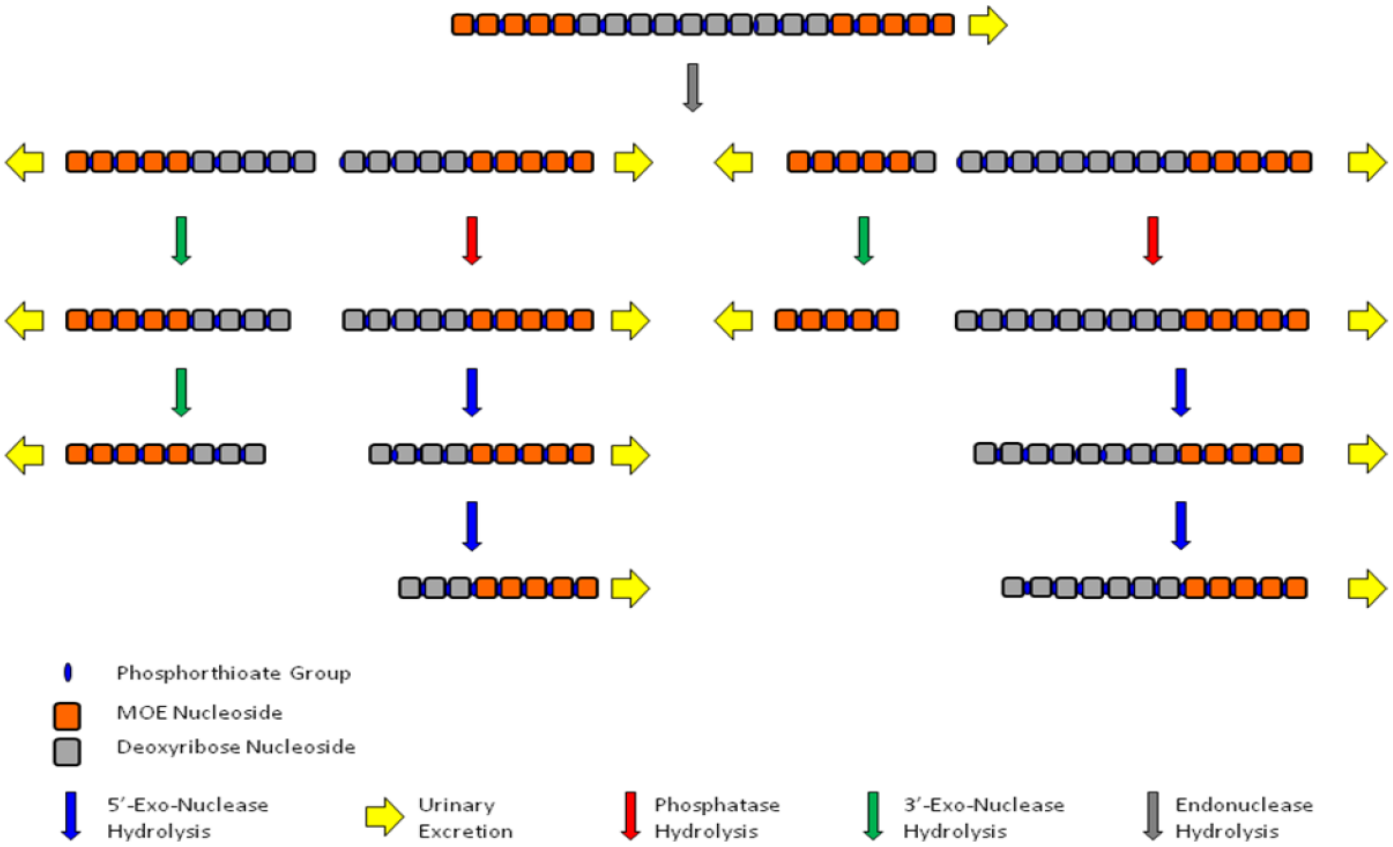
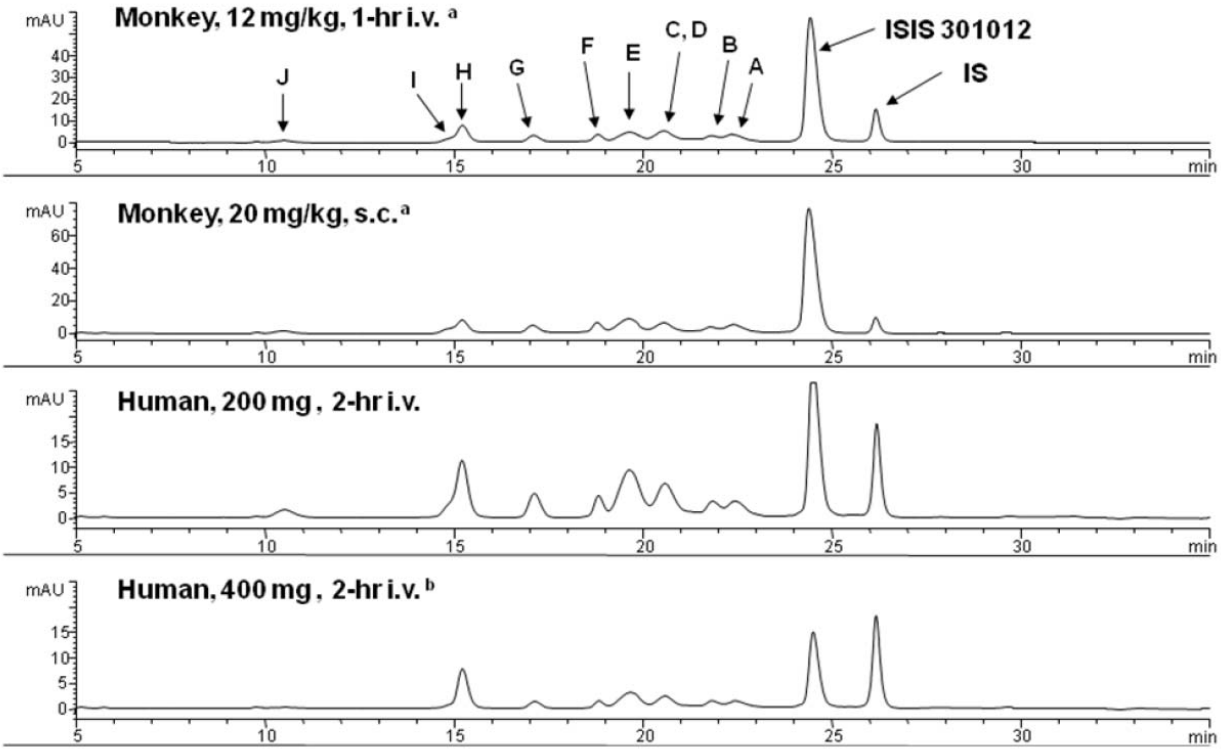


Figure 20: Representative Chromatogram of 0-24 Hours Urine Samples Collected from Monkey and Human Treated with Mipomersen (301012-IS07)



a: Monkey sample was diluted 1:10 prior to extraction and analysis.
b: Human sample was diluted 1:5 prior to extraction and analysis.
IS: Internal standard.
Refer to Table 11 for Peak identifications.

Table 11: Peak identifications of Mipomersen Metabolites Shown in Figure 20

Name	Length	Sequence [a]
Mipomersen	20	GCCTCAGTCTGCTTCGCACC
A	14	GTCTGCTTCGCACC
B	13	TCTGCTTCGCACC
C	11	TGCTTCGCACC
D	10	P(S)-GCTTCGCACC
E	10	GCTTCGCACC
F	10	GCCTCAGTCT
G	9	GCCTCAGTC
H	8	GCCTCAGT
I	8	TTCGCACC
J	7	GCCTCAG

[a] 2'-O-(2-methoxyethyl)-D-ribose (MOE) modified nucleotides are underlined.

EXCRETION

- Mipomersen is cleared from the body through urinary excretion of parent and chain-shortened metabolites (mouse, rat, monkey and human.
- In rats, ~32% of an administered dose of [^{3}H]-mipomersen was eliminated from the body within 2 weeks of a single IV dose (5 mg/kg): 26% in urine, 4% in feces. Less than 17% of the dose was eliminated within 24 h: 15% in urine, <1% in feces, and ~50% of the total radioactivity in urine was attributed to intact mipomersen.

Table 13-A: Excretion in Rat

Report Number:	301012-APK01			
Collection Time Period (h)	% of Dose (Mean ± SD) [a]			
	Urine	Faeces	Cage Wash	Total
0–24	14.084 ± 1.383	0.602 ± 0.249	N/A	14.685 ± 1.166
0–48	15.386 ± 1.170	1.092 ± 0.320	0.494 ± 0.288	16.972 ± 0.791
0–96	17.676 ± 1.178	1.830 ± 0.486	0.494 ± 0.288	20.000 ± 0.677
0–144	19.680 ± 1.158	2.441 ± 0.638	0.690 ± 0.327	22.811 ± 0.469
0–192	21.400 ± 1.268	2.996 ± 0.723	0.690 ± 0.327	25.086 ± 0.810
0–240	23.161 ± 1.468	3.452 ± 0.845	0.838 ± 0.383	27.451 ± 0.925
0–288	24.884 ± 1.484	3.851 ± 0.899	0.838 ± 0.383	29.574 ± 0.875
0–336	26.304 ± 1.791	4.217 ± 0.955	0.987 ± 0.434	31.507 ± 1.165
0–24 [b]	15.682 ± 0.465	0.341 ± 0.023	0.105 ± 0.063	16.128 ± 0.474

N/D = Not determined, TRA = total radioactivity.
[a] % of dose in radioactivity remained in carcass at 336 hours was 30.139 ± 1.587, resulting in 83% recovery of dosed radioactivity from the study.
[b] Data collected from a separate 24 hours mass balance subset of animals.

Table 12: Fraction of Mipomersen Dose Excreted (%) in Urine as Mipomersen and its Metabolites Over the Period of 0-24 Hours Following Intravenous and Subcutaneous Injection at Selected Doses Compared Across Species (301012-AS01PK, 301012-APK01, and 301012-AS02PK)

Parameter	Mouse		Rat [a]	Monkey	
Route of Administration	SC Injection		IV Bolus	1-hour IV Infusion	
Dose	5 mg/kg	25 mg/kg	5 mg/kg	2 mg/kg	12 mg/kg
fex, mipomersen	1.05%	22.2%	N/D	0.04%	2.35%
fex, metabolites	0%	1.5%	N/D	0.22%	0.54%
fex, total	1.05%	23.7%	16%	0.26%	2.89%

f_{ex} = Fraction of administered dose excreted in urine.
$f_{ex,mipo}$ = (% intact) x (% dose).
Relationship between $f_{ex,mipomersen}$, $f_{ex,metabolites}$, $f_{ex,total}$ is: $f_{ex,total} = f_{ex,\ mipomersen} + f_{ex,metabolites}$
[a] Fraction (%) of total radiolabeled dose excreted in urine

PHARMACOKINETIC DRUG INTERACTION

- Mipomersen is not metabolized by CYP450 enzymes, nor does it inhibit the activity of CYP450 enzymes commonly involved in metabolism of other drugs: CYP1A2, CYP2B6, CYP3A4, CYP2C9, CYP2C19 or CYP2D6.
- Mipomersen did not induce expression of CYP1A2, CYP2B6 or CYP3A4.
- Mipomersen is not a substrate for nor an inhibitor of P-glycoprotein.
- Mipomersen did not disrupt atorvastatin or simvastatin plasma protein binding, nor did the statins disrupt mipomersen binding to plasma protiens.

Table 15-A: Inhibition of Human CYP450 Isozymes

Report No:	301012-IS05				
Location in CTD:	4.2.2.6				
Type of Study:	*In vitro* evaluation of the potential of ISIS 301012 to inhibit the cytochromes P450 (CYP450), namely CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, in cryopreserved human hepatocytes (number of donors = 5)				
Method [a]:	CYP450 specific activity was determined from incubations containing appropriate CYP450 isozyme specific probe substrates for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Metabolites of the probe substrates were measured by LC/MS and LC-UV (for CYP3A4)				
ISIS 301012 Concentration (µg/mL)	**Specific Activity (pmol/min/million cells) [b]**				
	Acetaminophen Formation (CYP1A2)	**4'-Methylhydroxytolbutamide Formation (CYP2C9)**	**4'-Hydroxymephenytoin Formation (CYP2C19)**	**Dextrophan Formation (CYP2D6)**	**6β-Hydroxytestosterone Formation (CYP3A4)**
0 (Vehicle Control)	31.6 ± 1.28	30.2 ± 1.33	3.51 ± 0.177	15.3 ± 0.563	105 ± 10.2
8	31.6 ± 2.94	29.8 ± 1.77	3.84 ± 0.897	15.5 ± 0.694	104 ± 6.33
25	32.0 ± 1.46	30.5 ± 1.52	3.44 ± 0.0436	16.4 ± 0.501	112 ± 3.69
80	30.8 ± 0.629	30.9 ± 2.43	3.48 ± 0.200	14.5 ± 1.10	110 ± 2.66
250	29.7 ± 1.33	30.7 ± 1.14	3.64 ± 0.106	15.8 ± 1.16	111 ± 3.20
800	28.7 ± 0.589	33.2 ± 0.317	3.40 ± 0.145	14.7 ± 1.12	106 ± 0.900
Additional information: The IC_{50} of mipomersen, not reported in the study report because <20% inhibition was observed at the highest test concentration for all CYP450 isozymes evaluated, is considered >800 µg/mL for all CYP450 isozymes evaluated.					

[a] Enzyme activity of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 was determined by determining metabolite formation rate of phenacetin O-dealkylation, tolbutamide 4'-methylhydroxylation, S-mephenytoin 4'-hydroxylation, dextromethorphan O-demethylation, and testosterone 6β-hydroxylation, respectively.

[b] Mean ± SD from 5 human donors

Table 15-B: Induction of Human CYP450 Isozymes

Report No:	DMPK10-R015								
Location in CTD:	4.2.2.6								
Type of Study:	*In vitro* evaluation of the potential of ISIS 301012 to induce the CYP450 drug metabolizing enzymes, namely CYP1A2, CYP2B6, and CYP3A4, in plated cultures of cryopreserved human hepatocytes								
Method:	Catalytic CYP450 enzyme activity was determined from incubations containing appropriate probe substrates for CYP1A2, CYP2B6, and CYP3A4. Metabolites of the probe substrates were measured by LC-MS/MS. Changes in mRNA expression were determined by RT-PCR.								
Human Donor:	Hu0910, Hu8064, Hu4155								
	Percent of Positive Control (CYP450 Enzyme Activity [a])								
Positive Control / Concentration	**CYP1A2**			**CYP2B6**			**CYP3A4**		
	Hu0910	**Hu8064**	**Hu4155**	**Hu0910**	**Hu8064**	**Hu4155**	**Hu0910**	**Hu8064**	**Hu4155**
Rifampicin / 10 µM	NA	NA	NA	NA	NA	NA	100	100	100
Phenobarbital / 1000 µM	NA	NA	NA	100	100	100	NA	NA	NA
Omeprazole / 50 µM	100	100	100	NA	NA	NA	NA	NA	NA
ISIS 301012 / 500 µg/mL	-3.28	-0.806	-1.59	-0.782	0.628	-1.32	-0.167	0.956	-0.966
ISIS 301012 / 100 µg/mL	-1.59	-0.206	-0.699	2.26	-0.527	-0.883	3.91	0.945	-0.172
ISIS 301012 / 10 µg/mL	-0.775	0.779	-0.235	3.91	0.728	-0.389	4.75	2.1	-0.0262
ISIS 301012 / 1.0 µg/mL	-0.43	0.883	-0.416	5.24	1.36	1.09	2.58	1.64	0.317

Table 15-B: Induction of Human CYP450 Isozymes

Report No:	DMPK10-R015								
	Percent of Positive Control (mRNA Expression)								
Positive Control / Concentration	**CYP1A2**			**CYP2B6**			**CYP3A4**		
	Hu0910	**Hu8064**	**Hu4155**	**Hu0910**	**Hu8064**	**Hu4155**	**Hu0910**	**Hu8064**	**Hu4155**
Rifampicin / 10 µM	NA	NA	NA	NA	NA	NA	100	100	100
Phenobarbital / 1000 µM	NA	NA	NA	100	100	100	NA	NA	NA
Omeprazole / 50 µM	100	100	100	NA	NA	NA	NA	NA	NA
ISIS 301012 / 500 µg/mL	-1.53	1.43	-3.66	-0.759	2	-2.37	2.07	4.75	-0.83
ISIS 301012 / 100 µg/mL	-0.76	2.15	-2.11	-0.537	0.914	-0.116	3.1	4.28	1.07
ISIS 301012 / 10 µg/mL	-1.47	2.64	-0.425	0.584	2.03	0.205	4.82	3.12	1.35
ISIS 301012 / 1.0 µg/mL	-1.61	2.64	-0.549	-0.466	2.07	1.54	0.242	1.72	1.75

NA = Not applicable.

[a] Enzyme activity of CYP1A2, CYP2B6, and CYP3A4 was determined by determining metabolite formation rate of phenacetin O-dealkylation, efavirenz hydroxylation, and testosterone 6β-hydroxylation, respectively.

Table 15-C: Inhibition of Drug Transporters (Pgp Substrate and Inhibition)

Report No:	DMPK09-R037
Location in CTD:	4.2.2.6
Type of Study:	*In vitro* evaluation of P-glycoprotein involvement in MDCKII and MDCKII-MDR1 cells
Method:	All non-radiolabeled analytes (digoxin and control compounds) were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods. [^{3}H]-ISIS 301012 samples were quantitated using liquid scintillation counting method. To determine the substrate potential, the net efflux ratio of [^{3}H]-ISIS 301012 was determined. To determine the inhibition potential, the net efflux ratio of digoxin in the presence and absence of ISIS 301012 was determined.

Test Compound (Concentration)	**Net Efflux Ratio** [a]
[^{3}H]-ISIS 301012 (0.1 µM)	N/D [b]
[^{3}H]-ISIS 301012 (1 µM)	N/D [b]
[^{3}H]-ISIS 301012 (10 µM)	N/D [b]
Digoxin (2 µM)	6.5
Digoxin (2 µM) + ISIS 301012 (100 µM)	5.8

N/D = Not determined

[a] Net efflux ratio is determined by dividing the efflux ratio in MDCKII-MDR1 cells by the efflux ratio in MDCKII cells

[b] [^{3}H]-ISIS 301012 was not detected (LLOD = 0.001 µM) in receiver compartments at all tested concentrations, therefore, [^{3}H]-ISIS 301012 was not permeable under the conditions employed, and efflux ratio could not be determined.

Table 15-D: Displacement of Binding to Plasma Proteins (Interaction with Statins)

Report Number:	301012-IS04
Location in CTD:	4.2.2.6
Test Article:	[^{32}P]-ISIS 301012, [^{3}H]-Atorvastatin, [^{3}H]-Simvastatin
Type of Study:	*In vitro* plasma protein binding study
Method:	Plasma protein binding determined by ultrafiltration and analysis by liquid scintillation spectrometry
Species:	Human

Test Compound (Concentration)	**% Bound (Mean ± SD, n = 5 replicates)**		
	ISIS 301012	**Atorvastatin**	**Simvastatin**
[^{32}]P-ISIS 301012 (8 µg/mL) (Control)	97.97 ± 0.10	NA	NA
[^{32}]P-ISIS 301012 (8 µg/mL) + Atorvastatin (24.3 ng/mL)	97.59 ± 0.12	NA	NA
[^{32}]P-ISIS 301012 (8 µg/mL) + Atorvastatin (243 ng/mL)	97.68 ± 0.09	NA	NA
[^{32}]P-ISIS 301012 (80 µg/mL) (Control)	96.78 ± 0.10	NA	NA
[^{32}]P-ISIS 301012 (80 µg/mL) + Atorvastatin (24.3 ng/mL)	96.26 ± 0.22	NA	NA
[^{32}]P-ISIS 301012 (80 µg/mL) + Atorvastatin (243 ng/mL)	96.34 ± 0.15	NA	NA
[^{32}]P-ISIS 301012 (8 µg/mL) (Control)	97.97 ± 0.10	NA	NA
[^{32}]P-ISIS 301012 (8 µg/mL) + Simvastatin (2.94 ng/mL)	97.47 ± 0.11	NA	NA
[^{32}]P-ISIS 301012 (8 µg/mL) + Simvastatin (29.4 ng/mL)	97.54 ± 0.14	NA	NA
[^{32}]P-ISIS 301012 (80 µg/mL) (Control)	96.78 ± 0.10	NA	NA
[^{32}]P-ISIS 301012 (80 µg/mL) + Simvastatin (2.94 ng/mL)	96.27 ± 0.16	NA	NA
[^{32}]P-ISIS 301012 (80 µg/mL) + Simvastatin (29.4 ng/mL)	96.12 ± 0.14	NA	NA
[^{3}H]-Atorvastatin (24.3 ng/mL) (Control)	NA	94.85 ± 0.31	NA
[^{3}H]-Atorvastatin (24.3 ng/mL) + ISIS 301012 (8 µg/mL)	NA	95.48 ± 0.21	NA
[^{3}H]-Atorvastatin (24.3 ng/mL) + ISIS 301012 (80 µg/mL)	NA	95.71 ± 0.25	NA
[^{3}H]-Atorvastatin (243 ng/mL) (Control)	NA	95.62 ± 0.47	NA
[^{3}H]-Atorvastatin (243 ng/mL) + ISIS 301012 (8 µg/mL)	NA	95.73 ± 0.25	NA

[^{3}H]-Atorvastatin (243 ng/mL) + ISIS 301012 (80 µg/mL)	NA	95.64 ± 0.17	NA
[^{3}H]-Simvastatin (2.94 ng/mL) (Control)	NA	NA	96.45 ± 0.73
[^{3}H]-Simvastatin (2.94 ng/mL) + ISIS 301012 (8 µg/mL)	NA	NA	96.68 ± 0.35
[^{3}H]-Simvastatin (2.94 ng/mL) + ISIS 301012 (80 µg/mL)	NA	NA	96.72 ± 0.25
[^{3}H]-Simvastatin (29.4 ng/mL) (Control)	NA	NA	97.44 ± 0.20
[^{3}H]-Simvastatin (29.4 ng/mL) + ISIS 301012 (8 µg/mL)	NA	NA	96.91 ± 0.19
[^{3}H]-Simvastatin (29.4 ng/mL) + ISIS 301012 (80 µg/mL)	NA	NA	96.68 ± 0.44

NA = Not applicable

5.2 Toxicokinetics

See toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Species Study #	Dose	Notable Findings
301012-AS12 CD1 Mice 5/s/g GLP	Single dose, IV injection 7-day observation period 500, 1000, 1500, 2000 mg/kg Vehicle: PBS, pH 7.4 Volume: 5 to 10 mL/kg	Minimum lethal dose was 1500 mg/kg (1/5 ♀s). At 2000 mg/kg 1/5 ♂s and 2/5 ♀s died. Lethargy and piloerection in all dose groups, hunched posture at ≥ 1000 mg/kg, irregular breathing at ≥ 1500 mg/kg, palpebral closure at 2000 mg/kg.

6.2 Repeat-Dose Toxicity

The following studies have been previously reviewed under IND 70,969 and are included in this review by cross-reference:

1. 301012-AS01: 3-MONTH REPEAT-DOSE SUBCUTANEOUS TOXICITY STUDY OF ISIS 301012 IN CD-1 MICE WITH 3-MONTH RECOVERY
2. 301012-AS02: A 13-WEEK TOXICITY AND PHARMACOKINETIC STUDY OF ISIS 301012 ADMINISTERED BY INTRAVENOUS INFUSION OR SUBCUTANEOUS INJECTION TO CYNOMOLGUS MONKEYS, WITH A 4-WEEK RECOVERY PERIOD
3. 301012-AS09: A 5-WEEK TOXICITY STUDY OF ISIS 323358 ADMINISTERED BY SUBCUTANEOUS INJECTION ALONE OR IN COMBINATION WITH ATORVASTATIN ADMINISTERED BY ORAL GAVAGE TO CYNOMOLGUS MONKEYS, FOLLOWED BY A 2-MONTH RECOVERY PERIOD

Study title: 5-Month Repeat-Dose Subcutaneous Toxicity Study of ISIS 301012 in Sprague-Dawley Rats with a 3-Month Recovery

Study no.:	301012-AS21 ([(b) (4)] # 727-025)
Study report location:	4.2.3.2.1
Conducting laboratory and location:	[(b) (4)]
Date of study initiation:	2 August 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-006, 93.9%

Key Study Findings

- Immune stimulation -- minimal-mild lymphohistiocytic infiltration of non-immune tissues, lymphoid hyperplasia in the spleen [with splenomegaly] and histiocytosis in the lymph nodes [with lymphadenopathy], lymphoid depletion of the thymus, elevated chemokine/cytokine levels -- was seen primarily at doses ≥ 10 mg/kg/week. There are also changes in the levels of circulating leukocytes: Monocytes ↑61% in HD♂ (reversible), Eosinophils d.d. ↓ [tissue infiltration?] in ♂ & ♀ at all doses (max ↓ of 74% in HD♂), Basophils d.d. ↑ in ♂ & ♀ at all doses (max ↑ of 113% in HD♂), LUCs [typically representing lymphoblasts] ↑ at doses ≥ 30 mg/kg/week (max ↑ of 134% in HD♂).
- Dose-related reduction in food consumption and bodyweight gain after ~5 weeks of dosing, possibly related to worsening chronic progressive nephropathy (below).
- There was a d.d. ↓ in erythrocyte mass (2 to 17%) and in reticulocyte number (↓16 to 38%) in both sexes. This resulted in an increased incidence of extramedullary hematopoiesis in the spleen. Five HD animals had findings of aberrant red cell morphology at terminal sacrifice, including 1+ anisocytosis, 1+ to 2+ poikilocytosis, 2+ to 3+ echinocyte, 1+ polychromasia. One LMD♀ and one HDM♀ had comparable findings. Red cell morphology was normal in the controls. Some of these changes (poikilocytosis and echinocytes) likely reflect uremia, whereas anisocytosis and polychromasia may reflect increased RBC turnover.
- APTT was prolonged at doses ≥ 30 mg/kg/week (max ↑ of 30% in HD♂).
- There were multiple clinical chemistry findings consistent immunostimulation (↑ globulin), impaired renal function (↑BUN, ↑K, ↓Na) and perturbed hepatic function (↑ AST Bilirubin & lipids), primarily at doses ≥ 30 mg/kg/week.
- There was a worsening of chronic progressive nephropathy (CPN) in males (incidence & severity) at doses ≥ 30 mg/kg/week and in females (incidence) at 50 mg/kg/week. HD rats of both sex generally failed to recover following the 3-month recovery period, and ended the recovery period with worsening CPN. Two HD♂s had evidence of renal failure/uremia, presenting with vascular mineralization, polyarteritis, osteodystrophy and uremic hypogonadism. One HDr♂ was found dead with a CoD of CPN.

  - There is proteinuria by Day 29 in both sexes at ≥ 10 mg/kg/week and by Day 148 in the LD group (3 mg/kg/week).
  - Clinical chemistry is also consistent with impaired renal function.
- There were hyperplastic/neoplastic changes in some tissues
  - Squamous cell hyperplasia of the tongue in 2♀s at ≥ 30 mg/kg/week
  - Cervical fibromuscular hyperplasia in 1 HMD♀
  - 1 HD♂ had a kidney tubular cell adenoma and tubular hyperplasia
  - Kidney transitional cell hyperplasia in 1 HD♀
  - Bile duct hyperplasia (liver) in 5/10 HDr♀s
  - Mammary gland adenocarcinoma in 1 LMD♀
  - I HD♀ had transitional cell hyperplasia of the urinary bladder and ureter

Methods

Doses:	0, 3, 10, 30 & 50 mg/kg
Frequency of dosing:	Weekly
Route of administration:	SC injection
Dose volume:	10 mL/kg
Formulation/Vehicle:	Phosphate buffered saline, pH 7.4
Species/Strain:	Sprague-Dawley Rats
Number/Sex/Group:	20
Age:	~8 weeks of age at initiation of dosing
Weight:	♂: 236 to 280 g ♀: 174 to 211 g
Satellite groups:	Recovery: 10/s/g (C, LMD & HD only) TK: 24/s/g (LD & HD only)
Unique study design:	Total IgG & IgM levels were measured (C & HD) Cytokine/chemokine analysis (C, HMD, HD)
Deviation from study protocol:	None material to interpretation of the data.

Observations and Results

Mortality

I HDr♂ (# 5025) was found dead on Day 237 (2 days before scheduled recovery sacrifice) from chronic progressive nephropathy (CPN). While Sponsor considered this to be unrelated to mipomersen, it is likely that mipomersen contributed to the speed of onset and severity of CPN in this animal.

All other animals survived to scheduled sacrifice.

Clinical Signs

Unremarkable during the dosing phase; however, HD recovery animals exhibited multiple findings (reviewer's table below) during the recovery phase.

Clinical Signs During Recovery Period						
Sex	Male			Female		
Dose (mg/kg/week)	0	10	50	0	10	50
Hunched Posture	-	-	1 (W29-34)	1 (W25-26)	-	-
Teeth discolored, yellow	-	-	8 (W33-34)	-	-	1 (W33)
Thin	-	-	1 (W30-34)	1 (W25-26)	-	1 (W32-34)
Eye discolored, pale	-	-	1 (W24-29)	-	-	-
Piloerection	-	-	5 (W26-30)	-	-	4 (W26-30)
Skin discolored, pale	-	-	8 (W23-34)	-	-	4 (W31-34)
Unkempt	-	-	1 (W34)	-	-	-

Number of animals affected during recovery period (study Weeks with finding)
"-" finding not observed

Body Weights

Dose-dependent ↓ in bodyweight gain in both sexes (≥ 30 mkw in ♂s & ≥ 10 mkw in ♀s), mostly after Week 5.

Mean Body Weight Change (g) With Percent Decrease From Control Where Applicable (Weeks -1 to 21)		
Dose Group (mg/kg/week)	Male Mean Body Weight (g) Change Week -1 to 21 (%)	Female Mean Body Weight (g) Change Week -1 to 21 (%)
0 (PBS)	+385.3 (NA)	+154.7 (NA)
3 (ISIS 301012)	+363.9 (6)	+155.4 (NA)
10 (ISIS 301012)	+379.6 (1)	+136.4 (12)
30 (ISIS 301012)	+329.4 (15)	+116.0 (25)
50 (ISIS 301012)	+314.6 (18)	+88.1 (43)
NA – Not applicable		

Figure 1 Mean Body Weight Values – MALE

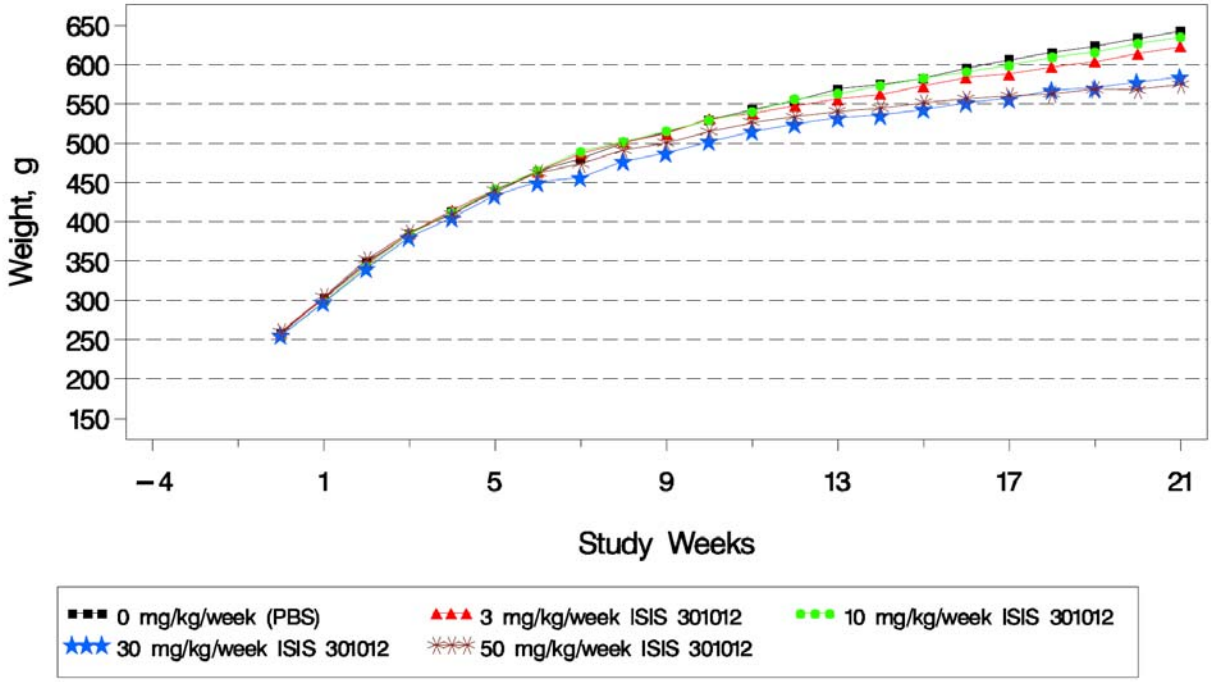
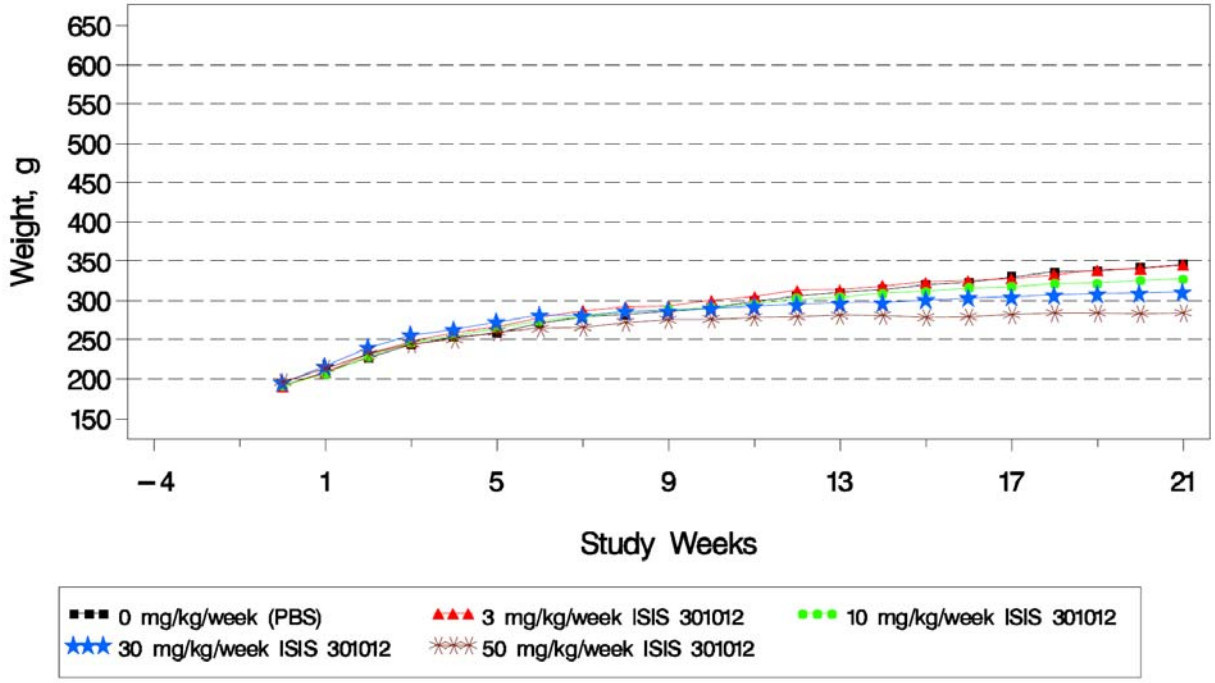


Figure 1A Mean Body Weight Values – FEMALE



Feed Consumption

↓ in FC generally correlated with the ↓ BW gain.

Ophthalmoscopy

No clearly treatment-related findings were noted in the ophthalmoscopic examinations. The few findings seen were reportedly typical of those commonly seen in rats of this age and strain.

Hematology

Erythrocyte mass (hemoglobin, hematocrit, and erythrocytes), MCV (mean corpuscular volume), and MCH (mean corpuscular hemoglobin) were ↓ 2 to 17% relative to control in a dose dependent manner at ≥30 mg/kg/week ISIS 301012 in both sexes. Reticulocytes were mildly to moderately (16 to 38%) s.s. ↓ in both sexes at all doses in ♂s and at doses ≥30 mg/kg/week ISIS 301012 in ♀s. RBC morphology was also abnormal in some animals at doses ≥ 10 mkw, especially at 50 mkw. Partial recovery was seen in reticulocyte & RBC parameters.

Various leukocytes were affected by treatment: monocytes s.s. ↑ 1.6x in HD♂, eosinophils were markedly and s.s. reduced (↓ 42 to 74%) in both sexes (≥ 10 mkw in ♂ & ≥ 30 mkw in ♀), Basophils and LUCs (large unstained cells) were ↑ 1.4x to 2.3x at doses ≥ 30 mkw. Recovery was partial to complete.

APTT was s.s. prolonged (1.2x to 1.3 x) in both sexes at doses ≥ 30 mkw. PT was s.s. shortened ~6% in ♂s at 50 mkw. Recovery was seen.

Hematology Group Means											
Sex		**Male**					**Female**				
ISIS 301012 m/k/w		**0**	**3**	**10**	**30**	**50**	**0**	**3**	**10**	**30**	**50**
Hemoglobin(g/dL)	Term	15.89	15.41	15.70	**14.52****	**13.26****	15.74	15.33	15.37	**14.60****	**13.67****
	Reco	15.04	-	15.17	-	**13.61***	15.20	-	14.50	-	**13.90****
RBC ($10^6/\mu L$)	Term	9.10	8.97	9.05	8.89	**8.22****	8.31	8.08	8.06	7.93	**7.65****
	Reco	8.63	-	8.59	-	**7.75***	8.00	-	7.57	-	**7.47***
Hematocrit (%)	Term	50.77	49.01	50.02	**46.60****	**42.70****	49.09	47.73	47.64	**45.23****	**42.80****
	Reco	47.42	-	47.88	-	**43.30***	47.01	-	44.67	-	**43.22****
Reticulocytes ($10^3/\mu L$)	Term	166.48	**139.80***	**125.91****	**109.61****	**103.87****	124.65	125.94	114.58	**93.82****	**77.98****
	Reco	204.79	-	137.39	-	151.00	123.64	-	130.27	-	119.76
MCV (fL)	Term	55.83	54.61	55.53	**53.44****	**52.00****	59.10	59.03	59.11	**57.05***	**56.55****
	Reco	55.04	-	55.81	-	55.97	58.75	-	59.06	-	57.93
MCH (pg)	Term	17.47	17.18	17.39	**16.36****	**16.18****	18.93	18.97	19.08	**18.44***	**18.08****
	Reco	17.43	-	17.72	-	17.57	19.01	-	19.16	-	18.66
Monocytes ($10^3/\mu L$)	Term	0.353	0.346	0.416	0.400	**0.567****	0.248	0.288	0.268	0.358	0.302
	Reco	0.479	-	0.380	-	0.356	0.314	-	0.302	-	0.356
Eosinophils ($10^3/\mu L$)	Term	0.188	0.161	**0.109****	**0.069****	**0.048****	0.138	0.120	0.087	**0.075****	**0.048****
	Reco	0.186	-	0.168	-	**0.099***	0.108	-	0.116	-	0.084

Basophils ($10^3/\mu L$)	Term	0.075	0.082	0.085	0.107	**0.160****	0.048	0.067	0.061	**0.095****	**0.095****
	Reco	0.058	-	0.055	-	**0.037***	0.043	-	0.038	-	0.036
LUCs ($10^3/\mu L$)	Term	0.110	0.130	0.123	**0.192***	**0.257****	0.082	0.098	0.090	**0.165****	**0.128****
	Reco	0.125	-	0.095	-	0.113	0.108	-	0.090	-	0.112
APTT (sec)	Term	20.21	20.01	21.97	**23.74****	**26.20****	17.19	18.86	17.63	**21.02****	**20.97****
	Reco	19.57	-	19.80	-	20.93	19.33	-	20.31	-	20.50
PT (sec)	Term	14.70	14.67	14.86	14.39	**13.82****	14.26	14.18	14.26	14.58	13.81
	Reco	15.30	-	15.48	-	14.47	15.16	-	15.12	-	14.49

Term = terminal, Reco = recovery
Bold indicates s.s. Δ from C
*p<0.05, **p<0.01, "-" = not measured

Individual Blood Cell Morphology Values - MALE
Terminal

Group, Animal Number	RBC Morphology	Aniso-cytosis	Poikilo-cytosis	Echnio-cyte	Polychro-masia	Platelet Estimate	WBC Morphology Observations
					(b) (4)		
10 mg/kg/week ISIS 301012							
3004	No finding					Adequate	No finding
50 mg/kg/week ISIS 301012							
5003	Findings					Adequate	No finding
5005	Findings					Adequate	No finding
5009	Findings					Adequate	No finding
5016	Findings					Adequate	No finding

Individual Blood Cell Morphology Values - FEMALE
Terminal

Group, Animal Number	RBC Morphology	Aniso-cytosis	Poikilo-cytosis	Echnio-cyte	Polychro-masia	Platelet Estimate	WBC Morphology Observations
					(b) (4)		
10 mg/kg/week ISIS 301012							
3513	Findings					Adequate	No finding
30 mg/kg/week ISIS 301012							
4514	Findings					Adequate	No finding
50 mg/kg/week ISIS 301012							
5501	Findings					Adequate	No finding

Clinical Chemistry

Clinical Chemistry Group Means											
Sex		**Male**					**Female**				
ISIS 301012 m/k/w		**0**	**3**	**10**	**30**	**50**	**0**	**3**	**10**	**30**	**50**
Sodium (mEq/L)	Term	146.3	146.0	145.9	145.3	**144.5****	144.2	143.0	143.0	**140.0****	**141.4***
	Reco	145.6	-	144.9	-	145.2	144.1	-	143.2	-	142.7
Potassium (mEq/L)	Term	6.32	**7.29***	7.29	**7.42***	**8.17****	7.50	7.86	8.36	**9.06****	**8.80****
	Reco	6.34	-	6.34	-	6.70	6.49	-	6.38	-	6.97
Alkaline Phosphatase (U/L)	Term	72.8	62.5	65.3	**57.8***	**52.3****	33.9	29.9	27.3	33.5	31.9
	Reco	71.0	-	59.9	-	59.9	29.7	-	30.2	-	31.3
Bilirubin (mg/dL)	Term	0.16	0.13	0.13	0.19	**0.25****	0.17	0.17	0.17	0.14	0.15
	Reco	0.13	-	0.10	-	0.17	0.18	-	0.15	-	0.20
AST (U/L)	Term	73.2	73.1	67.1	72.8	74.8	65.5	121.9	83.6	114.4	**145.4****
	Reco	76.5	-	66.8	-	**50.6****	122.6	-	89.6	-	267.1
ALT (U/L)	Term	30.9	32.5	31.4	31.9	34.0	29.7	79.3	44.9	71.3	89.6
	Reco	38.5	-	28.6	-	30.0	75.7	-	44.9	-	147.9
Urea Nitrogen (mg/dL)	Term	12.0	11.9	13.0	**15.0****	**17.6****	13.1	12.6	13.1	13.8	15.2
	Reco	11.8	-	11.8	-	39.8	13.2	-	12.9	-	14.0
Creatinine (mg/dL)	Term	0.40	0.41	**0.45***	0.44	0.43	0.47	0.47	0.51	0.47	0.44
	Reco	0.36	-	0.34	-	0.54	0.44	-	0.41	-	**0.33****
Total Protein (g/dL)	Term	6.90	6.74	7.02	7.03	**6.44***	7.86	7.88	8.12	7.56	**7.25****
	Reco	6.86	-	6.89	-	6.69	7.98	-	7.99	-	8.06
Albumin (g/dL)	Term	3.34	3.21	3.29	**3.09****	**2.41****	4.02	4.09	4.23	**3.60****	**3.24****
	Reco	3.38	-	3.38	-	**2.58****	4.27	-	4.22	-	**3.94****
Globulin (g/dL)	Term	3.56	3.53	3.73	**3.94****	**4.03****	3.84	3.79	3.89	3.96	4.01
	Reco	3.48	-	3.51	-	**4.11****	3.71	-	3.77	-	**4.12***
A:G Ratio	Term	0.95	0.92	**0.88***	**0.79****	**0.60****	1.05	1.08	1.08	**0.91****	**0.81****
	Reco	0.99	-	0.97	-	**0.64****	1.16	-	1.13	-	**0.96****
Triglycerides (mg/dL)	Term	78.1	**54.1***	62.4	**47.4****	**50.4****	50.3	49.3	43.5	**35.8***	41.7
	Reco	77.6	-	104.5	-	**261.6****	43.9	-	50.2	-	**59.6***
Cholesterol, total (mg/dL)	Term	77.0	63.7	65.1	85.7	**144.8****	96.9	100.1	96.1	108.6	**166.1****
	Reco	76.9	-	72.9	-	**308.0****	102.3	-	91.8	-	**169.4****
HDL (mg/dL)	Term	56.4	47.8	47.6	61.3	**101.2****	75.0	76.7	75.9	82.5	**124.5****
	Reco	54.4	-	51.5	-	**192.0****	77.3	-	69.6	-	**114.0****
LDL (mg/dL)	Term	4.7	4.1	5.1	6.0	**8.5****	3.6	3.4	3.4	4.3	**5.8****
	Reco	3.7	-	3.5	-	**25.6****	2.5	-	2.4	-	**6.1***
VLDL (mg/dL)	Term	15.8	**10.9***	12.5	**9.5****	**10.1****	10.1	9.9	8.7	**7.2***	8.3
	Reco	15.5	-	21.0	-	**52.1***	8.7	-	10.1	-	**12.0***
C-Reactive Protein (μg/mL)	Term	254.2	281.2	303.1	**339.4****	285.7	298.1	311.7	307.0	348.1	372.7
	Reco	703.8	-	533.5	-	429.5	536.0	-	530.3	-	**788.7****

Term = terminal, Reco = recovery
Bold indicates s.s. Δ from C
*p<0.05, **p<0.01, "-" = not measured

Urinalysis

No effect on urine volume, specific gravity or pH.

Protein to creatinine ratio was s.s. at the end of dosing in all dose groups. 24 h quantitative urine protein was elevated all doses ≥ 10 mkw. The 10 mkw dose may have been associated with recovery to some degree. There was no recovery at 50 mkw, indeed there was a marked deterioration in ♂s at this dose, with s.s. ↓ in urinary creatine and marked increases in 24 h quantitative urine protein. The progression in kidney deterioration may account for the poor clinical signs observed during the recovery period in the HD group.

Table 8 Summary of Urine Chemistry Values - MALE

Endpoint	Interval of Study	0 mg/kg/week (PBS) Mean	SD	N	3 mg/kg/week ISIS 301012 Mean	SD	N	10 mg/kg/week ISIS 301012 Mean	SD	N	30 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	Day 29	105.40	17.516	5	89.60	33.672	5	101.20	39.028	5	105.80	32.752	5
	Day 57	82.40	27.033	5	99.80	44.891	5	67.20	44.584	5	85.20	13.737	5
	Day 148	91.00	39.013	5	94.20	51.713	5	83.00	47.660	5	96.20	23.563	5
Creatinine mg/24hr	Day 29	14.400	5.5511	5	15.980	1.8900	5	15.480	2.0897	5	14.300	3.3272	5
	Day 57	19.400	2.9419	5	18.700	3.6919	5	15.740	5.2353	5	16.960	1.9655	5
	Day 148	20.240	4.8593	5	20.820	2.4046	5	20.240	2.6820	5	20.120	0.8871	5
Quanti-tative Urine Protein mg/dL	Day 29	250.4	176.96	5	157.6	95.16	5	268.8	120.19	5	560.8[a]	212.61	5
	Day 57	130.8	37.02	5	206.8	119.30	5	189.6	125.39	5	466.0[b]	93.89	5
	Day 148	136.4	55.55	5	206.4	96.89	5	263.0	136.89	5	881.0[b]	391.21	5
Quanti-tative Urine Protein mg/24hr	Day 29	28.20	6.907	5	26.60	8.569	5	40.92[a]	10.121	5	72.16[b]	10.045	5
	Day 57	31.48	8.003	5	37.24	8.978	5	43.86	16.192	5	92.90[b]	17.378	5
	Day 148	33.68	18.673	5	47.76	13.195	5	67.50[b]	15.895	5	184.12[b]	62.588	5
Protein/ Creatinine Ratio	Day 29	2.36	1.545	5	1.64	0.439	5	2.68	0.545	5	5.22[b]	1.069	5
	Day 57	1.60	0.187	5	2.02	0.409	5	2.78[b]	0.455	5	5.44[b]	0.577	5
	Day 148	1.58	0.606	5	2.28[a]	0.502	5	3.32[b]	0.471	5	9.26[b]	3.433	5

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Table 8 Summary of Urine Chemistry Values - MALE

Endpoint	Interval of Study	50 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	Day 29	69.80	18.526	5
	Day 57	78.80	33.132	5
	Day 148	50.80	10.941	5
Creatinine mg/24hr	Day 29	16.520	1.7167	5
	Day 57	17.220	3.5046	5
	Day 148	21.300	3.2488	5
Quanti-tative Urine Protein mg/dL	Day 29	412.4	177.35	5
	Day 57	488.0[b]	298.53	5
	Day 148	1643.6[b]	776.49	5
Quanti-tative Urine Protein mg/24hr	Day 29	93.88[b]	16.428	5
	Day 57	102.70[b]	32.407	5
	Day 148	730.22[b]	437.714	5
Protein/ Creatinine Ratio	Day 29	5.80[b]	1.539	5
	Day 57	5.94[b]	1.379	5
	Day 148	35.22[b]	20.969	5

N - Number of measures used to calculate mean
SD - Standard Deviation
[b]Significantly different from control; ($p<0.01$)

Table 8 Summary of Urine Chemistry Values - FEMALE

Endpoint	Interval of Study	0 mg/kg/week (PBS) Mean	SD	N	3 mg/kg/week ISIS 301012 Mean	SD	N	10 mg/kg/week ISIS 301012 Mean	SD	N	30 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	Day 29	65.00	37.195	5	82.00	23.270	5	67.00	36.125	5	89.40	49.263	5
	Day 57	75.80	21.638	5	51.40	20.305	5	72.00	27.459	5	46.00	30.290	5
	Day 148	79.00	14.933	5	65.20	40.146	5	51.40	27.574	5	47.60	18.942	5
Creatinine mg/24hr	Day 29	8.760	2.4521	5	8.000	2.5407	5	8.280	2.2016	5	7.000	1.6508	5
	Day 57	9.740	2.5175	5	9.580	2.4509	5	8.760	2.2334	5	9.940	1.2522	5
	Day 148	11.580	2.5004	5	9.460	1.0407	5	10.340	2.5657	5	8.360	2.3860	5
Quanti-tative Urine Protein mg/dL	Day 29	29.4	18.88	5	49.2	28.98	5	65.0	33.82	5	254.6[b]	167.44	5
	Day 57	58.4	30.64	5	34.4	12.34	5	108.4	81.35	5	153.4	125.75	5
	Day 148	41.8	8.41	5	62.0	48.91	5	100.8	49.77	5	236.2[b]	133.72	5
Quanti-tative Urine Protein mg/24hr	Day 29	4.08	2.362	5	4.40	1.861	5	8.46[b]	3.066	5	18.62[b]	2.872	5
	Day 57	7.50	4.099	5	6.78	2.451	5	11.72	2.580	5	30.70[b]	8.266	5
	Day 148	6.52	3.087	5	8.90	3.444	5	20.46[b]	5.178	5	40.90[b]	15.984	5
Protein/ Creatinine Ratio	Day 29	0.46	0.207	5	0.58	0.268	5	1.04[b]	0.288	5	2.82[b]	1.033	5
	Day 57	0.76	0.321	5	0.68	0.130	5	1.40[b]	0.406	5	3.10[b]	0.791	5
	Day 148	0.54	0.219	5	0.94[a]	0.329	5	2.02[b]	0.370	5	4.78[b]	0.873	5

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Table 8 Summary of Urine Chemistry Values - FEMALE

Endpoint	Interval of Study	50 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	Day 29	53.60	20.983	5
	Day 57	65.00	33.504	5
	Day 148	58.80	17.697	5
Creatinine mg/24hr	Day 29	9.460	1.5900	5
	Day 57	9.740	1.8338	5
	Day 148	9.400	2.1875	5
Quanti-tative Urine Protein mg/dL	Day 29	180.0[b]	124.48	5
	Day 57	266.4[a]	170.87	5
	Day 148	744.2[b]	372.71	5
Quanti-tative Urine Protein mg/24hr	Day 29	29.06[b]	6.782	5
	Day 57	38.02[b]	7.512	5
	Day 148	120.66[b]	51.602	5
Protein/ Creatinine Ratio	Day 29	3.18[b]	0.998	5
	Day 57	3.96[b]	0.780	5
	Day 148	12.60[b]	4.022	5

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Table 8 Summary of Urine Chemistry Values - MALE

Endpoint	Interval of Study	0 mg/kg/week (PBS) Mean	SD	N	10 mg/kg/week ISIS 301012 Mean	SD	N	50 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	Recovery	128.82	29.159	5	114.60	29.617	5	67.70	48.483	4
Creatinine mg/24hr	Recovery	24.380	3.1348	5	18.940	6.6771	5	13.225[a]	3.1553	4
Quanti-tative Urine Protein mg/dL	Recovery	270.4	141.46	5	203.6	78.54	5	3396.5[a]	1705.03	4
Quanti-tative Urine Protein mg/24hr	Recovery	57.66	46.528	5	32.38	9.773	5	748.68[b]	329.929	4
Protein/ Creatinine Ratio	Recovery	2.28	1.641	5	1.76	0.483	5	55.78[b]	15.682	4

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Table 8 Summary of Urine Chemistry Values - FEMALE

Endpoint	Interval of Study	0 mg/kg/week (PBS) Mean	SD	N	10 mg/kg/week ISIS 301012 Mean	SD	N	50 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	Recovery	60.42	10.400	5	57.36	39.493	5	54.38	25.796	5
Creatinine mg/24hr	Recovery	12.840	5.0545	5	8.460	6.7077	5	9.180	3.3929	5
Quanti-tative Urine Protein mg/dL	Recovery	61.6	46.40	5	140.4	200.19	5	1095.6	1237.61	5
Quanti-tative Urine Protein mg/24hr	Recovery	12.28	8.035	5	29.23	44.355	4	139.80	127.205	5
Protein/ Creatinine Ratio	Recovery	1.02	0.817	5	4.78	8.023	4	15.98[a]	16.495	5

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)

Gross Pathology

Epididymides
Small: 1 HD♂ (moderate)

Kidney
Cyst (mild-moderate): none at terminal sac; 3 HDr♂
Enlarged (moderate): 1 HD♂; 1HDr♂
Discolored, tan (mild): 1 HD♂, 1 HMD♀, 1 HD♀
Dilatation, pelvic (mild): 1 HD♀; 1 HDr♀
Irregular surface (moderate): none at terminal sac; 1 HDr♂

Liver
Enlarged (mild): 1 HD♂
Discoloration, black (mild): none at terminal sac; 1 HDr♂

Lung
Focus/foci (white) (mild): 1 HD♂

Lymph Node (mandibular)
Enlarged (mild): 1 HD♂

Lymph Node (mediastinal)
Enlarged (moderate): 1 HD♂

Skin/Subcutis
Discoloration, white (moderate): none at terminal sac; 1 HDr♂

Spleen
Enlarged (mild-moderate): 5 HD♂
Discoloration, white (moderate): none at terminal sac; 1 HDr♂

Testes
Small (moderate): 1 HD♂
Discoloration, white (moderate): none at terminal sac; 1 HDr♂

Thymus
Small (mild-moderate): 3 HD♂, 2 HMD♀, 1 HD♀

Organ Weights

Numerous s.s. organ weight effects were seen, but the majority of these were deemed to arise from the ↓ bodyweight of treated animals, compared to controls. Those changes judged likely to be related to mipomersen and independent of the effects on bodyweight are captured below. All organs in the table except the thyroid gland had macroscopic and microscopic findings consistent with the observed changes in weight. The changes in thyroid weight may be spurious, but it is notable that absolute thymus weight went up at all doses <50 mkw, even while bodyweight went down.

Organ Weight -- Fold Changes from Control

Organs		Dose (mg/kg/week)							
		3		10		30		50	
		M	F	M	F	M	F	M	F
Body weight	Term	1.0	1.0	1.0	0.9*	0.9*	0.9*	0.9*	0.8*
	Reco	-	-	1.0	1.1	-	-	0.7*	0.9*
Kidney (BWt%)	Term	1.0	1.1*	1.0	1.1*	1.0	1.2*	1.1*	1.4*
	Reco	-	-	1.1	1.1	-	-	2.0*	1.5*
Liver (BWt%)	Term	1.0	1.0	1.1	1.1*	1.2	1.3*	1.4*	1.5*
	Reco	-	-	1.0	1.1	-	-	1.7*	1.4*
Spleen (BWt%)	Term	1.0	1.1	1.2*	1.3*	1.8*	2.1*	2.3*	2.3*
	Reco	-	-	1.2	1.0	-	-	2.0*	1.5*
Thymus (BWt%)	Term	1.1	1.0	0.9	0.8*	0.7*	0.6*	0.7*	0.6*
	Reco	-	-	0.9	1.1	-	-	0.5*	1.2
Thyroid	Term	1.3*	1.3*	1.4*	1.3*	1.4*	1.4*	1.0	1.0
	Reco	-	-	1.0	1.0	-	-	0.9	1.0

* Statistically significant compared to controls
"-" = no data

Histopathology

Adequate Battery

Yes

Peer Review

No

Histological Findings

Proinflammatory changes were observed in the adrenal glands, choroid plexus of the brain, coagulating gland, epididymides, heart, injection sites, kidneys, larynx, lung, lymph nodes, mediastinum, ovaries, oviducts, pancreas, pituitary gland, prostate gland, seminal vesicles, skin, spleen, testes, tongue, urinary bladder, uterus with cervix, and vagina. Findings were primarily lymphohistiocytic infiltrates in non-lymphoid tissues, lymphohistiocytic infiltrates and lymphoid hyperplasia in the spleen, and sinus histiocytosis in lymph nodes (see Sponsor's tables below). Other immune effects include lymphocytic infiltration in the kidneys, increased incidence of alveolar histiocytosis, ymphoid or lymphocyte/plasmacyte hyperplasia in lymph nodes and generalized thymic lymphoid depletion (see Reviewer's table below).

The "lymphohistiocytic infiltrates" were characterized as generally being comprised of macrophages containing a basophilic granular material indicative of oligo uptake. Likewise the "sinus histiocytosis" observed in multiple lymph nodes represented accumulation of oligo-laden macrophages in the sinuses of lymph nodes. Similarly basophilic granules in the Kupffer cells of the liver and proximal tubular cells of the kidney are thought to represent uptake and accumulation of oligo in these cells.

At terminal sacrifice male rats show an increased incidence and severity of chronic progressive nephropathy (CPN) at doses ≥ 30 mkw (minimal- moderate). HD♀s also appear to have an increased incidence of minimal CPN. HD recovery animals (both sexes) show a further worsening (not recovery) of CPN, along with other findings suggestive of deteriorating kidney health and function. The two HDr♂s that had the most severe CPN (#5024 had the highest BUN at recovery sampling [222 vs. 15 for rest of cohort], #5025 died on study with CoD of CPN) had findings likely to be secondary to kidney failure/uremia:

- 5024: polyarteritis (epididymides, seminal vesicles, testes), mineralization (aorta, testes tubules), oligospermia/germ cell debris (epididymides), depletion secretory (seminal vesicles), degeneration/atrophy, seminiferous tubules (testes).
- 5025: vascular mineralization (aorta, heart, kidney), osteodystrophy.

Other notable kidney findings include lymphocytic infiltration (not lymphohistiocytic) at doses ≥ 30 mkw at terminal sacrifice and hyperplastic/neoplastic findings in two HD recovery animals (1 ♂ & 1 ♀):

- #5029 had tubular cell hyperplasia (minimal) and adenoma
- #5525 had transitional cell hyperplasia (mild)

Vascular events (other than those noted above) include polyarteritis (rectum) in HD♂ 5020, aneurysm (aorta) in HDr♀ 5521, and thrombus (brain) in HDr♂ 5024. Given the low incidence, the relationship between these findings and the test item are unclear. Interestingly, doses ≥ 30 mkw appear to be associated with a reduction in the incidence of cardiomyopathy (minimal).

Liver findings (aside from basophilic granules in Kupffer cells) are limited to a decrease in the incidence of minimal mononuclear cell infiltration. This effect persists after the recovery period. While intriguing, this is of no known toxicological significance. Following recovery sacrifice there is minimal bile duct hyperplasia in 5/10 HDr♀s, possible increase in incidence in focal necrosis in HDr♀s, and centrilobular or midzonal vacuolation in HDr♂s.

Other findings captured in reviewer's table below are of uncertain relationship to the test item.

Incidence and Severity of Treatment-Related Proinflammatory Effects								
	3 mg/kg/week ISIS 301012		10 mg/kg/week ISIS 301012		30 mg/kg/week ISIS 301012		50 mg/kg/week ISIS 301012	
	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20
Lymphohistiocytic infiltrates								
• **Adrenal gland**					20 (min)	18 (min)	20 (min)	20 (min)
• **Choroid plexus**					18 (min)	20 (min)	20 (min)	20 (min)
• **Coagulating gland**					20 (min)		19 (min)	
• **Epididymides**					20 (min)		20 (min)	
• **Heart**			2 (min)		19 (min)	19 (min)	20 (min-mild)	20 (min)
• **Injection site 1**		1 (min)	9 (min)	4 (min)	7 (min)	16 (min-mild)	13 (min)	10 (min)
• **Injection site 2***	7 (min)	1 (min)		8 (min)	11 (min)	15 (min)	14 (min)	13 (min)
• **Injection site 3**	2 (min)			1 (min)	1 (min)	4 (min)	3 (min)	4 (min)
• **Injection site 4**	4 (min)	1 (min)	1 (min)		2 (min)	5 (min)	13 (min-mild)	4 (min)

Incidence and Severity of Treatment-Related Proinflammatory Effects, continued								
	3 mg/kg/week ISIS 301012		10 mg/kg/week ISIS 301012		30 mg/kg/week ISIS 301012		50 mg/kg/week ISIS 301012	
	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20
• **Lung**					1 (min)			
• **Ovaries**						20 (min)		19 (min)
• **Oviducts**						20 (min)		18 (min)
• **Pancreas**			16 (min)	11 (min)	20 (min)	20 (min)	18 (min)	20 (min)
• **Pituitary**					2 (min)		9 (min)	
• **Prostate**					5 (min)		3 (min)	
• **Seminal Vesicle**					20 (min)		20 (min)	
• **Skin**		1 (min)	1 (min)	1 (min)	4 (min-mild)	6 (min)	10 (min-mild)	9 (min-mild)
• **Spleen**						6 (min)	17 (min)	16 (min)
• **Testes**			13 (min)		20 (min)		20 (min)	
• **Tongue**			13 (min)		20 (min)		17 (min)	1 (min)
• **Urinary Bladder**						16 (min)		20 (min)
• **Uterus with Cervix**					5 (min)	20 (min)	11 (min)	20 (min)
• **Vagina**						1 (min)		9 (min)

min - minimal

* Lymphohistiocytic infiltrates were noted in 6 control animals. These infiltrates were reactive to the injection and did not appear to contain oligonucleotide material.

Incidence and Severity of Treatment-Related Proinflammatory Effects								
	3 mg/kg/week ISIS 301012		10 mg/kg/week ISIS 301012		30 mg/kg/week ISIS 301012		50 mg/kg/week ISIS 301012	
	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20
Histiocytosis sinus								
• **Lymph node axillary**					19 (min-mild)	20 (min-mild)	20 (min-mild)	20 (min-mild)
• **Lymph node inguinal**					20 (min-mild)	20 (min-mild)	20 (min-mild)	20 (min-mild)
• **Lymph node Mandibular**			15 (min-mild)	7 (min-mild)	19 (min-mild)	20 (min-mild)	20 (min-mild)	18 (min)
• **Lymph node mediastinal**		2 (min)		19 (min-mild)		19 (min-mild)	1 (min)	19 (min-mild)
• **Lymph node Mesenteric**			19 (min-mild)		20 (min-mild)		20 (min-mild)	

min - minimal

Incidence and Severity of Treatment-Related Proinflammatory Effects								
	3 mg/kg/week ISIS 301012		10 mg/kg/week ISIS 301012		30 mg/kg/week ISIS 301012		50 mg/kg/week ISIS 301012	
	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20	M N=20	F N=20
Lymphoid hyperplasia								
• **Spleen**			14 (min)	3 (min)	20 (min-mild)	20 (min-mild)	20 (min-mild)	20 (min-mild)

min - minimal

Histopathology Terminal Sacrifice-- Incidence (Mean Severity)										
Sex	**Male**					**Female**				
ISIS 301012 (mg/kg/week)	**0**	**3**	**10**	**30**	**50**	**0**	**3**	**10**	**30**	**50**
Epididymides										
Oligospermia/germ cell debris, bilateral	0/20 -	-	-	0/20 -	1/20 (4.0)					
Heart										
Cardiomyopathy	8/20 (1.0)	12/20 (1.1)	8/20 (1.0)	1/20 (1.0)	2/20 (1.0)	2/20 (1.0)	3/20 (1.0)	0/20 -	1/20 (1.0)	0/20 -
Kidney										
Basophilic granules, Tubular cell	0/20 -	20/20 (1.0)	20/20 (1.0)	20/20 (1.1)	20/20 (2.0)	0/20 -	18/20 (1.0)	20/20 (1.3)	20/20 (2.6)	20/20 (2.6)
Infiltration, lymphocytic	1/20	4/20	0/20	2/20	9/20	0/20	0/20	0/20	4/20	5/20

	(1.0)	(1.0)	-	(1.0)	(1.0)	-	-	-	(1.2)	(1.0)
Nephropathy, chronic progressive	10/20 (1.0)	7/20 (1.0)	8/20 (1.0)	14/20 (1.1)	20/20 (1.6)	2/20 (1.0)	0/20 -	2/20 (1.0)	2/20 (1.0)	6/20 (1.0)
Liver										
Basophilic granules, Kupffer cell	0/20 -	18/20 (1.0)	20/20 (1.1)	20/20 (2.0)	20/20 (3.0)	0/20 -	20/20 (1.0)	19/20 (1.1)	20/20 (2.0)	20/20 (3.0)
Infiltration, mononuclear cell	20/20 (1.0)	20/20 (1.0)	20/20 (1.0)	3/20 (1.0)	0/20 -	15/20 (1.0)	14/20 (1.0)	7/20 (1.0)	5/20 (1.0)	2/20 (1.0)
Lung										
Histiocytosis, alveolar	5/20 (1.0)	-	-	9/20 (1.0)	12/20 (1.0)	2/20 (1.0)	-	-	7/20 (1.0)	5/20 (1.0)
Infiltration, lymphoid, perivascular	0/20 -	-	-	0/20 -	2/20 (1.5)	0/20 -	-	-	1/20 (1.0)	0/20 -
Lymph Node (axillary)										
Hyperplasia, lymphoid, paracortex	0/20 -	-	-	0/20 -	2/20 (2.0)	0/19 -	-	-	0/20 -	0/20 -
Lymph Node (mandibular)										
Hyperplasia, lymphocyte/plasmacyte	0/20 -	0/20 -	0/20 -	0/20 -	2/20 (2.0)	0/20 -	0/20 -	0/20 -	0/20 -	0/20 -
Lymph Node (mediastinal)										
Hyperplasia, lymphoid, paracortex	-	-	-	-	1/1 (2.0)	-	-	-	-	-
Rectum										
Polyarteritis	0/20 -	-	-	0/20 -	1/20 (1.0)	0/20 -	-	-	0/20 -	0/20 -
Salivary Gland (sublingual)										
Atrophy	0/20 -	-	-	0/20 -	1/20 (3.0)	0/20 -	-	-	0/20 -	0/20 -
Spleen										
Hematopoiesis, extramedullary ↑	0/20 -	0/20 -	1/20 (1.0)	3/20 (1.0)	7/20 (1.0)	0/20 -	0/20 -	0/20 -	1/20 (1.0)	0/20 -
Testes										
Degeneration/atrophy, seminiferous tubules, bilateral	0/20 -	0/20 -	0/20 -	0/20 -	1/20 (4.0)					
Thymus										
Depletion, lymphoid, generalized	0/20 -	-	-	20/20 (3.0)	19/20 (3.2)	0/20 -	-	-	19/20 (2.9)	20/20 (3.0)
Tongue										
Hyperplasia, squamous cell	0/20 -	-	-	0/20 -	0/20 -	0/20 -	-	-	1/20 (2.0)	1/20 (2.0)
Vagina										
Hyperplasia, cervical, fibromuscular						0/20 -	-	-	1/20 (2.0)	0/20 -

Histopathology Recovery Sacrifice -- Incidence (Mean Severity)						
Sex	**Male**			**Female**		
ISIS 301012 (mg/kg/week)	**0**	**10**	**50**	**0**	**10**	**50**
Adrenal glands						
Infiltration, lymphohistiocytic	0/10 -	-	2/10 (1.0)	0/10 -	-	1/10 (1.0)
Vacuolation, focal	0/10 -	-	2/10 (1.0)	1/10 (1.0)	-	0/10 -
Aorta						
Aneurysm	0/10 -	-	0/10 -	0/10 -	-	1/10 (2.0)
Mineralization	0/10 -	-	2/10 (1.5)	0/10 -	-	0/10 -
Bone (femur)						
Osteodystrophy	0/10	0/10	1/10	0/10	-	0/10

	-	-	(1.0)	-		-
Bone (sternum)						
Osteodystrophy	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Brain						
Thrombus	0/10 -	-	1/10 (3.0)	0/10 -	-	0/10 -
Choroid plexus						
Infiltration, lymphohistiocytic	-	-	1/1 (1.0)	-	-	1/1 (1.0)
Epididymides						
Infiltration, lymphohistiocytic	0/10 -	-	10/10 (1.0)			
Oligospermia/germ cell debris, bilateral	0/10 -	-	1/10 (4.0)			
Polyarteritis	0/10 -	-	1/10 (1.0)			
Heart						
Infiltration, lymphohistiocytic	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Mineralization, vascular	0/10 -	0/10 -	1/10 (3.0)	0/10 -	0/10 -	0/10 -
Injection site 1						
Fibrosis	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Kidney						
Adenoma, tubular cell, benign, primary, bilateral	0/10 -	0/10 -	1/10 (P)	0/10 -	0/10 -	0/10 -
Basophilic granules, tubular cell	0/10 -	9/10 (1.0)	8/10 (1.2)	0/10 -	10/10 (1.0)	10/10 (2.0)
Cyst	0/10 -	1/10 (1.0)	1/10 (2.0)	0/10 -	0/10 -	0/10 -
Hyperplasia, transitional cell	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	1/10 (2.0)
Hyperplasia, tubular	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Mineralization, vascular	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Nephropathy, chronic progressive	9/10 (1.0)	6/10 (1.0)	10/10 (2.6)	1/10 (1.0)	2/10 (1.0)	8/10 (1.2)
Liver						
Basophilic granules, Kupffer cell	0/10 -	9/10 (1.0)	10/10 (1.5)	0/10 -	10/10 (1.0)	10/10 (1.2)
Hyperplasia, bile duct	2/10 (1.0)	3/10 (1.0)	1/10 (1.0)	0/10 -	0/10 -	5/10 (1.0)
Infiltration mononuclear cell	9/10 (1.0)	9/10 (1.0)	3/10 (1.0)	7/10 (1.0)	4/10 (1.0)	1/10 (1.0)
Necrosis, focal	1/10 (1.0)	0/10 -	0/10 -	1/10 (1.0)	0/10 -	3/10 (1.0)
Vacuolation, centrilobular	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Vacuolation, midzonal	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -
Lymph node (axillary)						
Histiocytosis, sinus	0/10 -	-	10/10 (1.0)	0/10 -	-	9/10 (1.1)
Lymph node (inguinal)						
Histiocytosis, sinus	0/9 -	-	10/10 (1.0)	0/10 -	-	7/10 (1.0)

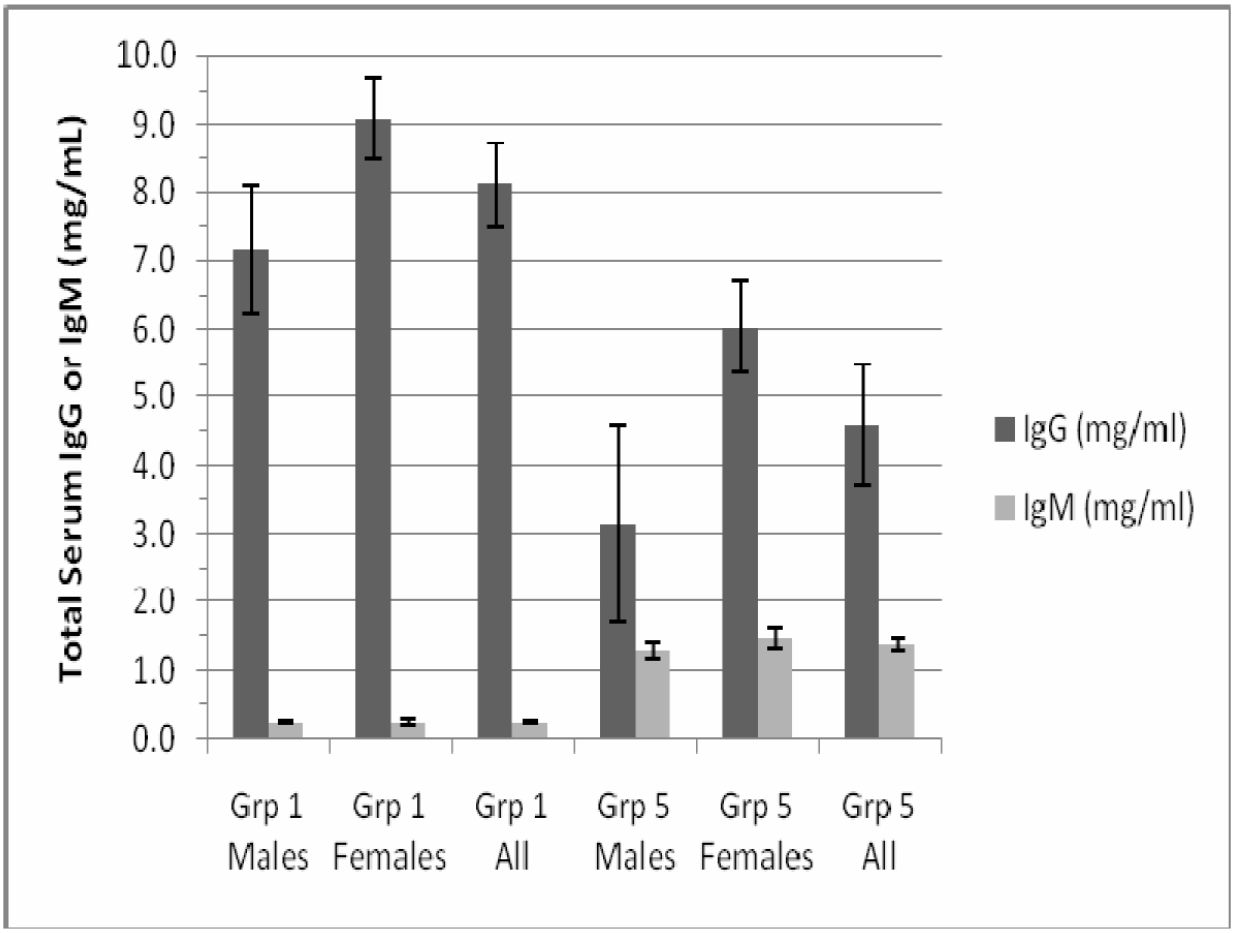
Lymph node (mandibular)						
Histiocytosis, sinus	0/10 -	0/10 -	7/10 (1.0)	0/10 -	0/10 -	7/10 (1.0)
Lymph node (mesenteric)						
Histiocytosis, sinus	0/10 -	1/10 (1.0)	7/10 (1.0)	0/10 -	1/10 (1.0)	9/10 (1.1)
Mammary gland						
Adenocarcinoma, malignant, primary				0/10 -	1/2 (P)	0/10 -
Seminal vesicles						
Depletion, secretory	0/10 -	-	1/10 (4.0)			
Infiltration, lymphohistiocytic	0/10 -	-	1/10 (1.0)			
polyarteritis	0/10 -	-	1/10 (1.0)			
Spleen						
Hyperplasia, lymphoid, generalized	0/10 -	0/10 -	8/10 (1.2)	0/10 -	0/10 -	7/10 (1.0)
Testes						
Degeneration/atrophy, seminiferous tubules, unilateral	0/10 -	0/10 -	1/10 (4.0)			
Infiltration, lymphohistiocytic	0/10 -	0/10 -	10/10 (1.0)			
Mineralization, tubular	0/10 -	0/10 -	1/10 (3.0)			
Polyarteritis	0/10 -	0/10 -	1/10 (1.0)			
Thymus						
Depletion, lymphoid, generalized	0/10 -	-	9/10 (3.2)	0/10 -	-	9/10 (3.1)
Thyroid						
Depletion, lymphoid, generalized	0/10 -	-	0/10 -	0/10 -	-	1/10 (1.0)
Tongue						
Hyperplasia, squamous cell	0/10 -	-	0/10 -	1/10 (2.0)	-	0/10 -
Ureters						
Dilatation	0/10 -	-	0/10 -	0/10 -	-	1/10 (3.0)
Hyperplasia, transitional cell	0/10 -	-	0/10 -	0/10 -	-	1/10 (2.0)
Inflammation, subacute/chronic	0/10 -	-	0/10 -	0/10 -	-	1/10 (2.0)
Urinary bladder						
Hyperplasia, simple transitional cell	0/10 -	-	0/10 -	0/10 -	-	1/10 (3.0)
Inflammation, subacute/chronic	0/10 -	-	0/10 -	0/10 -	-	1/10 (2.0)

Special Evaluation

Total Immunoglobulin

At HD IgM levels were ↑ ~5.5-fold, while IgG levels were ↓ by ~57%, compared to control. No explanation was provided, but this may indicate a deficiency in class switching.

Total Serum IgG and IgM Levels at Terminal Necropsy (Day 150)



Values represent group means ± standard error of the mean

Cytokine/Chemokine Analysis

A multiplex ELISA approach was used to measure the levels of IL-1α, IL-1β, IL-6, TNF-α, MCP-1, MIP-1α, GRO (functional homolog to IL-8) and IFN-γ in samples from the C, HMD & HD animals at the end of the dosing period.

Insufficient information was provided in the study report to fully assess the extent to which the assay system is suitable for the intended analysis. The assay is not validated.

MCP-1, IL1α, IL-10, IFNγ and MIP1α all showed dose-related increases. There was no clear effect on GRO or TNFα.

Mean Cytokine/Chemokine Levels

Dose (mg/kg/week)	MCP1 (pg/mL)	GRO(IL-8) (pg/mL)	IL1α (pg/mL)	IL-10 (pg/mL)	IFNγ (pg/mL)	TNFα (pg/mL)	MIP1α (pg/mL)
0	2128	no Δ	6.0	<1.6	43.9	no Δ	10.8
30	5422		8.8	10.6	79.4		19.4
50	8438		8.2	19.6	100.6		30.3

Toxicokinetics

Plasma TK was only determined for the LD (3 mkw) and the HMD (30 mkw). Exposure increased hyperproportionally between these two doses. T_{max} was 0.5 to 2 hr. Terminal half-life ranged from 2.4 days (following the 1st 3 mg/kg dose) to 22.4 days (following the last 30 mg/kg dose).

Table 3. Plasma pharmacokinetic parameters in rats following subcutaneous administration(s) of ISIS 301012 for Study 301012-AS21

Dose (Group)	Study Day	C_{max} [a] (µg/mL)	T_{max} (hr)	$AUC_{0\rightarrow 48\ hr}$ (µg*hr/mL)	$AUC_{0\rightarrow\tau}$ (µg*hr/mL)	$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	$t_{1/2\alpha}$ [b] (hr)	$t_{1/2\beta}$ [b] (days)	$t_{1/2\lambda z}$ (days)
3 mg/kg	1	2.88 ± 0.571	0.50	9.94	10.1	10.2 [d]	1.54 ± 0.46	0.41 ±0.17 [c]	2.40 [c,d]
(Group 6)	148	3.75 ± 0.513	2.00	21.5	25.1	NC	2.31 ± 0.79	16.3 ± 3.6	17.5 [d]
30 mg/kg	1	45.6 ± 6.30	0.50	231	234	234 [d]	2.55 ± 0.16	2.59 ± 0.58 [c]	1.77 [c]
(Group 7)	148	49.1 ± 0.928	1.00	395	455	NC	3.35 ± 0.42	21.5 ± 4.1	22.4

[a] Data presented are mean ± standard deviation (n=3)

[b] Data presented are mean ± standard error of the estimate

[c] General estimate of apparent plasma elimination because of limited sampling times.

[d] Values are reported for information purposes only and should be interpreted with caution because of poor regression when determining λ_z (rsq<0.8).

NC Not calculated.

The kidney was the major site of distribution, followed by the liver. While plasma exposure increased hyperproportionally to dose (above), tissue exposures increased with dose hypoproportionally.

Table 4. ISIS 301012 and total oligonucleotide tissue concentrations (µg/g) in numerous tissues collected 48 hr following 5 months of treatment (Day 150). Male and female rats were combined (n= 10).

Organ	Group	Dose (mg/kg/week)	N	ISIS 301012	Total Oligonucleotide	%Intact
				Mean ± SD	Mean ± SD	Mean ± SD
Kidney	2	3	10	467 ± 84	537 ± 89.3	86.8 ± 3.1
	3	10	10	720 ± 225	875 ± 277	82.6 ± 2.96
	4	30	10	1130 ± 645	1354 ± 784	83.6 ± 4.17
	5	50	10	1407 ± 535	1714 ± 660	83.1 ± 11
Liver	2	3	10	79.5 ± 21.4	114 ± 34.7	71.4 ± 12.6
	3	10	10	243 ± 75	306 ± 98.8	80.1 ± 7.84
	4	30	10	671 ± 170	746 ± 170	89.8 ± 6.33
	5	50	10	847 ± 271	941 ± 345	91.5 ± 6.09

Data presented are mean ± standard deviation.

N= number of animals

The elimination half-lives from the tissue was relatively slow, ranging between ~8 h for liver following the last 3 mg/kg dose and ~35 h for the kidney following the final 30 mg/kg dose.

Table 5. Estimated tissue half-lives for ISIS 301012 following 5 months of s.c. injections of ISIS 301012 to rats

Group	Dose	Organ	$t_{1/2}$ (day)
6	3 mg/kg/week	Kidney	24.8
		Liver	8.13
7	30 mg/kg/week	Kidney	34.8 [a]
		Liver	21.5

[a] Organ half-life estimation may not be accurate due to sampling time less than two half-lives.

Dosing Solution Analysis

Solution was stable during the study, and complied with nominal concentration.

Study title: 6 - Month Repeat-Dose Subcutaneous Toxicity Study of ISIS 301012 and ISIS 147764 in CD-1 Mice with 3-Month Recovery

Study no.:	301012-AS14 ([(b) (4)] # 727-022)
Study report location:	4.2.3.2.1
Conducting laboratory and location:	[(b) (4)]
Date of study initiation:	January 6, 2006
GLP compliance:	Yes
QA statement:	Present & signed
Drug, lot #, and % purity:	ISIS 301012, CA301012-001, 92.8%
Drug, lot #, and % purity:	ISIS 301012, CA301012-003, 91.9%
Drug, lot #, and % purity:	ISIS 147764, CA147764-001, 86.6%

Key Study Findings

- Proinflammatory effects were apparent at doses ≥ 10 mkw. This was characterized by lymphohistiocytic infiltration (minimal to mild) in numerous tissues, an increased incidence of lymphoid depletion from the thymus (with decreased thymus weight), as well as increased levels of the cytokine MCP-1. Increased granulocytic hyperplasia in the bone marrow is also likely secondary to the proinflammatory effect of the oligos (ISIS 301012 and ISIS 147764).
- A dose-dependent decrease (↓ 13-27%) in RBC parameters (red cell count, hematocrit and hemoglobin) was seen in both sexes at doses ≥ 10 mkw. A dose-related ↑ in platelet count was seen in ♂s at doses ≥ 25 mkw. These effects were not reversible, and were not seen with the mouse surrogate. There was increased extramedullary hematopoiesis in the spleen (and increased spleen weight), presumably reflecting a compensatory response to the reduction in red cells.

- Consistent with tissue distribution of the oligos, the liver was a target tissue. Liver weight as a % of bodyweight was increased in both sexes, and this was correlated with the incidence/severity of basophilic granules in Kupffer cells. ALT was ↑ ~3- to 6-fold at 75 mkw (for both oligos) in both sexes. AST was ↑ ~60-70 % at 75 mkw (both sexes) and ALP was ↑ ~2-fold in HD♂s. In addition albumin was mildly decreased (↓ 14-23%) at doses ≥ 25 mkw. Karyomegaly (minimal-mild) was seen in 13/16 surrogate-treated ♀s. This same dose group had 2/16 mice with minimal single hepatocyte necrosis.
- Other than the presence of basophilic granules in tubule cells (though to represent drug accumulation), there were no clear effects on the kidney, despite relatively high exposure levels in this tissue.
- At doses ≥ 10 mkw, ISIS 301012 was associated with the presence of "foreign material" in the skin at time of histopathological examination, with 14/16 HD♂s exhibiting this finding. Sponsor provides no additional characterization of the foreign material, nor an explanation for this finding.
- The NOAEL is considered to be 2 mg/kg/week.

Methods

Doses ISIS 301012:	0, 2, 10, 25, and 75 mg/kg
Doses ISIS 147764(mouse surrogate):	75 mg/kg
Frequency of dosing:	Weekly
Route of administration:	SC Injection
Dose volume:	10-15 mL/kg
Formulation/Vehicle:	Phosphate buffered saline
Species/Strain:	CD-1 Mouse
Number/Sex/Group:	16
Age:	~8 weeks at initiation of dosing
Weight:	♂: 27.4 to 33.4 g ♀: 21.7 to 27.2 g
Satellite groups:	6/s/g (C, LMD & HD only) [recovery] 30♂s/g (HMD & HD only) [TK]
Unique study design:	None
Deviation from study protocol:	No material deviations

Group Assignments				
Group Number	Dose Level (mg/kg/week)	Test Article	Number of Animals	
			Male	Female
1	0	ISIS 301012	22	22
2	2	ISIS 301012	16	16
3	10	ISIS 301012	22	22
4	25	ISIS 301012	16	16
5	75	ISIS 301012	22	22
6	75	ISIS 147764	16	16
7[a]	10	ISIS 301012	30	0
8[a]	75	ISIS 301012	30	0
[a]Pharmacokinetic Animals				

Observations and Results

Mortality

No test item related effect.

Clinical Signs

No test item related effect.

Body Weights

There were slight, generally dose-related increases in mean body weight gain when compared to controls throughout the study, which appeared to be related to increased food consumption in those groups.

Figure 1 Mean Body Weight Values – MALE

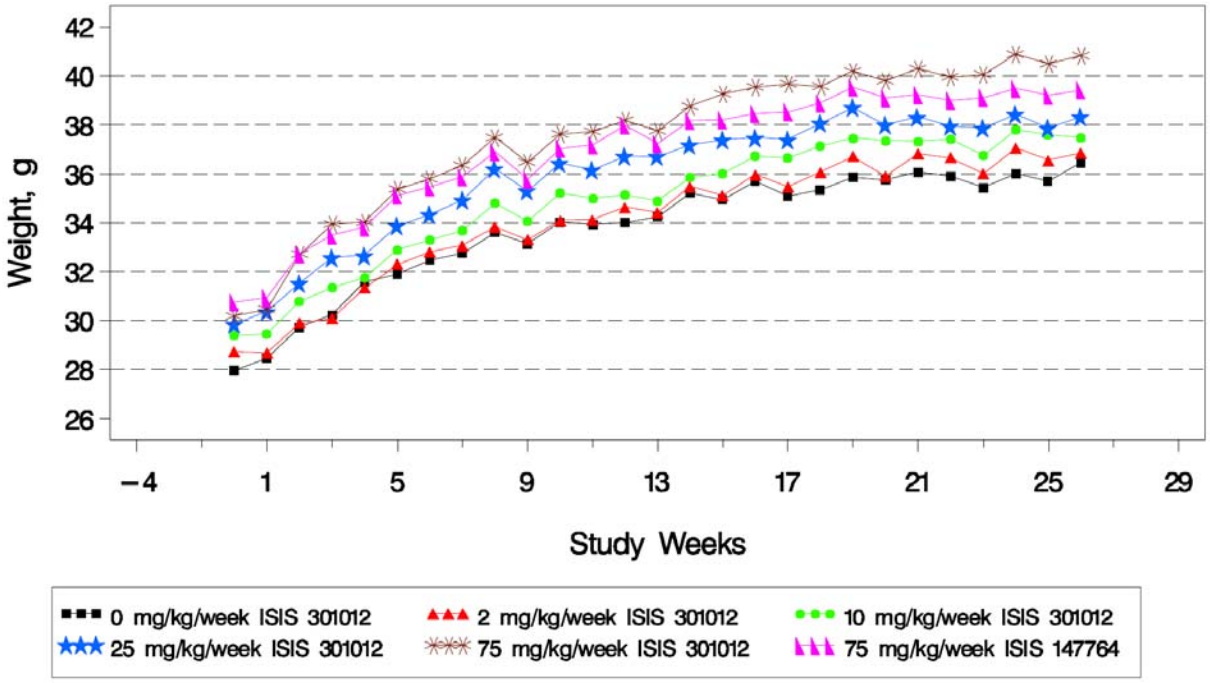
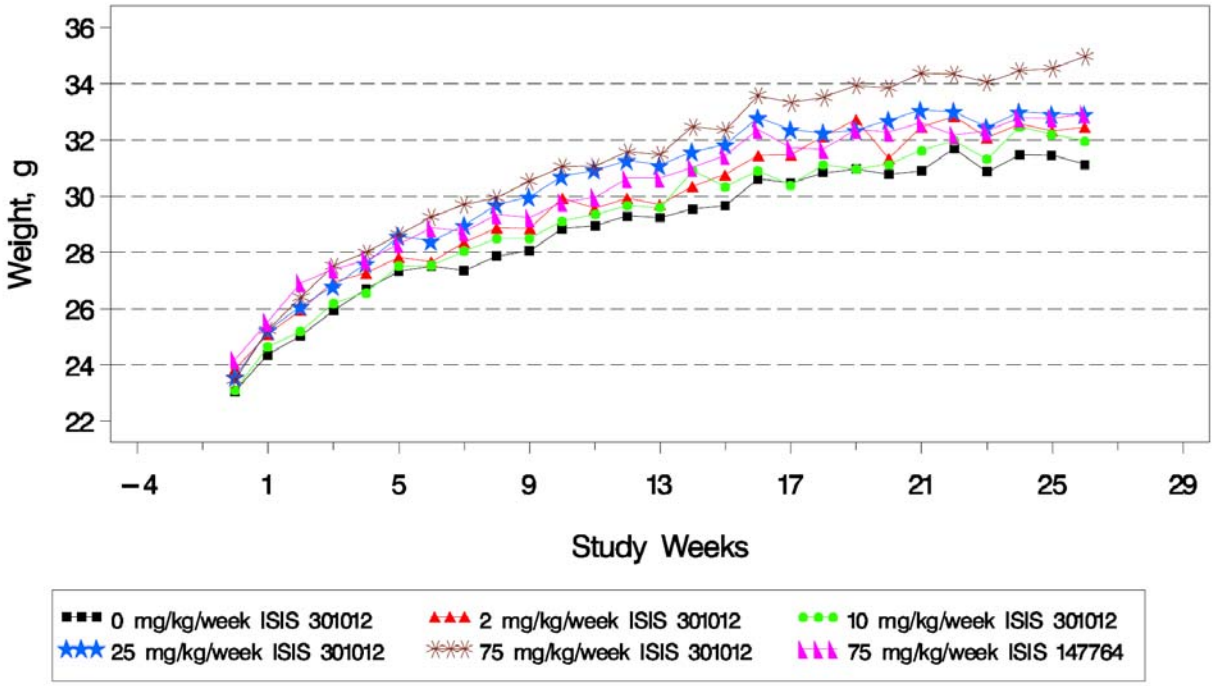


Figure 1A Mean Body Weight Values – FEMALE



Feed Consumption

Figure 2 Mean Food Consumption Values – MALE

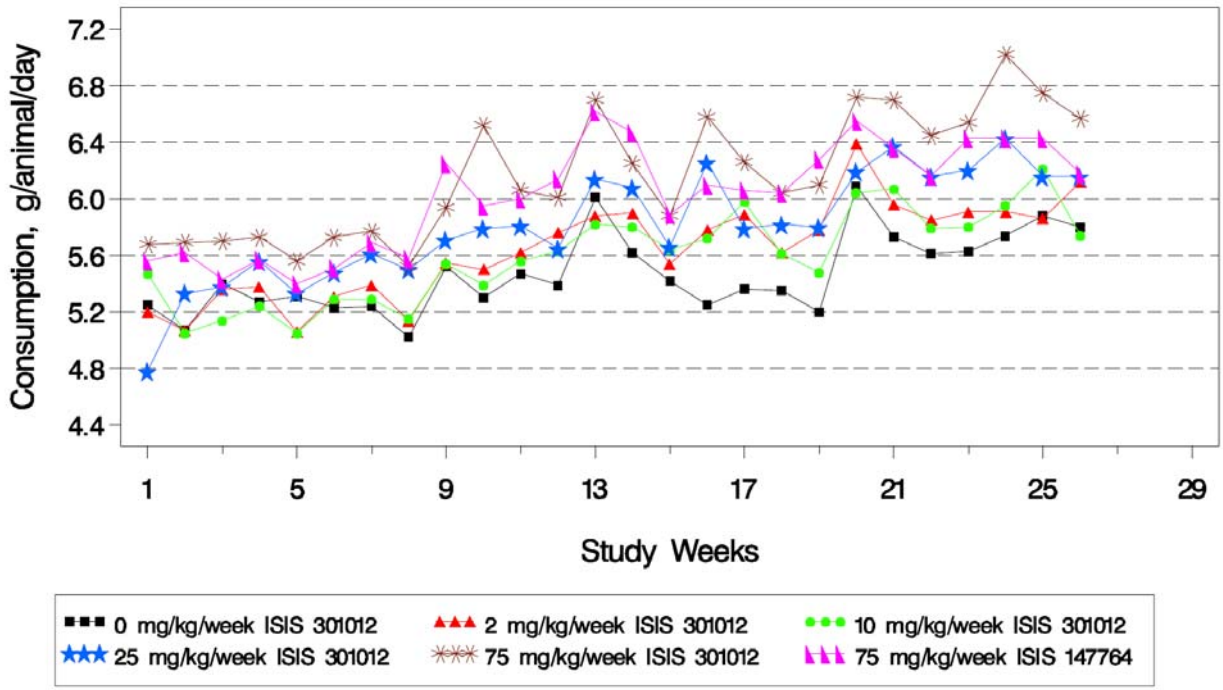
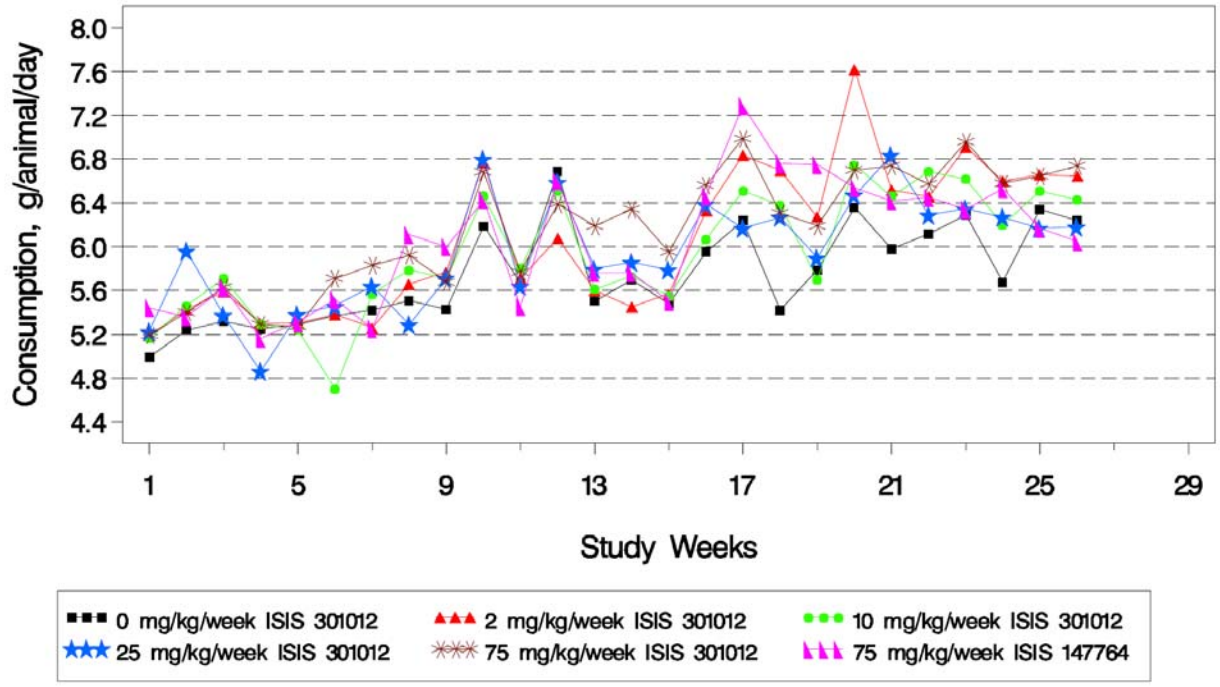


Figure 2A Mean Food Consumption Values – FEMALE



Ophthalmoscopy

No treatment-related ophthalmoscopic findings were observed at the examinations conducted prior to the terminal and recovery sacrifices. The few findings seen were typical of those commonly seen in mice of this age and strain.

Hematology: 8/s/g terminal and recovery sac

ISIS 301012 was associated with a dose-related ↓ in RBC mass in both sexes and a dose-related ↑ in platelet count in ♂s. Reversibility was incomplete by 3 months; absence of values in Sponsor's table indicates lack of s.s. (*NB* n=3 for recovery groups), not reversal of the numerical difference. The murine surrogate (ISIS 147764) was without effect on these parameters.

Coagulation parameters were not assessed.

Summary of ISIS 301012 Effects on Hematology Parameters

		ISIS 301012†				
		10 mg/kg/week	25 mg/kg/week	75 mg/kg/week	10 mg/kg/week Recovery	75 mg/kg/week Recovery
Erythrocytes	M	-	83%	79%	-	83%
	F	83%	81%	75%	-	74%
Hemoglobin	M	-	79%	73%	85%	77%
	F	83%	81%	75%	-	73%
Hematocrit	M	87%*	81%	75%	-	81%
	F	82%	80%	73%	-	75%
Platelet Counts	M	-	1.49	1.61	-	-
	F	-	-	-	-	-

*statistically significant change relative to control value for males(M). ($p<0.05$)
†statistically significant change relative to control values for males(M) and females(F). ($p<0.01$)
Mean treated value < control mean value displayed as % of control.
Mean treated value > control mean value displayed as fold increase.
- : Not statistically significant

Clinical Chemistry: 8/s/g terminal and recovery sac

In addition to the findings captured in Sponsor's table below:

- Dose-dependent ↓ in total bilirubin in F, s.s. at ≥ 25 mkw (52%↓ at HD)
- Chloride s.s. ↑ in HDr♂, n.s.s. ↑ in HDr♀

Summary of ISIS 301012 and ISIS 147764 Effects on Clinical Chemistry Parameters				
		25 mg/kg/week ISIS 301012	75 mg/kg/week ISIS 301012	75 mg/kg/week ISIS 147764
ALP	M	-	1.93†	-
	F	-	-	-
AST	M	-	1.63*	-
	F	-	1.83	-
ALT	M	-	3.16†	2.86†
	F	-	3.70	6.28†
Total Protein	M	-	92%†	90%†
	F	90%*	86%†	-
Albumin	M	-	78%†	80%†
	F	86%†	77%†	-
A:G Ratio	M	-	73%†	81%†
	F	-	80%*	-

*statistically significant change relative to control value for males(M) and females(F). ($p<0.05$)
†statistically significant change relative to untreated control for males(M) and females(F). ($p<0.01$)
Mean treated value < control mean value displayed as % of control.
Mean treated value > control mean value displayed as fold increase.
-: Not statistically significant

Urinalysis

Not performed.

Gross Pathology

Apparent effects on the immune system (consistent with a proinflammatory effect) and the skin (consistent with local irritation).

Gross Pathology -- Incidence (mean severity)												
Sex	**Male**						**Female**					
ISIS 301012 (mg/kg/week)	**0**	**2**	**10**	**25**	**75**	**-**	**0**	**2**	**10**	**25**	**75**	**-**
ISIS 147764 (mg/kg/week)	**-**	**-**	**-**	**-**	**-**	**75**	**-**	**-**	**-**	**-**	**-**	**75**
All Tissues w/i Normal Limits	14	11	14	11	9	11	10	13	8	12	7	11
Lymph node, mandibular												
Enlarged	-	-	-	1 (1.0)	1 (2.0)	-	n.e.					
Skin												
Abrasion	-	-	-	2 (2.5)	1 (3.0)	2 (2.0)	-	-	1 (2.0)	-	1 (3.0)	-
Thymus												
Small	-	1 (2.0)	1 (2.0)	1 (2.0)	2 (3.0)	1 (2.0)	-	-	-	-	2 (2.5)	-

n.e. = no effect
"-" = zero (not observed)

Organ Weights

Dose-dependent ↑ in body weight (all doses in ♂s, ≥ 25 mkw in ♀s) somewhat confounds interpretation of organ weight changes. For example, ↑s in kidney weight (10% in ♀ & 20% in ♂) (not shown) appear to be related to the increase in body weight, rather than a direct effect of the oligos on the kidney, although the ↑ may also reflect drug uptake by the kidney (tubule cell basophilic granules). An apparent ↑ in thyroid weight in ♂s had no dose-dependence and is not considered to be drug-related in the absence any correlating histopathology findings. The following changes are considered to be possibly drug-related:

Heart: ↑ (most notably in ♂s), correlated with lymphohistiocytic infiltration
Liver: ↑ (♂ & ♀), correlated with basophilic granules in Kupffer cells
Spleen: ↑ (♂ & ♀), correlated with extramedullary hematopoiesis
Thymus: ↓ (♂ & ♀), correlated with lymphoid depletion

Organ Weights -- % Change from Control												
Sex	**Male**						**Female**					
ISIS 301012 (m/k/w)	**0**	**2**	**10**	**25**	**75**	**-**	**0**	**2**	**10**	**25**	**75**	**-**
ISIS 147764 (m/k/w)	**-**	**-**	**-**	**-**	**-**	**75**	**-**	**-**	**-**	**-**	**-**	**75**
Body Weight (g)	28.5	30.2	**31.0****	**31.2****	**32.7****	**31.6****	25.6	26.5	25.4	26.8	**28.4****	26.5
Heart												
Absolute (g)	0.180	**↑17%***	↑14%	**↑27%****	**↑22%****	**↑16%***	0.174	↑8%	↑10%	↑10%	↑9%	↓5%
% BW	0.632	↑10%	↑5%	**↑17%****	↑7%	↑5%	0.684	-	↑11%	-	-	↓8%
BrW ratio	0.387	↑17%	↑8%	**↑25%****	**↑20%***	↑11%	0.360	↑10%	↑12%	↑5%	↑9%	↓5%
Recovery (% BW)	0.649	n.d.	↑14%	n.d.	-	n.d.	0.594	n.d.	↑11%	n.d.	↑18%	n.d.
Liver												
Absolute (g)	1.311	↑5%	**↑14%***	**↑20%****	**↑48%****	**↑50%****	1.172	↑10%	↑8%	**↑22%***	**↑54%****	**↑43%****
% BW	4.589	-	↑6%	**↑11%***	**↑29%****	**↑35%****	4.582	↑6%	↑8%	**↑16%***	**↑38%****	**↑37%****
BrW ratio	2.806	↑5%	↑9%	**↑19%****	**↑45%****	**↑43%****	2.422	↑11%	↑10%	↑16%	**↑54%****	**↑43%****
Recovery (% BW)	4.612	n.d.	-	n.d.	**↑12%****	n.d.	4.843	n.d.	-	n.d.	↑20%	n.d.
Spleen												
Absolute (g)	0.072	↑14%	↑18%	↑53%	↑28%	**↑61%***	0.089	↓6%	-	-	**↑49%****	↑29%
% BW	0.253	↑7%	↑7%	↑41%	↑10%	↑47%	0.350	↓10%	-	↓5%	**↑32%****	↑23%
BrW ratio	0.157	↑11%	↑10%	↑50%	↑23%	↑54%	0.186	↓7%	-	-	**↑47%****	↑28%
Recovery (% BW)	0.332	n.d.	**↓35%***	n.d.	↓5%	n.d.	0.306	n.d.	↑8%	n.d.	↑10%	n.d.
Thymus												
Absolute (g)	0.024	-	↓29%	↓33%	↓38%	↑25%	0.028	↑14%	↓18%	↓18%	↓36%	**↓36%***
% BW	0.084	-	↓35%	↓40%	↓47%	↑12%	0.108	↑11%	↓16%	↓22%	**↓41%***	**↓38%***
BrW ratio	0.050	↑6%	↓31%	↓34%	↓37%	↑17%	0.057	↑14%	↓15%	↓22%	↓33%	**↓36%***
Recovery (% BW)	0.069	n.d.	↓6%	n.d.	↓20%	n.d.	0.082	n.d.	↓5%	n.d.	↓19%	n.d.
Thyroid/Parathyroid												
Absolute (g)	0.005	**↑33%***	↑28%	**↑33%***	**↑35%***	**↑41%****	0.006	-	↓5%	↓10%	↑9%	↑9%
% BW	0.016	↑25%	↑20%	↑21%	↑19%	**↑28%***	0.023	-	-	↓15%	-	↑6%
BrW ratio	0.010	**↑31%***	↑22%	↑28%	**↑32%***	**↑33%****	0.012	-	↓5%	↓16%	↑7%	↑7%
Recovery (% BW)	0.017	n.d.	-	n.d.	-	n.d.	0.019	n.d.	↑16%	n.d.	↑19%	n.d.

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
"-" = no effect (<5% change)
n.d. = no data

Histopathology

Adequate Battery

Yes

Peer Review

None conducted

Histological Findings

Lymphohistiocytic infiltrates (macrophages containing basophilic granular material) in multiple tissues (minimal-mild), lymph node sinus histiocytosis (minimal to mild) are considered to reflect proinflammatory effects and cellular uptake of the test item (see Sponsor's tables below). Likewise basophilic granules in the kidney tubule cells (minimal to mild) and in liver Kupffer cells (minimal to moderate) is thought to represent test item uptake/accumulation. Lymphoid depletion in the thymus (moderate-severe in ♂s and mild to severe in ♀s) may also be related to the proinflammatory activity of the oligos.

Increased granulocytic hyperplasia in the bone marrow is likely a secondary response to the proinflammatory effects the oligos.

Some evidence of injury and regeneration at the injection sites.

75 mkw of the surrogate (ISIS 147764) was associated with (minimal-mild) Karyomegaly in the liver in ♀s. Two of these animals also exhibited (minimal) single cell necrosis. These findings were also associated with elevations in ALT and AST. Sponsor notes that there was no evidence of steatosis.

Sponsor provides no explanation for the "foreign material" (minimal-mild) that is seen dose-dependently in the skin of ♂s at doses ≥ 10 mkw.

Increased extramedullary hematopoiesis in the spleen is likely secondary to decreases in red cell mass.

Histopathology -- Incidence (mean severity)												
Sex	**Male**						**Female**					
ISIS 301012 (m/k/w)	**0**	**2**	**10**	**25**	**75**	**-**	**0**	**2**	**10**	**25**	**75**	**-**
ISIS 147764 (m/k/w)	**-**	**-**	**-**	**-**	**-**	**75**	**-**	**-**	**-**	**-**	**-**	**75**
Bone Marrow, femur												
Hyperplasia, granuloctyic	1/16 (1.0)	1/1 (3.0)	-	0/1 -	4/16 (1.5)	-	0/16 -	-	-	-	1/16 (3.0)	-
Bone Marrow, sternum												
Hyperplasia, granuloctyic	1/16 (1.0)	2/16 (2.0)	1/16 (1.0)	2/16 (3.0)	4/16 (1.5)	4/16 (1.8)	0/16 -	0/16 -	3/16 (1.7)	0/16 -	1/16 (3.0)	0/16 -
Injection site, L shoulder												
Hyperplasia, epidermal	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	1/16 (1.0)	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -
Injection site, R shoulder												
Erosion/Ulcer	0/16 -	0/16 -	0/16 -	0/16 -	1/16 (1.0)	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -
Hyperplasia, epidermal	0/16 -	0/16 -	0/16 -	0/16 -	1/16 (1.0)	1/16 (1.0)	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -

Heart												
Polyarteritis	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	1/16 (2.0)	0/15 -	0/16 -
Kidneys												
Basophilic granules, tubular cells	0/16 -	0/16 -	1/16 (1.0)	1/16 (1.0)	7/17 (1.0)	16/16 (1.2)	0/16 -	0/16 -	0/16 -	0/16 -	6/16 (1.5)	16/16 (1.3)
Liver												
Basophilic granules, Kupffer cells	0/16 -	0/16 -	16/16 (1.0)	16/16 (1.7)	16/16 (2.9)	16/16 (1.7)	0/16 -	0/16 -	15/16 (1.0)	16/16 (1.6)	16/16 (2.8)	16/16 (1.8)
Karyomegaly	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	13/16 (1.2)
Necrosis, Individual hepatocyte	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	2/16 (1.0)
Skin												
Foreign material	0/16 -	0/16 -	3/16 (1.0)	4/16 (1.0)	14/16 (1.1)	1/16 (1.0)	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -	0/16 -
Spleen												
↑ Extramedullary hematopoiesis	1/16 (1.0)	3/16 (1.3)	5/16 (1.0)	10/16 (1.3)	10/16 (1.1)	15/16 (1.2)	5/16 (1.0)	1/16 (1.0)	3/16 (1.0)	2/16 (1.0)	11/16 (1.3)	13/16 (1.0)
Thymus												
Lymphoid depletion	1/16 (4.0)	2/2 (3.0)	0/1 -	2/2 (3.5)	15/15 (3.5)	1/1 (2.0)	3/16 (2.3)	-	-	-	10/15 (2.9)	-

"-" = not assessed

Incidence and Severity of Treatment-Related Proinflammatory Effects								
	10 mg/kg/week		25 mg/kg/week		75 mg/kg/week ISIS 301012		75 mg/kg/week ISIS 147764	
	M N=16	F N=16	M N=16	F N=16	M N=16	F N=16	M N=16	F N=16
Lymphohistiocytic infiltrates								
• **Epididymides**			1 (min)		16 (min)			
• **Heart**	16 (min)	14 (min)	16 (min-mild)	16 (min)	16 (min-mild)	15 (min-mild)	16 (min-mild)	16 (min)
• **Injection site, left flank**	4 (min)	16 (min)	15 (min)	16 (min)	16 (min)	15 (min)	15 (min)	16 (min)
• **Injection site, left shoulder**	3 (min)	8 (min)	15 (min)	14 (min)	16 (min)	11 (min)	16 (min)	9 (min)
• **Injection site, right flank**	2 (min)	16 (min)	13 (min)	16 (min)	14 (min)	13 (min-mild)	15 (min)	15 (min)
• **Injection site, right shoulder**	2 (min)	5 (min)	15 (min)	14 (min)	16 (min)	11 (min)	16 (min)	11 (min)
• **Ovaries**						6 (min)		2 (min)
• **Pancreas**	16 (min)	8 (min)	16 (min)	16 (min)	16 (min)	16 (min)	16 (min)	16 (min)
• **Prostate**			1 (min)		15 (min)			
• **Skin**	7 (min)	15 (min)	16 (min)	16 (min)	15 (min-mild)	14 (min)	15 (min)	11 (min)
• **Spleen**		1 (min)	1 (min)	2 (min)	15 (min-mild)	16 (min)	16 (min)	15 (min-mild)
• **Testes**			13 (min)		15 (min)		16 (min)	

min - minimal

Incidence and Severity of Treatment-Related Proinflammatory Effects								
	10 mg/kg/week		25 mg/kg/week		75 mg/kg/week ISIS 301012		75 mg/kg/week ISIS 147764	
	M N=16	F N=16	M N=16	F N=16	M N=16	F N=16	M N=16	F N=16
Lymphohistiocytic infiltrates								
• **Tongue**					14 (min)	10 (min)		
• **Urinary bladder**			1 (min)		16 (min)	16 (min)		
• **Uterus with cervix**		1 (min)		16 (min)		16 (mild)		16 (min)
• **Vagina**						10 (min)		12 (min)
Sinus histiocytosis								
• **Inguinal LN**			1 (min)		13 (min)	14 (min)		
• **Mandibular LN**	6 (min)		12 (min)	14 (min)	16 (min-mild)	13 (min)	13 (min)	14 (min)
• **Mesenteric LN**	8 (min)	5 (min)	15 (min)	16 (min)	15 (min-mild)	14 (min-mild)	16 (min)	16 (min)
Increased extramedullary hematopoiesis								
• **Spleen**	5 (min)	3 (min-mod)	10 (min-mod)	2 (min)	10 (min-mild)	11 (min-mod)	15 (min-mild)	13 (min)

min - minimal
mod – moderate

Special Evaluation

Immunoglobulin

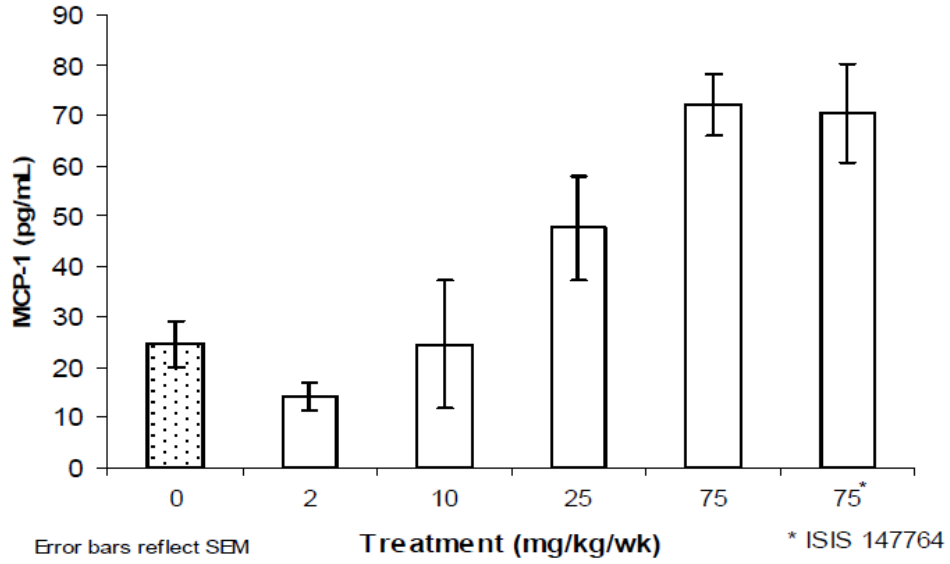
No effect of test item on total serum IgM or IgG.

Cytokine/Chemokine Analysis

A multiplex ELISA approach was used to measure the levels of IL-1α, IL-1β, IL-6, TNF-α, MCP-1 (JE), MIP-1α, RANTES, IL-8 and IFN-γ.

Insufficient information was provided in the study report to fully assess the extent to which the assay system is suitable for the intended analysis. The assay is not validated. The only parameter that appeared to demonstrate a clear relationship to test item was MCP-1 (JE), which showed a dose-dependent (sequence independent) increase at doses ≥ 25 mg/kg.

Plasma MCP-1 Expression After 6 Months of Treatment (ELISA)



Toxicokinetics

ISIS 301012 is rapidly cleared from plasma, presumably reflecting distribution to tissues. By Day 94 steady state plasma levels appear to have been achieved at HD (75 mkw); however at a lower dose (10 mkw) moderate accumulation continues after Day 94. (See Sponsor's tables 2 & 3, below.)

Exposure (AUC and C_{max}) increase roughly dose proportionally in plasma. Tissue exposures increase with dose, but generally hypoproportionally. The major sites of distribution are the kidney, liver and spleen, with the liver predominating at higher doses and the kidney at lower doses. In the assessed tissues, at most doses, intact ISIS 301012 represented >75% of measured oligonucleotide. (See Sponsor's tables 4 & 5, below.)

Table 2. **Summary of the observed ISIS 301012 concentrations (μg/mL) in plasma for Study 301012-AS14**

Group	Dose (mg/kg/week)	No. Doses	Study Day	Time Point	N	ISIS 301012 Concentrations[a] in Plasma (μg/mL)
7	10	1	1	15 min	3	4.64 ± 1.38
				30 min	3	8.15 ± 2.31
				60 min	3	5.09 ± 0.96
				2 hr	3	2.25 ± 0.86
				4 hr	3	0.373 ± 0.0394
				8 hr	3	0.183 ± 0.0329
				24 hr	3	0.00968 ± 0.00181
				48 hr	1[b]	0.00764
		16	94	48 hr	3	0.0158 ± 0.0043
		29	185	48 hr	3	0.0185 ± 0.0020
8	75	1	1	15 min	3	47.1 ± 12.9
				30 min	3	32.6 ± 2.4
				60 min	3	39.0 ± 3.4
				2 hr	3	17.8 ± 3.5
				4 hr	3	6.43 ± 1.23
				8 hr	3	1.48 ± 0.45
				24 hr	3	0.190 ± 0.033
				48 hr	3	0.0514 ± 0.0075
		16	94	48 hr	3	0.275 ± 0.058
		29	185	48 hr	3	0.236 ± 0.026

[a] Values are mean ± standard deviation

[b] Two out of three measurements were not reported due to insufficient volume was collected for analysis.

N= number of animals

Table 3. **Plasma pharmacokinetic parameters in mice following subcutaneous administration(s) of ISIS 301012 for Study 301012-AS14**

Parameters	Group 7 10 mg/kg/week	Group 8 75 mg/kg/week
Observed C_{max} (μg/mL)[a]	8.15 ± 2.31	47.1 ± 12.9
T_{max} (hr)	0.50	0.25
$AUC_{0\rightarrow 48\ hr}$ (μg*hr/mL)	14.6	118
$AUC_{0\rightarrow\infty}$ (μg*hr/mL)	14.7	119
CL/F (mL/hr/kg)[b]	679	630
Vz/F (mL/kg)[c]	7612	7706
$t_{1/2\lambda z}$ (hr)	7.77	8.48

[a] Data presented are mean ± standard deviation.

[b] CL/F and [c]Vz/F reported for s.c. dosing.

Table 4. ISIS 301012 and total oligonucleotide tissue concentrations (µg/g) in numerous tissues collected 48 hr after the last subcutaneous doses on Day 185. Male and female mice were combined (n= 2 to 10).

Organ	Group	Dose (mg/kg/week)	N	ISIS 301012	Total Oligonucleotide	%Intact
Ileum	2	2	10	2.90 ± 4.72	5.60 ± 12.71	83.5 ± 33.0
	3	10	10	23.0 ± 8.4	23.0 ± 8.4	100.0 ± 0.0
	4	25	10	43.8 ± 11.5	50.4 ± 18.5	90.1 ± 14.3
	5	75	10	65.0 ± 25.5	68.8 ± 23.3	93.4 ± 10.5
Kidney	2	2	10	46.5 ± 20.8	48.5 ± 21.5	96.6 ± 5.8
	3	10	10	83.7 ± 36.7	84.4 ± 36.8	99.1 ± 2.7
	4	25	10	155 ± 51	182 ± 65	87.9 ± 13.4
	5	75	9[b]	197 ± 77	236 ± 104	87.1 ± 12.6
Liver	2	2	10	18.4 ± 8.8	35.9 ± 17.9	57.3 ± 19.2
	3	10	10	84.5 ± 22.9	85.3 ± 23.2	99.2 ± 2.6
	4	25	10	243 ± 62	278 ± 79	88.6 ± 8.1
	5	75	10	699 ±184	908 ± 239	77.4 ± 8.6
Spleen[a]	2	2	2	9.70	13.5	78.6
	3	10	2	53.0	75.8	72.0
	4	25	2	89.6	89.6	100.0
	5	75	2	263	375	70.0

N= number of animals or animal pools

Data presented are mean ± standard deviation (SD not calculated for n<3)

[a] n=2, represents the average of one male (pooled from 3 males) and one female (pooled from 3 females).

[b] n=9, concentration for one sample (Animal 2253, ISIS 301012 conc. was 4467 µg/g) was >20-fold higher than the mean of the other nine samples, the cause of this abnormally high value was not identified. The values for mean ± SD with the outlier included are 624 ± 1352 µg/g and 734 ± 1579 for ISIS 301012 and total oligonucleotide concentrations, respectively.

Table 5. **ISIS 301012 and total oligonucleotide tissue concentrations (µg/g) in numerous tissues collected 48 hr after the last subcutaneous dose on Day 185 and 91 days after the last subcutaneous dose on Day 274 (3-month recovery). Male and female mice were combined** (n= 1 to 10).

	Group 3, 10 mg/kg/week		Group 5, 75 mg/kg/week	
Organ	**Day 185**	**Day 274**	**Day 185**	**Day 274**
Intact ISIS 301012:				
Brain	BLQ	BLQ	0.816 ± 1.124[c]	BLQ
Heart[a]	15.5	BLQ	50.7	20.4
Ileum	23.0 ± 8.4	0.96[a]	65.0 ± 25.5	19.1[a]
Kidney	83.7 ± 36.7	5.23 ± 3.39	197 ± 77[b]	39.8 ± 6.5
Liver	84.5 ± 22.9	3.53	699 ± 184	186 ± 235
Lung[a]	6.63	BLQ	53.8	20.6
Mes. Lymph[a]	37.1	3.82	210	36.9
Ovaries[a]	22.7	BLQ	139	13.9
Spleen[a]	53.0	3.75	263	29.0
Testes[a]	18.0	5.29	147	95.7
Uterus[a]	29.1	BLQ	181	6.56
Total Oligonucleotides:				
Brain	BLQ	BLQ	0.816 ± 1.124	BLQ
Heart[a]	15.5	BLQ	61.3	23.6
Ileum	23.0 ± 8.4	0.960	68.8 ± 23.3	20.7
Kidney	84.4 ± 36.8	10.5 ± 9.66	236 ± 104[b]	39.8 ± 6.5
Liver	85.3 ± 23.2	7.57	908 ± 239	211 ± 308
Lung[a]	6.63	BLQ	63.9	22.9
Mes. Lymph[a]	37.1	16.3	210	45.7
Ovaries[a]	28.0	BLQ	158	13.9
Spleen[a]	75.8	3.75	375	29.0
Testes[a]	32.0	19.3	218	137
Uterus[a]	41.9	37.2	223	6.56

Data presented are mean ± standard deviation (SD not calculated for n<3)

BLQ= below the limit of quantitation (LOQ = 1.52 µg/g)

[a] Samples were pooled by gender per group per time point.

[b] n=9, concentration for one sample (Animal 2253, ISIS 301012 conc. was 4467 µg/g) was >20-fold higher than the mean of the other nine samples, the cause of this abnormally high value was not identified. The values for mean ± SD with the outlier included are 624 ± 1352 µg/g and 734 ± 1579 for ISIS 301012 and total oligonucleotide concentrations, respectively.

[c] Four out of ten samples had measurable values (>LOQ) and six of the ten samples were BLQ.

Pharmacodynamic Analysis

The expression level of ApoB mRNA in the liver was assessed as a means of testing whether the mouse surrogate (ISIS 147764) was demonstrating the intended

pharmacology (reduction in ApoB message level). ApoB mRNA levels were ↓ ~30% at 75 mg/kg/week of ISIS 14776 after 6 months of treatment.

Dosing Solution Analysis

A homogeneity assessment was not conducted as the dosing formulation is a solution.

Stability was assessed by the Sponsor. The end-of-study assay values of the drug product show that concentrations of material remained within specification over the course of the study, and that ISIS 301012 was stable under the storage and handling conditions employed.

Study title: A One Year Toxicity Study of ISIS 301012 Administered by Subcutaneous Injection to Cynomolgus Monkeys

Study no.:	301012-AS15 ((b) (4) # ADQ00023)
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 12, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-003, 91.9%

Key Study Findings

- 1 HD♀ (# 5506) was moribund sacrificed in Week 39. Cause of death was considered to be widespread multifocal hemorrhage (secondary to thrombocytopenia), and multifocal necrosis (possibly secondary to local tissue anoxia associated with anemia and altered tissue perfusion). Sponsor considered this death to be of questionable relationship to the test item. However, given the plausible role of thrombocytopenia and anemia in this animals moribund state, and clear relationship between the test item and decreased platelet counts, this reviewer considers the death to be test item-related.
- The early sacrifice of a LMD♀ (#3504) in Week 43 due to apparent sepsis is not considered by the Sponsor to be test item related. This reviewer agrees that the proximal cause of morbidity in this animal (sepsis) was not likely to be mipomersen-related. However, this animal also had marked activation of the monocyte phagocytosis system. ~60% of marrow cells in this monkey were mature monocytes, which exhibited marked erythrophagocytosis. This animal was judged by the clinical pathologist reviewing the bone marrow cytology to have extravascular immune-mediated hemolytic anemia and immune-mediated thrombocytopenia. This reviewer hypothesizes that the combination of mipomersen-mediated lymphohistiocytosis and ongoing bacterial infection resulted in a condition similar to hemophagocytic lymphohistiocytosis (HLH).

Two other monkeys (#s 5505 [interim sacrifice] and 5506 [moribund sacrifice noted above]), both HD monkeys, also had evidence of immune-mediated (macrophage/histiocyte) extravascular destruction of RBCs and/or platelets, and may also have had an HLH-like syndrome.

- Thrombocytopenia was elicited in 3 HD monkeys, requiring dosing holidays of various durations. Platelet counts recovered following drug withdrawal with the exception of HD♀ # 5506, which was subsequently sacrificed moribund (above). Notably, the other 2 HD monkeys developing thrombocytopenia sufficient to require a dosing holiday were found to have vascular lesions at necropsy (below). Splenomegaly, with sequestration of platelets, may have contributed to the thrombocytopenia, although an immune-mediated loss of platelets (due to HLH and/or complement activation) cannot be ruled out.
- Mipomersen initiated a dose-related proinflammatory response, as evidenced by biomarkers of the acute phase response, expansion of lymphoid tissues, infiltration of lymphocytes into multiple tissues, establishment of lymphoid follicles (tertiary lymphoid tissue) in multiple tissues and hypertrophy/hyperplasia of histiocytes. Histiocytes in multiple tissues were found to contain basophilic granules, considered to represent mipomersen or its metabolites.
- Although the greatest concentrations of mipomersen were found in the kidney and liver, aside from the inflammatory and lymphoproliferative responses noted above, there was little demonstrably adverse effect of mipomersen treatment on the liver, with the exception of a possible increase in the incidence of minimal cytoplasmic vacuolation in hepatocytes at HD. In the kidney, doses of mipomersen of $\geq$ 10 mg/kg/week were associated with minimal-moderate cytoplasmic vacuolation of the tubular epithelium and minimal-slight degeneration of the tubular epithelium.
- Two HD monkeys, ♂ # 5007 and ♀ #5603 had vascular lesions in multiple tissues. These lesions were characterized by basophilic intimal thickening (hyperplasia) and infiltration of lymphocytes (including plasma cells), occasionally with eosinophils. The degree of intimal thickening was pronounced in some cases, sometimes nearly occluding the lumen. Although originally characterized as vasculitis, subsequent expert review states that the lesions, should not be characterized as a “vasculitis;” nonetheless the changes noted are considered to be adverse.
- In this reviewer’s opinion, the HD♀ sacrificed moribund (5506) should also be considered as suffering from vascular lesions. The finding of anemia, coupled with the presence of schistocytes is suggestive of microangiopathic anemia, even though this was not documented as a histopathology finding in this animal.

Methods

Doses:	0, 1, 3, 10 & 30 mg/kg
Frequency of dosing:	Weekly (except for 2 week loading period, during which doses were administered twice a week)
Route of administration:	Subcutaneous Injection
Dose volume:	0.2 mL/kg
Formulation/Vehicle:	Phosphate buffered saline, pH 7.4
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	Terminal sac: 3
Age:	2.8 to 4.6 years old
Weight:	♂: 2.0 to 3.3 kg ♀: 1.9 to 2.9 kg
Source:	China
Satellite groups:	Recovery (6 month): 2 (C & HD only) Interim sac (6 month): 2/s/g
Unique study design:	Complement (C3 and Bb) were assessed. Cytokine/chemokine levels were assessed. Acute Phase markers were assessed.
Deviation from study protocol:	Several HD monkeys received dosing holidays due to low platelet counts: HD♂ 5007: Days 211-232 & 309 to 330; HD♀ 5506: Day 246 to unscheduled sac on Day 271; HD♀ 5603: Day 309. After Day 134 the number of injection sites was increased from 4 to 8 to avoid reinjection of sites that were swollen and or red from the previous injection.

Group No.	Number of M/F	Dose Level[a] (mg/kg/week)[b]	Dose Solution Conc. (mg/mL)	Number Euthanized		
				Interim 6-Month/ Week 27/ Day 185	Terminal 12-Month/ Week 52/ Day 360	Recovery 18-Month/ Week 78/ Day 540/542
1	7/7	0 (control)	0	2/2	3/3	2/2
2	5/5	1	5	2/2	3/3	NA
3	5/5	3	15	2/2	3/2[c]	NA
4	5/5	10	50	2/2	3/3	NA
5	7/7	30	150	2/2	3/3	2/1[d]

M/F = male/female; NA = not applicable

[a] Weekly dose level after the loading dose period; during the loading dose period, monkeys were dosed at this dose level twice weekly for 2 weeks.

[b] Dose volume = 0.2 mL/kg

[c] One Group 3 female was euthanized moribund on Day 299

[d] One Group 5 female was euthanized moribund on Day 271

Observations and Results

Mortality

HD♀ 5506 was sac'd moribund on Day 271 (Week 39). At the time of sacrifice, this animal was on a dosing holiday due to marked thrombocytopenia and anemia (last dose was on Day 239). In the weeks prior to euthanasia, the monkey appeared to be dehydrated and was treated with SC Lactated Ringer's Solution and fruit supplementation. On Day 249 this animal was noted to have sustained a cage injury that resulted in severe swelling and bruising of one of its arms. On Day 251 this animal was noted to have a mass on both legs (~2 x 1 centimeter), which persisted until Day 268. Following blood collection on Day 269, it was noted that that the venipuncture site bled for ~30 min after sample collection and became severely swollen. The animal was lethargic thereafter, and was sacrificed on Day 271 after failing to respond to IV Lactated Ringer's Solution/dextrose and hetastarch. Postmortem analysis revealed widespread multifocal hemorrhage (presumably secondary to thrombocytopenia), and multifocal necrosis (possibly secondary to local tissue anoxia associated with anemia and altered tissue perfusion). Sponsor considered that thrombocytopenia, cage injury and anemia were the major factors of morbidity leading to euthanasia of this animal. Sponsor concluded that the relationship of the ISIS 301012 was unclear.

LMD♀ 3504 was sac'd moribund on Day 299 (Week 43) because of reduced body temperature and rapidly deteriorating condition. This monkey had a history of diarrhea and watery or red feces associated with mucus on 96 of 299 days on study; however, the incidence was reduced in the month preceding necropsy. Beginning on Week 41, this monkey had a severely swollen face. From Days 296 to 299 the animal continued to have facial swelling, reduced activity, hunched appearance, low food consumption and hypothermia. Necropsy revealed fluid accumulation in the abdominal cavity, which upon cytologic examination was revealed to be purulent exudate with a predominance of neutrophils (90%) along with intra- and extra-cellular bacteria. Histopathologic examination of the gastrointestinal tract showed widespread submucosal edema, with marked inflammation in the cecum that extended through the mucosa and may have resulted in the observed purulent exudate. The kidney was remarkable for diffuse membranous glomerulonephritis. Sponsor considered that the moribund state of this animal could be attributed to large intestinal inflammation (with probable consequent sepsis) and glomerulonephritis (possibly secondary to chronic gastroenteritis and sepsis). Sponsor concluded that while a contribution of ISIS 301012 administration to the moribund sate of this animal was unlikely, it could not be completely ruled out.

Clinical Signs

Other than the clinical signs noted above associated with the early decedents, clinical signs were generally limited to the injection sites: transient, generalized raised and circular redness and slight to moderate (occasionally severe) swelling. These signs were seen in all groups, but their severity and incidence were dose-related.

Body Weights

No test item-related effect.

Feed Consumption

No test item-related effect.

Ophthalmoscopy

No test item-related effect.

ECG

No test item-related effect.

Hematology

Sponsor-provided group mean data a statistical analyses combine both sexes.

≤ *3 mg/kg:* no test item-related effect.

10 mg/kg: Minimal increase in lymphocytes in males

30 mg/kg: ↓ platelet counts in both sexes (↓19-31% throughout the study), ↓ in neutrophils (♂&♀) and sporadic ↑ in lymphocytes, eosinophils, basophils and/or unclassified cells, especially in ♂s, suggests immune perturbation in some animals. 1 HD♀ (5506) had severe thrombocytopenia that likely contributed to early sacrifice, as discussed above. Monkey 5506 was also notable for the presence of abnormal RBCs, beginning Day 233, anisocytosis & spherocytes; Day 245, polychromasia; Day 250, microcytosis & nucleated red cells; and Day 269, hypochromasia, macrocytosis & schistocytes. By Day 250, monkey 5506 was moderately anemic (RBCs 55% of baseline), and by Day 269 she was severely anemic (RBCs 13% of baseline). The presence of the schistocytes is suggestive of microangiopathic anemia. The etiology of the ↓ in platelets is unclear, but does not appear to be due to reduced production, as analysis of bone marrow finds normal or elevated levels of magakaryocytes. Also there is a correlation between reduced platelet count and increased mean platelet volume (MPV) in affected animals, indicating increased thrombopoiesis.

Splenic sequestration may account for some of the ↓ in platelet count, since the spleen is enlarged in HD animals, especially so in HD monkeys 5007, 5506 and 5603, which experienced some of the greatest ↓s and had the largest spleens (~3.5x the rest of their dose cohort). Alternatively, platelets can be activated (and thereby consumed) by complement activation. Acquired hemophagocytic lymphohistiocytosis (LHL) may also have played a role in platelet, RBC and/or neutrophil destruction. This would be consistent with the increased number and activity of lymphocytes and histiocytes in treated monkeys and increased erythrophagocytosis documented in some monkeys.

Parameter: Plt 10^3/uL

GROUP	STATISTIC	PRESTUDY	WEEK 1	WEEK 14	WEEK 27	WEEK 39	WEEK 51	WEEK 65: RECOVERY
1	Mean	428.5	441.6	395.6	396.6	393.7	398.6	410.3
	SD	99.1	99.8	109.3	98.7	116.9	139.6	67.6
	N	14	14	14	14	10	10	4
	Statistical Sig							
2	Mean	454.4	447.1	419.5	413.9	405.8	417.7	0.0
	SD	111.7	105.4	98.4	106.1	141.9	125.0	0.0
	N	10	10	10	10	6	6	0
	Statistical Sig							
3	Mean	421.1	420.5	391.4	402.5	416.5	393.2	0.0
	SD	60.7	52.6	66.4	45.6	69.8	72.3	0.0
	N	10	10	10	10	6	5	0
	Statistical Sig							
4	Mean	386.8	413.4	383.7	378.9	398.0	403.0	0.0
	SD	64.8	89.5	78.6	99.6	65.3	69.6	0.0
	N	10	10	10	10	6	6	0
	Statistical Sig							
5	Mean	402.9	372.8	321.3	298.7	273.6	303.7	366.7
	SD	86.2	58.2	79.1	85.6	132.8	91.2	137.3
	N	14	14	14	14	10	9	3
	Statistical Sig				*			

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: Neut /uL

GROUP	STATISTIC	PRESTUDY	WEEK 1	WEEK 14	WEEK 27	WEEK 39	WEEK 51	WEEK 65: RECOVERY
1	Mean	7859.7	5133.6	5226.9	5024.0	3699.5	5526.5	4989.3
	SD	4645.2	2679.8	2119.5	1897.1	1075.9	1888.9	2358.6
	N	14	14	14	14	10	10	4
	Statistical Sig							
2	Mean	5488.3	4798.8	4798.2	3480.6	5518.3	4717.5	0.0
	SD	2313.1	1511.8	2416.7	1112.5	2523.1	1941.5	0.0
	N	10	10	10	10	6	6	0
	Statistical Sig							
3	Mean	4673.5	5318.6	4593.1	3591.9	4905.3	4315.6	0.0
	SD	2469.1	1642.4	2909.0	1504.5	1731.0	1648.8	0.0
	N	10	10	10	10	6	5	0
	Statistical Sig							
4	Mean	4369.0	3351.8	4171.8	2936.8	3706.3	3633.8	0.0
	SD	2469.9	1663.3	2567.9	1432.8	2146.9	1332.8	0.0
	N	10	10	10	10	6	6	0
	Statistical Sig				*			
5	Mean	5335.1	3969.5	3392.7	2779.7	2598.4	2627.3	2693.3
	SD	3083.2	1449.7	1629.7	1679.3	2040.6	2461.2	1020.2
	N	14	14	14	14	10	9	3
	Statistical Sig				*		*	

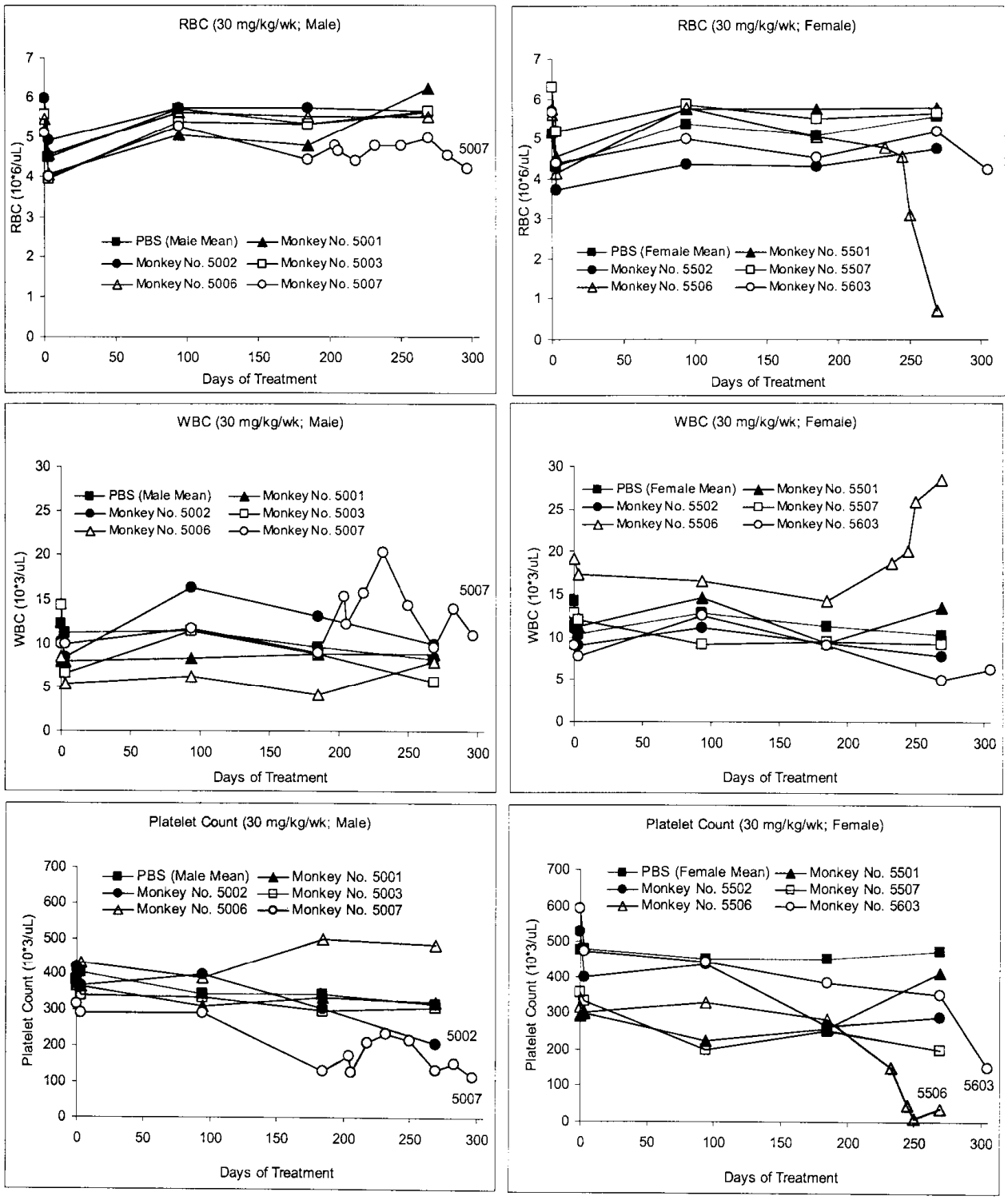
* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: Unc /uL

GROUP	STATISTIC	PRESTUDY	WEEK 1	WEEK 14	WEEK 27	WEEK 39	WEEK 51	WEEK 65: RECOVERY
1	Mean	73.9	80.6	85.6	72.1	64.2	68.8	68.8
	SD	44.8	46.6	46.3	28.1	23.1	50.1	36.0
	N	14	14	14	14	10	10	4
	Statistical Sig							
2	Mean	90.4	72.5	87.0	70.5	81.5	70.5	0.0
	SD	34.2	33.0	35.6	18.6	36.9	35.6	0.0
	N	10	10	10	10	6	6	0
	Statistical Sig							
3	Mean	66.5	67.9	66.6	68.5	71.8	60.4	0.0
	SD	23.0	31.8	24.3	26.7	17.8	10.1	0.0
	N	10	10	10	10	6	5	0
	Statistical Sig							
4	Mean	84.2	66.6	78.7	80.6	101.7	81.7	0.0
	SD	52.6	39.9	34.1	41.9	30.1	19.8	0.0
	N	10	10	10	10	6	6	0
	Statistical Sig							
5	Mean	69.1	64.9	86.7	105.2	127.8	85.2	73.0
	SD	18.2	17.7	31.9	45.3	128.4	27.0	27.9
	N	14	14	14	14	10	9	3
	Statistical Sig				*			

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Figure 4 **Red Blood Cells, White Blood Cells and Platelets in Monkeys Treated with 30 mg/kg/wk of ISIS 301012 for 10 months.**



Coagulation

Group mean data were not provided by the Sponsor, only individual data.

No clear effect of test item on PT, APTT or Fibrinogen.

On Days 352 & 353 (24 and 48 h after most recent dose) Sponsor measured the percentage of circulating platelets (CD61 positive cells) that are activated (double positive for CD62P and CD61) in samples from groups 1, 4 & 5. None of the C group samples gave values within the positive control range, while 1/6 group 4 sample was and 2/9 group 5 samples were. This may indicate an effect of test item to activate platelets.

	Dose Level ISIS 301012	Platelets[a] 10^3/uL	CD61/CD62P % Double Positive
Positive Control	N/A	N/A	3 - 9
Group 1	0 mg/kg	241 - 733	1 - 2
Group 4	10 mg/kg	311 - 509	1 - 6[b]
Group 5	30 mg/kg	163 - 486	2 - 9[c]

[a]Platelet data from hematology

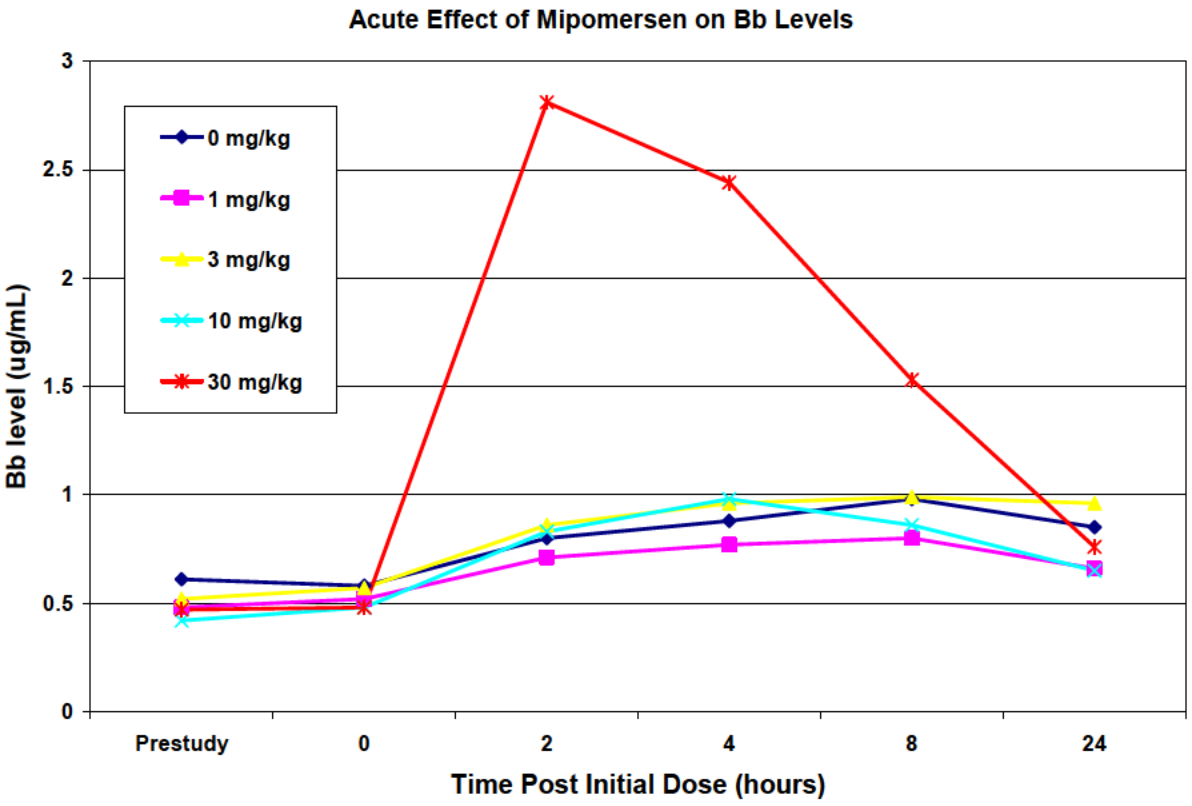
[b]One sample is within the positive control % double positive range

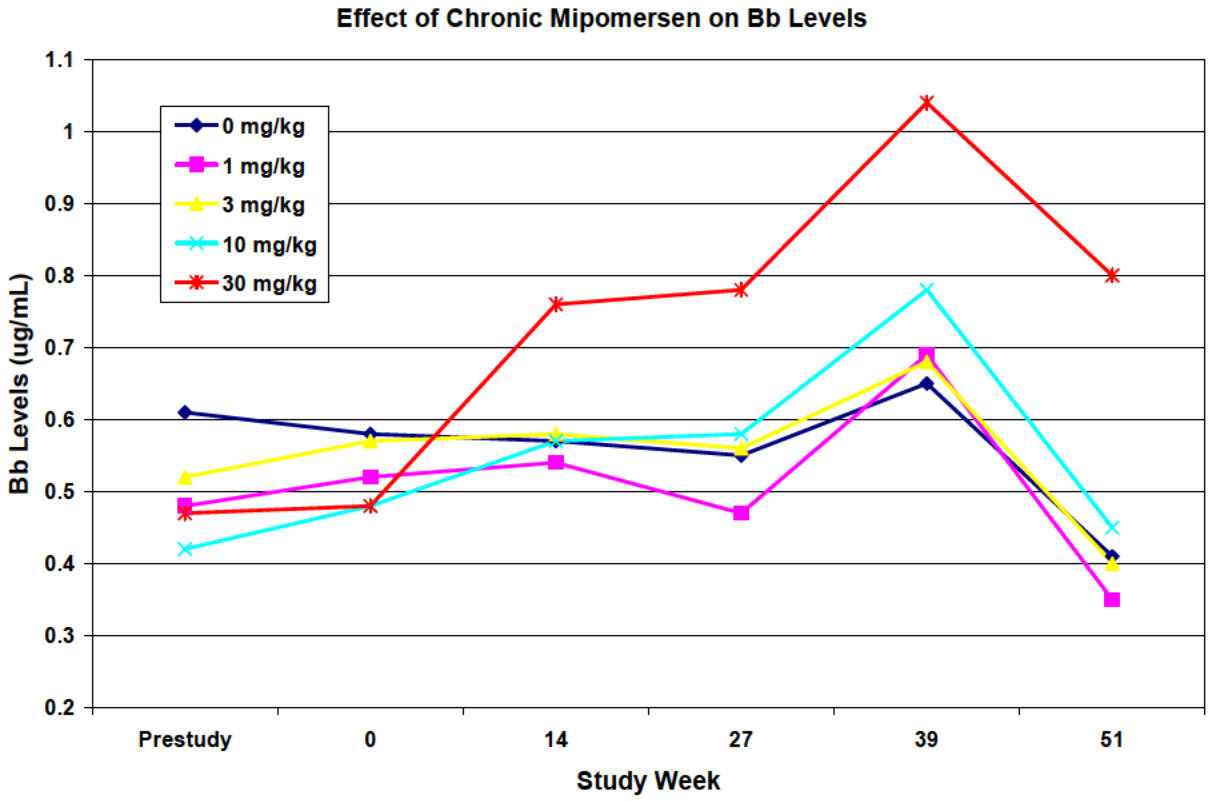
[c]Two samples are within the positive control % double positive range

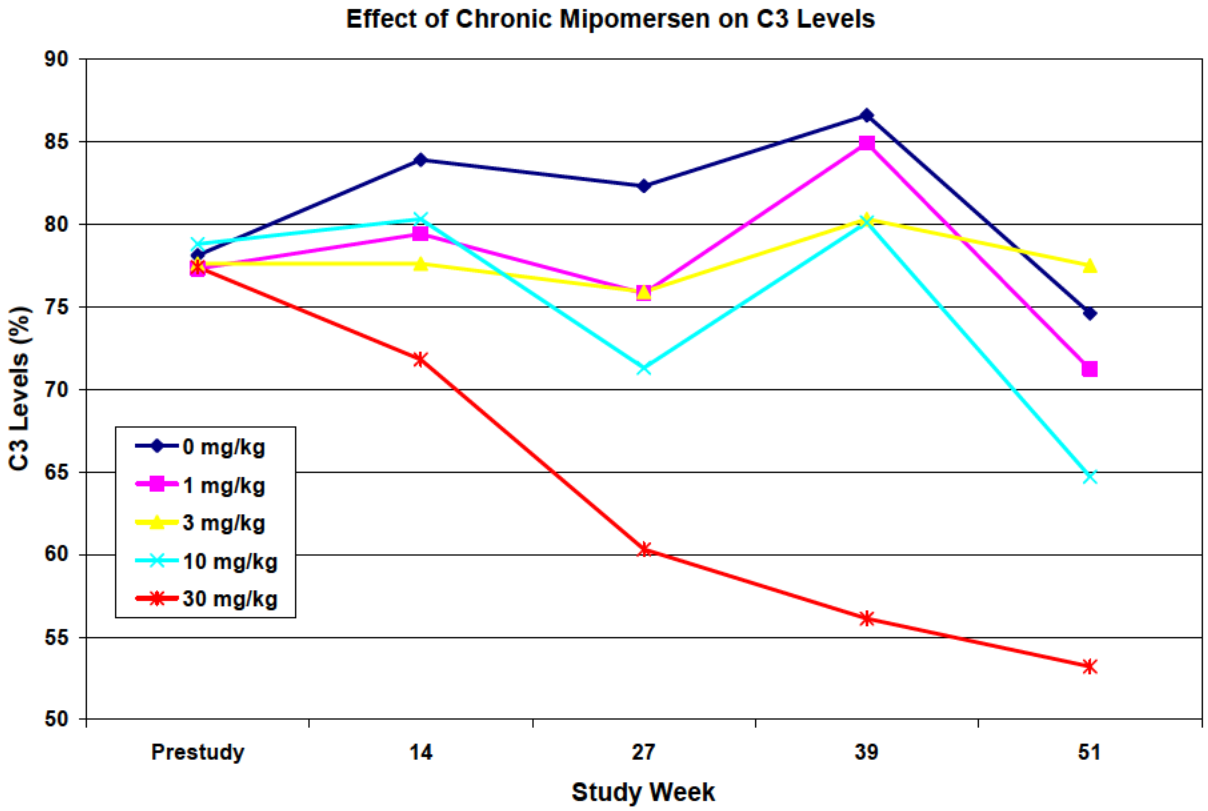
Complement

C3 (intact) and the split product of B (Bb) were measured.

The HD (30 mkw) was associated with acute and chronic activation of the alternative pathway for complement activation (generation of the Bb split product of factor B and consumption of C3). This is consistent with the known propensity for phosphorothioate oligonucleotides to activate the alternative complement pathway. While less clear, there is some indication that the HMD (10 mkw) may activate complement chronically to a lesser extent.


Acute Effect of Mipomersen on Bb Levels
Bb level (ug/mL)
0 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
0
0.5
1
1.5
2
2.5
3
Prestudy
0
2
4
8
24
Time Post Initial Dose (hours)


Effect of Chronic Mipomersen on Bb Levels
Bb Levels (ug/mL)
0 mg/kg
1 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Prestudy
0
14
27
39
51
Study Week



Cytokines/Chemokines

The levels of the following cytokines/chemokines was measured using a multiplex ELISA approach: IFN-γ, IL-1β, IL-6, IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, and TNFα.

Insufficient information was provided in the study report to fully assess the extent to which the assay system is suitable for the intended analysis. The assay is not validated. There is extremely large intragroup variability that limits the interpretability of intergroup changes.

There is no indication of an acute response following the first dose of mipomersen with IL-1β, IL-8, MCP-1, RANTES, MIP-1α, TNF-α. There was a transient increase in MCP-1, but this was also seen in the control group.

At 30 mkw there were minimal elevations (peaking at 4 h postdose) in response to the first dose for IL-6 (~10x ↑ over baseline vs. 3x for C); IFNγ (~40% ↑ over baseline vs. no change for C); MIP-1β (~2.5x ↑ over baseline vs. no change for C).

IL-1β was elevated at 30 mkw beginning around Day 183 and was s.s. ↑ on the last day of dosing 4 and 48 h after dosing. MIP-1β also had sporadic s.s. ↑s over C at later timepoints.

Parameter: IL-1b pg/mL

GROUP	STATISTIC	DAY 1 PRE	DAY 1 4 HR	DAY 1 8 HR	DAY 1 48 HR	DAY 92 PRE	DAY 183 PRE	DAY 267 PRE
1	Mean	8.42	7.54	10.85	7.16	8.82	6.75	4.90
	SD	6.78	5.02	11.91	5.11	7.05	4.08	0.00
	N	14	14	14	14	14	14	10
	Statistical Sig							
2	Mean	5.91	4.98	7.50	5.56	8.74	8.26	4.90
	SD	2.39	0.25	5.51	1.40	6.70	6.66	0.00
	N	10	10	10	10	10	10	6
	Statistical Sig							
3	Mean	6.69	5.28	5.64	5.28	5.19	5.81	38.38
	SD	5.66	1.20	2.34	1.20	0.92	2.88	82.02
	N	10	10	10	10	10	10	6
	Statistical Sig							
4	Mean	4.90	4.90	6.52	6.57	11.55	9.02	4.90
	SD	0.00	0.00	5.12	3.59	15.44	7.97	0.00
	N	10	10	10	10	10	10	6
	Statistical Sig							
5	Mean	8.32	8.17	7.47	9.17	10.52	29.58	20.06
	SD	5.65	6.96	4.29	7.96	9.35	50.82	26.54
	N	14	14	14	14	14	14	10
	Statistical Sig							

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: IL-1b pg/mL

GROUP	STATISTIC	DAY 358 PRE	DAY 358 4 HR	DAY 358 8 HR	DAY 358 48 HR
1	Mean	4.90	4.90	4.90	4.90
	SD	0.00	0.00	0.00	0.00
	N	10	10	10	10
	Statistical Sig				
2	Mean	11.10	11.68	8.68	4.90
	SD	10.63	12.52	9.27	0.00
	N	6	6	6	6
	Statistical Sig				
3	Mean	4.90	4.90	5.14	4.90
	SD	0.00	0.00	0.39	0.00
	N	5	5	5	5
	Statistical Sig				
4	Mean	10.43	12.75	11.70	10.67
	SD	13.55	19.23	16.46	9.68
	N	6	6	6	6
	Statistical Sig				
5	Mean	31.91	30.79	37.90	38.82
	SD	41.14	41.63	47.63	49.02
	N	9	9	9	9
	Statistical Sig		*		*

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: MIP-1b pg/mL

GROUP	STATISTIC	DAY 1 PRE	DAY 1 4 HR	DAY 1 8 HR	DAY 1 48 HR	DAY 92 PRE	DAY 183 PRE	DAY 267 PRE
1	Mean	46.48	46.21	36.06	42.67	49.19	44.77	20.42
	SD	47.34	52.20	45.16	56.29	48.93	47.29	12.45
	N	14	14	14	14	14	14	10
	Statistical Sig							
2	Mean	59.04	50.78	56.34	58.09	50.40	58.34	14.65
	SD	64.66	59.07	62.77	65.98	61.11	50.71	16.72
	N	10	10	10	10	10	10	6
	Statistical Sig							
3	Mean	18.05	15.55	13.83	16.55	16.60	27.08	28.48
	SD	20.59	17.46	19.07	19.02	17.99	23.31	41.02
	N	10	10	10	10	10	10	6
	Statistical Sig							
4	Mean	38.99	47.67	49.19	46.10	61.98	110.71	44.70
	SD	41.20	45.08	49.94	38.09	50.86	174.51	51.36
	N	10	10	10	10	10	10	6
	Statistical Sig							
5	Mean	59.02	149.54	70.72	58.47	103.18	129.51	77.91
	SD	50.55	189.56	67.78	49.37	84.85	100.40	45.64
	N	14	14	14	14	14	14	10
	Statistical Sig						*	*

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: MIP-1b pg/mL

GROUP	STATISTIC	DAY 358 PRE	DAY 358 4 HR	DAY 358 8 HR	DAY 358 48 HR
1	Mean	113.99	166.07	128.03	107.12
	SD	113.62	184.08	162.78	128.58
	N	10	10	10	10
	Statistical Sig				
2	Mean	75.40	77.53	71.90	64.77
	SD	32.41	30.79	22.59	27.13
	N	6	6	6	6
	Statistical Sig				
3	Mean	68.84	86.68	76.70	73.60
	SD	27.78	19.87	32.78	37.29
	N	5	5	5	5
	Statistical Sig				
4	Mean	114.78	209.83	144.05	103.65
	SD	85.31	161.16	100.68	71.16
	N	6	6	6	6
	Statistical Sig				
5	Mean	122.57	198.44	148.96	117.12
	SD	38.30	155.81	92.33	40.92
	N	9	9	9	9
	Statistical Sig				

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Serum Immunoglobulins

All monkeys were assessed for serum IgG and IgM levels. There was no effect of mipomersen except at HD. It is notable in particular that monkey 3504 (moribund sac'd on Day 299) did not have elevated IgG or IgM compared to baseline. At 30 mkw, IgG levels (but not IgM) were elevated 2-3-fold compared to concurrent control or baseline. This effect was s.s. at Weeks 39 and 51, and was reversible.

Serum Immunoglobulin Values

Study Number: ADQ00023

Animal Number	Sex	Total IgG mg/mL						
		Prestudy	Day 94	Day 185	Day 269	Day 353	Day 450	Day 540
Group 1: Control (0 mg/kg)								
1001	M	27.8	9.7	10.4	13.3	10.7	NEC	NEC
1002	M	9.7	10.8	9.4	8.0	8.8	NEC	NEC
1003	M	12.9	14.5	29.7	12.1	10.3	NEC	NEC
1004	M	21.4	17.2	18.3	NEC	NEC	NEC	NEC
1005	M	18.8	16.7	14.4	NEC	NEC	NEC	NEC
1006	M	17.1	13.2	9.8	8.0	10.7	34.5	19.8
1007	M	13.7	11.8	16.2	12.5	11.7	22.6	12.3
Mean		**17.3**	**13.4**	**15.5**	**10.8**	**10.4**	**28.6**	**16.1**
S.D.		6.0	2.9	7.2	2.6	1.1	8.4	5.3
1501	F	16.7	19.2	25.7	11.7	15.6	NEC	NEC
1502	F	17.3	17.6	22.5	13.1	13.1	NEC	NEC
1504	F	22.7	16.0	15.4	NEC	NEC	NEC	NEC
1505	F	12.7	24.3	26.6	NEC	NEC	NEC	NEC
1506	F	10.7	14.7	11.7	8.5	7.3	20.5	17.3
1507	F	11.1	7.6	7.1	9.0	11.9	28.0	12.3
1603	F	NS	29.6	26.5	12.4	16.1	NEC	NEC
Mean		**15.2**	**18.4**	**19.4**	**10.9**	**12.8**	**24.3**	**14.8**
S.D.		4.6	7.0	7.9	2.1	3.5	5.3	3.5

Serum Immunoglobulin Values

Study Number: ADQ00023

Animal Number	Sex	Total IgG mg/mL						
		Prestudy	Day 94	Day 185	Day 269	Day 353	Day 450	Day 540
		Group 5: ISIS 301012 (30 mg/kg)						
5001	M	12.4	10.7	14.4	24.7	27.8	NEC	NEC
5002	M	3.4	4.1	5.7	15.2	38.7	NEC	NEC
5003	M	3.7	3.5	3.5	12.2	14.5	32.8	19.1
5004	M	4.3	4.2	3.8	NEC	NEC	NEC	NEC
5005	M	4.8	4.6	6.0	NEC	NEC	NEC	NEC
5006	M	2.5 [a]	3.4	3.1	11.4	10.7	27.4	17.3
5007	M	10.1	24.1	25.6	26.3	21.0	NEC	NEC
Mean		**5.9**	**7.8**	**8.9**	**18.0**	**22.5**	**30.1**	**18.2**
S.D.		3.8	7.6	8.3	7.0	11.1	3.8	1.3
5501	F	11.2	11.1	11.6	14.6	21.0	NEC	NEC
5502	F	9.9	12.3	18.4	20.3	13.8	NEC	NEC
5504	F	14.1	12.7	15.7	NEC	NEC	NEC	NEC
5505	F	12.7	12.2	5.0	NEC	NEC	NEC	NEC
5506	F	14.5	28.6	29.3	29.0	UNEC	UNEC	UNEC
5507	F	9.1	17.5	13.3	14.4	18.7	28.7	12.9
5603	F	NS	17.3	18.1	28.2	57.0	NEC	NEC
Mean		**11.9**	**16.0**	**15.9**	**21.3**	**27.6**	**28.7**	**12.9**
S.D.		2.2	6.1	7.5	7.1	19.8	0.0	0.0

[a] 2.5 mg/mL represents an assigned value at the lower limit of the assay detection limit and is used in the statistical analysis
NEC = animal necropsied (scheduled)
NS = no sample

Clinical Chemistry

Sponsor-provided group mean data a statistical analyses combine both sexes.

CRP was markedly elevated (≥ 2 mg/dL) at one or more timepoints in the majority of HD animals (10/14). This finding was rare in other dose groups: 2/14 in C, 0/10 in LD, 2/10 in LMD and 1/10 in HMD.

8/14 HD monkeys (♂&♀) had ≥ 20% ↓ in albumin at one or more timepoints (mostly later in the study. With the exception of a single LMD monkey, this was not seen in any other animals on the study. 7/14 HD monkeys (♂&♀) had a ≥ 20% ↑ in serum globulin. A comparable decrease was not seen in any other animals on the study. The affected animals also exhibited slight to marked ↓s in the A:G ratio, with 6/14 animals exhibiting ↓s of ≥ 50%.

There was no effect of test item on serum transaminase levels.

No convincing effect of mipomersen on lipid levels (CHOL or LDL-C) was apparent in these normolipidemic animals at any dose.

Parameter: A:G ratio

GROUP	STATISTIC	PRESTUDY	WEEK 1	WEEK 14	WEEK 27	WEEK 39	WEEK 51	WEEK 65: RECOVERY
1	Mean	0.94	0.99	0.94	0.94	0.95	0.93	0.88
	SD	0.13	0.14	0.17	0.14	0.14	0.16	0.13
	N	14	14	14	14	10	10	4
	Statistical Sig							
2	Mean	0.96	1.03	0.99	0.98	1.00	0.87	0.00
	SD	0.13	0.11	0.12	0.12	0.17	0.12	0.00
	N	10	10	9	10	6	6	0
	Statistical Sig							
3	Mean	1.01	1.05	1.05	1.01	0.93	0.94	0.00
	SD	0.12	0.12	0.07	0.07	0.21	0.05	0.00
	N	10	10	10	10	6	5	0
	Statistical Sig							
4	Mean	0.97	1.07	1.00	0.96	0.92	0.85	0.00
	SD	0.14	0.13	0.12	0.11	0.18	0.10	0.00
	N	10	10	10	10	6	6	0
	Statistical Sig							
5	Mean	0.95	0.98	0.86	0.77	0.68	0.64	0.87
	SD	0.11	0.13	0.12	0.17	0.17	0.25	0.21
	N	14	14	14	14	10	9	3
	Statistical Sig				*	*	*	

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: Alb g/dL

GROUP	STATISTIC	PRESTUDY	WEEK 1	WEEK 14	WEEK 27	WEEK 39	WEEK 51	WEEK 65: RECOVERY
1	Mean	3.36	3.34	3.33	3.20	3.40	3.30	3.03
	SD	0.27	0.29	0.30	0.23	0.40	0.42	0.15
	N	14	14	14	14	10	10	4
	Statistical Sig							
2	Mean	3.47	3.44	3.24	3.25	3.37	3.37	0.00
	SD	0.22	0.23	0.23	0.20	0.35	0.46	0.00
	N	10	10	9	10	6	6	0
	Statistical Sig							
3	Mean	3.63	3.38	3.51	3.28	3.38	3.52	0.00
	SD	0.24	0.23	0.23	0.24	0.73	0.13	0.00
	N	10	10	10	10	6	5	0
	Statistical Sig							
4	Mean	3.44	3.45	3.31	3.13	3.27	3.17	0.00
	SD	0.25	0.21	0.19	0.23	0.41	0.31	0.00
	N	10	10	10	10	6	6	0
	Statistical Sig							
5	Mean	3.51	3.43	3.04	2.94	2.89	2.69	3.10
	SD	0.19	0.22	0.20	0.30	0.56	0.53	0.26
	N	14	14	14	14	10	9	3
	Statistical Sig			*	*		*	

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Parameter: Glob g/dL

GROUP	STATISTIC	PRESTUDY	WEEK 1	WEEK 14	WEEK 27	WEEK 39	WEEK 51	WEEK 65: RECOVERY
1	Mean	3.64	3.42	3.56	3.46	3.66	3.60	3.45
	SD	0.36	0.23	0.41	0.36	0.30	0.25	0.44
	N	14	14	14	14	10	10	4
	Statistical Sig							
2	Mean	3.61	3.38	3.32	3.30	3.50	3.83	0.00
	SD	0.40	0.21	0.33	0.37	0.40	0.48	0.00
	N	10	10	9	10	6	6	0
	Statistical Sig							
3	Mean	3.63	3.21	3.35	3.23	3.65	3.68	0.00
	SD	0.28	0.21	0.26	0.35	0.29	0.24	0.00
	N	10	10	10	10	6	5	0
	Statistical Sig							
4	Mean	3.54	3.29	3.34	3.29	3.68	3.75	0.00
	SD	0.35	0.27	0.35	0.34	0.43	0.27	0.00
	N	10	10	10	10	6	6	0
	Statistical Sig							
5	Mean	3.77	3.55	3.59	4.01	4.32	4.46	3.57
	SD	0.39	0.36	0.48	0.76	0.49	1.26	0.35
	N	14	14	14	14	10	9	3
	Statistical Sig				*	*		

* ANOVA with Dunnett's/Dunn's (p <= 0.05)

Acute Phase Markers

Sponsor identified 6 HD monkeys (#s 5001, 5002, 5007, 5501, 5506 & 5603) having evidence of an acute phase response. This was defined as two or more of the following concurrent changes: 1) $\geq$ 20% $\downarrow$ in serum albumin from baseline, 2) $\geq$ 20% $\uparrow$ in serum globulin from baseline, 3) CRP $\geq$ 2 mg/dL, 4) $\geq$ 50% $\uparrow$ in plasma fibrinogen from baseline or 5) β2-microglobulin of $\geq$ 100 nmol/L (see table below). One C (# 1005) and one HMD (# 4505) monkey also showed signs of an acute phase response.

Dose-dependent $\uparrow$ in serum β2-microglobulin measured at Week 39. Serum β2-microglobulin was not measured at any other timepoint. Urinary β2-microglobulin (expressed as a ratio to creatinine) was generally unaffected by treatment at all assessed timepoints (Weeks 17, 27, 39 & 51); however 1 HD♂ (# 5007) had unusually high values at Week 27 (1.03) and Week 39 (4.75), which correspond to high serum levels in this animal. No other animal in the study have a ratio of greater than one at any timepoint. Notably though, # 5007's values returned to the normal range by Week 51.

β2-Microglobulin Week 39 (nmol/L)												
Control			1 mg/kg/week			3 mg/kg/week			10 mg/kg/week			30 mg/kg/week		
1001	M	88.75	2001	M	NEC	3001	M	94.20	4001	M	NEC	5001	M	191.18
1002	M	61.65	2002	M	NEC	3002	M	108.75	4002	M	NEC	5002	M	119.53
1003	M	71.43	2003	M	81.25	3003	M	104.21	4003	M	109.30	5003	M	140.49
1004	M	NEC	2004	M	99.75	3004	M	NEC	4004	M	158.49	5004	M	NEC
1005	M	NEC	2005	M	98.07	3005	M	NEC	4005	M	122.35	5005	M	NEC
1006	M	94.93	2501	F	NEC	3501	F	NEC	4502	F	NEC	5006	M	140.53
1007	M	104.34	2502	F	NEC	3502	F	NEC	4503	F	99.39	5007	M	421.24
1501	F	83.16	2503	F	110.94	3503	F	88.93	4504	F	116.21	5501	F	139.49
1502	F	126.30	2504	F	85.16	3504	F	105.16	4505	F	106.84	5502	F	156.26
1504	F	NEC	2505	F	96.21	3505	F	109.94	4601	F	NEC	5504	F	NEC
1505	F	NEC										5505	F	NEC
1506	F	126.89										5506	F	112.16
1507	F	72.61										5507	F	228.01
1603	F	79.56										5603	F	171.59
Mean		**90.96**			**95.23**			**101.87**			**118.76**			**182.05**
S.D.		22.38			10.71			8.43			21.00			90.82

NEC = animal necropsied

Fecal Culture

Fecal culturing was performed on samples from HD monkeys 5006 (Day 324), 5501 (Day 346), 5603 (Days 306 & 333) and 5507 (Days 315 & 359). Monkey 5507 was found to be positive for *Campylobacter sp.* on Day 315, and monkey 5603 was found to be positive for *Shigella sp.* Both animals were treated with Baytril® at 5mg/kg for 1 week, and were subsequently negative for the respective bacteria. All other cultures were negative for *Campylobacter sp, Shigella sp, and Salmonella sp.*

Urinalysis

HD♂ #5007 was positive for protein in the urine (+1 to +3) and occult blood (small to large) by dipstick, and had an elevated microprotein-to-creatinine ratio beginning on Study Day 269. This same animal also had an pronounced elevations of serum β2-microglobulin at this time and urine β2-microglobulin on Days 185, 248 and 269. Urine β2-microglobulin was near normal on Day 353. Other HD monkeys were unremarkable.

Group Sex	Animal Number	Day Number	Urine Crea mg/dL	Urine M-TP mg/dL	Urine M-TP/Cre ratio
5m	5007	-6	64.9	8.0	0.10
		3	91.3	15.0	0.20
		94	66.2	13.0	0.20
		119	11.5	5.9*	0.50
		185	26.5	10.0	0.40
		248	44.9*	26.0*	0.60*
		269	20.5	32.0	1.60
		304	103.6*	160.0*	1.50*
		316	55.7*	64.0*	1.10*
		330	21.1*	40.0*	1.90*
		353	10.9	26.0	2.40

Urine β2-microglobulin in HD♂ 5007

Day 119	<2.54 nmol/L	
Day 185	26.82 nmol/L	
Day 248	26.37 nmol/L	
Day 269	97.34 nmol/L	(serum = 421.24 nmol/L on this day)
Day 353	3.39 nmol/L	

Gross Pathology

In the absence of Sponsor-provided summary tables, the following is reproduced from the pathologists report.

5.1. Gross Pathology

5.1.1. Interim Necropsy Animals (Day 185)

Macroscopic findings attributed to the subcutaneous injection of ISIS 301012 in cynomolgus monkeys euthanized at Day 185 (after 27 weeks of dosing) were limited to the enlargement of one or more lymph nodes (inguinal, axillary, sublumbar, iliac, bronchial, and cervical) in 1 of 4, 1 of 4, and 2 of 4 monkeys dosed with 1, 3, and 30 mg/kg/week ISIS 301012, respectively. Lymph node enlargement was attributed to lymphoid hyperplasia, medullary, or subcapsular sinus histiocytosis (accumulation of macrophage-like cells) and/or histiocyte hypertrophy. Although lymphoid hyperplasia and sinus histiocytosis were present in control article and test article-treated monkeys, these findings were generally more frequent and/or severe in monkeys dosed with ISIS 301012. The histologic finding of histiocyte hypertrophy (with intracellular basophilic granules) was only identified in monkeys dosed with ISIS 301012. Lymphoid hyperplasia, sinus histiocytosis, histiocyte hypertrophy, and intracellular basophilic granules have been observed previously with oligonucleotide administration and, as such, were considered an effect of the test article in the present study. All other

macroscopic findings were randomly distributed across control and test article-treated monkeys, or were considered common incidental findings for cynomolgus monkeys.

5.1.2. Unscheduled Necropsy Animals

Two monkeys were euthanized moribund prior to the Day 360 terminal necropsy. Monkey 3504-MF23342F (Group 3 female, 3 mg/kg/week ISIS 301012) was euthanized in moribund condition on Day 299/Week 43 because of reduced body temperature and rapidly deteriorating condition. Monkey 5506-MF4071F (Group 5 female, 30 mg/kg/week ISIS 301012) was euthanized moribund on Day 271/Week 39. Prior to euthanasia, the monkey was weak and was determined to be anemic and thrombocytopenic. Gross findings and associated histologic correlates are presented in the Individual Animal Data Reports that constitute Table 14.

Macroscopic findings (and histologic/cytologic correlates) in monkey 3504 consisted of fluid accumulation in the abdominal cavity (purulent exudate based on cytologic evaluation); increased size of the adrenals (cortical hyperplasia); a red focus on the cecum (acute inflammation); decreased thickness of the colon (no histologic correlate); red discoloration of the ileum (hemorrhage); increased thickness of injection sites 1, 2, 3, and 4 (subcutaneous edema); pale discoloration of the kidney (glomerulonephritis); decreased size of the thymus (involution); and pale discoloration of the thyroid (no histologic correlate). None of the above findings were consistent with typical changes in cynomolgus monkeys dosed with oligonucleotides. Gross findings that were associated with an oligonucleotide-based histologic
correlate were limited to an accentuated lobular pattern, increased size, and friability of the liver; all three macroscopic observations were correlated to Kupffer cell hypertrophy/hyperplasia.

Gross observations (and associated histologic correlates) in monkey 5506 included red discoloration of the bone marrow, heart (hemorrhage), multiple lymph nodes (erythrophagocytosis), and gastric mucosa (hemorrhage), pale discoloration of the kidney (multifocal cellular infiltrates), and liver (multifocal extramedullary hematopoiesis), and an increase in size of multiple lymph nodes (extramedullary hematopoiesis and/or plasmacytosis/plasma cell hyperplasia). With the exception of an increase in size of lymph nodes associated with plasmacytosis, none of the above gross findings were considered typical test article-associated gross observations.

5.1.3. Terminal Necropsy Animals (Day 360)

Macroscopic findings ascribed to the subcutaneous administration of ISIS 301012 in cynomolgus monkeys euthanized at Day 360 (after 52 weeks of dosing) consisted of lymph node enlargement, accentuation of the follicular pattern in the spleen, and an increase in the thickness of the subcutaneous tissue in one or more of injection sites 1, 2, 3, and 4 (left and right thigh area). Enlargement of one or more lymph nodes (axillary, cervical, iliac, inguinal, mandibular, mesenteric, pancreatic, popliteal, sublumbar, and tracheobronchial) was identified in 1 of 5, 3 of 6, and 6 of 6 monkeys dosed with 3, 10, and 30 mg/kg/week ISIS 301012, respectively. Lymph node enlargement was most consistently associated with microscopic findings of lymphoid hyperplasia and plasmacytosis, although sinus histiocytosis and histiocyte hypertrophy contributed to the

increase in size of a small number of lymph nodes. Accentuation of the follicular pattern in the spleen was observed in a single control monkey and in 4 of 6 monkeys dosed at 30 mg/kg/week ISIS 301012. Accentuation of the follicular pattern in the spleen correlated to lymphoid hyperplasia; the single occurrence in a control monkey was consistent with the background incidence at this facility. Enlargement of lymph nodes and accentuation of the follicular pattern in the spleen were attributed to immune stimulation secondary to ISIS 301012 administration. Increased thickness of the subcutaneous tissue in one or more of injection sites 1 thru 4 was identified in 1 of 5, 1 of 6, and 3 of 6 monkeys from the 3, 10, and 30 mg/kg/week ISIS 301012 groups, was attributed to fibrosis and/or edema, and considered secondary to ISIS 301012-induced inflammation. All other macroscopic findings were randomly distributed across control and test article-treated monkeys, or were considered incidental.

5.1.4. Recovery Necropsy Animals (Day 540/542)

There were no gross observations in monkeys at the Recovery Necropsy that were attributed to ISIS 301012 administration. All macroscopic findings were considered incidental.

Organ Weights

Liver, kidney, spleen and testis exhibited s.s. ↑s in mass, while testis exhibited a n.s.s. ↓. Comparable effects were also apparent following the interim sacrifice. Effects on organ weight are partially reversed following the recovery period.

12-month Changes in Organ to Body Weight Ratio (fold over controls; ♂&♀ combined)

Organ	Dose Level			
	1 mg/kg/week	3 mg/kg/week	10 mg/kg/week	30 mg/kg/week
Kidney	1.01	1.18	1.18	1.43*
Liver	1.01	1.05	1.11	1.43*
Lung	0.97	1.07	1.21*	1.17*
Spleen	1.33	1.25	1.61*	6.30*
Testis	1.43	0.46	0.18	0.39
Inguinal Lymph Node	1.12	1.36	2.14	4.41*

* Statistically significant compared to controls

Histopathology

Adequate Battery Yes

Peer Review Yes

Histological Findings

The major toxicologically significant target appears to be the immune system. Lymphoid tissue throughout the body (*e.g.* lymph nodes, spleen, Peyer's patches) exhibit dose-dependent minimal-marked lymphoid hyperplasia. Lymph nodes also exhibit minimal-moderate histiocyte hypertrophy (presumably reflecting activation of histiocytes), minimal-mild sinus histiocytosis, and minimal-moderate accumulation of basophilic granules in the histiocytes (reflecting drug accumulation). These effects are seen at all doses.

Immune stimulation is disseminated as well. Multiple (non-immune) tissues have evidence of an ongoing proinflammatory response, primarily at HMD & HD (see notable exceptions below). These tissues had one or more of the following findings: the presence of lymphocytic infiltration, lymphoid follicles (neogenesis of tertiary lymphoid tissue), lymphoid hyperplasia, infiltration of mononuclear cells, sinus histiocytosis and histiocyte hypertrophy. Severity was generally minimal to mild. Nearly all examined tissues at HD showed some degree of lymphoid/monocyte immune infiltration/activation, which was partially reversible (these findings appear in their own table after the general histopathology findings).

The injection sites also showed dose-dependent immune cell infiltration (lymphocytes, histiocytes, neutrophils) and inflammation. Minimal-marked edema, minimal-moderate fibroplasia/fibrosis, minimal-marked hyperplasia (epidermis) was also observed.

Some tissues, particularly those with high levels of mipomersen, also have infiltration of histiocytes containing basophilic granules. This is thought to represent uptake of mipomersen by tissue resident macrophages (histiocytes). This finding showed partial reversibility.

Of potential interest are inflammatory effects in the nervous system and the vasculature (considered in more detail below). For example, minimal-mild lymphocytic infiltration of the choroid plexus is seen at all dose levels:

(♂ & ♀ together): C = 0, LD = 3, LMD = 2, HMD = 3, HD = 4

This finding (minimal) was still apparent in 2/3 HD recovery animals. Nonetheless it is not clear that this finding is adverse, and may reflect immune response to uptake of mipomersen by the choroid plexus.

Perivascular and intimal cellular infiltrates, often accompanied by intimal thickening in one or more arteries, in multiple tissues was seen in some mipomersen treated monkeys at terminal sacrifice, but were not seen at the interim (or recovery) sacrifice. Arterial intimal cellular infiltrates consisted of mononuclear (lymphocytes and plasma cells) or mixed (lymphocytes, plasma cells and eosinophils) cells immediately beneath the endothelium; similar cells were occasionally observed within the vascular media of some affected arteries. Arterial perivascular infiltrates occupied the loose adventitial connective tissue around affected vessels and were of a similar mononuclear or mixed cell character to infiltrates present in the intima. Intimal thickening was usually associated with intimal/perivascular cell infiltrates and consisted of increased basophilic matrix intermixed with rare to abundant spindle cells in the subendothelial space.

It is notable that similar intimal thickening was seen in a few of the control animals, but was considered incidental due to the lack of concurrent perivascular or intimal cellular infiltrates. Per the reviewing pathologist, nonspecific, but apparently immune-based arterial vasculitis (similar cell infiltrates in the intima, media and/or perivascular adventitia) is occasionally observed in cynomolgus monkeys and often considered incidental due to the infrequent occurrence and the common limited distribution to kidney, heart and gastrointestinal tissues. Because of the appearance of intimal thickening in some of the control animals, Sponsor considered intimal thickening to be drug related only when it was associated with intimal and/or perivascular inflammatory cell infiltrates.

The animals most clearly positive by this criteria were HD monkeys 5007 and 5603, which had findings (arterial perivascular and intimal mononuclear and/or mixed cell infiltrates with intimal thickening) in multiple tissues, including (but not limited to) the GI tract, liver, heart, sex organs, lymph nodes and pancreas (see Reviewer's table, below). Sponsor argues that the extent of the findings in these animals can be correlated with changes in the measured inflammatory biomarkers (C3, Bb, CRP, IgG, IgM, β2-microglobulin, and cytokines/chemokines); however, with the exception of β2-microglobulin in HD♂ 5007, and TNF-α in HD♀ 5603, the inflammatory biomarker values recorded for these animals are not necessarily remarkable for their dose cohort. In fact a better correlation exists between the inflammatory vascular findings and low platelet counts (both monkeys 5007 and 5603 had dosing holidays due to low platelet counts) and a high percentage of activated platelets, as monkeys 5007 and 5603 had the highest percentage of activated platelets seen in the study. This observation is not meant to imply causality, but does suggest a common etiology to both low platelet counts and the vascular lesions.

An additional HD♀ monkey (5502) and a single HMD♀ monkey (4504) exhibited very limited arterial cellular infiltrates in gastrointestinal tissues (cecum, colon and/or rectum) only at 12-months without associated intimal thickening, and (according to the Sponsor) lacked clearly-associated changes in inflammatory markers similar to those observed in Monkeys 5007 and 5603. As such, Sponsor concluded that a clear association of the limited vascular cell infiltrates in Monkeys 4504 and 5502 to mipomersen administration could not be made.

Animals/Tissues Affected By Vascular Effects of Mipomersen (Severity)					
		Dose (mg/kg/week)			
		10	30		
Finding	Animal #	4504	5502	5007	5603
Hyperplasia, intima, artery	Focal				Stomach(2)
	Multifocal			Cecum(1) Liver(3) Mes. Lymph Node(3) Heart(2) Pancreas(2) Prostate(3) Seminal Vesicle(3) Stomach(2) Testes(2)	Aorta Cecum(3) Cervix(3) Colon(3) Gallbladder(2) Heart(3) Ileum(1) Jejunum(1) Kidney(3) Lung(3) Mes. Lymph Node(2) Pan. Lymph Node(2)

					Injection Site(1) Ovary(2) Pancreas(2) Rectum(2) Trachea(3) Uterus(1) Vagina(3)
Hyperplasia, media, artery	Focal			Urinary Bladder(1) Pancreas(1)	
	Multifocal			Kidney(2)	
Perivasculitis, artery	Focal	Cecum(2)	Rectum(1)	Pancreas(1) Skeletal Muscle(1) Tongue(2)	Cecum(3) Injection Site(1) Trach. Lymph Node(2)
	Multifocal			Heart(1) Mes. Lymph Node(2) Pancreas(1) Pan. Lymph Node(2) Prostate(1) Seminal Vesicle(1) Stomach(2) Testes(1)	Cervix(2) Colon(2) Gallbladder(2) Heart(2) Ileum(2) Jejunum(2) Kidney(2) Liver(2) Ovary(1) Pancreas(2) Rectum(3) Stomach(2) Trachea(2) Uterus(2) Vagina(2)
Vasculitis, artery	Focal	Cecum(1)	Rectum(1)	Cecum(1) Pancreas(1) Skeletal Muscle(1) Tongue(1)	Injection Site(1) Trach. Lymph Node(1) Stomach(1)
	Multifocal			Heart(1) Liver(1) Mes. Lymph Node(1) Pancreas(1) Panc. Lymph Node(2) Prostate(1) Seminal Vesicle(1) Stomach(1) Testes(1)	Cecum(2) Cervix(2) Colon(1) Gallbladder(1) Heart(1) Liver(1) Mes. Lymph Node(1) Pan. Lymph Node(1) Ovary(1) Pancreas(1) Rectum(1) Trachea(1) Uterus(1) Vagina(1)

It should be noted that the slides showing vascular injury were subsequently reviewed by two experts outside of Isis and the CRO. Dr. Kerns is considered to be an expert in drug-induced vascular injury, while Dr. Palate an expert in the pathology of the macaque. Both external experts concluded that the vascular lesions referenced above cannot accurately be characterized as a vasculitis/perivasculitis, citing minimal medial changes (*e.g.*, no medial fibrinoid necrosis or fibrin leakage). The absence of perivascular hemorrhages was also noted. Both reviewers agreed that the vascular changes seen animals 5007 and 5603 were drug-related. Dr. Palate thought that the observed changes may indicate a chronic intimal injury with ongoing insult, suggested by infiltration of mixed inflammatory cells and cellular debris. Dr. Palate suggested that

the basophilic appearance of the intima could result from influx and proliferation of smooth muscle cells. The findings in other animals were considered likely to be incidental.

Arising largely from the vascular findings noted above, sections from the heart and kidney were stained with antibodies to C3, IgG or IgM to determine the distribution of these molecules and assess what role (if any) they played in the etiology of the vascular lesions.

C3 Kidney: No positive staining in any of the C samples. Positive in 1/2 HMD and 11/13 HD monkeys. Positive staining was of the cortical tubular epithelial cells.

C3 Heart: No positive staining in any of the samples examined from any dose group.

IgG Kidney & Heart: No positive staining in any of the samples examined from any dose group.

IgM Kidney: 2/14 C and 7/13 HD monkeys were positive. Positive cells identified as very rare to rare plasma cells within interstitial and/or subcapsular lymphoid cell aggregates/follicles.

IgM Heart: 4/13 HD monkeys were positive. Positive cells identified as very rare plasma cells within lymphoid cell aggregates in the myocardium.

-- No specific vascular-associated staining was detected with anti-C3, IgG or IgM

These same tissues were also stained for the presence of mipomersen (ISIS 301012) using an anti-mipomersen antibody. The results of this investigation were reported as follows:

Kidney:

ISIS 301012 staining was seen in kidney from ISIS 301012 treated animals. The intense staining was limited in proximal convoluted tubules in cortex. This was an expected finding as proximal tubular epithelium is a known site of oligonucleotide accumulation. Weak staining was also noticed in distal tubules. Of those 7 animals, two animals (Monkey Nos. 5007 and 5603) present with focal vascular inflammatory lesion (moderate arierial media thickening with mononuclear cell infiltration) in the juction between cortex and medulla. No or only very weak ISIS 301012 staining was detected in the arterial wall within the lesion area in both kidneys.

Heart:

Minimal ISIS 301012 staining was detected in myocardium from drug treated animal. Similar to kidney findings, focal inflammatory lesions of small to medium sized arteries were also present in Monkey Nos. 5007 and 5603. For Monkey 4601, mutiple sections of heart were cut but the intimal thickning of the epicardial coronary artery in the right heart with accompanying minimal mononuclear cells infiltrates was not found. Weak ISIS 301012 staining was observed in the arterial wall (tunica media and endothelial cell) within the lesion area. The only postive staining was observed in resident macrophages and in perivascular infiltrating macrophage were detected in both heart sections.

Selected Histopathology Findings -- Incidence (mean severity)											
	Sex	Male					Female				
	Dose (mkw)	0	1	3	10	30	0	1	3	10	30
Tissue/Finding	Interval										
Aorta											
Basophilic granules, Histiocyte, lymphoid tissue	Terminal	0/3 -	0/3 -	0/3 -	1/3 (2.0)	1/3 (2.0)	0/3 -	0/3 -	0/2 -	1/3 (2.0)	1/3 (2.0)
	Recovery	0/2 -				1/2 (1.0)	0/2 -				0/1 -
Cecum											
Hemosiderin	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	1/3 (1.0)	0/3 -	0/3 -	0/2 -	0/3 -	0/3 -
Dilated/Cystic Gland, Mucosa, focal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	1/3 (1.0)	0/2 -	1/3 (1.0)	0/3 -
Dilated/Cystic Gland, Mucosa, multifocal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	1/3 (1.0)
Hemorrhage, Lymphoid Follicle, submucosa	Recovery	0/2 -				1/2 (1.0)	0/2 -				0/1 -
Duodenum											
Hemorrhage, Mucosa	Terminal	0/3 -	1/3 (1.0)	0/3 -	0/3 -	1/3 (1.0)	0/3 -	0/3 -	1/2 (1.0)	1/3 (1.0)	1/3 (1.0)
	Recovery	0/2 -				0/2 -	0/2 -				1/1 (1.0)
Esophagus											
Basophilic granules, Histiocyte, lymphoid tissue	Terminal	0/3 -	0/3 -	0/3 -	2/3 (2.0)	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	0/3 -
Heart											
Recanalization, coronary artery, right, focal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	1/3 (P)
Ileum											
Hemorrhage, Mucosa	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	1/2 (1.0)	0/3 -	1/3 (1.0)
Jejunum											
Hemorrhage, Mucosa	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	1/3 (2.0)
Kidney											
Basophilic granules, Histiocyte, lymphoid tissue	Interim	0/2 -	2/2 (1.0)	2/2 (1.0)	2/2 (2.5)	2/2 (2.5)	0/2 -	2/2 (1.0)	2/2 (1.0)	2/2 (2.5)	2/2 (3.0)
	Terminal	0/3 -	3/3 (1.0)	3/3 (1.3)	3/3 (2.0)	3/3 (2.3)	0/3 -	3/3 (1.0)	2/2 (1.5)	3/3 (2.0)	3/3 (2.7)
	Recovery	0/2 -				0/2 -	0/2 -				1/1 (1.0)
Hemorrhage, Interstitium	Interim	0/2	0/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2

focal		-	-	(1.0)	-	(1.0)	-	-	-	-	-
Hemorrhage, mesangium. Glomerulus, focal	Interim	0/2 -	0/2 -	1/2 (2.0)	0/2 -	0/2 -	0/2 -	0/2 -	0/2 -	0/2 -	0/2 -
Hemorrhage, Tubules, multifocal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	1/3 (1.0)	0/3 -	0/3 -	0/2 -	0/3 -	0/3 -
Hypertrophy, Mesangial cell, Glomerulus, focal	Interim	0/2 -	0/2 -	1/2 (2.0)	1/2 (2.0)	0/2 -	0/2 -	0/2 -	1/2 (2.0)	0/2 -	0/2 -
Vacuolation, Cytoplasm, Tubular epithelium, multifocal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	3/3 (2.0)	0/3 -	0/3 -	0/2 -	1/3 (3.0)	3/3 (1.7)
	Recovery	0/2 -				0/2 -	0/2 -				1/1 (1.0)
Degeneration, Tubular epithelium, multifocal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	3/3 (1.0)	0/3 -	0/3 -	0/2 -	1/3 (2.0)	1/3 (1.0)
Cyst, Tubules, Cortex, multifocal	Recovery	0/2 -				1/2 (3.0)	0/2 -				0/1 -
Thickening, Mesangium, diffuse	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	1/3 (2.0)
Liver											
Hypertrophy/Hyperplasia, Kupffer cell, diffuse	Interim	0/2 -	0/2 -	1/2 (1.0)	2/2 (2.0)	2/2 (3.0)	0/2 -	0/2 -	0/2 -	2/2 (2.5)	2/2 (3.0)
	Terminal	0/3 -	0/3 -	2/3 (1.5)	3/3 (1.7)	3/3 (3.3)	0/3 -	0/3 -	2/2 (1.0)	3/3 (3.0)	3/3 (2.7)
Hypertrophy/Hyperplasia, Kupffer cell, multifocal	Interim	0/2 -	0/2 -	1/2 (1.0)	0/2 -	0/2 -	0/2 -	0/2 -	2/2 (1.0)	0/2 -	0/2 -
Basophilic granules, Histiocyte, lymphoid tissue	Interim	0/2 -	0/2 -	2/2 (1.5)	2/2 (3.0)	2/2 (3.0)	0/2 -	0/2 -	2/2 (2.0)	2/2 (3.0)	2/2 (3.0)
	Terminal	0/3 -	1/3 (1.0)	3/3 (1.3)	3/3 (1.7)	3/3 (3.0)	0/3 -	2/3 (1.0)	2/2 (1.0)	3/3 (3.0)	3/3 (2.7)
Hyperplasia, Bile duct, multifocal	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	1/3 (2.0)
Vacuolation, cytoplasm, hepatocyte	Terminal	1/3 (1.0)	0/3 -	1/3 (1.0)	0/3 -	2/3 (1.0)	0/3 -	0/3 -	0/2 -	0/3 -	0/3 -
	Recovery	0/2 -				1/2 (2.0)	0/2 -				0/1 -
Lymph Node (Inguinal)											
Basophilic granules, Histiocyte, lymphoid tissue	Interim	0/2 -	1/2 (2.0)	1/2 (1.0)	2/2 (2.0)	2/2 (1.5)	0/2 -	2/2 (2.0)	2/2 (2.0)	2/2 (2.0)	2/2 (2.0)
	Terminal	0/3 -	1/3 (1.0)	2/3 (1.5)	3/3 (2.7)	3/3 (2.3)	0/3 -	1/3 (2.0)	2/2 (1.0)	3/3 (2.0)	3/3 (2.0)
	Recovery	0/2 -				1/2 (2.0)	0/2 -				0/1 -
Plasmacytosis	Terminal	1/3 (1.0)	2/3 (1.5)	1/3 (1.0)	3/3 (1.0)	1/3 (2.0)	0/3 -	3/3 (1.7)	1/3 (2.0)	1/3 (1.0)	3/3 (2.0)
Lymph Node (Mandibular)											
Basophilic granules, Histiocyte, lymphoid tissue	Interim	0/2 -	0/2 -	0/2 -	2/2 (2.5)	1/2 (1.0)	0/2 -	0/2 -	0/2 -	2/2 (2.5)	2/2 (2.5)
	Terminal	0/3 -	0/3 -	1/3 (1.0)	3/3 (2.0)	3/3 (2.7)	0/3 -	0/3 -	1/2 (1.0)	3/3 (2.0)	3/3 (2.3)
Lymph Node (Mesenteric)											
Basophilic granules, Histiocyte, lymphoid tissue	Interim	0/2 -	2/2 (1.5)	1/2 (2.0)	2/2 (2.0)	2/2 (2.0)	0/2 -	0/2 -	2/2 (2.0)	2/2 (2.0)	2/2 (3.0)
	Terminal	0/3 -	0/3 -	2/3 (2.0)	3/3 (2.0)	3/3 (2.0)	0/3 -	1/3 (1.0)	2/2 (1.0)	3/3 (2.3)	3/3 (2.7)
	Recovery	0/2 -				2/2 (2.0)	0/2 -				1/1 (1.0)
Plasmacytosis	Terminal	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	1/3 (2.0)
Paracortical expansion	Recovery	0/2 -				1/2 (1.0)	0/2 -				0/1 -
Pancreas											

Ectopic tissue, Spleen	Terminal	0/3 -	0/3 -	0/3 -	1/3 (P)	1/3 (P)	0/3 -	0/3 -	0/2 -	1/3 (P)	0/3 -
Basophilic granules, Histiocyte, lymphoid tissue	Terminal	0/3 -	0/3 -	0/3 -	3/3 (1.7)	1/3 (2.0)	0/3 -	0/3 -	0/2 -	2/3 (2.0)	1/3 (2.0)
	Recovery	0/2 -				2/2 (2.0)	0/2 -				1/1 (3.0)
Thymus											
Basophilic granules, Histiocyte, lymphoid tissue	Terminal	0/3 -	0/3 -	0/3 -	1/3 (?)	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	2/3 (2.5)
Urinary Bladder											
Basophilic granules, Histiocyte, lymphoid tissue	Interim	0/2 -	0/2 -	0/2 -	2/2 (2.0)	2/2 (2.0)	0/2 -	0/2 -	0/2 -	1/2 (2.0)	2/2 (2.0)
	Terminal	0/3 -	0/3 -	0/3 -	3/3 (1.3)	3/3 (2.3)	0/3 -	0/3 -	0/2 -	3/3 (2.0)	3/3 (2.0)
	Recovery	0/2 -				2/2 (1.5)	0/2 -				0/1 -
Sclerosis, Artery	Interim	0/2 -	0/2 -	0/2 -	1/2 (3.0)	0/2 -	0/2 -	0/2 -	0/2 -	0/2 -	0/2 -
	Terminal	0/3 -	0/3 -	0/3 -	1/3 (3.0)	0/3 -	0/3 -	0/3 -	0/2 -	0/3 -	0/3 -

Immunostimulatory Findings (excluding vascular infiltrations noted above) at Terminal Sacrifice -- Incidence										
Sex	**Male**					**Female**				
Dose (mg/kg/week)	**0**	**1**	**3**	**10**	**30**	**0**	**1**	**3**	**10**	**30**
Aorta										
Hypertrophy, Histiocyte, Lymphoid tissues					1/3				1/3	1/3
Bone Marrow										
Infiltrate, Lymphocyte, focal			2/3					1/2		
Lymphoid Follicle		2/3		2/3	2/3			1/2	1/3	1/3
Hyperplasia, Lymphoid, Lymphoid Follicle					1/3					1/3
Bone, Femur										
Lymphoid Follicle, marrow				2/3	2/3					
Hyperplasia, Lymphoid, Lymphoid Follicle, marrow										1/3
Bone, Rib										
Lymphoid Follicle, Marrow										2/3
Brain										
Infiltrate, Mononuclear cell, Perivascular, Parenchyma		1/3			1/3					
Infiltrate, Lymphocyte, Choroid plexus		1/3	1/3	2/3	2/3		2/3	1/2	1/3	2/3
Infiltrate, Lymphocyte, Meninges		1/3		1/3					1/3	
Infiltrate, Histiocyte, Choroid plexus					1/3					
Hyperplasia, Lymphoid, Lymphoid Follicle, Choroid plexus										1/3
Cervix										
Infiltrate, Mononuclear cell, Muscularis										1/3
Duodenum										
Infiltrate, Mononuclear cell, Muscularis					2/3					1/3
Epididymis										
Infiltrate, Lymphocyte, Interstitium					1/3					
Infiltrate, Mononuclear cell, Interstitium					1/3					
Esophagus										
Infiltrate, Lymphocyte, Muscularis	1/3		1/3	1/3	1/3					1/3
Infiltrate, Lymphocyte, Submucosa					2/3					
Infiltrate, Mixed cell, Submucosa			1/3							
Hypertrophy, Histiocyte, Lymphoid tissues				2/3						
Lymphoid Follicle			1/3							
Eye										

Infiltrate, Lymphocyte, Choroid					1/3					1/3
Infiltrate, Lymphocyte, Ciliary body		1/3		1/3	1/3					1/3
Infiltrate, Lymphocyte, Sclera					1/3					
Lymphoid Follicle, Sclera					1/3					
Gallbladder										
Infiltrate, Mononuclear cell, Submucosa					2/3				1/3	
Lymphoid Follicle, Submucosa					1/3			1/2		
Heart										
Infiltrate, Mononuclear cell, Myocardium	1/3	2/3	3/3	3/3	3/3	2/3	2/2	1/2	2/3	3/3
Infiltrate, Mononuclear cell, Epicardium			1/3		1/3		1/3	1/2		2/3
Infiltrate, Mononuclear cell, Endocardium			1/3		1/3		1/3			1/3
Lymphoid Follicle, Myocardium					1/3					
Lymphoid Follicle, Epicardium					1/3					
Injection Site 1 (representative of all sites)										
Infiltrate, Mixed cell, Intramuscular										1/3
Infiltrate, Mixed cell, Dermis										1/3
Infiltrate, Mononuclear cell, Intramuscular					2/3				1/3	1/3
Infiltrate, Mononuclear cell, Subcutaneous									1/3	
Infiltrate, Mononuclear cell, Perivascular					2/3				1/3	1/3
Infiltrate, Histiocyte, Intramuscular					1/3					1/3
Infiltrate, Histiocyte, Subcutaneous					1/3				1/3	2/3
Infiltrate, Histiocyte, Dermis					1/3				1/3	
Inflammation, Mixed, Subcutaneous					1/3				1/3	1/3
Inflammation, Neutrophilic, Subcutaneous					1/3					1/3
Lymphoid Follicle, Subcutaneous									1/3	
Jejunum										
Hyperplasia, Lymphoid, Peyer's patches			1/3	1/3	1/3		1/3	1/2		
Kidney										
Infiltrate, Mononuclear Cell, Capsule, diffuse					1/3					
Infiltrate, Mononuclear Cell, Interstitium	1/3	3/3	2/3	2/3	3/3	2/3	3/3	2/2	3/3	3/3
Lymphoid Follicle, Interstitium										1/3
Lymphoid Follicle, Subcapsular				1/3	2/3					
Lymphoid Follicle, Hilus				1/3	1/3		1/3			1/3
Inflammation, Mixed cell, Hilus					1/3					1/3
Liver										
Hypertrophy/Hyperplasia, Kupffer cell		1/3	3/3	3/3	3/3		2/3	2/2	3/3	3/3
Infiltrate, Mononuclear Cell, Periportal	1/3			1/3	2/3		1/3	1/2	1/3	2/3
Infiltrate, Mononuclear Cell, Periductal					1/3					
Lymphoid Follicle, Parenchyma				2/3				1/2	1/3	1/3
Lung										
Lymphoid Follicle, Alveolar wall					1/3					1/3
Infiltrate, Lymphocyte, Alveolar wall					1/3					
Lymph Node, Mesenteric (representative of LNs)										
Hypertrophy, Histiocyte			2/3	3/3	3/3			2/2	3/3	3/3
Sinus Histiocytosis			1/3	3/3	3/3		1/3	1/2	2/3	1/3
Hyperplasia, Lymphoid		2/3	3/3	2/3	3/3		3/3	2/2	3/3	2/3
Plasmacytosis										1/3
Lymphoid Follicle, Mesentery					1/3					
Nerve, Sciatic										
Infiltrate, Mononuclear cell, Epineurium				2/3	2/3					1/3
Infiltrate, Mononuclear cell, Endoneurium, Multifocal					1/3					1/3
Infiltrate, Mixed cell, Perineural										1/3
Lymphoid Follicle, Epineurium					1/3					
Pancreas										
Hyperplasia, Lymphoid, Lymphoid tissue, Mesentery			1/3	2/3	1/3					
Sinus Histiocytosis, Lymphoid tissue				1/3	1/3				1/3	1/3
Hypertrophy, Histiocyte, Lymphoid tissue					1/3					

Lymphoid Follicle, Interstitium				1/3	1/3					
Pituitary										
Infiltrate, Mononuclear cell				3/3	1/3				1/3	
Prostate										
Infiltrate, Mononuclear cell, Interstitium, Multifocal					2/3					
Infiltrate, Mononuclear cell, Perieurthral					1/3					
Lymphoid Follicle, Interstitium					1/3					
Rectum										
Hyperplasia, Lymphoid, Submucosa		3/3	3/3	2/3	1/3	1/3		1/2	2/3	2/3
Infiltrate, Lymphocyte, Muscularis					1/3					
Infiltrate, Neutrophil, Duct, Intramuscular										1/3
Salivary Gland, Mandibular										
Hyperplasia, Lymphoid, Lymphoid follicle, Interstitium			1/3		2/3				1/3	1/3
Seminal Vesicle										
Infiltrate, Mononuclear cell, Interstitium				1/3	2/3					
Hyperplasia, Lymphoid, Lymphoid follicle, Interstitium					1/3					
Skeletal Muscle (representative of muscle)										
Infiltrate, Mixed cell										1/3
Infiltrate, Mononuclear cell					2/3					
Skin										
Infiltrate, Mononuclear cell, Subcutaneous					2/3					
Infiltrate, Mononuclear cell, Dermis					2/3			1/2		
Spleen										
Hyperplasia, Lymphoid		3/3	2/3	1/3	3/3	2/3	1/3	2/2	3/3	3/3
Hypertrophy, Lymphoid Follicle			2/3	1/3				1/2		
Stomach										
Infiltrate, Lymphocyte, Muscularis, Fundus				1/3	2/3					
Infiltrate, Lymphocyte, Submucosa, Fundus					1/3					
Infiltrate, Lymphocyte, Perivascular					1/3					
Infiltrate, Lymphocyte, Mesentery					1/3					
Infiltrate, Mononuclear cell, Muscularis										1/3
Hypertrophy, Histiocyte, Lymphoid tissue									1/3	
Thymus										
Hypertrophy, Histiocyte, Lymphoid tissue				1/3						2/3
Hyperplasia, Lymphoid, Lymphoid tissue, Mediastinal										2/3
Plasmacytosis, Lymphoid Follicle, Mediastinal										1/3
Thyroid										
Lymphoid Follicle, Interstitium		1/3			1/3					
Hyperplasia, Lymphoid, Lymphoid Follicle, Interstitium										1/3
Tongue										
Infiltrate, Mononuclear cell, Muscularis		1/3			2/3					1/3
Trachea										
Hypertrophy, Histiocyte, Lymphoid tissue					1/3					1/3
Urinary Bladder										
Infiltrate, Histiocyte, Submucosa				3/3	3/3				3/3	3/3
Infiltrate, Mononuclear cell, Muscularis					1/3					
Lymphoid Follicle, Submucosa		1/3	1/3							
Uterus										
Infiltrate, Mononuclear cell, Myometrium									1/3	1/3

Unless otherwise indicated, findings with variable distribution (*i.e.,* focal, multifocal or diffuse) have been combined into a single entry for brevity.

Toxicokinetics

Dose-related, hyperproportional increases were seen in plasma AUC exposure. Accumulation is apparent for all doses at 6 months, compared to Day 1. Plasma trough levels, measured 7 days after dosing, increased 2- to 3-fold with repeated dosing following 27 weeks of dosing, compared to Day 22, and essentially plateaued thereafter.

Tissue levels increased dose-dependently, but generally hypoproportionally, especially at higher doses. The highest concentrations were measured in the kidney and liver. There were no consistent increases in tissue levels at 12 months, compared to 6 months, suggesting that tissue steady state may have been reached by 6 months.

Table 2. Plasma pharmacokinetic parameters of ISIS 301012 on Days 1, 183 and 358 following subcutaneous treatment(s) with ISIS 301012 to cynomolgus monkeys for up to 1 year.

Group	Dose (mg/kg/week)	Day	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0\rightarrow 48\ hr}$ (µg*hr/mL)	$AUC_{0\rightarrow\infty}$ (µg*hr/mL)	$t_{1/2\lambda z}$* (hr)
			Mean ± SD	Median (Min-Max)	Mean ± SD	Mean ± SD	Mean ± SD
2	1	1	0.817 ± 0.230	2 (1-2)	5.16 ± 1.04	5.25 ± 1.06	9.51 ± 1.82
		183	1.31 ± 0.45	1 (1-2)	7.30 ± 1.62	8.02 ± 2.10	14.6 ± 4.8
		358	1.05 ± 0.40	2 (1-4)	8.88 ± 2.14	9.45 ± 2.22	12.9 ± 1.5
3	3	1	3.82 ± 1.25	2 (2-2)	18.5 ± 2.9	18.7 ± 2.9	9.35 ± 1.08
		183	5.54 ± 1.42	2 (1-4)	26.7 ± 6.6	28.4 ± 8.1	14.9 ± 4.2
		358	3.24 ± 1.33	2 (1-2)	19.3 ± 4.1	20.4 ± 3.8	14.1 ± 3.6
4	10	1	15.8 ± 3.9	2 (1-4)	112 ± 42	113 ± 42	8.00 ± 2.88
		183	23.2 ± 4.5	2 (1-2)	127 ± 33	128 ± 35	10.0 ± 2.5
		358	17.4 ± 5.8	2 (1-4)	156 ± 40	162 ± 45	10.7 ± 2.4
5	30	1	46.6 ± 12.0	2 (1-2)	518 ± 119	522 ± 118	6.60 ± 1.36
		183	53.1 ± 10.0	2 (1-4)	627 ± 180	638 ± 194	7.46 ± 1.48
		358	33.9 ± 9.9	2 (1-4)	448 ± 116	466 ± 121	10.2 ± 2.4

Data represented are mean ± standard deviation or median (range) (N=5 to 14).

* The last three time points were used to define λ_z where t_{last} = 48 hr.

Table 3. Plasma pharmacokinetic parameters of ISIS 301012 in recovery animals following 30 mg/kg/week dose on Day 358.

Animal No.	Gender	Cmax (µg/mL)	Tmax (hr)	$AUC_{0\rightarrow\tau}$ (µg*hr /mL)	$AUC_{0\rightarrow\infty}$ (µg*hr /mL)	$t_{1/2\lambda z}$* (day)	Accumulation Index**
5003	M	41.8	2	724	945	37.3	8
5006	M	29.3	2	594	744	23.7	5
5507	F	28.8	2	488	884	28.1	6
Mean ± SD		**33.3 ± 7.4**	**2 ± 0**	**602 ± 118**	**858 ± 103**	**29.7 ± 6.9**	**7 ± 1**

* The last three time points were used to define λ_z where t_{last} = 92 days (2208 hours).

** Accumulation index = $1/(1-e^{(-\lambda z^*\tau)})$.

Table 4. Plasma trough ISIS 301012 concentrations (ng/mL) collected 7 days after dose following 1, 3, 6 and 12 months of treatment to cynomolgus monkeys with ISIS 301012 administered subcutaneously

				Plasma Trough ISIS 301012 Conc. (ng/mL)			
Group	Dose Level mg/kg/week	Study Day	N	Mean ± SD	SE	Median	Min-Max
2	1	22	7	4.74 ± 2.35	0.89	5.67	(1.62-7.34)
		92	9	10.9 ± 9.8	3.3	6.0	(2.5-30.9)
		183	8	15.7 ± 11.3	4.0	13.2	(4.5-35.3)
		267	4	17.9 ± 10.0	5.0	17.2	(6.3-30.7)
		295	6	16.0 ± 10.0	4.1	15.1	(4.9-30.9)
		323	4	17.2 ± 6.6	3.3	15.9	(11.6-25.4)
		358	6	15.0 ± 6.2	2.5	14.8	(5.0-22.5)
3	3	22	10	11.7 ± 3.4	1.1	11.2	(7.2-16.9)
		92	10	15.1 ± 7.4	2.3	15.3	(3.8-27.9)
		183	9	53.2 ± 42.0	14.0	31.4	(14.6-138.1)
		267	6	32.4 ± 19.4	7.9	27.7	(10.2-66.8)
		295	6	48.8 ± 60.0	24.5	26.6	(10.4-170.2)
		323	5	24.1 ± 5.4	2.4	25.0	(18.4-30.3)
		358	5	16.4 ± 5.6	2.5	17.6	(10.4-23.2)
4	10	22	10	33.1 ± 10.6	3.3	32.3	(21.3-57.2)
		92	10	43.9 ± 7.7	2.4	41.3	(35.0-55.6)
		183	9	107 ± 72	24	82	(43-276)
		267	6	166 ± 50	21	174	(74-221)
		295	6	178 ± 54	22	185	(100-247)
		323	6	182 ± 55	23	183	(115-266)
		358	6	218 ± 133	54	199	(84-451)
5	30	22	14	147 ± 64	17	116	(81-261)
		92	14	225 ± 150	40	206	(80-670)
		183	14	543 ± 947	253	258	(138-3805)
		267	9	475 ± 287	96	351	(221-1147)
		295	9	449 ± 257	86	392	(169-1030)
		323	9	667 ± 283	94	622	(356-1197)
		358	9	325 ± 111	37	307	(150-507)

N= number of observations

Table 5. ISIS 301012 and total oligonucleotide concentrations (µg/g) in major tissues collected 2 days following 6 months (on Day 185) and 1 year (on Day 360) of treatment to cynomolgus monkeys with ISIS 301012 administered subcutaneously.

			6-Month		1-Year	
Organ	Group	Dose (mg/kg/week)	ISIS 301012 (µg/g)	Total Oligonucleotide (µg/g)	ISIS 301012 (µg/g)	Total Oligonucleotide (µg/g)
Ileum	2	1	2.21 ± 0.83	7.18 ± 7.44	0.690 ± 1.080	2.15 ± 2.77
	3	3	9.28 ± 3.88	18.8 ± 18.9	13.3 ± 15.5	16.8 ± 20.7
	4	10	49.2 ± 30.4	73.6 ± 38.8	24.6 ± 4.5	29.0 ± 8.5
	5	30	62.1 ± 11.9	87.7 ± 54.6	52.9 ± 23.6	75.4 ± 40.4
Kidney Cortex	2	1	181 ± 37	218 ± 79	198 ± 29	210 ± 35
	3	3	425 ± 151	504 ± 295	373 ± 117	474 ± 130
	4	10	1248 ± 256	1477 ± 288	985 ± 565	1081 ± 643
	5	30	1821 ± 403	2041 ± 334	1898 ± 342	2271 ± 421
Kidney Medulla	2	1	89.1 ± 40.0	112 ± 63	117 ± 37	124 ± 44
	3	3	223 ± 89	246 ± 98	176 ± 128	185 ± 135
	4	10	639 ± 169	706 ± 229	507 ± 385	648 ± 509
	5	30	519 ± 174	519 ± 174	958 ± 601	1259 ± 791
Liver	2	1	40.2 ± 8.6	79.5 ± 15.2	42.8 ± 11.5	70.3 ± 12.6
	3	3	278 ± 161	376 ± 140	169 ± 39	298 ± 42
	4	10	599 ± 139	742 ± 182	547 ± 138	638 ± 177
	5	30	838 ± 152	1088 ± 578	896 ± 431	1112 ± 370

Data represented are mean ± standard deviation (N=4 to 6).

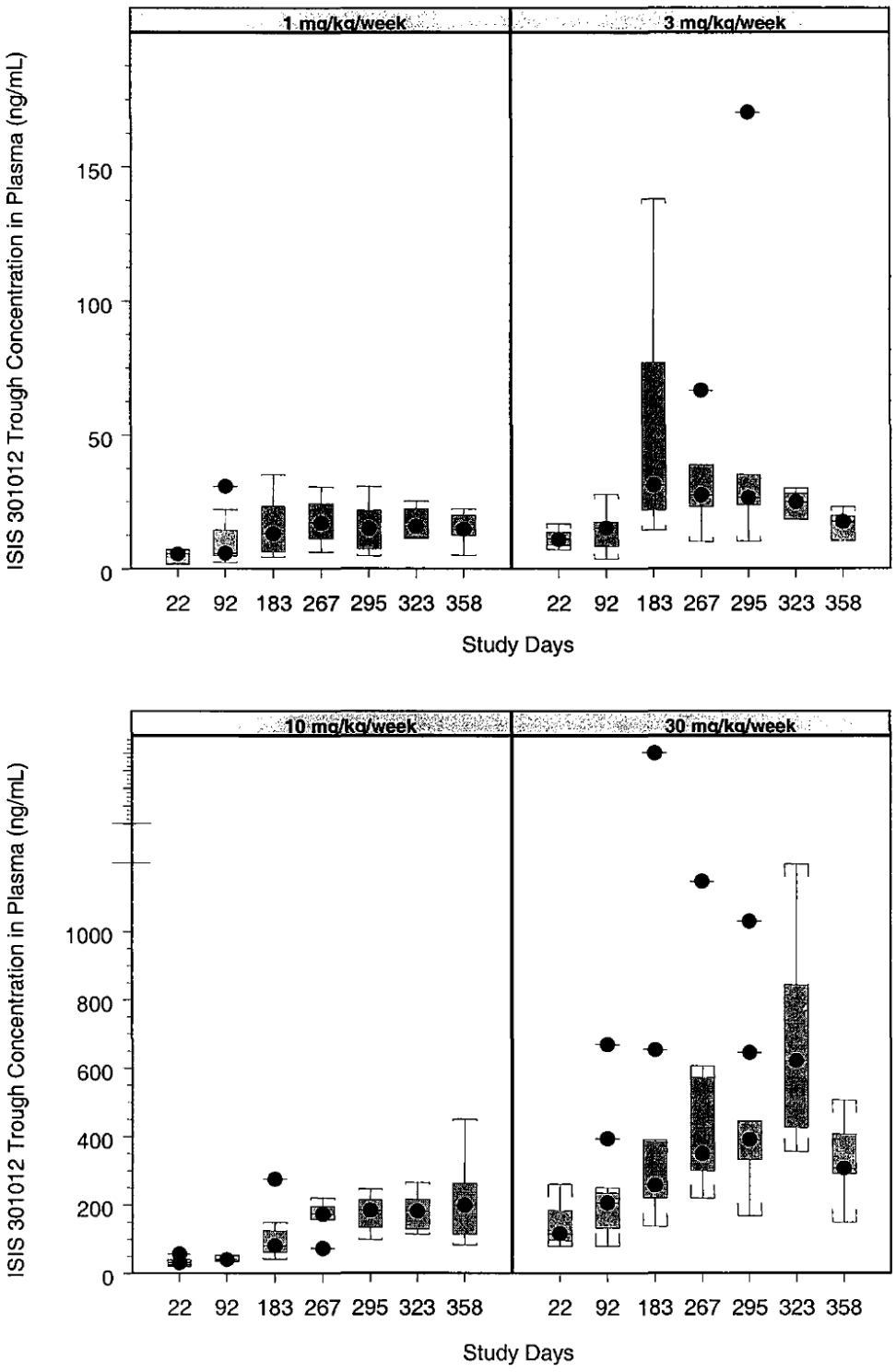


Figure 5. Plasma trough concentrations of ISIS 301012 as a function of treatment days by dose groups following s.c. administration up to 1 year.

Definition: The line in the box is median, the box boundaries represent 25th and 75th percentile, and the whiskers represent 75th percentile + (1.5 * IQR) (upper cap) and 25th percentile - (1.5 * IQR) (lower cap), respectively. The inter-quartile range, or IQR, is defined to be the 75th percentile minus the 25th percentile. The IQR (inter-quartile range) is essentially the height of the box.

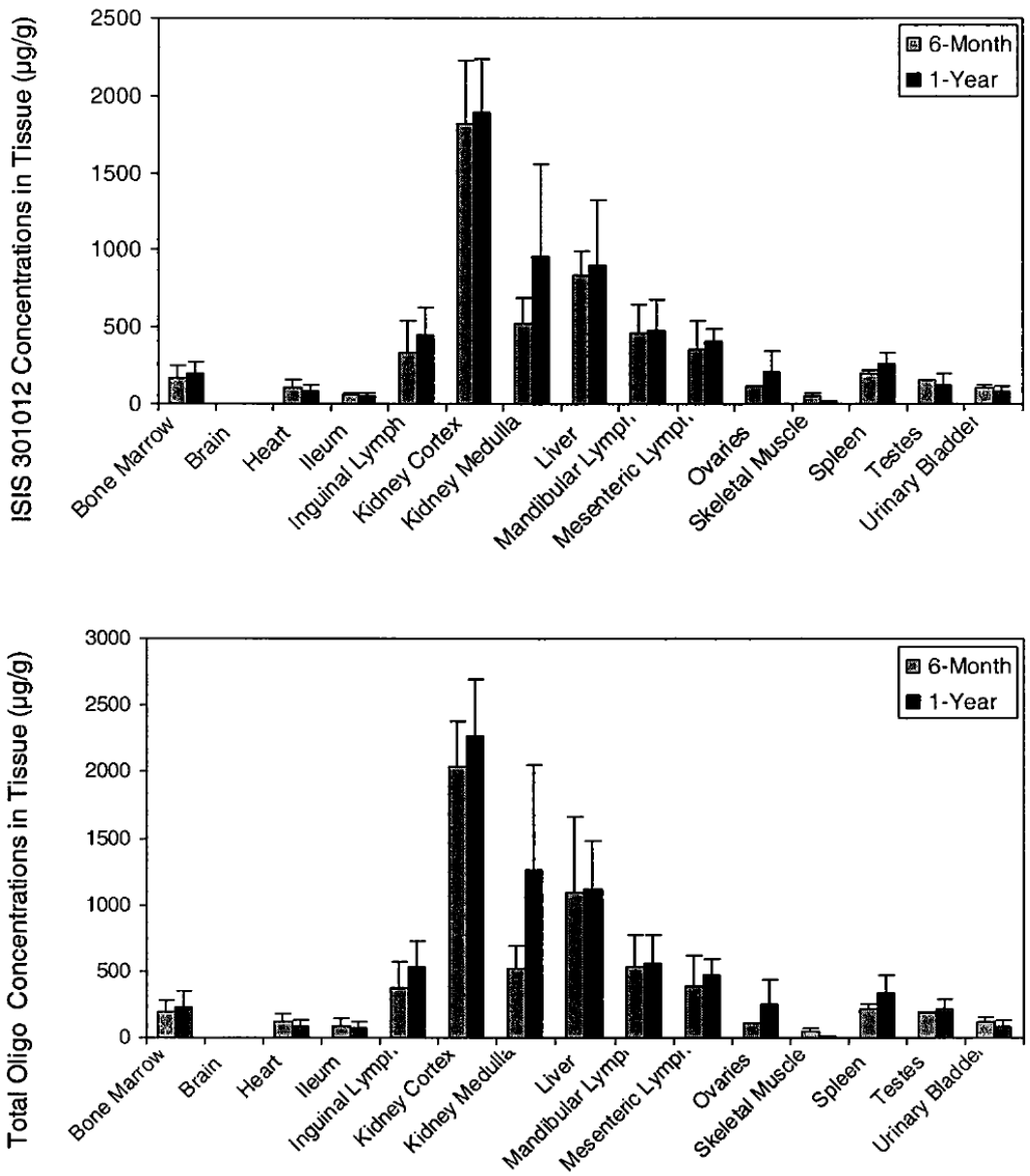


Figure 6. **Tissue distribution of ISIS 301012 (top) and total oligonucleotide (bottom) (µg/g) in monkey tissues collected 2 days following 6 months and 1 year treatment with 30 mg/kg/week ISIS 301012 administered subcutaneously (n=1 to 6). Error bars represent standard deviation. No error bar is shown if n<3.**

PD Marker

Mipomersen treatment caused a dose-dependent reduction in ApoB expression level in the liver at 6 and 12 months. Effects on plasma ApoB expression level was less clear, but HD appeared to cause decreased expression at 6 & 12 months.

Immunogenicity

Anti-mipomersen antibodies were measured using a validated ELISA method involving binding of ADA to plate-immobilized biotinylated mipomersen. Eleven samples exhibited a positive signal upon initial screening. Of these, seven were positive in a confirmatory binding assay, and were assessed for specificity. Specificity was determined in a competition assay by spiking in either mipomersen (ISIS 301012) or two sequence-unrelated ISIS oligonucleotides. Samples demonstrating a $\geq$ 50% reduction in signal with mipomersen, but not the other two oligonucleotides were considered to be specific for mipomersen. Of the seven samples screened, three were considered to be specific for mipomersen. However, all samples with positive binding activity were found to be of very low titer (1:100). It seems unlikely that these low titer antibodies materially affected the study or contributed to any of the toxicity findings noted above.

Specificity Results for Anti-ISIS 301012

(b) (4) (UUQ2) ID	Animal #	Study Day	Sample Date	Re-run Result*	Re-run Conclusion	1uM 301012 Result*	10uM 301012 Result*	Sample Diluent Result*	301012 Conclusion	1uM 13920 Result*	10uM 13920 Result*	Sample Diluent Result*	13920 Conclusion	1uM 3521 Result*	10uM 3521 Result*	Sample Diluent Result*	3521 Conclusion
55	2005	1	12/14/2005	0.020	N	0.031	0.036	0.019	N/A	0.039	0.045	0.021	N/A	0.039	0.048	0.025	N/A
56	2005	94	3/17/2006	0.044	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
57	2005	185	6/16/2006	0.106	P	0.072	0.044	0.106	S	0.232	0.205	0.133	U	0.257	0.240	0.133	R
187	2005	269	9/8/2006	0.069	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
225	2005	360	12/8/2006	0.043	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
85	3005	1	12/15/2005	0.024	N	0.045	0.041	0.019	N/A	N/A	N/A	N/A	N/A	0.023	0.023	0.014	N/A
86	3005	94	3/18/2006	0.021	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
87	3005	185	6/17/2006	0.139	P	0.159	0.120	0.141	T	N/A	N/A	N/A	N/A	0.125	0.097	0.068	Q
127	4505	1	12/15/2005	0.022	N	0.020	0.035	0.022	N/A	0.040	0.040	0.024	N/A	0.046	0.045	0.030	N/A
128	4505	94	3/18/2006	0.021	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
129	4505	185	6/17/2006	0.113	P	0.099	0.059	0.104	T	N/A	N/A	N/A	N/A	0.451	0.457	0.247	Q
202	4505	269	9/9/2006	0.206	P	0.136	0.063	0.197	S	0.565	0.581	0.336	U	0.639	0.686	0.442	R
239	4505	360	12/9/2006	0.101	P	0.070	0.038	0.108	S	0.390	0.383	0.155	U	0.412	0.454	0.205	R
151	5007	1	12/15/2005	0.014	N	0.031	0.032	0.020	N/A	N/A	N/A	N/A	N/A	0.039	0.036	0.017	N/A
152	5007	94	3/18/2006	0.134	RP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
153	5007	185	6/17/2006	0.307	P	0.232	0.203	0.370	T	N/A	N/A	N/A	N/A	0.636	0.634	0.354	Q
207	5007	269	9/9/2006	0.467	P	0.443	0.290	0.537	T	N/A	N/A	N/A	N/A	0.810	0.863	0.543	Q
166	5506	1	12/15/2005	0.034	N	0.033	0.043	0.023	N/A	0.041	0.053	0.025	N/A	0.025	0.033	0.016	N/A
167	5506	94	3/18/2006	0.036	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
168	5506	185	6/17/2006	0.203	P	0.140	0.066	0.185	S	0.477	0.436	0.199	U	0.294	0.292	0.153	R
210	5506	269	9/9/2006	0.247	P	0.186	0.066	0.246	S	0.299	0.302	0.260	U	0.222	0.236	0.197	R

Specificity Results for Anti-ISIS 301012 (cont)

(b) (4) ID (UUQ2)	Animal #	Study Day	Sample Date	Re-run Result*	Re-run Conclusion	1uM 301012 Result*	10uM 301012 Result*	Sample Diluent Result*	301012 Conclusion	1uM 13920 Result*	10uM 13920 Result*	Sample Diluent Result*	13920 Conclusion	1uM 3521 Result*	10uM 3521 Result*	Sample Diluent Result*	3521 Conclusion
169	5507	1	12/15/2005	0.014	N	0.029	0.039	0.014	N/A	N/A	N/A	N/A	N/A	0.035	0.044	0.025	N/A
170	5507	94	3/18/2006	0.034	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
171	5507	185	6/17/2006	0.044	RN	0.129	0.109	0.095	T	N/A	N/A	N/A	N/A	0.242	0.181	0.169	Q
211	5507	269	9/9/2006	0.122	RP	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
133	5001	1	12/13/2005	0.011	N	0.021	0.027	0.012	N/A	0.023	0.028	0.015	N/A	0.023	0.024	0.014	N/A
134	5001	94	3/16/2006	0.011	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
135	5001	185	6/15/2006	0.020	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
203	5001	269	9/7/2006	0.046	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
240	5001	360	12/7/2006	0.074	P	0.102	0.078	0.063	T	0.262	0.253	0.061	O	0.213	0.209	0.069	Q

Legend:

N	Negative.
P	Confirmed positive.
N/A	Not applicable.
S	Sample exposed to ISIS 301012 demonstrates a decrease in response compared to sample that is not exposed to the drug; therefore, the potentially positive result could be specific to ISIS 301012 and needs to be tested further.
T	Sample exposed to ISIS 301012 demonstrates similar response compared to sample that is not exposed to the drug; therefore, the potentially positive result could be considered non-specific to ISIS 301012 and no further testing is needed.
RP	Potentially positive result for re-run; however, was initially negative.
RN	Negative result for re-run; however, was initially potentially positive.
U	Sample exposed to ISIS 13920 demonstrates similar response compared to sample that is not exposed to the drug; therefore, the potentially positive result could be considered specific to ISIS 301012.
R	Sample exposed to ISIS 3521 demonstrates similar response compared to sample that is not exposed to the drug; therefore, the potentially positive result could be considered specific to ISIS 301012.
O	Sample exposed to ISIS 13920 demonstrates similar response compared to sample that is not exposed to the drug; since exposure to ISIS 301012 was non-specific, potentially positive result could be considered non-specific to ISIS 301012.
Q	Sample exposed to ISIS 3521 demonstrates similar response compared to sample that is not exposed to the drug; since exposure to ISIS 301012 was non-specific, potentially positive result could be considered non-specific to ISIS 301012.
*	All results recorded as an OD value.

Titration Results for Anti-ISIS 301012

(b) (4) ID (UUQ2)	Animal #	Study Day	Sample Date	Sample Dilution	Result (OD)	UUQ2 Run ID	Run Cutpoint (OD)	Conclusion	Reportable Titer
57	2005	185	06/16/2006	Dil 1	0.104	23	0.098	P	1
57	2005	185	06/16/2006	Dil 2	0.029	23	0.098	N	
57	2005	185	06/16/2006	Dil 4	0.029	23	0.098	N	
57	2005	185	06/16/2006	Dil 8	0.032	23	0.098	N	
57	2005	185	06/16/2006	Dil 16	0.030	23	0.098	N	
168	5506	185	06/17/2006	Dil 1	0.167	25	0.097	P	1
168	5506	185	06/17/2006	Dil 2	0.024	25	0.097	N	
168	5506	185	06/17/2006	Dil 4	0.025	25	0.097	N	
168	5506	185	06/17/2006	Dil 8	0.026	25	0.097	N	
168	5506	185	06/17/2006	Dil 16	0.031	25	0.097	N	
202	4505	269	09/09/2006	Dil 1	0.282	23	0.098	P	1
202	4505	269	09/09/2006	Dil 2	0.025	23	0.098	N	
202	4505	269	09/09/2006	Dil 4	0.032	23	0.098	N	
202	4505	269	09/09/2006	Dil 8	0.029	23	0.098	N	
202	4505	269	09/09/2006	Dil 16	0.032	23	0.098	N	
210	5506	269	09/09/2006	Dil 1	0.314	25	0.097	P	1
210	5506	269	09/09/2006	Dil 2	0.027	25	0.097	N	
210	5506	269	09/09/2006	Dil 4	0.028	25	0.097	N	
210	5506	269	09/09/2006	Dil 8	0.028	25	0.097	N	
210	5506	269	09/09/2006	Dil 16	0.027	25	0.097	N	
239	4505	360	12/09/2006	Dil 1	0.130	25	0.097	P	1
239	4505	360	12/09/2006	Dil 2	0.024	25	0.097	N	
239	4505	360	12/09/2006	Dil 4	0.025	25	0.097	N	
239	4505	360	12/09/2006	Dil 8	0.027	25	0.097	N	
239	4505	360	12/09/2006	Dil 16	0.027	25	0.097	N	

Legend:

N Negative.

P Positive.

Dosing Solution Analysis

Dosing formulation is a true solution, was stable under the conditions of study and conformed to the label claim upon analysis at beginning-of-study and end-of-study.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Study of ISIS 301012 in the *Salmonella typhimurium/Escherichia coli* Plate Incorporation Assay	
Study no.:	301012-IS01
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10 July 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-001, 92.8%

Key Study Findings

- Mipomersen was negative in the Ames assay for genetic toxicity.

Methods

Strains:	See Sponsor's Table Below
Concentrations in definitive study:	
Basis of concentration selection:	
Negative control:	
Positive control:	
Formulation/Vehicle:	
Incubation & sampling time:	

Study Validity

The study is judged to be valid.

Results

Table 12: Bacterial Reverse Mutation Study of ISIS 301012 in the *Salmonella typhimurium/Escherichia coli* Plate Incorporation Assay

Test for Induction of: Reverse mutations	**No. of Independent Assays:** 2	**Study No.** 301012-IS01	**Test Article:** mipomersen sodium	
		Location in CTD: Section 4.2.3.3.1		
Strains: TA98, TA100, TA1535, TA1537, and WP2 *uvr*A	**No. of Replicate Cultures:** 2 (1st assay)/ 3 (2nd assay)	**GLP Compliance:** Yes		
Metabolizing System: Aroclor 1254-induced rat liver S9	**No. of Cells Analyzed/Culture:** N/A	**Date of Treatment:** July 10, 2003		
Vehicles: For Test Article: Phosphate buffered 0.9% saline (PBS)	**For Positive Controls:** dimethyl sulfoxide or water (sodium azide)			
Treatment: plate incorporation for 48-72 hours				
Cytotoxic Effects: negative in both the presence and absence of Aroclor-induced rat liver S9				
Genotoxic Effects: negative in both the presence and absence of Aroclor-induced rat liver S9				

Metabolic Activation	**Test Article**	**Dose Level (µg/plate)**	**Assay**				
			(#1) Revertant per Plate (mean ± SD)				
			TA98	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
With Activation	Vehicle	0	30±1	207±23	15±1	10±4	20±2
	ISIS 301012	2.5	23±1	204±7	15±1	6±2	15±0
		7.5	27±1	204±20	19±1	8±2	14±3
		25	28±7	244±18	13±1	9±6	19±1
		75	22±4	210±16	14±9	4±0	16±1
		200	31±0	196±6	16±6	7±0	16±0
		600	30±10	205±28	17±1	8±5	21±4
		1800	24±1	219±8	14±1	5±1	19±0
		5000	29±5	196±5	12±4	4±0	19±4
	2-aminoanthracine[a]	1.0	632±20	747±78	154±4	85±0	-
		10	-	-	-	-	983±78

Table 12: Bacterial Reverse Mutation Study of ISIS 301012 in the *Salmonella typhimurium/Escherichia coli* Plate Incorporation Assay — **Study No.** 301012-IS01 (continued)

Metabolic Activation	**Test Article**	**Dose Level (µg/plate)**	**Assay**				
			(#1) Revertant per Plate (mean ± SD)				
			TA98	**TA100**	**TA1535**	**TA1537**	**WP2 *uvr*A**
Without Activation	Vehicle	0	15 ± 4	165 ± 13	15 ± 6	5 ± 1	11 ± 1
	ISIS 301012	2.5	18 ± 2	153 ± 9	16 ± 1	6± 1	14 ± 6
		7.5	20 ± 0	156 ±6	15± 2	5±1	17±2
		25	17±4	191±16	10±2	6±1	16±4
		75	16±6	185±5	16±0	8±2	16±0
		200	16±0	174±3	15±4	8±2	11±0
		600	15±4	183±21	15±4	9±2	20±0
		1800	13±7	171±6	15±7	5±1	11±6
		5000	15±1	166±11	11±4	6±1	16±1
	2-nitrofluorene[a]	1.0	181±27	-	-	-	-
	Sodium azide[a]	1.0	-	662±19	468±4	-	-
	9-aminoacridine[a]	75	-	-	-	905±54	-
	Methyl methanesulfonate[a]	1000	-	-	-	-	118±2

Table 12: Bacterial Reverse Mutation Study of ISIS 301012 in the *Salmonella typhimurium/Escherichia coli* Plate Incorporation Assay			Study No. 301012-IS01 (continued)				
Metabolic Activation	Test Article	Dose Level (µg/plate)	Assay				
			(#2) Revertants per Plate (mean ± SD)				
			TA98	TA100	TA1535	TA1537	WP2 *uvrA*
With Activation	Vehicle	0	15 ± 2	173 ± 16	10 ± 1	4 ± 2	13 ±1
	ISIS301012	75	12 ± 2	141 ± 8	11 ± 1	6 ± 3	12 ± 1
		200	13 ± 1	149 ± 7	9 ± 3	4 ± 2	12 ± 1
		600	13 ± 2	145 ± 11	11 ± 1	5 ± 2	14 ± 1
		1800	14 ± 2	138 ± 16	9 ± 1	5 ± 1	13 ± 2
		5000	15 ± 2	136 ± 8	8 ± 3	5 ± 1	14 ± 1
	2-aminoanthracine	1.0	328 ± 41	643 ± 16	101 ± 8	57 ± 6	-
		10	-	-	-	-	242 ± 86
Without Activation	Vehicle	0	12 ± 3	157 ± 7	11 ± 2	8 ± 2	16 ± 1
	ISIS301012	75	12 ± 1	152 ± 19	11 ± 1	6 ± 2	14 ± 3
		200	12 ± 2	151 ± 11	10 ± 2	3 ± 1	15 ± 3
		600	11 ± 0	136 ± 8	11 ± 1	5 ± 3	15 ± 3
		1800	10 ± 1	170 ± 3	10 ± 0	5 ± 2	11 ± 1
		5000	11 ± 2	176 ± 11	12 ± 1	5 ± 2	11 ± 2
	2-nitrofluorene	1.0	220 ± 126	-	-	-	-
	Sodium azide	1.0	-	662 ± 43	586 ± 6	-	-
	9-aminoacridine	75	-	-	-	489 ± 97	-
	Methyl methanesulfonate	1000	-	-	-	-	145 ± 19

[a] Positive Control

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* L5178Y/TK+/- Mouse Lymphoma Cell Gene Mutation Assay of ISIS 301012	
Study no.:	301012-IS02
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3 July 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-001, 92.8%

Key Study Findings

- Mipomersen was negative for genetic toxicity in the mouse lymphoma assay.

Methods

Cell line:	See Sponsor's Table Below
Concentrations in definitive study:	
Basis of concentration selection:	
Negative control:	
Positive control:	
Formulation/Vehicle:	
Incubation & sampling time:	

Study Validity

Study is judged to be valid.

Results

Table 13: *In Vitro* L5178Y/TK+/- Mouse Lymphoma Cell Gene Mutation Assay of ISIS 301012

Test for Induction of: lymphoma mutagenesis		**No. of Independent Assays:** 2			**Study No.** 301012-IS02	
					Test Article: mipomersen sodium	
Strains: L5178Y/TK$^{+/-}$		**No. of Replicate Cultures:** 2			**Location in CTD:** Section 4.2.3.3.1	
Metabolizing System: Aroclor 1254-induced rat liver S9		**No. of Cells Analyzed/Culture:** N/A			**GLP Compliance:** Yes	
					Date of Treatment: July 8, 2003	
Vehicles: For Test Article: Phosphate buffered saline (PBS)			**For Positive Controls:** not reported			
Treatment: continuous treatment for 4 hours with and without S9; 24 hours without S9						
Cytotoxic Effects: no toxic effects in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay						
Genotoxic Effects: negative in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay						
Metabolic Activation	**Test Article**	**Exposure Time (hours)**	**Concentration (µg/mL)**	**Mutant Frequency**	**Induced Mutant Frequency**	**Total Growth (%)**
Without Activation	Vehicle	4	0	66	-	-
				40	-	-
	ISIS 301012		1000	43	-10	118
				41	-12	98
			2000	75	22	90
				36	-18	100
			3000	41	-12	111
				28	-25	99
			4000	40	-14	115
				29	-24	109
			5000	60	6	99
				34	-19	102
	Positive Control[a]		10	96	43	43
			20	258	205	14

Table 13: *In Vitro* L5178Y/TK+/- Mouse Lymphoma Cell Gene Mutation Assay of ISIS 301012				**Study No.** 301012-IS02 (continued)		
Metabolic Activation	**Test Article**	**Exposure Time (hours)**	**Concentration (µg/mL)**	**Mutant Frequency**	**Induced Mutant Frequency**	**Total Growth (%)**
With Activation	Vehicle	4	0	33	-	-
				20	-	-
	ISIS 301012		1000	49	22	117
				16	-11	102
			2000	90	64	109
				22	-5	115
			3000	38	11	127
				33	7	109
			4000	71	44	113
				48	21	112
			5000	41	14	118
				38	12	117
	Positive Control[b]		2.5	109	82	53
			4	477	451	15

Table 13: *In Vitro* L5178Y/TK+/- Mouse Lymphoma Cell Gene Mutation Assay of ISIS 301012				Study No. 301012-IS02 (continued)		
Metabolic Activation	**Test Article**	**Exposure Time (hours)**	**Concentration (µg/mL)**	**Mutant Frequency**	**Induced Mutant Frequency**	**Total Growth (%)**
Without Activation	Vehicle	24	0	78	-	-
				31	-	-
	ISIS 301012		1000	53	-2	112
				35	-20	106
			2000	29	-25	117
				30	-25	96
			3000	30	-25	102
				18	-36	116
			4000	45	-10	123
				24	-31	103
			5000	34	-21	112
				20	-34	123
	Positive Control[a]		2.5	244	190	64
			5	243	188	52

[a] Methyl Methanesulfonate
[b] 7,12 Dimethylbenz (a) anthracene

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

Study title: Mammalian Erythrocyte Micronucleus Assay of ISIS 301012 in ICR Mice	
Study no:	301012-AS12
Study report location:	4.2.3.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	18 April 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-001, 92.8%

Key Study Findings

- Mipomersen was negative for genetic toxicity in the mouse micronucleus assay.

Methods

Doses in definitive study:	See Sponsor's Table Below
Frequency of dosing:	
Route of administration:	
Dose volume:	
Formulation/Vehicle:	
Species/Strain:	
Number/Sex/Group:	
Satellite groups:	
Basis of dose selection:	
Negative control:	
Positive control:	

Study Validity

Study is judged to be valid.

Results

Table 14: Mammalian Erythrocyte Micronucleus Assay of ISIS 301012 in ICR Mice

Test for Induction of: micronucleated polychromatic erythrocytes in bone marrow		**Treatment Schedule:** single dose		**Study No.** 301012-AS12	
				Test Article: mipomersen sodium	
Species/Strain: ICR mice		**Sampling Time:** 24 and 48 hours after dose administration		**Location in CTD:** Section 4.2.3.3.2	
				GLP Compliance: Yes	
Age: 6 to 8 weeks old		**Method of Administration:** IV injection		**Date of Dosing:** 18 April 2005	
Cells Evaluated: micronucleated polychromatic erythrocytes (PCEs)		**Vehicle/Formulation:** **For Test Article:** Phosphate bufferred saline (PBS) **For Positive Controls:** 0.9% sodium chloride for injection (saline)			
No. of Cells Analyzed/Animal: 2000 polychromatic erythrocytes per animal					
Special Features: NA					
Toxic/Cytotoxic Effects: no inhibition of erythropoiesis					
Genotoxic Effects: negative in the mouse micronucleus assay					
Evidence of Exposure: NA					
Test Article	**Dose (mg/mg)**	**Time after dose administration (hours)**	**No. of Animals**	**PCE/Total Erythrocytes (Mean +/- SD)**	**Number/1000 PCEs (Mean +/- SD)**
Vehicle	0	24	5	M: 0.502 ± 0.05 F: 0.445 ± 0.07	M: 0.1 ± 0.22 F: 0.2 ± 0.27
ISIS 301012	300		5	M: 0.523 ± 0.05 F: 0.497 ± 0.06	M: 0.2 ± 0.45 F: 0.2 ± 0.27
	600		5	M: 0.514 ± 0.06 F: 0.509 ±0.07	M: 0.1 ± 0.22 F: 0.2 ± 0.27
	1200		5	M: 0.522 ± 0.06 F: 0.474 ± 0.05	M: 0.3 ± 0.27 F: 0.2 ± 0.27

Table 14: Mammalian Erythrocyte Micronucleus Assay of ISIS 301012 in ICR Mice				**Study No.** 301012-AS12 (continued)	
Test Article	**Dose (mg/mg)**	**Time after dose administration (hours)**	**No. of Animals**	**PCE/Total Erythrocytes (Mean +/- SD)**	**Number/1000 PCEs (Mean +/- SD)**
Positive Control [a]	50	48	5	M: 0.433 ± 0.04 F: 0.444 ± 0.05	M: 10.8 ± 1.86 F: 14.0 ± 3.39
Vehicle	0		5	M: 0.519 ± 0.03 F: 0.559 ± 0.03	M: 0.3 ± 0.45 F: 0.2 ± 0.27
ISIS 301012	300		5	M: 0.487 ± 0.08 F: 0.567 ± 0.05	M: 0.0 ± 0.00 F: 0.4 ±0.55
	600		5	M: 0.495 ± 0.03 F: 0.508 ± 0.05	M: 0.2 ± 0.27 F: 0.4 ± 0.22
	1200		5	M: 0.523 ± 0.07 F: 0.537 ± 0.04	M: 0.1 ± 0.22 F: 0.3 ± 0.45

NA - Not Applicable
[a] Cyclophosphamide

7.4 Other Genetic Toxicity Studies

None.

8 Carcinogenicity

Study title: 2-YEAR SUBCUTANEOUS CARCINOGENICITY STUDY OF ISIS 301012 AND ISIS 147764 IN CD-1 MICE

Study no.:	GT-348-TX-1
Study report location:	4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	05-17-2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-002, 93.6% ISIS 301012, CA301012-007, 91.6% ISIS 301012, CA301012-008, 93.6% ISIS 301012, CA301012-010, 93.9% ISIS 301012, CA301012-012, 88.3% ISIS 147764, CA147764-001, 86.6%
CAC concurrence:	Yes. Memo indicating ECAC concurrence with Sponsor's dosing counter proposal filed in DARRTS on 06/06/2007. IR letter issued 12/19/2008 indicates concurrence with discontinued dosing at n=20 and early sacrifice at n=15.

Key Study Findings

- Apart from the neoplastic findings, which are discussed below, ISIS 301012 was associated with adverse effects primarily on the cardiovascular system at doses ≥ 20 mg/kg/week that resulted in increased mortality. The NOAEL was 5 mg/kg/week.
    - ↑ incidence and severity of cardiac thrombus (both sexes)
    - worsening of cardiomyopathy (♀)
    - atrial/ventricular dilatation (♀) [secondary to thrombus?]
    - endocarditis (♀)
- Contrary to what was observed in the rat study (reviewed below), there is no evidence of oligonucleotide induced kidney toxicity. Indeed, oligonucleotides may be protective of the mouse kidney, as the incidence and/or severity of multiple spontaneous kidney findings were dose-dependently reduced: chronic progressive nephropathy, tubular mineralization, hydronephrosis, cysts & hyaline droplets.
- Females exhibited a dose-dependent ↑ in the incidence/severity of lobular hyperplasia in the mammary gland; however, there was no increase in mammary tumors.

Adequacy of Carcinogenicity Study

The protocol was approved by ECAC. Interpretation of the study is somewhat confounded by early termination of dosing and early sacrifice in several dose groups. ECAC was consulted and conferred with Sponsor's plans prior to early termination of dosing and early sacrifice. While on the one hand the excess deaths confounds analysis of the effect of the test item on age-related tumor development, it does indicate that the study adequately explored the potential carcinogenic effect of maximally tolerated doses of the test item. The study is judged to be adequate, pending ECAC concurrence.

Appropriateness of Test Models

The mouse is a commonly used test model for carcinogenesis. The route and frequency of dosing was consistent with the intended clinical use of mipomersen.

Evaluation of Tumor Findings

There are 3 tumor types that appear likely to be related to administration of test item: 1) fibrosarcoma in ♂s at 60 mg/kg QW (also s.s. ↑ for mouse surrogate); 2) hepatocellular adenoma in ♀s at 60 mg/kg QW (also s.s. ↑ for mouse surrogate); and 3) hemangiosarcoma in ♀s at 60 mg/kg QW. Other tumors types that showed s.s. ↑s are considered to likely be unrelated to the test item on the basis on lack of dose response and/or marginal ↑s over concurrent and/or historical controls.

Methods

Doses (ISIS 301012, clinical candidate):	5, 20, 60, mg/kg/week or 80 mg/kg/month
Doses (ISIS 147764, mouse surrogate):	60 mg/kg/week
Frequency of dosing:	see above
Dose volume:	6.4 to 16.7 mL/kg
Route of administration:	SC injection to the back
Formulation/Vehicle:	soln. in phosphate buffered saline
Basis of dose selection:	MTD
Species/Strain:	Crl:CD-1 Mouse
Number/Sex/Group:	70
Age:	~8 weeks old at dosing initiation
Animal housing:	Individually
Paradigm for dietary restriction:	*ad libitum*
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Sentinel animals (3-5/sex) were scarified at 6 month intervals to check for various infectious agents
Deviation from study protocol:	None material to study interpretation

Group Assignments

Group Number	Dose Level (mg/kg)	Vehicle or Test Article	Number of Animals Male	Number of Animals Female
1	0[a]	PBS	70	70
2	5[a]	ISIS 301012	70	70
3	20[a]	ISIS 301012	70	70
4	60[a]	ISIS 301012	70	70
5	80[b]	ISIS 301012	70	70
6	60[a]	ISIS 147764	70	70

[a] Animals were dosed once weekly
[b] Animals were dosed once monthly

Observations and Results

Mortality

Test item was associated with increased mortality at HD (♂&♀) and in MD ♀, requiring early cessation of dosing when surviving animals in dose group dropped to 20, and early sacrifice (when surviving animal number dropped to 15).

60 mg/kg/week
M dosing stopped at week 95, sac'd Day 672
F dosing stopped at week 83, sac'd Day 614
20 mg/kg/week

F dosing stopped at week 98, sac'd Day 717

Sponsor considers that there was no definitive determination of the cause of early death. Heart atrial thrombi and undetermined were the two most commonly recorded causes of death (see Sponsor's table, below). Notably there was a dose-related ↑ in probable fibrosarcoma-related deaths in ♂s: 0, 0, 1, 4, 0, 1 (C, LD, MD1, HD, MD2, MS).

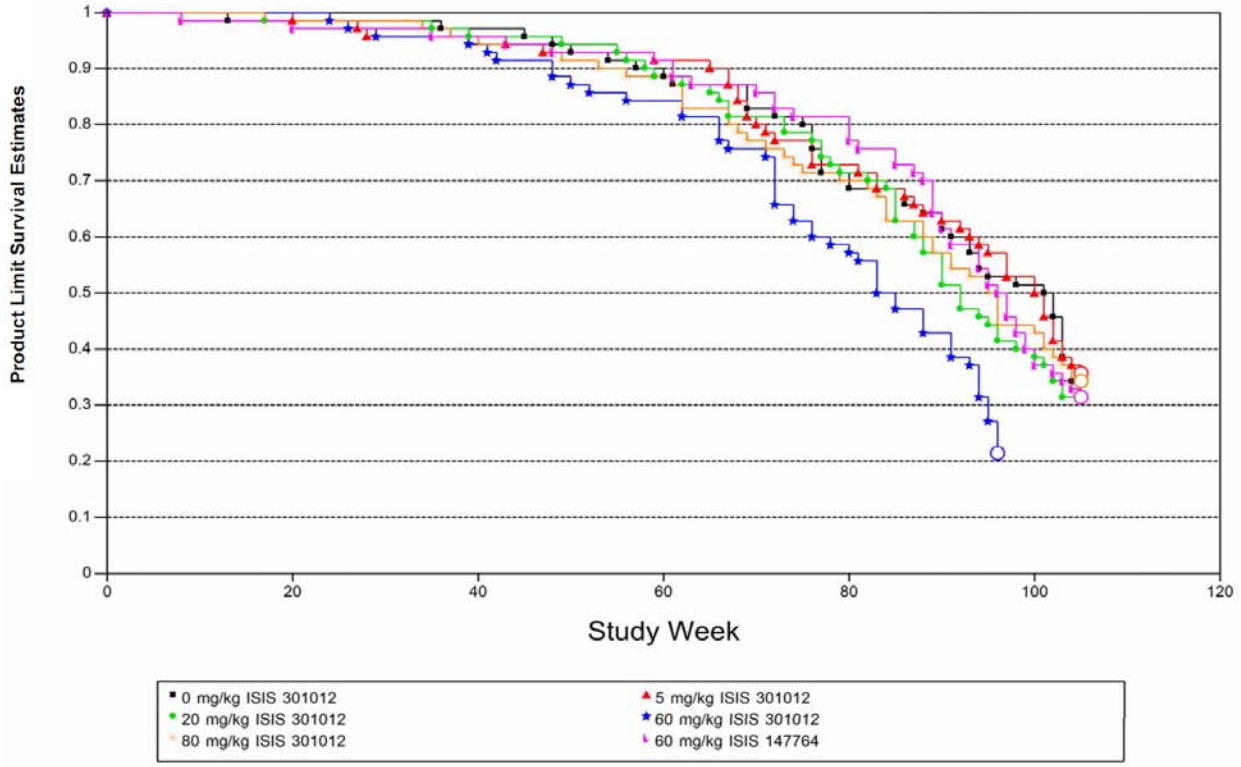
The ♀ mice receiving the mouse surrogate (ISIS 147764) appeared to have better survival than the C group.

Table 1 Number of Animals Surviving to Scheduled Terminal Necropsy

The number of animals surviving to the scheduled terminal necropsy (Week 105)*		
Dose Level	Male	Female
0	24 (34%)	24 (34%)
5 mg/kg/week ISIS 301012	25 (37%)	20 (33%)
20 mg/kg/week ISIS 301012	22 (31%)	0 (0%)
60 mg/kg/week ISIS 301012	0 (0%)	0 (0%)
80 mg/kg/month ISIS 301012	24 (34%)	19 (29%)
60 mg/kg/week ISIS 147764	22 (33%)	28 (43%)
*Respective survival percentage calculations include/reflect either death or necropsy at Week 105 of the study for groups surviving to scheduled terminal necropsy (see mean survival data in Table 1 of this report)		

Summary of Survival Estimates - MALE

Figure 1



Summary of Survival Estimates - FEMALE

Figure 1A

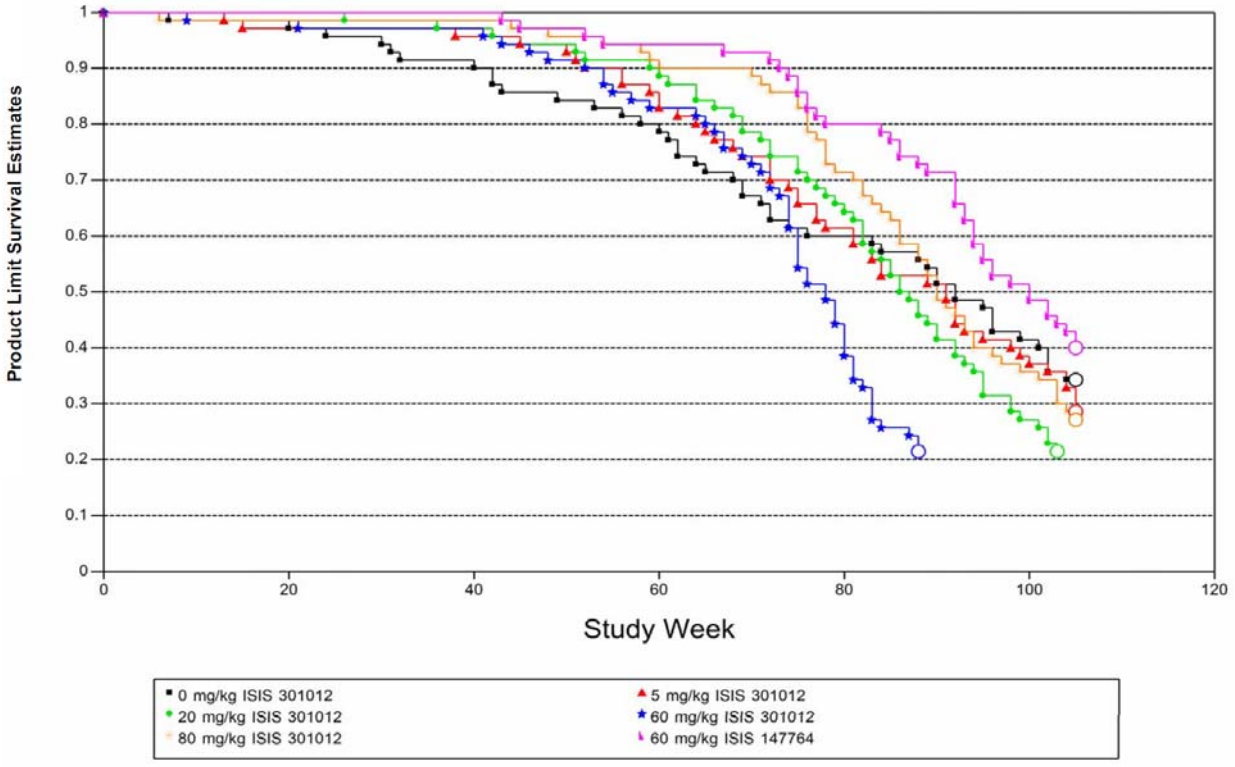


Table 8 **Summary of Probable Cause of Death - MALE**

Cause of Death	0 mg/kg ISIS 301012	5 mg/kg ISIS 301012	20 mg/kg ISIS 301012	60 mg/kg ISIS 301012
Number of Animals	70	70	70	70
Summary of Animal Disposition				
accidental death	1	0	0	0
died prior to euthanasia	2	0	2	1
euthanized *in extremis*	22	21	27	19
found dead	21	24	19	35
terminal necropsy	24	25	22	15
Cause of Death				
accidental injury	1	1	0	0
amyloidosis	1	1	0	0
cardiomyopathy	0	0	1	0
chronic progressive nephropathy/uremia	1	0	1	0
fibrosarcoma/fibroma	0	0	1	4
heart failure/atrial thrombus	2	3	6	11
heart inflammation/necrosis	2	0	0	0
heart mineralization, myofiber	1	0	0	0
heart ventricular thrombus.	0	0	0	0
hemangiosarcoma/hemangioma	5	2	5	4
hemorrhage	0	1	0	0
histiocytic sarcoma	1	2	0	1
inflammation/septicemia	1	0	0	0
leukemia	2	0	0	0

Table 8 **Summary of Probable Cause of Death - MALE**

Cause of Death	80 mg/kg ISIS 301012	60 mg/kg ISIS 147764
Number of Animals	70	70
Summary of Animal Disposition		
accidental death	0	0
died prior to euthanasia	1	0
euthanized *in extremis*	18	20
found dead	27	28
terminal necropsy	24	22
Cause of Death		
accidental injury	0	0
amyloidosis	0	0
cardiomyopathy	0	0
chronic progressive nephropathy/uremia	0	0
fibrosarcoma/fibroma	0	1
heart failure/atrial thrombus	7	10
heart inflammation/necrosis	1	0
heart mineralization, myofiber	0	0
heart ventricular thrombus.	0	1
hemangiosarcoma/hemangioma	5	4
hemorrhage	0	0
histiocytic sarcoma	1	0
inflammation/septicemia	1	0
leukemia	0	1

Table 8 **Summary of Probable Cause of Death - MALE**

Cause of Death	0 mg/kg ISIS 301012	5 mg/kg ISIS 301012	20 mg/kg ISIS 301012	60 mg/kg ISIS 301012
Number of Animals	70	70	70	70
Cause of Death				
liver anomaly, developmental	0	1	0	0
liver cyst, biliary	0	0	0	0
liver tumor	1	1	3	1
lung tumor	5	1	1	0
lymphoid tumor	1	4	3	3
obstruction/impaction, gastrointestinal tract	0	0	1	0
odontodysplasia/periodontitis	0	0	1	0
possible accidental based upon lung hemorrhage.	0	0	0	0
schwannoma	1	0	0	0
skin inflammation/necrosis	1	0	3	6
spinal cord hemorrhage/necrosis/degeneration	0	0	0	0
undetermined	5	7	6	10
urinary bladder tumor	0	0	0	0
urogenital inflammation/obstruction/calculi	15	21	16	15

Table 8 **Summary of Probable Cause of Death - MALE**

Cause of Death	80 mg/kg ISIS 301012	60 mg/kg ISIS 147764
Number of Animals	70	70
Cause of Death		
liver anomaly, developmental	0	0
liver cyst, biliary	1	0
liver tumor	1	2
lung tumor	1	0
lymphoid tumor	3	0
obstruction/impaction, gastrointestinal tract	0	0
odontodysplasia/periodontitis	0	0
possible accidental based upon lung hemorrhage.	0	1
schwannoma	0	0
skin inflammation/necrosis	6	2
spinal cord hemorrhage/necrosis/degeneration	0	1
undetermined	7	9
urinary bladder tumor	1	0
urogenital inflammation/obstruction/calculi	11	16

Table 8 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	0 mg/kg ISIS 301012	5 mg/kg ISIS 301012	20 mg/kg ISIS 301012	60 mg/kg ISIS 301012
Number of Animals	70	70	70	70
Summary of Animal Disposition				
died prior to euthanasia	1	0	1	0
euthanized *in extremis*	21	20	15	13
found dead	24	30	39	42
terminal necropsy	24	20	15	15
Cause of Death				
accidental injury	1	2	0	0
amyloidosis	0	0	0	0
bone proliferation	0	0	0	0
brain hemorrhage/necrosis	1	0	0	0
chronic progressive nephropathy/uremia	2	4	5	1
fibrosarcoma/fibroma	0	1	0	1
gastrointestinal tympanites	0	1	0	0
harderian gland tumor	0	0	0	2
heart failure/atrial thrombus	4	3	11	10
heart inflammation/necrosis	0	1	0	0
hemangiosarcoma/hemangioma	1	5	2	4
hemorrhage	0	1	0	0
histiocytic sarcoma	2	2	1	0
inflammation/septicemia	0	0	1	2
kidneys glomerulosclerosis	1	0	1	1

Table 8 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	80 mg/kg ISIS 301012	60 mg/kg ISIS 147764
Number of Animals	70	70
Summary of Animal Disposition		
died prior to euthanasia	2	0
euthanized *in extremis*	19	17
found dead	30	25
terminal necropsy	19	28
Cause of Death		
accidental injury	0	0
amyloidosis	0	1
bone proliferation	0	1
brain hemorrhage/necrosis	0	0
chronic progressive nephropathy/uremia	3	3
fibrosarcoma/fibroma	1	1
gastrointestinal tympanites	0	0
harderian gland tumor	1	0
heart failure/atrial thrombus	5	8
heart inflammation/necrosis	0	1
hemangiosarcoma/hemangioma	7	0
hemorrhage	0	0
histiocytic sarcoma	1	0
inflammation/septicemia	1	0
kidneys glomerulosclerosis	1	0

Table 8 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	0 mg/kg ISIS 301012	5 mg/kg ISIS 301012	20 mg/kg ISIS 301012	60 mg/kg ISIS 301012
Number of Animals	70	70	70	70
Cause of Death				
leiomyoma/leiomyosarcoma	2	0	0	0
leukemia	0	2	1	0
lipoma/liposarcoma	0	0	0	0
liver tumor	0	0	0	0
lung inflammation/necrosis	0	0	1	0
lung tumor	1	1	1	1
lymphoid tumor	6	9	6	5
mammary tumor	0	1	1	0
ovarian cyst/hemorrhage	3	3	1	0
pancreas tumor	1	0	0	0
pituitary tumor	2	1	1	0
polyarteritis	1	0	0	0
skin inflammation/necrosis	2	4	1	2
skin tumor	0	0	0	0
undetermined	13	7	18	23
urogenital inflammation/obstruction/calculi	0	0	0	0
uterus hemorrhage	0	0	0	0
uterus prolapse	0	0	0	1
uterus tumor	1	2	3	2
uterus with cervix angiectasis	1	0	0	0
uterus with cervix hyperplasia, cystic endometrial	1	0	0	0

Table 8 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	80 mg/kg ISIS 301012	60 mg/kg ISIS 147764
Number of Animals	70	70
Cause of Death		
leiomyoma/leiomyosarcoma	0	0
leukemia	3	0
lipoma/liposarcoma	0	1
liver tumor	1	0
lung inflammation/necrosis	0	0
lung tumor	2	2
lymphoid tumor	4	6
mammary tumor	1	0
ovarian cyst/hemorrhage	6	5
pancreas tumor	0	0
pituitary tumor	0	0
polyarteritis	0	0
skin inflammation/necrosis	0	1
skin tumor	2	0
undetermined	8	7
urogenital inflammation/obstruction/calculi	0	3
uterus hemorrhage	0	1
uterus prolapse	1	1
uterus tumor	3	0
uterus with cervix angiectasis	0	0
uterus with cervix hyperplasia, cystic endometrial	0	0

Clinical Signs

There were several findings (*e.g.,* distended abdomen) that exhibited a reduction in incidence at HD (data not shown), most likely as a consequence of high intercurrent death and early sacrifice of the HD group (group 4), and a normally late age of onset of the finding.

Both sexes have increased hair sparseness, abrasions and scabbing on the trunk and neck, possibly related to injection site reactions.

Clinical Signs -- Instances/Animals Affected												
Sex	Male						Female					
Dose Group	1	2	3	4	5	6	1	2	3	4	5	6
Day of Terminal Sacrifice	735	735	735	672	735	735	735	735	717	614	735	735
PELAGE/SKIN												
Cervical region												
Abrasion(s)	45/2	47/4	93/3	85/10	59/3	24/3	18/3	47/3	6/2	50/5	0/0	35/2
Hair absent	0/0	0/0	0/0	34/2	7/1	0/0	0/0	0/0	12/1	0/0	0/0	8/1
Hair sparse	40/4	70/4	5/2	216/18	48/4	50/5	8/2	18/3	62/4	72/9	0/0	43/3
Scabbed area	22/2	33/5	70/7	296/22	25/3	86/7	28/4	29/3	33/5	95/11	11/4	55/3
Dorsal surface												
Abrasion(s)	0/0	37/2	30/3	9/2	48/5	10/3	1/1	12/2	6/1	2/1	0/0	0/0
Hair sparse	2/1	8/3	58/5	46/5	21/2	0/0	12/3	12/2	42/3	93/9	0/0	0/0
Scabbed area	4/1	3/1	31/5	61/10	41/5	26/6	8/4	39/12	35/6	59/8	6/3	2/1
Lumbar region												
Hair sparse	0/0	1/1	36/2	4/1	15/1	26/3	35/2	113/12	80/8	341/20	48/7	248/14
Scabbed area	0/0	1/1	4/1	7/2	0/0	4/2	0/0	0/0	0/0	5/1	0/0	0/0
Sacral region												
Hair sparse	8/1	1/1	0/0	0/0	7/1	0/0	0/0	6/3	71/	39/5	14/3	12/2
Thoracic region												
Abrasion(s)	0/0	0/0	0/0	17/4	9/1	8/1	0/0	9/2	8/2	16/1	0/0	0/0
Hair absent	0/0	0/0	0/0	0/0	2/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Hair sparse	17/1	1/1	1/1	111/10	9/2	0/0	0/0	6/2	7/2	40/5	3/1	24/1
Scabbed area	0/0	23/4	1/1	91/13	0/0	109	6/1	10/1	1/1	38/4	13/2	11/1

Mass Findings

No definitive treatment-related trends in mass findings were noted during the study.

Table 3 Summary of Mass Findings[+]- MALE
Weeks 1 to 105

Observation	0 mg/kg ISIS 301012	5 mg/kg ISIS 301012	20 mg/kg ISIS 301012	60 mg/kg ISIS 301012	80 mg/kg ISIS 301012	60 mg/kg ISIS 147764
Number of Animals Alive at Start of Interval	70	70	70	70	70	70
Number of Normal Animals	70	70	69	67	69	67
Mass						
Mass 1, Large >or=4 cm, Cervical region	0/0	0/0	1/1	0/0	0/0	0/0
Mass 1, Medium 2-3.9 cm, Cervical region	0/0	0/0	3/1	0/0	0/0	0/0
Mass 1, Medium 2-3.9 cm, Lumbar region	0/0	0/0	0/0	4/1	0/0	0/0
Mass 1, Medium 2-3.9 cm, Thoracic region	0/0	0/0	0/0	7/1	0/0	1/1
Mass 1, Small 1-1.9 cm, Anogenital region	0/0	0/0	0/0	0/0	8/1	0/0
Mass 1, Small 1-1.9 cm, Cervical region	0/0	0/0	2/1	0/0	0/0	0/0
Mass 1, Small 1-1.9 cm, Inguinal region/right	0/0	0/0	0/0	0/0	0/0	51/1
Mass 1, Small 1-1.9 cm, Shoulder/left	0/0	0/0	0/0	2/1	0/0	0/0
Mass 1, Small 1-1.9 cm, Thoracic region	0/0	0/0	0/0	5/1	0/0	5/2
Mass 1, Ulcerated, medium 2-3.9 cm, Thoracic region	0/0	0/0	0/0	0/0	0/0	1/1
Mass 1, Ulcerated, small 1-1.9 cm, Shoulder/left	0/0	0/0	0/0	1/1	0/0	0/0

Table 3 Summary of Mass Findings[+]- FEMALE
Weeks 1 to 105

Observation	0 mg/kg ISIS 301012	5 mg/kg ISIS 301012	20 mg/kg ISIS 301012	60 mg/kg ISIS 301012	80 mg/kg ISIS 301012	60 mg/kg ISIS 147764
Number of Animals Alive at Start of Interval	70	70	70	70	70	70
Number of Normal Animals	69	65	66	69	66	65
External Appearance						
Ear/portion of ear missing, Ear/left	1/1	0/0	0/0	0/0	0/0	0/0
Ear/portion of ear missing, Ear/right	1/1	0/0	0/0	0/0	0/0	0/0
Mass						
Mass 1, Medium 2-3.9 cm, Anogenital region	0/0	8/2	0/0	0/0	0/0	0/0
Mass 1, Medium 2-3.9 cm, Axillary region/left	0/0	0/0	0/0	0/0	0/0	1/1
Mass 1, Medium 2-3.9 cm, Cervical region	0/0	0/0	0/0	0/0	0/0	4/1
Mass 1, Medium 2-3.9 cm, Dorsal surface	0/0	2/1	0/0	0/0	0/0	0/0
Mass 1, Medium 2-3.9 cm, Shoulder/left	0/0	6/1	0/0	0/0	0/0	0/0
Mass 1, Medium 2-3.9 cm, Thoracic region	0/0	0/0	0/0	3/1	4/1	1/1
Mass 1, Small 1-1.9 cm, Anogenital region	0/0	10/2	0/0	0/0	0/0	0/0
Mass 1, Small 1-1.9 cm, Axillary region/left	1/1	0/0	0/0	0/0	3/1	2/1
Mass 1, Small 1-1.9 cm, Axillary region/right	0/0	0/0	0/0	0/0	0/0	6/1
Mass 1, Small 1-1.9 cm, Cervical region	0/0	4/1	0/0	0/0	3/1	1/1
Mass 1, Small 1-1.9 cm, Dorsal surface	0/0	3/1	0/0	0/0	0/0	0/0
Mass 1, Small 1-1.9 cm, Inguinal region/left	0/0	0/0	1/1	0/0	0/0	0/0
Mass 1, Small 1-1.9 cm, Inguinal region/right	0/0	0/0	1/1	0/0	0/0	0/0
Mass 1, Small 1-1.9 cm, Shoulder/left	0/0	4/1	0/0	0/0	0/0	2/1
Mass						
Mass 1, Small 1-1.9 cm, Shoulder/right	0/0	0/0	1/1	0/0	0/0	0/0
Mass 1, Small 1-1.9 cm, Thoracic region	0/0	0/0	1/1	0/0	3/2	4/1
Mass 1, Ulcerated, medium 2-3.9 cm, Dorsal surface	0/0	1/1	0/0	0/0	0/0	0/0

Body Weights

No consistent s.s., dose-related effect of test item was seen in males; however, females exhibited a dose-related, frequently s.s. increase in body weight gain with test item treatment.

Percent Differences in Mean Body Weight (g) from Control – Males

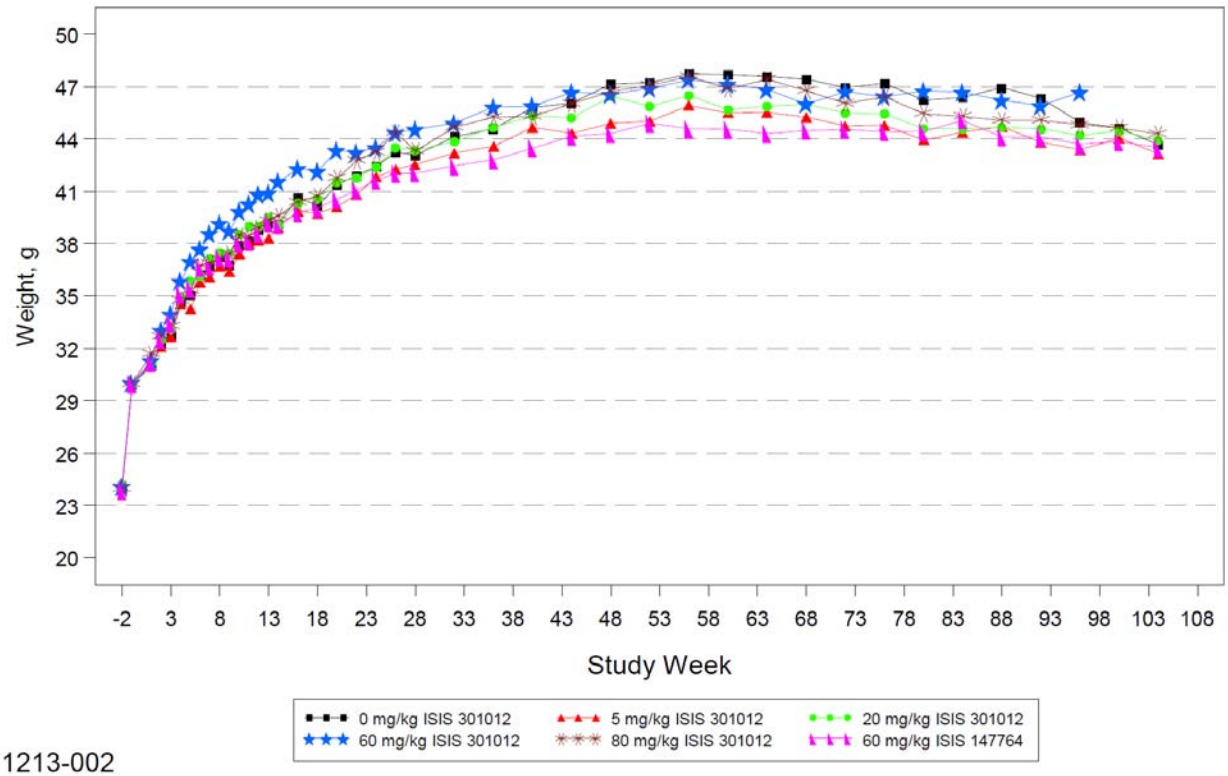
Interval	Dose Level					
	0*	5 mg/kg/week ISIS 301012	20 mg/kg/week ISIS 301012	60 mg/kg/week ISIS 301012	80 mg/kg/month ISIS 301012	60 mg/kg/week ISIS 147764
Week 4	34.65	↓0.29	0.95	3.46	0.23	1.39[a]
Week 13	39.15	↓2.17	0.89	4.47	0.41	0.05
Week 26	43.22	↓2.20	0.67	2.64	2.66	↓2.78
Week 52	47.23	↓4.72	↓2.86	↓0.70	↓0.36	↓4.91
Week 64	47.57	↓4.31	↓3.55	↓1.53	↓0.29	↓6.87
Week 76	47.20	↓5.13	↓3.69	↓1.63	↓1.69	↓5.91
Week 88	46.94	↓4.77	↓4.84	↓1.56	↓4.03	↓5.99
Week 104	43.65	↓1.17	0.62	NA	1.49	↓0.34

* Control value is expressed in grams.
[a]Statistically significant at the 0.05 level

Percent Differences in Mean Body Weight (g) from Control - Females						
Interval	Dose Level					
	0*	5 mg/kg/week ISIS 301012	20 mg/kg/week ISIS 301012	60 mg/kg/week ISIS 301012	80 mg/kg/month ISIS 301012	60 mg/kg/week ISIS 147764
Week 4	27.91	0.68	1.22	3.65	0.93	2.76
Week 13	30.60	2.65	3.27[a]	6.41[a]	2.61[a]	3.86[a]
Week 26	33.62	0.57	2.35	6.01	2.17	1.19
Week 52	35.67	4.74[a]	5.41[a]	12.70[a]	6.59[a]	5.19[a]
Week 64	36.66	3.16[a]	8.43[a]	11.78[a]	4.77[a]	4.83[a]
Week 76	36.79	5.82[a]	11.50[a]	12.23[a]	5.57[a]	4.10[a]
Week 88	37.53	4.45	11.00	NA	1.17	4.64
Week 104	38.11	4.41	NA	NA	↓3.41	2.94

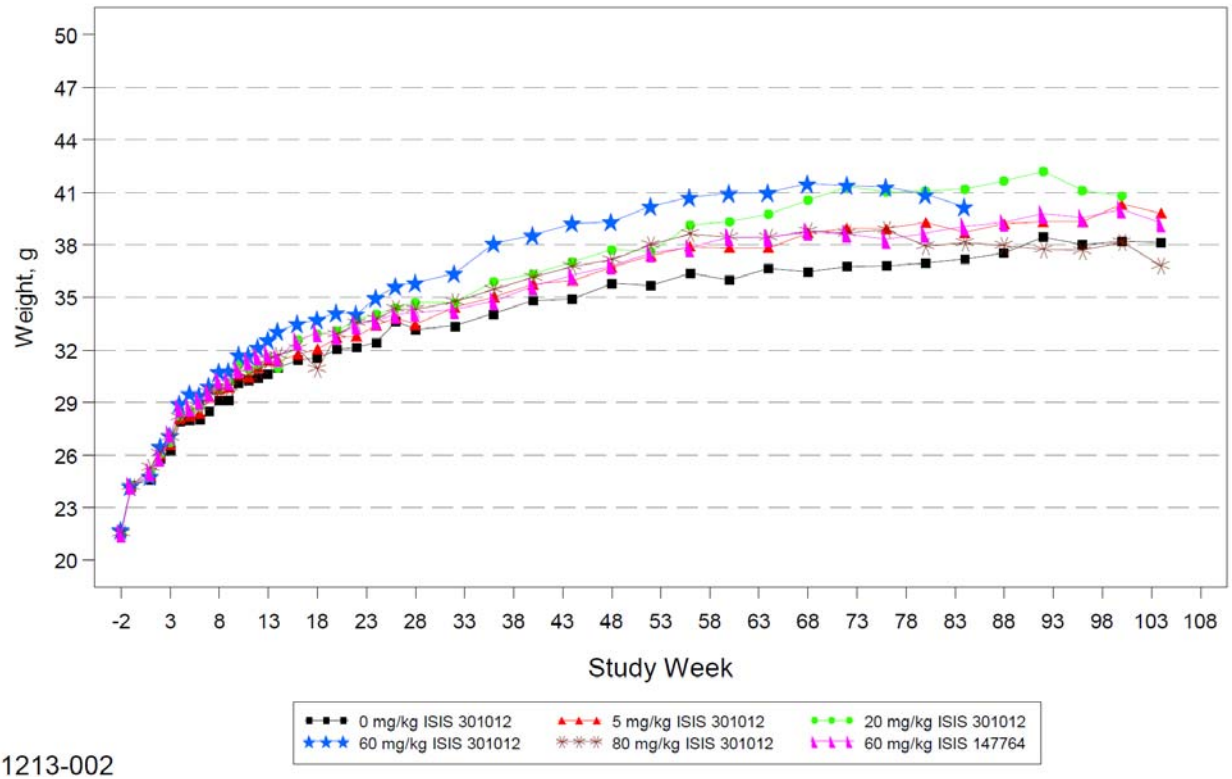
* Control value is expressed in grams.
[a]Statistically significant at the 0.05 level

Figure 2 Mean Body Weight Values - MALE



1213-002

Figure 2A Mean Body Weight Values - FEMALE



1213-002

Feed Consumption

No clear effect of test item on food consumption.

Ophthalmoscopy

Unremarkable

Hematology

There were mild test item related decreases in red cell mass (erythrocytes, hemoglobin, and hematocrit) (12 to 33%) in both sexes at all dose levels in those animals receiving ISIS 301012 relative to controls. Sporadic s.s. changes in some immune cell levels were also recorded; however the toxicological significance of these findings is unclear due to either their small magnitude, sporadic nature, presence in only a single sex and/or absence of a dose response.

Notably, especially in light of the apparent dose-dependent increase in fatal cardiac thrombotic events, there was no assessment of coagulation parameters.

Hematology -- % Change from Control												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
Leukocytes ($10^3/\mu L$)	4.50	↑11	↑32	↑70	n.e.	36	8.86	↓30	↓19	↑17	↓55	↓41
Erythrocytes ($10^6\mu L$)	8.93	↓13	**↓27****	**↓29****	**↓22****	↓10	8.18	**↓23***	**↓26***	**↓29****	**↓21***	n.e.
Hemoglobin (g/dL)	13.73	↓12	**↓28****	**↓33****	**↓20****	↓12	12.91	**↓22****	**↓27****	**↓29****	**↓19***	n.e.
Hematocrit (%)	46.39	↓14	**↓29****	**↓33****	**↓21****	↓13	43.61	**↓25****	**↓26****	**↓30****	**↓20***	n.e.
Lymphocytes ($10^3/\mu L$)	1.859	↑44	↑20	**↑165***	↑15	↑136	4.849	↓14	n.e.	↑48	↓53	↓45
Monocytes ($10^3/\mu L$)	0.089	n.e.	↑24	↑28	n.e.	↓62	0.080	↑30	↑15	**↑295****	n.e.	n.e.
Eosinophils ($10^3/\mu L$)	0.64	↑41	↑102	↑144	↑84	↑27	0.105	↓36	↑71	↓56	↓41	↓9
Basophils ($10^3/\mu L$)	0.005	n.e.	↑80	**↑220***	↑140	n.e.	0.066	↓48	↓90	↓79	↓85	↓76
Other Cells ($10^3/\mu L$)	0.023	↑140	↑160	**↑1152**	↑317	n.e.	0.192	↓32	↓61	↓36	↓71	↓68

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
n.e. = no effect

Clinical Chemistry

In animals (♂ & ♀) receiving 60 mg/kg/week ISIS 147764 (mouse surrogate) there were increases in AST (134 to 156%) and ALT (210 to 283%) relative to controls. There were also milder increases in AST (82 to 106%) and ALT (50 to 67%) in animals receiving 80 mg/kg/month ISIS 301012 (♂ & ♀). These changes appear to be test article-related in both groups. Urea nitrogen was increased 64% relative to controls in ♂s receiving 60 mg/kg/week ISIS 147764, a similar change was not observed in ♀s. The intra-group consistency and magnitudes of change observed strongly suggest a test article-related effect. There were other statistically significant alterations among various clinical chemistry parameters that were not considered toxicologically relevant due to their small magnitude, sporadic/inconsistent nature, and/or lack of a dose response.

Clinical Chemistry -- % Change from Control												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
Bilirubin (mg/dL)	0.22	↓9	**↓41****	**↓45****	↓9	**↓32***	0.18	↑17	↓33	n.e.	n.e.	↑72
AlkPhos (U/L)	77.2	↓21	n.e.	↓5	n.e	↑14	92.8	↑31	↑16	↑52	↑20	**↑132***
AST (U/L)	117.8	↑62	↑53	n.e.	↑82	**↑134****	225.8	↓18	↓41	↓12	↑106	↑156
ALT (U/L)	83.1	↑100	↑37	↓29	↑67	**↑283****	111.2	↓40	↓39	↓17	↑50	↑210
Albumin (mg/dL)	3.06	↓14	↓6	**↓22****	n.e.	**↓15***	3.00	n.e.	↓15	n.e.	n.e.	n.e.
A:G Ratio	1.10	↓10	↓7	**↓18***	n.e.	↓13	1.09	n.e.	↓8	↓6	↑9	n.e.
Triglyceride (mg/dL)	64.5	-	-	**↑146***	-	↓34	57.7	-	↑47	↓23	-	↓37
BUN (mg/dL)	32.6	↓13	↓10	↓30	n.e.	**↑64***	31.1	↑14	↓20	↑7	n.e.	↑9
HDL-C (mg/dL)	106.9	-	-	↓10	-	n.e.	71.4	-	n.e.	↑39	-	**↑65***

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
n.e. = no effect
"-" not available

Immunoglobulin

No effect of test item on total serum IgM or IgG.

Cytokine/Chemokine Analysis

A multiplex ELISA approach (b) (4) was used to measure the levels of IL-1α, IL-1β, IL-6, TNF-α, MCP-1, MIP-1α, RANTES, IL-8 and IFN-γ.

Insufficient information was provided in the study report to fully assess the extent to which the assay system is suitable for the intended analysis. The assay is not validated. There is extremely large intragroup variability that limits the interpretability of intergroup changes.

Cytokine/Chemokine -- Group Mean ± SD												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
IL-1α (pg/mL)	5.1 NC	10.3 ± 3.7	22.6 ± 3.5	NC NC	6.7 ± 8.4	19.4 ± 29.0	8.0 ± 6.6	18.7 ± 25.7	264 NC	48.0 NC	4.4 ± 4.3	118.3 ± 224.6
IL-1β (pg/mL)	3.6 ± 1.4	3.7 ± 3.6	23.0 NC	NC NC	5.1 ± 7.1	12.7 ± 17.8	9.7 ± 13.8	3.7 ± 3.0	34.9 NC	30.8 NC	10.6 ± 11.9	15.4 ± 12.2
IL-6 (pg/mL)	46.8 ± 61.0	43.8 ± 49.6	38.9 ± 47.9	25.4 ± 7.4	76.2 ± 53.9	78.4 ± 158	36.7 ± 44.5	104 ± 176	3927 ± 8719	198 ± 204	62.8 ± 85.2	130 ± 210
TNF-α (pg/mL)	55.2 ± 79.1	13.5 ± 9.8	21.5 ± 42.6	45.0 ± 74.1	44.1 ± 87.5	19.1 ± 24.6	42.2 ± 82.7	17.8 ± 10.9	23.5 ± 29.6	130 ± 139	15.3 ± 9.8	76.7 ± 110
MCP-1 (pg/mL)	39.7 ± 36.0	55.9 ± 59.9	35.4 ± 20.5	33.4 ± 28.9	25.0 ± 15.4	252 ± 617	43.3 ±42.7	52.4 ± 57.6	22.2 ± 20.6	163 ± 155	23.8 ± 23.8	59.1 ± 53.2
MIP-1α (pg/mL)	4.1 ± 3.7	1.6 ± 1.1	1.2 ± 0.9	5.4 ± 8.6	2.3 ±2.4	2.0 ± 2.0	2.2 ±2.6	1.2 ± 1.0	5.0 ± 6.0	14.2 ± 20.8	0.4 ± 0.5	6.4 ± 6.4
RANTES (pg/mL)	6.5 ± 5.5	8.3 ± 7.7	6.3 ± 5.8	5.6 ± 4.6	8.6 ± 6.2	13.6 ± 20.3	9.5 ± 4.1	7.9 ±3.5	290 ± 640	34.0 ± 36.4	9.0 ± 6.8	16.9 ± 22.3
IL-8 (pg/mL)	4.0 ± 2.4	2.8 ± 1.8	2.9 ± 3.8	1.9 ±1.0	2.6 ± 2.2	3.5 ± 3.5	4.2 ± 3.5	14.0 ± 25.5	38.9 ± 74.2	55.0 ± 65.1	4.1 ± 2.8	10.5 ± 8.6
IFN-γ (pg/mL)	11.2 ± 10.1	4.5 NC	16.8 ± 17.7	13.0 ± 12.4	47.0 NC	NC NC	50.6 ± 11.4	72.9 ± 74.6	6980 ± 9870	1823 ± 1302	66.9 ± 44.8	379 ± 424

NC = not calculable
"-" not available

Gross Pathology

See table below. For discussion of these findings, please refer to the Non-neoplastic Histopathology section.

Macroscopic Observations – Incidence (Mean Severity of Affected Animals)												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
Cavity, Thoracic												
Fluid, Red	3 (2.0)	3 (2.7)	9 (3.2)	6 (2.5)	5 (2.6)	1 (2.0)	3 (2.3)	6 (2.7)	4 (2.5)	11 (2.5)	8 (2.5)	2 (2.5)
Liver												
Enlarged	0 -	2 (4.0)	0 -	2 (3.0)	3 (3.3)	1 (3.0)	2 (3.0)	2 (2.5)	0 -	4 (3.0)	1 (3.0)	4 (3.2)
Mass	17 (P)	11 (P)	20 (P)	17 (P)	15 (P)	25 (P)	6 (P)	3 (P)	8 (P)	11 (P)	6 (P)	22 (P)
Nodule	4 (P)	0 (P)	1 (P)	2 (P)	1 (P)	2 (P)	0 (P)	1 (P)	2 (P)	3 (P)	1 (P)	3 (P)
Lung												
Focus/Foci, Red	0 -	1 (3.0)	0 -	1 (2.0)	0 -	0 -	0 -	0 -	1 (2.0)	1 (2.0)	0 -	0 -
Focus/Foci, Tan	0 -	0 -	1 (2.0)	1 (2.0)	0 -	0 -	0 -	1 (2.0)	2 (2.5)	2 (2.0)	0 -	3 (2.7)
Focus/Foci, White	0 -	3 (2.0)	0 -	3 (2.3)	0 -	4 (2.0)	1 (2.0)	0 -	3 (2.0)	0 -	0 -	0 -
Skin												
Abrasion/Scab	2 (3.0)	3 (2.7)	4 (2.5)	8 (2.4)	9 (2.7)	3 (2.3)	3 (2.3)	3 (2.7)	3 (2.3)	9 (2.6)	2 (2.0)	2 (2.0)
Skin/Subcutis												
Mass	0 -	1 (P)	2 (P)	4 (P)	0 -	4 (P)	2 (P)	5 (P)	3 (P)	1 (P)	4 (P)	7 (P)
Testes												
Small	0 -	1 (3.0)	1 (2.0)	2 (3.5)	2 (2.0)	4 (2.2)						

"-" not applicable

Organ Weights

↑ liver weight at HD (♂ & ♀), MD (♀) and with the mouse surrogate appear to be related to test article administration. Their may be an ↑ in heart weight in ♀s that is not accounted for by ↑ bodyweight.

Organ Weights – % Change from Control												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
Body Weight (g)	36.2	n.e.	n.e.	↑**22****	n.e.	n.e.	31.5	n.e.	↑**25****	n.e.	↓7	n.e.
Heart												
Absolute (g)	0.263	n.e.	n.e.	↑**16****	↑8	n.e.	0.209	↑7	↑**34****	↑**16***	↑7	↑12
% of BW	0.729	n.e.	↑8	n.e.	↑8	n.e.	0.669	n.e.	↑7	↑**15***	↑**15***	↑10
Ratio to BrW	0.543	n.e.	↑6	↑14	↑6	n.e.	0.423	↑6	↑**31****	↑14	↑6	↑11
Liver												
Absolute (g)	1.780	n.e.	n.e.	↑**77****	n.e.	↑**40****	1.805	↓8	↑**46***	↑23	↓14	↑**55****
% of BW	4.889	n.e.	↑6	↑**44****	n.e.	↑**47****	5.612	↓10	↑18	↑27	↓7	↑**55****
Ratio to BrW	3.692	n.e.	n.e.	↑**72****	n.e.	↑**43****	3.642	↓9	↑42	↑22	↓16	↑**56****

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
n.e. = no effect

Histopathology

Peer Review: None conducted.

Neoplastic

Neoplastic Findings												
Sex	Male						Female					
Dose Group	1	2	3	4	5	6	1	2	3	4	5	6
Day of Terminal Sacrifice	735	735	735	672	735	735	735	735	717	614	735	735
Adrenal glands												
adenoma, cortical (B, P)	2	1	-	-	2	2	-	-	-	-	1	1
adenoma, subcapsular cell (B, P)	3	4	1	-	1	2	4	1	3	-	2	1
carcinoma, subcapsular cell (M, P)	-	1	-	-	-	-	-	-	-	-	-	-
pheochromocytoma (B, P)	-	-	-	-	-	-	-	-	-	-	1	1
Brain												
carcinoma, pars distalis (M, S)	-	-	-	-	-	-	1	1	-	-	-	-
schwannoma (M, S)	1	-	-	-	-	-	-	-	-	-	-	-
Cavity, abdominal												
adenocarcinoma (M, S)	-	-	-	-	-	-	-	1	-	-	-	-
fibrosarcoma (M, S)	-	-	-	-	-	-	-	-	-	-	-	1
Leiomyosarcoma (M, S)	-	-	-	-	-	-	1	-	-	-	-	-
liposarcoma (M, S)	-	-	-	-	-	-	-	-	-	-	-	1
Cavity, thoracic												
carcinoma, bronchiolar alveolar (M, S)	1	-	-	-	1	-	-	-	-	-	-	-
liposarcoma (M, S)	-	-	-	-	-	-	-	-	-	-	-	1
osteosarcoma (M, P)	-	-	-	-	-	-	-	-	1	-	-	-
Coagulating glands												
adenoma (B, P)	-	-	-	-	-	1						
Epididymides												
adenoma (B, P)	-	-	-	-	-	1						
adenoma, interstitial cell (B, P)	-	-	-	-	-	1						
leiomyoma (B, P)	1	-	-	-	-	-						
Esophagus												
papilloma, squamous cell (B, P)	-	-	-	-	-	1	-	-	-	-	-	-
Gallbladder												
leiomyoma (B, P)	-	-	-	-	-	-	-	-	-	-	1	-
Harderian glands												
adenocarcinoma (M, P)	-	1	-	-	2	-	1	3	-	-	3	1
adenoma (B, P)	9	15	6	3	6	4	1	3	3	4	4	3
adenomas + adenocarcinomas + carcinomas	9	16	6	3	8	4	2	6	3	4	7	4
Head												
fibrosarcoma (M, P)	-	-	-	-	-	-	-	-	-	1	-	-

schwannoma (M, P)	1	-	-	-	-	-	-	-	-	-	-	-
Heart												
carcinoma, bronchiolar alveolar (M, S)	1	-	-	-	-	-	-	-	-	-	-	-
Injection site 4												
fibrosarcoma (M, P)	-	-	-	1	-	-	-	-	-	-	-	-
Kidneys												
carcinoma, bronchiolar alveolar (M, S)	1	-	-	-	-	-	-	-	-	-	-	-
carcinoma, tubular cell (M, P)	1	-	-	-	-	-	-	-	-	-	-	-
Liver												
adenocarcinoma (M, S)	-	-	-	-	-	-	-	1	-	-	-	-
adenoma, hepatocellular (M, P)	13	8	10	11	10	22	4	-	5	11	5	23
carcinoma, hepatocellular (M, P)	1	2	8	2	1	4	-	-	-	2	-	2
adenomas + carcinomas	14	10	18	13	11	26	4	-	5	13	5	25
fibrosarcoma (M, S)	-	-	1	-	-	-	-	-	-	-	-	-
Lung												
adenocarcinoma (M, S)	-	-	-	-	-	-	-	-	1	-	1	-
adenoma, bronchiolar alveolar (B, P)	16	15	10	11	18	8	10	9	17	11	9	8
carcinoma, bronchiolar alveolar (M, P)	10	3	6	3	6	3	5	4	4	3	5	5
bronchiolar/alveolar adenomas + carcinomas	26	18	16	14	24	11	15	13	21	14	14	13
carcinoma, hepatocellular (M, S)	-	-	2	-	-	-	-	-	-	-	-	-
fibrosarcoma (M, S)	-	-	-	1	-	-	-	-	-	-	-	1
leiomyosarcom (M, S)	-	-	-	-	-	-	1	-	-	-	-	-
Mammary gland												
adenoacanthoma (M, P)							-	-	1	-	-	-
adenocarcinoma (M, P)							2	4	2	2	1	4
adenoma, benign (B, P)							-	-	-	-	-	1
adenomas + adenocarcinomas + adenoacanthomas							2	4	3	2	1	5
Multicentric neoplasm												
hemangioma (B)	-	-	-	-	-	-	1	-	1	1	-	2
hemangiosarcoma (M)	10	7	9	11	10	16	2	8	6	11	9	2
hemangiomas + hemangiosarcomas	10	7	9	11	10	16	3	8	7	12	9	4
leukemia, granulocytic (M)	2	1	-	-	-	1	-	2	2	-	3	-
lymphoma (M)	1	5	3	3	9	-	13	15	6	7	7	9
sarcoma, histiocytic (M)	2	2	-	2	1	-	4	2	2	1	1	2
Ovaries												
cystadenoma (B, P)							2	4	1	3	-	4
granulosa cell tumor (B, P)							1	1	-	-	-	-
leiomyosarcoma (M, S)							1	-	-	-	-	-
luteoma (B, P)							1	-	-	1	1	-
Pancreas												
adenoma, islet cell (B, P)	-	1	-	-	1	-	2	-	-	-	-	-
carcinoma, islet cell (M, P)	-	-	-	-	-	-	1	-	-	-	-	-
leiomyosarcoma (M, S)	-	-	-	-	-	-	1	-	-	-	-	-
liposarcoma (M, S)	-	-	-	-	-	-	-	-	-	-	-	1
Pituitary gland												
adenoma, pars distalis (B, P)	-	1	1	1	-	-	7	4	8	4	6	6
adenoma, pars intermedia (B, P)	-	-	-	-	-	-	-	1	-	1	-	-
carcinoma, pars distalis (M, P)	-	-	-	-	-	-	1	1	-	-	-	-
anterior lobe, adenomas + carcinomas	-	1	1	1	-	-	8	6	8	5	6	6
Prostate gland												

adenocarcinoma (M, P)	1	-	-	-	-	-						
Skeletal muscle												
carcinoma, bronchiolar alveolar (M, S)	1	2	1	-	-	-	-	-	-	-	-	-
osteosarcoma (M, P)	-	-	-	-	-	-	-	-	-	-	1	-
Skin, subcutis												
carcinoma, bronchiolar alveolar (M, S)	1	-	-	-	-	-	-	-	-	-	-	-
carcinoma, squamous cell (M, P)	-	-	-	-	-	-	-	-	-	-	2	-
hair follicle tumor (B, P)	-	-	-	-	-	-	-	-	-	-	1	1
fibrosarcoma (M, P)	-	-	1	4	-	3	-	1	-	-	1	1
fibrous histiocytoma (M, P)	-	-	-	-	-	-	-	1	-	-	-	-
liposarcoma (M, P)	-	-	-	-	-	-	-	-	-	-	-	1
osteoma (B, P)	-	-	-	-	-	-	-	-	-	-	-	1
sarcomas + fibrosarcomas + liposarcomas + rhabdomyosarcomas	-	-	1	4	-	3	-	1	-	-	1	2
Small intestine, duodenum												
adenocarcinoma (M, P)	-	-	-	-	1	-	-	-	-	-	-	-
adenoma (B, P)	-	-	-	-	1	-	-	-	-	-	-	-
Stomach, nonglandular												
carcinoma, squamous cell (M, P)	-	-	-	-	-	-	-	-	-	-	-	1
leiomyosarcoma (M, S)	-	-	-	-	-	-	1	-	-	-	-	-
papilloma, squamous cell (B, P)	-	-	-	-	-	-	-	-	1	-	-	-
Testes												
adenoma, interstitial cell (B, P)	-	2	4	-	2	1						
Sertoli cell tumor (B, P)	-	1	-	-	-	-						
Thyroid gland												
adenoma, follicular cell (B, P)	-	-	-	-	-	-	2	-	-	-	-	-
carcinoma, follicular cell (M, P)	-	-	-	-	1	-	-	-	-	-	1	1
follicular adenomas + carcinomas	-	-	-	-	1	-	2	-	-	-	1	1
Tongue												
carcinoma, squamous cell (M, P)	-	-	-	-	-	-	-	-	-	-	1	-
Urinary bladder												
carcinoma, transitional cell (M, P)	-	-	-	-	1	-	-	-	-	-	-	-
mesenchymal tumor (B, P)	2	-	-	-	-	1	-	-	-	-	-	-
Uterus with cervix												
adenocarcinoma (M, P)							-	-	-	-	1	-
leiomyoma (B, P)							1	2	3	2	4	-
leiomyosarcoma (M, P)							4	1	4	-	-	2
leiomyomas + leiomyosarcomas							5	3	7	2	5	2
polyp, glandular (B, P)							1	2	2	1	-	1
polyp, stromal (B, P)							4	6	4	4	3	9
sarcoma, stromal (M, P)							1	-	-	2	2	1
Vagina												
leiomyosarcoma (M, P)							1	-	-	-	-	-

"-" = not observed in dose group
B = benign, M = malignant, P = primary, S = secondary

Sponsor's statistical analysis: *quoted from study report*

Males

There were no statistically significant increases in any type of neoplasm in the male mice with either the Fisher Exact test for pair-wise comparisons, the Cochran-Armitage Trend test, the Peto test, the Onset rate test, or the Poly-3 Trend test. The Poly-3 pair-wise analysis gave the following positive results (s.s. findings in blue):

hepatocellular carcinoma:

LD_{5QW}: 2/70
MD_{20QW}: 8/70 or 11.43% overall rate, 16.27% adjusted rate
MD_{80QM}: 1/70
HD_{60QW}: 2/70
$S_{60QW:}$ 4/70
C: 1/70 or 1.43% overall rate, 2.04% adjusted rate
HC: 0-10%, mean incidence of 3.8% (common tumor)

malignant lymphoma:

LD_{5QW}: 5/70
MD_{20QW}: 3/70
MD_{80QM}: 9/70 or 12.86% overall rate, 18.50% adjusted rate
HD_{60QW}: 3/70
$S_{60QW:}$ 0/70
C: 1/70 or 1.43% overall rate, 2.00% adjusted
HC: 0-13.3%, mean incidence of 6.2% (common tumor)

fibrosarcoma:

LD_{5QW}: 0/70
MD_{20QW}: 1/70
MD_{80QM}: 0/70
HD_{60QW}: 4/70 or 5.71% overall rate, 11.05% adjusted rate
$S_{60QW:}$ 3/70 or 4.29% overall rate, 5.99% adjusted
C: 0/70 or 0.00% overall rate and adjusted rate
HC: 0-3.3%, mean incidence of 0.4% (rare tumor)

Females

hepatocellular adenoma:

LD_{5QW}: 0/70
MD_{20QW}: 5/70
MD_{80QM}: 5/70
HD_{60QW}: 11/67 or 16.42% overall rate, 34.56% adjusted rate
$S_{60QW:}$ 23/69 or 33.33% overall rate, 41.51% adjusted rate
C: 4/70 or 5.71% overall rate, 9.38% adjusted rate
HC: 0-5.7%, mean incidence of 1.4% (common tumor)

Blue s.s. by one or more: Cochran-Armitage trend test, the Peto test, the Poly-3 trend test, the Fisher Exact and the Poly-3 pair-wise comparisons

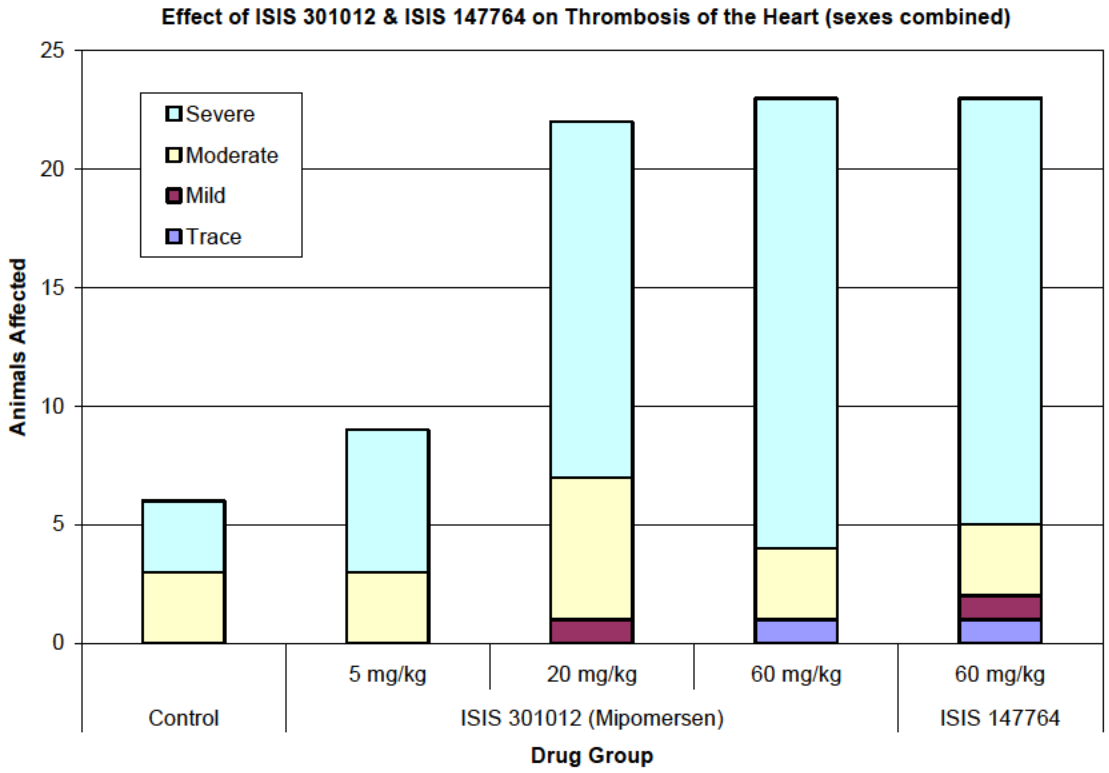
hemangiosarcoma:

LD_{5QW}: 8/70
MD_{20QW}: 6/70
MD_{80QM}: 9/70
HD_{60QW}: 11/70 or 15.71% overall rate, 32.79% adjusted rate

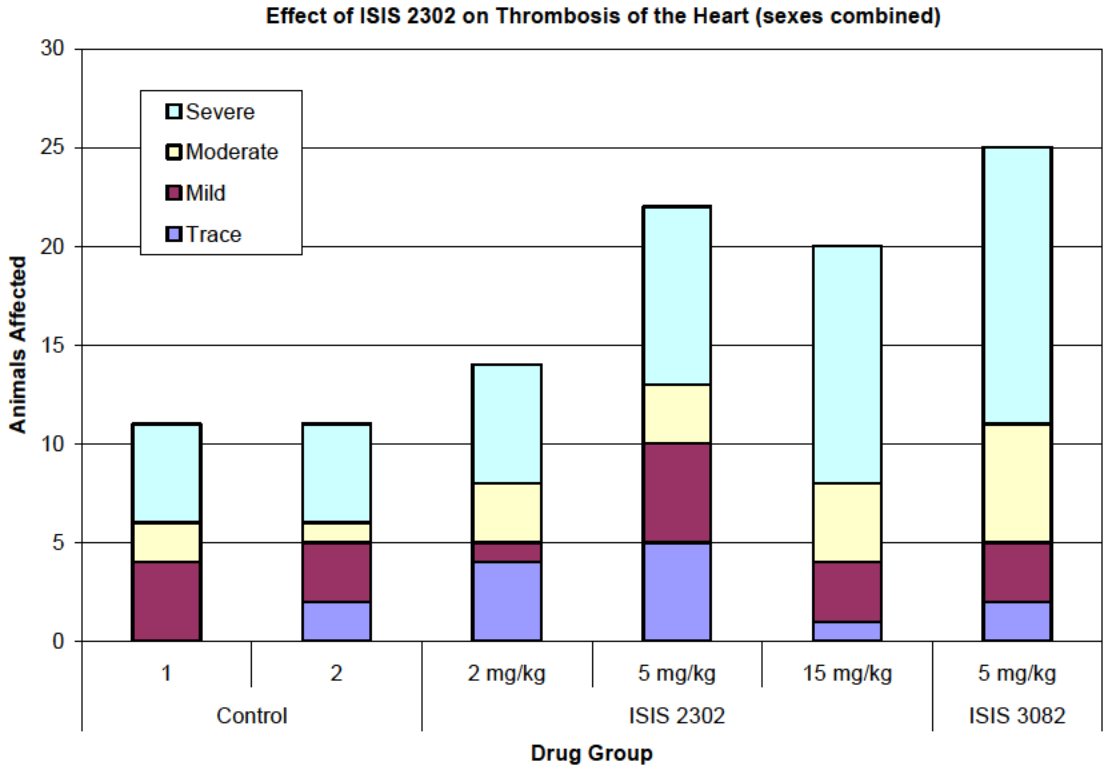
S_{60QW}: 2/70
C: 2/70 or 2.86% overall rate, 4.67% adjusted rate
HC: 0-15.0%, mean incidence of 4.6% (common tumor)
Blue s.s. by the Poly-3 pair-wise comparisons

Non Neoplastic

Mipomersen (and the mouse surrogate) were associated with cardiac findings. Perhaps most striking was a generally dose dependent increase in the incidence and severity of cardiac thrombosis at doses ≥ 20 mg/kg/injection.



Sponsor believes that this finding is spurious and argues that the incidence in the control groups was lower than the (b) (4) historical control incidence (captured below); however, it is notable that another phosphorothioate oligonucleotide (ISIS 2302, antisense to ICAM-1), and its murine surrogate, were also associated with an increased incidence and severity of thrombosis (see below). This reviewer concludes that phosphorothioate oligonucleotides increase the incidence and severity of cardiac thrombosis in mice. The ↑ incidence of chronic passive congestion of the lung seen in both sexes at doses ≥ 20 mg/kg is likely to be secondary to left atrial thrombosis.



Other coronary findings were seen primarily in females: slightly worsened spontaneous cardiomyopathy in MD & HD, atrial/ventricular dilatation in MD & HD and endocarditis in females treated with the surrogate.

As opposed to rats (see below), there is no evidence of oligonucleotide induced kidney toxicity. Indeed, oligonucleotides may be protective of the kidney, as the incidence and/or severity of multiple spontaneous kidney findings were dose-dependently reduced: chronic progressive nephropathy, tubular mineralization, hydronephrosis, cysts, hyaline droplet formation.

At HD, the test item was associated with increased epidermal hyperplasia in the skin and the injection sites.

Females exhibited a dose-dependent ↑ in the incidence/severity of lobular hyperplasia in the mammary gland.

Single cell necrosis (minimal-moderate) of hepatocytes is increased vs. control for most oligonucleotide-treated groups, with the exception of HD♂s. The ↑ incidence is dose-dependent in ♀s.

Lymphohistiocytic infiltrates in many tissues, sinus histiocytosis in lymph nodes, and basophilic granules in Kupffer cells of the liver and in the cytoplasm of kidney tubule epithelial cells were common findings in mice from all treated groups with a clear dose response relationship for the number of tissues affected and/or the severity of the

infiltrates. Per the Sponsor, in all cases, these findings represent uptake of the oligonucleotide test article into affected cells.

A number of findings exhibited an inverse relationship to test item dose. These are spontaneous findings whose incidence/severity generally increases with age. In some cases, the apparent decrease in the incidence/severity of these findings (depicted by green text in reviewer's table below) is considered an artifact of early death/terminal sacrifice in the ISIS 301012-treated animals. However, in some cases, these decreases were seen in dose groups that survived to the scheduled terminal sacrifice. In these cases, the decreased incidence/severity may reflect a protective effect of the oligonucleotide (mechanism unknown). Examples include: multiple findings in the kidney (discussed above); monocytic infiltrates and pigmented macrophages in the liver; dilatation of the seminal vesicles.

Increased extramedullary hematopoiesis was observed in multiple tissues (especially the liver) in all treatment groups. This is presumably a homeostatic response to the decreased red cell mass noted above.

Non-neoplastic Histopathology – Incidence (Mean Severity of Affected Animals)												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
Adrenal Glands												
Ceroid, increased	1/69 (1.0)	4/70 (1.0)	7/69 (1.0)	6/69 (1.0)	3/69 (1.3)	4/70 (1.0)	33/70 (1.4)	43/70 (1.5)	48/69 (1.4)	56/70 (1.4)	48/69 (1.3)	47/70 (1.4)
Hyperplasia, focal, cortical	0/69 -	0/70 -	1/69 (1.0)	2/69 (2.0)	0/69 -	2/70 (2.0)	0/70 -	0/70 -	0/69 -	0/70 -	0/69 -	0/70 -
Hypertrophy, focal, cortical	2/69 (1.5)	1/70 (2.0)	2/69 (2.0)	1/69 (2.0)	5/69 (2.0)	7/70 (1.9)	0/70 -	1/70 (1.0)	0/69 -	0/70 -	0/69 -	1/70 (1.0)
Bone Marrow, Femur												
Hyperplasia, granulocytic	2/70 (2.0)	10/70 (1.8)	11/70 (2.0)	12/70 (2.0)	12/70 (2.1)	8/70 (2.0)	6/70 (1.9)	12/70 (2.1)	11/70 (2.1)	7/70 (2.0)	3/69 (1.7)	9/70 (2.0)
Bone Marrow, Sternum												
Hyperplasia, granulocytic	2/68 (2.0)	10/70 (1.7)	11/70 (1.7)	13/70 (1.8)	11/69 (1.9)	8/70 (2.0)	10/70 (1.9)	11/70 (2.1)	10/70 (2.0)	7/70 (2.0)	3/69 (1.3)	8/69 (1.9)
Ears												
Erosion/Ulcer	1/1 (2.0)	2/2 (3.0)	2/3 (2.0)	5/5 (3.2)	4/4 (2.2)	3/4 (2.3)	2/2 (3.0)	0/0 -	0/0 -	2/2 (2.5)	0/1 -	1/1 (3.0)
Epididymides												
Dilatation	1/70 (2.0)	1/70 (3.0)	2/70 (2.0)	4/70 (1.8)	1/70 (2.0)	1/70 (2.0)						
Oligospermia/Germ cell debris, unilateral	3/70 (4.0)	3/70 (3.7)	4/70 (3.8)	6/70 (4.0)	6/70 (3.7)	9/70 (3.8)						
Heart												
Cardiomyopathy	40/70 (1.0)	44/70 (1.2)	44/69 (1.2)	42/69 (1.1)	43/70 (1.2)	55/70 (1.2)	24/69 (1.0)	28/70 (1.0)	39/69 (1.3)	36/70 (1.4)	34/69 (1.0)	40/70 (1.1)
Dilatation, ventricular/atrial	0/70 -	0/70 -	0/69 -	0/69 -	0/70 -	0/70 -	0/69 -	0/70 -	2/69 (2.5)	2/70 (2.0)	0/69 -	1/70 (2.0)
Endocarditis, valvular, vegetative	0/70 -	0/70 -	0/69 -	0/69 -	0/70 -	0/70 -	0/69 -	0/70 -	0/69 -	0/70 -	2/69 (3.5)	2/70 (3.0)
Thrombus	2/70 (3.5)	4/70 (3.5)	9/69 (3.8)	13/69 (3.7)	9/70 (4.0)	13/70 (3.8)	4/69 (3.5)	4/70 (3.8)	11/69 (3.5)	10/70 (3.8)	6/69 (3.5)	10/70 (3.5)
Injection Site 1												

Crust, serocellular	0/70 -	0/70 -	0/70 -	5/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/70 -	0/69 -	0/70 -
Erosion/Ulcer	0/70 -	1/70 (1.0)	0/70 -	2/70 (2.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/69 (2.0)	0/70 -
Exudate, epidermal surface	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/69 -	0/70 -
Fibrosis	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/69 -	0/70 -
Hyperplasia, epidermal	0/70 -	2/70 (1.5)	0/70 -	10/70 (1.5)	1/70 (2.0)	1/70 (2.0)	0/70 -	1/70 (2.0)	1/70 (2.0)	4/70 (2.0)	2/69 (1.5)	0/70 -
Injection Site 2												
Crust, serocellular	0/70 -	0/70 -	0/70 -	3/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/69 -	0/70 -
Erosion/Ulcer	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/70 -	2/70 (1.5)	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/69 -	0/70 -
Exudate, epidermal surface	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/69 -	0/70 -
Hemorrhage	0/70 -	0/70 -	1/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/69 -	2/70 (2.0)
Hyperplasia, epidermal	0/70 -	0/70 -	1/70 (1.0)	9/70 (1.8)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	6/70 (1.7)	1/69 (2.0)	0/70 -
Injection Site 3												
Erosion/Ulcer	0/70 -	0/70 -	0/70 -	1/70 (3.0)	1/70 (2.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/69 -	0/70 -
Hemorrhage	0/70 -	0/70 -	0/70 -	1/70 (2.0)	2/70 (1.0)	1/70 (2.0)	0/70 -	0/70 -	0/70 -	2/70 (2.0)	0/69 -	2/70 (2.0)
Injection Site 4												
Hemorrhage	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/70 -	1/70 (1.0)	0/70 -	0/70 -	0/70 -	1/70 (1.0)	1/69 (1.0)	1/70 (1.0)
Hyperplasia, epidermal	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/69 (1.0)	1/70 (2.0)
Kidney												
Basophilic granules, tubular cell	0/70 -	6/70 (1.0)	29/70 (1.3)	39/70 (1.2)	14/70 (1.4)	55/70 (1.8)	0/70 -	15/70 (1.4)	30/69 (1.1)	49/70 (1.4)	10/69 (1.2)	65/70 (1.6)
Cyst	35/70 (1.6)	23/70 (1.5)	14/70 (1.1)	7/70 (1.3)	22/70 (1.5)	28/70 (1.4)	20/70 (1.3)	4/70 (1.0)	6/69 (1.3)	4/70 (1.2)	8/68 (1.1)	12/70 (1.3)
Hyaline, droplets, increased	0/70 -	1/70 (2.0)	0/70 -	0/70 -	0/70 -	0/70 -	4/70 (2.2)	2/70 (2.0)	1/69 (2.0)	0/70 -	1/69 (1.0)	0/70 -
Hydronephrosis, bilateral	15/70 (2.5)	1/70 (2.0)	2/70 (3.0)	1/70 (3.0)	4/70 (2.0)	10/70 (2.5)	3/70 (2.3)	0/70 -	0/69 -	0/70 -	0/69 -	8/70 (2.2)
Mineralization, tubular	40/70 (1.0)	30/70 (1.0)	22/70 (1.0)	5/70 (1.0)	31/70 (1.0)	28/70 (1.0)	5/70 (1.0)	0/70 -	1/69 (1.0)	0/70 -	0/69 -	1/69 (1.0)
Nephropathy, chronic progressive	52/70 (1.1)	40/70 (1.1)	33/70 (1.1)	29/70 (1.0)	44/70 (1.1)	35/70 (1.0)	42/70 (1.4)	40/70 (1.5)	36/69 (1.5)	21/70 (1.4)	34/69 (1.5)	35/70 (1.5)
Liver												
Basophilic granules, Kupffer cells	0/70 -	61/70 (1.0)	66/70 (2.0)	68/70 (2.9)	66/70 (1.6)	69/70 (2.1)	1/70 (1.0)	50/70 (1.0)	66/68 (1.6)	66/67 (2.8)	64/69 (1.3)	67/69 (2.0)
Cyst, biliary	2/70 (3.0)	1/70 (2.0)	3/70 (2.0)	2/70 (2.5)	8/70 (2.9)	6/70 (2.3)	2/70 (2.0)	2/70 (2.0)	5/68 (2.4)	2/67 (2.5)	4/69 (2.8)	9/69 (2.3)
Focus of cellular alteration, basophilic	1/70 (1.0)	5/70 (2.0)	4/70 (2.2)	6/70 (1.7)	4/70 (1.2)	9/70 (1.4)	1/70 (1.0)	1/70 (1.0)	2/68 (1.5)	2/67 (1.5)	1/69 (2.0)	9/69 (1.3)
Focus of cellular alteration, eosinophilic	1/70 (2.0)	2/70 (2.0)	1/70 (1.0)	4/70 (2.2)	3/70 (1.0)	8/70 (1.6)	0/70 -	1/70 (1.0)	1/68 (3.0)	10/67 (1.8)	0/69 -	9/69 (1.8)
Hypertrophy, eosinophilic, biliary	0/70 -	0/70 -	0/70 -	1/70 (2.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/68 -	0/67 -	0/69 -	0/69 -
Hypertrophy, hepatocyte, centrilobular	0/70 -	0/70 -	0/70 -	1/70 (2.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/68 -	0/67 -	0/69 -	0/69 -
Hypertrophy, hepatocyte, panlobular	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (2.0)	0/70 -	0/70 -	0/68 -	0/67 -	0/69 -	1/69 (2.0)
Infiltration,	29/70	21/70	23/70	9/70	16/70	24/70	31/70	15/70	13/68	8/67	14/69	33/69

mononuclear cell	(1.0)	(1.0)	(1.0)	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.2)	(1.1)	(1.4)
Macrophages, pigmented	11/70 (1.0)	8/70 (1.0)	12/70 (1.1)	1/70 (1.0)	9/70 (1.0)	16/70 (1.2)	30/70 (1.1)	8/70 (1.0)	16/68 (1.0)	7/67 (1.4)	9/69 (1.0)	37/69 (1.2)
Necrosis, individual hepatocyte	3/70 (1.0)	10/70 (1.0)	9/70 (1.0)	2/70 (1.0)	13/70 (1.0)	28/70 (1.2)	1/70 (1.0)	6/70 (1.0)	7/68 (1.1)	12/67 (1.2)	5/69 (1.4)	12/69 (1.1)
Lung												
Congestion, chronic passive	9/70 (2.1)	7/70 (1.4)	12/70 (1.6)	13/70 (1.3)	10/70 (1.5)	15/70 (1.8)	8/70 (2.0)	8/70 (1.8)	17/70 (1.9)	18/70 (1.8)	16/69 (1.6)	11/70 (1.8)
Lymph node, mandibular												
Depletion, lymphoid	0/69 -	0/69 -	0/67 -	0/68 -	1/68 (3.0)	1/69 (2.0)	0/67 -	0/70 -	2/67 (3.0)	1/67 (3.0)	1/66 (3.0)	1/66 (4.0)
Erythrocytosis/ erythrophagocytosis, sinus	2/69 (1.5)	1/69 (1.0)	1/67 (1.0)	3/68 (1.7)	1/68 (1.0)	3/69 (1.3)	3/67 (1.3)	5/70 (1.8)	8/67 (1.2)	6/67 (1.5)	5/66 (1.2)	9/69 (1.3)
Lymph node, mesenteric												
Congestion	0/70 -	0/69 -	0/67 -	2/66 (2.5)	0/69 -	0/70 -	0/67 -	0/69 -	0/65 -	6/66 (2.2)	0/65 -	0/67 -
Depletion, lymphoid	0/70 -	1/69 (3.0)	4/67 (3.2)	2/66 (3.0)	1/69 (3.0)	2/70 (3.5)	1/67 (3.0)	4/69 (2.5)	7/65 (3.3)	0/66 -	1/65 (3.0)	2/67 (3.0)
Depletion, lymphoid generalized	0/70 -	1/69 (3.0)	4/67 (3.2)	2/66 (3.0)	1/69 (3.0)	2/70 (3.5)	0/67 -	0/69 -	2/65 (3.5)	1/66 (2.0)	1/65 (3.0)	0/67 -
Erythrocytosis/ erythrophagocytosis, sinus	13/70 (1.2)	8/69 (1.4)	15/67 (1.5)	17/66 (1.7)	9/69 (1.4)	18/70 (1.4)	11/67 (1.4)	13/69 (1.5)	22/65 (1.7)	29/66 (1.7)	19/65 (1.3)	21/67 (1.4)
Mammary gland												
Hyperplasia, lobular							21/69 (1.5)	23/70 (1.7)	46/70 (1.8)	49/70 (1.9)	32/69 (1.7)	40/70 (1.8)
Nerve, sciatic												
Degeneration, axonal/myelin	46/70 (1.3)	37/70 (1.2)	34/70 (1.2)	25/70 (1.0)	34/70 (1.2)	41/70 (1.2)	43/70 (1.6)	36/70 (1.4)	42/70 (1.3)	28/70 (1.1)	45/70 (1.3)	52/70 (1.5)
Ovaries												
Angiectasis							0/69 -	0/70 -	1/69 (2.0)	1/69 (2.0)	0/68 -	3/70 (1.7)
Cyst							53/69 (2.3)	52/70 (2.5)	44/68 (2.1)	39/69 (2.1)	51/68 (2.4)	49/70 (2.6)
Seminal vesicles												
Dilatation	31/70 (2.4)	23/70 (2.2)	18/70 (2.2)	8/70 (2.1)	17/70 (2.3)	17/70 (2.1)						
Skin												
Crust, serocellular	0/70 -	0/70 -	1/70 (2.0)	3/70 (2.0)	1/70 (2.0)	1/70 (2.0)	0/70 -	1/70 (2.0)	0/70 -	1/70 (1.0)	0/70 -	1/70 (2.0)
Erosion/Ulcer	4/70 (2.2)	1/70 (3.0)	4/70 (2.8)	7/70 (2.7)	8/70 (3.0)	3/70 (3.0)	2/70 (3.0)	4/70 (3.2)	2/70 (2.5)	4/70 (2.8)	2/69 (2.0)	1/70 (2.0)
Hyperplasia, epidermal	1/70 (3.0)	1/70 (1.0)	3/70 (2.0)	7/70 (2.6)	2/70 (2.0)	3/70 (2.7)	3/70 (2.3)	3/70 (2.7)	2/70 (2.0)	5/70 (2.0)	2/69 (2.0)	1/70 (2.0)
Testes												
Degeneration/atrophy, seminiferous tubules bilateral	17/70 (2.5)	11/69 (3.1)	12/70 (2.4)	8/70 (3.1)	8/70 (2.8)	22/70 (2.8)						
Ureters												
Dilatation	13/69 (2.5)	14/70 (2.6)	11/70 (2.1)	6/68 (2.7)	9/66 (2.3)	5/70 (2.4)	1/69 (1.0)	0/68 -	0/68 -	0/68 -	0/69 -	3/69 (2.7)
Urinary bladder												
Infiltration, lymphocytic	5/70 (1.0)	3/70 (1.0)	8/70 (1.0)	1/70 (1.0)	3/70 (1.0)	4/70 (1.0)	22/70 (1.1)	19/70 (1.0)	18/70 (1.0)	11/70 (1.0)	17/69 (1.0)	25/70 (1.0)
Zymbal's gland												
Infiltration, lymphocytic	2/64 (1.0)	8/65 (1.0)	10/63 (1.1)	15/61 (1.0)	9/67 (1.0)	12/69 (1.0)	10/66 (1.0)	6/63 (1.0)	10/67 (1.0)	9/65 (1.0)	5/64 (1.0)	13/68 (1.0)

Green text depicts findings that exhibited an inverse relationship to test item.
The columns for groups 5 & 6 are slightly shaded to indicate that they should be analyzed separately from groups 1-4.

Lymphohistiocytic Infiltrates – Incidence (Mean Severity of Affected Animals)												
Sex	Male						Female					
Dose Group	1	2	3	4	5	6	1	2	3	4	5	6
Day of Terminal Sacrifice	735	735	735	672	735	735	735	735	717	614	735	735
Clitoral glands							0/70 -	0/70 -	0/70 -	23/69 (1.0)	3/69 (1.0)	5/70 (1.0)
Coagulating glands	0/70 -	0/70 -	0/70 -	7/69 (1.0)	0/70 -	3/70 (1.3)						
Epididymides	0/70 -	5/70 (1.0)	61/70 (1.0)	65/70 (1.5)	56/70 (1.0)	67/70 (1.2)						
Harderian Glands	0/70 -	0/70 -	0/70 -	2/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	1/68 (1.0)	1/70 (1.0)	0/69 -	0/70 -
Heart	1/70 (4.0)	50/70 (1.0)	65/69 (1.9)	66/69 (2.3)	63/70 (1.2)	65/70 (1.8)	0/69 -	32/70 (1.0)	60/69 (1.3)	67/70 (2.3)	58/69 (1.0)	67/70 (1.5)
Injection Site 1	0/70 -	20/70 (1.0)	64/70 (1.0)	66/70 (1.0)	31/70 (1.0)	62/70 (1.0)	0/70 -	16/70 (1.0)	53/70 (1.0)	63/70 (1.0)	27/69 (1.0)	55/70 (1.0)
Injection Site 2	1/70 (1.0)	17/70 (1.0)	60/70 (1.0)	64/70 (1.0)	34/70 (1.0)	62/70 (1.0)	0/70 -	19/70 (1.0)	46/70 (1.0)	63/70 (1.0)	30/69 (1.0)	51/70 (1.0)
Injection Site 3	1/70 (1.0)	36/70 (1.0)	67/70 (1.0)	67/70 (1.0)	39/70 (1.0)	65/70 (1.0)	0/70 -	35/70 (1.0)	59/70 (1.0)	67/70 (1.0)	38/69 (1.0)	60/70 (1.0)
Injection Site 4	1/70 (1.0)	35/70 (1.0)	66/70 (1.0)	68/70 (1.0)	46/70 (1.0)	66/70 (1.0)	0/70 -	36/70 (1.0)	61/70 (1.0)	66/70 (1.0)	41/69 (1.0)	63/70 (1.0)
Joint, Tibiofemoral	0/70 -	0/70 -	0/70 -	3/70 (1.0)	1/70 (1.0)	0/70 -	0/70 -	1/70 (1.0)	0/70 -	3/70 (1.0)	0/69 -	1/70 (1.0)
Kidney	0/70 -	1/70 (1.0)	16/70 (1.0)	29/70 (1.0)	5/70 (1.0)	4/70 (1.0)	0/70 -	0/70 -	6/69 (1.2)	30/70 (1.1)	4/69 (1.0)	0/70 -
Lacrimal glands, exorbital	0/70 -	0/70 -	0/70 -	2/70 (1.0)	0/70 -	0/70 -	0/69 -	0/68 -	0/70 -	1/69 (1.0)	0/69 -	0/70 -
Larynx	0/69 -	0/70 -	0/70 -	0/68 -	0/68 -	1/68 (1.0)	0/70 -	0/70 -	1/70 (1.0)	2/68 (1.0)	0/65 -	2/69 (1.0)
Lung, alveolar (histiocytosis)	5/70 (1.0)	7/70 (1.0)	11/70 (1.2)	27/70 (1.1)	7/70 (1.0)	7/70 (1.0)	6/70 (1.5)	15/70 (1.1)	13/70 (1.5)	26/70 (1.2)	14/69 (1.0)	15/70 (1.1)
Lymph node, mandibular sinus (histiocytosis)	0/69 -	2/69 (1.0)	21/67 (1.0)	53/68 (1.1)	8/68 (1.0)	49/69 (1.0)	0/67 -	4/70 (1.0)	16/67 (1.1)	47/67 (1.1)	14/66 (1.0)	36/69 (1.0)
Lymph node, mesenteric sinus (histiocytosis)	0/70 -	3/69 (1.0)	21/67 (1.0)	43/66 (1.2)	9/69 (1.0)	37/69 (1.1)	0/67 -	4/69 (1.0)	11/65 (1.2)	42/66 (1.1)	8/65 (1.0)	27/69 (1.0)
Pancreas	0/70 -	36/69 (1.0)	53/69 (1.0)	58/69 (1.3)	38/69 (1.0)	54/70 (1.1)	0/68 -	8/69 (1.0)	41/68 (1.0)	53/67 (1.1)	25/67 (1.0)	53/67 (1.0)
Preputial glands	0/70 -	2/70 (1.0)	25/70 (1.0)	56/70 (1.2)	17/69 (1.1)	31/70 (1.0)						
Prostate gland	0/70 -	0/70 -	16/70 (1.0)	47/69 (1.1)	6/70 (1.0)	22/70 (1.0)						
Seminal vesicles	0/70 -	0/70 -	3/70 (1.0)	10/70 (1.0)	0/70 -	1/70 (1.0)						
Skin	0/70 -	27/70 (1.0)	63/70 (1.0)	65/70 (1.0)	38/70 (1.0)	61/70 (1.0)	0/70 -	22/70 (1.0)	46/70 (1.0)	61/70 (1.1)	28/69 (1.0)	54/70 (1.0)
Spleen	0/70 -	0/67 -	7/70 (1.0)	25/67 (1.1)	1/68 (1.0)	30/70 (1.0)	0/70 -	0/70 -	4/69 (1.0)	17/63 (1.1)	1/66 (1.0)	29/68 (1.0)
Testes	0/70 -	7/69 (1.0)	57/70 (1.0)	64/70 (1.5)	54/70 (1.0)	66/70 (1.0)						
Thyroid	0/70 -	0/69 -	13/67 (1.0)	30/69 (1.0)	7/70 (1.0)	12/70 (1.0)	0/70 -	0/70 -	12/69 (1.0)	31/70 (1.0)	8/68 (1.0)	12/70 (1.0)
Tongue	0/70 -	0/70 -	0/70 -	2/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/70 -	1/70 (1.0)	0/69 -	0/70 -
Urinary bladder	0/70 -	4/70 (1.0)	54/70 (1.0)	56/70 (1.0)	35/70 (1.0)	52/70 (1.0)	0/70 -	5/70 (1.0)	35/70 (1.0)	57/70 (1.1)	32/69 (1.0)	60/70 (1.0)
Uterus with cervix							0/70 -	2/70 (1.0)	12/70 (1.0)	58/70 (1.3)	11/69 (1.0)	39/70 (1.1)

Vagina							0/69 -	1/70 (1.0)	11/70 (1.0)	35/70 (1.0)	7/69 (1.0)	44/70 (1.0)

The columns for groups 5 & 6 are slightly shaded to indicate that they should be analyzed separately from groups 1-4.

Extramedullary Hematopoiesis – Incidence (Mean Severity of Affected Animals)												
Sex	**Male**						**Female**					
Dose Group	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
Day of Terminal Sacrifice	**735**	**735**	**735**	**672**	**735**	**735**	**735**	**735**	**717**	**614**	**735**	**735**
Harderian Glands	0/70 -	0/70 -	1/70 (1.0)	2/70 (1.0)	0/70 -	0/70 -	0/70 -	0/70 -	0/68 -	0/70 -	0/69 -	0/70 -
Kidney	0/70 -	0/70 -	0/70 -	1/70 (1.0)	1/70 (1.0)	0/70 -	0/70 -	0/70 -	0/69 -	0/70 -	0/69 -	0/70 -
Liver	2/70 (1.0)	3/70 (1.0)	6/70 (1.0)	12/70 (1.4)	3/70 (1.3)	8/70 (1.1)	3/70 (1.0)	8/70 (1.2)	6/68 (1.3)	14/67 (1.0)	7/69 (1.0)	6/69 (1.5)
Lymph node, hepatic	0/4 -	0/4 -	0/2 -	1/11 (1.0)	0/7 -	4/9 (1.0)	0/3 -	0/2 -	0/2 -	0/6 -	0/2 -	0/11 -
Lymph node, mesenteric	0/70 -	0/69 -	1/67 (1.0)	4/66 (1.2)	3/69 (1.3)	4/70 (1.0)	2/67 (1.5)	4/69 (1.2)	1/65 (1.0)	3/66 (1.3)	3/65 (1.3)	8/67 (1.1)
Spleen	37/70 (1.2)	39/67 (1.2)	49/70 (1.3)	27/67 (1.6)	49/68 (1.2)	51/70 (1.4)	39/70 (1.5)	46/70 (1.6)	40/69 (1.4)	34/63 (1.4)	44/66 (1.5)	55/68 (1.3)

The columns for groups 5 & 6 are slightly shaded to indicate that they should be analyzed separately from groups 1-4.

Selected Non-neoplastic Historical Control Data

Heart

Female

Study		A	A	B	C	D
Experimental Start Date		1/10/2001	1/10/2001	4/6/2001	4/26/2002	9/29/2004
Experimental Termination Date		1/13/2003	1/13/2003	4/9/2003	4/13/2004	9/29/2006
Route of Administration		Dermal	Dermal	Oral Gavage	Intravaginal	Oral Gavage
Intervals Used		Terminal	Terminal	0 to Termination	Terminal	Terminal
Control Group		C-1	C-2	C-1	C-1	C-1
Number of Animals		70	70	60	50	60
Heart		(70)	(70)	(60)	(50)	(60)
Bacterial colonies		0	0	0	1	0
	-minimal	0	0	0	0	0
	-mild	0	0	0	0	0
	-moderate	0	0	0	0	0
	-severe	0	0	0	1	0
Cardiomyopathy		20	9	32	11	16
	-minimal	20	8	27	10	14
	-mild	0	1	5	1	2
	-moderate	0	0	0	0	0
Dilatation		*No data reported*				
Endocarditis, valvular, vegetative		*No data reported*				
Thrombus		3	5	5	4	3
	-minimal	0	1	2	0	0
	-mild	3	2	0	1	2
	-moderate	0	1	2	0	1
	-severe	0	1	1	3	0

Study		E	F	G	H	H
Experimental Start Date		3/23/2004	12/7/2004	11/8/2000	5/9/2001	5/9/2001
Experimental Termination Date		3/17/2006	9/14/2006	10/3/2002	5/12/2003	5/12/2003
Route of Administration		Oral Gavage	Oral Gavage	Subcutaneous	Subcutaneous	Subcutaneous
Intervals Used		Terminal	Terminal	0 to Termination	Terminal	Terminal
Control Group		C-1	C-1	C-1	C-1	C-2
Number of Animals		60	60	37	65	65
Heart		(60)	(60)	(37)	(65)	(65)
Bacterial colonies		2	2	0	1	0
	-minimal	1	0	0	0	0
	-mild	0	1	0	1	0
	-moderate	0	1	0	0	0
	-severe	1	0	0	0	0
Cardiomyopathy		13	8	16	11	13
	-minimal	13	8	14	11	13
	-mild	0	0	1	0	0
	-moderate	0	0	1	0	0
Dilatation	*No data reported*					
Endocarditis, valvular, vegetative	*No data reported*					
Thrombus		1	1	3	6	4
	-minimal	0	0	0	0	2
	-mild	0	0	0	3	1
	-moderate	1	1	1	1	1
	-severe	0	0	2	2	0

Male

Study		A	A	B	D	E
Experimental Start Date		1/10/2001	1/10/2001	4/6/2001	9/29/2004	3/23/2004
Experimental Termination Date		1/13/2003	1/13/2003	4/9/2003	9/29/2006	3/17/2006
Route of Administration		Dermal	Dermal	Oral Gavage	Oral Gavage	Oral Gavage
Intervals Used		Terminal	Terminal	0 to Termination	Terminal	Terminal
Control Group		C-1	C-2	C-1	C-1	C-1
Number of Animals		70	70	60	60	60
Heart		(70)	(70)	(60)	(60)	(60)
Cardiomyopathy		22	28	36	14	34
	-minimal	21	27	29	9	26
	-mild	1	1	5	5	8
	-moderate	0	0	2	0	0
Thrombus		13	3	7	7	3
	-minimal	1	0	1	1	0
	-mild	3	0	0	4	0
	-moderate	8	3	0	0	2
	-severe	1	0	6	2	1

Study		F	G	H	H
Experimental Start Date		12/7/2004	11/8/2000	5/9/2001	5/9/2001
Experimental Termination Date		9/14/2006	10/3/2002	5/12/2003	5/12/2003
Route of Administration		Oral Gavage	Subcutaneous	Subcutaneous	Subcutaneous
Intervals Used		Terminal	0 to Termination	Terminal	Terminal
Control Group		C-1	C-1	C-1	C-2
Number of Animals		60	37	65	65
Heart		(60)	(37)	(65)	(65)
Cardiomyopathy		16	22	28	26
	-minimal	15	13	24	20
	-mild	1	9	4	6
	-moderate	0	0	0	0

Thrombus		5	4	10	10
	-minimal	0	0	0	0
	-mild	1	0	2	6
	-moderate	1	0	4	1
	-severe	3	4	4	3

Toxicokinetics

Oligonucleotides are rapidly removed from circulation, and therefore plasma oligo levels are not a meaningful means of assessing exposure. Sponsor collected samples from the liver (target organ), kidney and spleen which were flash frozen. The oligonucleotide concentration in these organs was subsequently determined by a validated method. ISIS 301012 was recoverable in all three tissues assessed, and concentrations exhibited a generally dose-dependent increase.

Pharmacodynamic Analysis

In order to confirm that the mouse surrogate (SIS 147764) was having the anticipated pharmacological effect during the course of the study, Sponsor collected liver samples (10/sex) from ISIS 147764-treated mice and analyzed them for apoB mRNA level. A statistically significant 37% decrease in hepatic apoB mRNA was observed, compared to control.

Dosing Solution Analysis

Stability analyses conducted by ISIS Pharmaceuticals showed that the test article solutions provided for this study remained stable throughout their period of use at the range of concentrations utilized in this study.

Study title: 2-YEAR SUBCUTANEOUS CARCINOGENICITY STUDY OFISIS 301012 IN SPRAGUE-DAWLEY RATS

Study no.:	GT-348-TX-2
Study report location:	4.2.3.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01-08-2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-002, 93.6% ISIS 301012, CA301012-007, 91.6% ISIS 301012, CA301012-008, 93.6% ISIS 301012, CA301012-010, 93.9% ISIS 301012, CA301012-012, 88.3% ISIS 147764, CA147764-001, 86.6%
CAC concurrence:	No

Key Study Findings

- Apart from the neoplastic findings, which are discussed below, ISIS 301012 was associated with adverse effects on health and survival in all dose groups. The NOAEL was 3 mg/kg.
- The most profound effect was on kidney function. Urinalysis data show impaired function (proteinuria) as early as 4 months after initiation of dosing and impaired creatinine filtration by 12 months at HD (only treatment group assessed). Adverse effects on kidney function were also apparent by clinical chemistry, gross pathology and histopathology at doses $\geq$ 10 mg/kg. Sponsor has interpreted the kidney findings as being a worsening of spontaneous chronic progressive nephropathy.
- Multiple organs/tissues were subject to changes secondary to renal failure and resultant uremia. Indeed, most of the histopathology findings seen in this study are attributable to renal failure.
- The liver also appears to be a target organ of ISIS 301012, with increased incidence and severity of centrilobular hepatocellular vacuolation/necrosis

Adequacy of Carcinogenicity Study

Although ECAC was not asked to comment on Sponsor's rat study, the study appears to be adequate for assessing test item related carcinogenesis in the rat. The study is somewhat confounded by early termination of dosing and early sacrifice in most dose groups. While on the one hand this confounds analysis of the effect of the test item on age-related spontaneous tumors, it does indicate that the study adequately explored the potential carcinogenic effect of maximally tolerated doses of the test item. The study is judged to be adequate.

Appropriateness of Test Models

The rat is a commonly used model for assessment of carcinogenicity. The route and frequency of administration used in this study is the same as that intended clinically.

Evaluation of Tumor Findings

A few tumor types exhibited s.s. increases at doses ≥ 10 mg/kg. Of particular interest are malignant fibrous histiocytomas of the skin/subcutis, as these were s.s. ↑ in both sexes at doses ≥ 10 mg/kg. Sponsor attributes this increase to multiple SC injections; however this explanation does not account for the apparent dependence on ISIS 301012, nor that the finding, while associated with the skin/subcutis, was not associated with the injection sites.

Fibrosarcomas of the skin/subcutis were also increased in ♀s, s.s. at HD. ♀s also exhibited an apparent dose-related increase in benign granular cell tumors of the uterus (s.s. at HD).

The relationship of the test item to other tumors identified by the Sponsor as showing s.s. ↑s is uncertain.

Methods

Doses ISIS 301012 (clinical candidate):	♂: 3, 10, 30↓25↓20 mg/kg (HD ↓ to 25 m/k at Week 2 & 20 m/k at Week 25) ♀: 3, 10, 25↓20 mg/kg (HD ↓ to 20 m/k at Week 25)
Doses ISIS 147768 (rat surrogate):	♂ & ♀: 10 mg/kg
Frequency of dosing:	Weekly
Dose volume:	1.6 to 5 mL/kg
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	Soln. in phosphate buffered saline
Basis of dose selection:	MTD
Species/Strain:	Crl:CD®(SD) Rats
Number/Sex/Group:	60
Age:	8 weeks at initiation of dosing
Animal housing:	Individually
Paradigm for dietary restriction:	*ad libitum*
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Sentinel animals (5/sex) were scarified at 6 month intervals to check for various infectious agents (serological health screen)
Deviation from study protocol:	None material to study interpretation

Group Assignments					
Group Number	Dose Level (mg/kg/week)		Dose Material	Number of Animals	
	Male	Female		Male	Female
1	0	0	PBS	60	60
2	3	3	ISIS 301012	60	60
3	10	10	ISIS 301012	60	60
4	30/25/20[a]	25/20[a]	ISIS 301012	60	60
5[b]	10	10	ISIS 147768	60	60

[a]The dose level for male animals was reduced to 25 mg/kg/week during Week 2 and for male and female animals to 20 mg/kg/week during Week 25.
[b]Dosing for animals was initiated during Week 8.

Observations and Results

Mortality

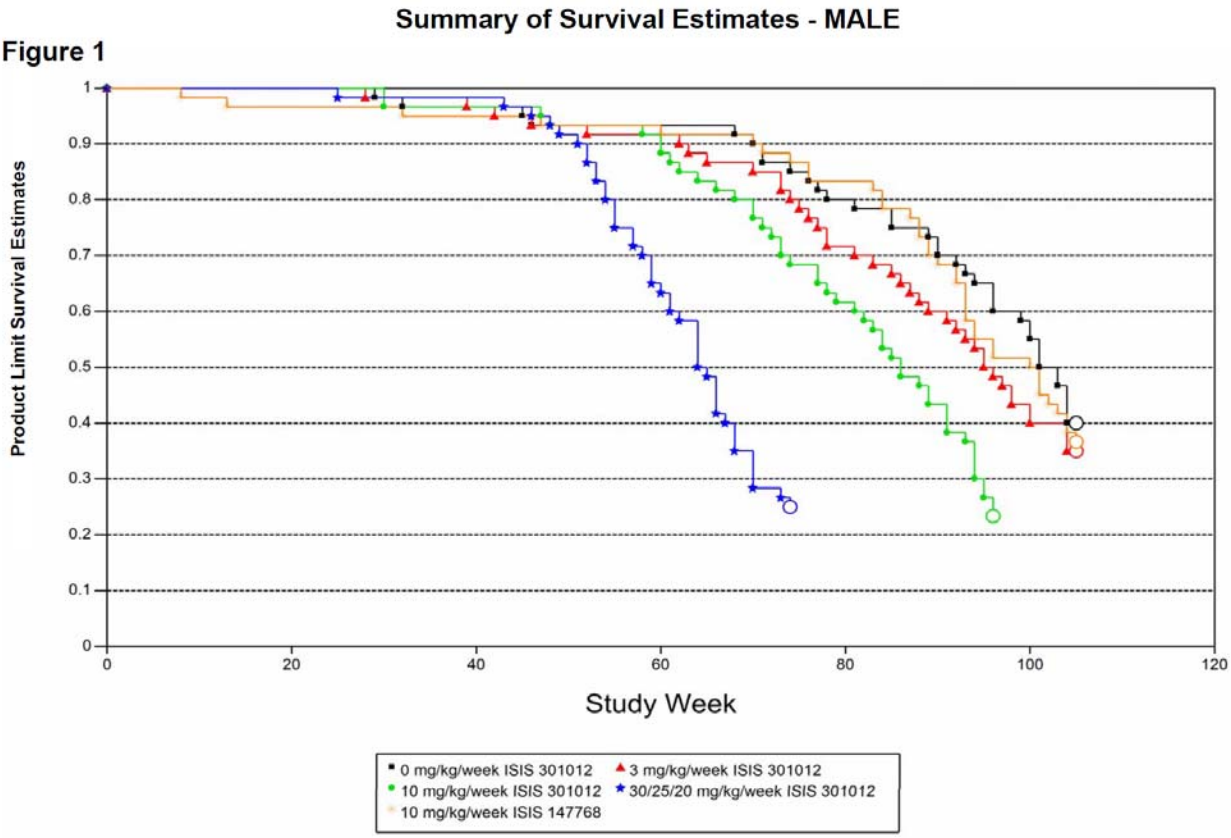
Test item was associated with increased mortality in both sexes. With the exception only of LD♂s, all ISIS 301012 treatment groups experienced early discontinuation of dosing and early sacrifice of surviving members of each dose cohort. Dosing was discontinued when surviving animals in a dose group dropped to 20, and all surviving members of the dose group were sacrificed when the surviving animal number dropped to 15.

30↓25↓20 (M), 25↓20 (F) mg/kg/week
M dosing stopped at week 70, sac'd Day 517
F dosing stopped at week 88, sac'd Day 641
10 mg/kg/week
M dosing stopped at week 94, sac'd Day 666
F dosing stopped at week 98, Day 697
3 mg/kg/week
F dosing stopped at week 96, sac'd Day 681

COD included an exacerbation of chronic progressive nephropathy and associated uremia in ♂s at MD & HD and in HD♀s. Fibrosarcoma/Fibroma and fibrous histiocytoma as a COD was higher in treated ♀s. Fatal cardiac thrombosis was also seen in HD♀s.

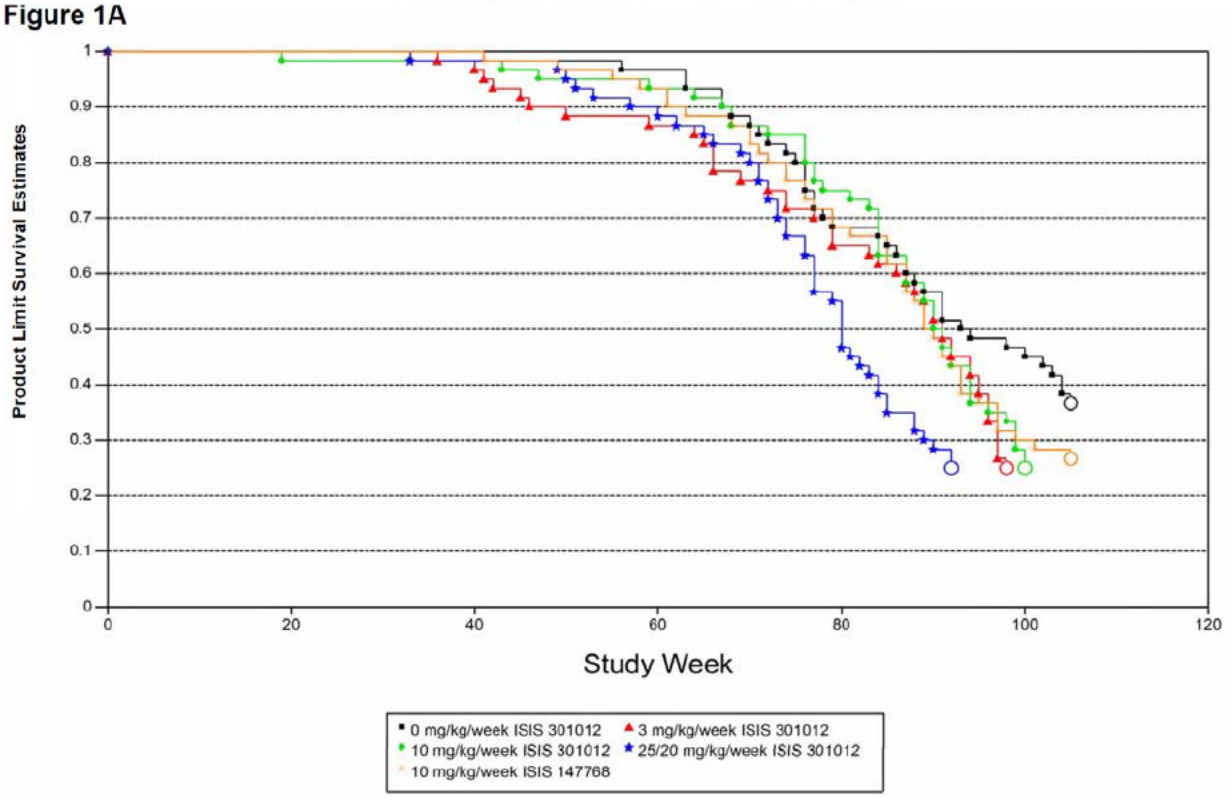
The number of animals surviving to the scheduled terminal necropsy (Week 105)[a]		
Dose Level	Male	Female
0 mg/kg/week	24 (40%)	22 (38%)
3 mg/kg/week ISIS 301012	21 (35%)	0 (0%)
10 mg/kg/week ISIS 301012	0 (0%)	0 (0%)
30/25/20 mg/kg/week ISIS 301012 (male), 25/20 mg/kg/week ISIS 301012 (female)	0 (0%)	0 (0%)
10 mg/kg/week ISIS 147764	22 (38%)	16 (28%)
[a]Respective survival percentage calculations include/reflect either death or necropsy at Week 105 of the study for groups surviving to scheduled terminal necropsy (see mean survival data in Table 1 of this report).		

Figure 1



Summary of Survival Estimates - FEMALE

Figure 1A



(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - MALE**

Cause of Death	0 mg/kg/week ISIS 301012	3 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 301012	30/25/20 mg/kg/week ISIS 301012
Number of Animals	60	60	60	60
Summary of Animal Disposition				
died after dosing	0	0	0	0
died prior to euthanasia	1	1	1	1
euthanized *in extremis*	21	23	17	20
found dead	14	15	28	24
terminal necropsy	24	21	14	15
Cause of Death				
accidental injury	0	0	0	0
adrenal glands necrosis	1	0	0	0
brain hemorrhage/necrosis	0	0	2	0
brain tumor	1	0	0	1
chronic progressive nephropathy/uremia	0	0	23	37
fibrosarcoma/fibroma	1	2	0	0
fibrous histiocytoma	0	1	2	3
gastrointestinal tumor	0	1	0	0
gastrointestinal ulceration	1	0	1	0
heart failure/atrial thrombus	0	0	0	0
heart inflammation/necrosis	0	1	1	0
hemangiosarcoma/hemangioma	0	1	0	0
hibernoma	1	0	1	1

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - MALE**

Cause of Death	0 mg/kg/week ISIS 301012	3 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 301012	30/25/20 mg/kg/week ISIS 301012
Number of Animals	60	60	60	60
Cause of Death				
histiocytic sarcoma	0	1	0	0
kidney tumor	0	0	0	0
lipoma/liposarcoma	0	1	0	0
liver tumor	0	1	0	0
lung tumor	1	0	0	0
lymphoid tumor	1	1	1	0
mammary tumor	2	0	0	0
nose/oral inflammation/ulceration	2	1	2	0
oropharyngeal tissue carcinoma, sebaceous cell	0	1	0	0
osteoarthritis/pododermatitis	3	1	0	0
pituitary hemorrhage/necrosis	1	0	0	0
pituitary tumor	14	22	7	0
polyarteritis	0	0	1	0
schwannoma	0	0	1	0
skin inflammation/necrosis	0	1	0	0
undetermined	6	3	4	1
undifferentiated carcinoma	0	0	0	1
urogenital inflammation/obstruction/calculi	1	0	0	1

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - MALE**

Cause of Death	10 mg/kg/week ISIS 147768
Number of Animals	60
Summary of Animal Disposition	
died after dosing	1
died prior to euthanasia	1
euthanized *in extremis*	16
found dead	20
terminal necropsy	22
Cause of Death	
accidental injury	1
adrenal glands necrosis	0
brain hemorrhage/necrosis	0
brain tumor	1
chronic progressive nephropathy/uremia	1
fibrosarcoma/fibroma	2
fibrous histiocytoma	0
gastrointestinal tumor	0
gastrointestinal ulceration	0
heart failure/atrial thrombus	1
heart inflammation/necrosis	0
hemangiosarcoma/hemangioma	0
hibernoma	1

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - MALE**

Cause of Death	10 mg/kg/week ISIS 147768
Number of Animals	60
Cause of Death	
histiocytic sarcoma	2
kidney tumor	2
lipoma/liposarcoma	1
liver tumor	0
lung tumor	0
lymphoid tumor	0
mammary tumor	0
nose/oral inflammation/ulceration	0
oropharyngeal tissue carcinoma, sebaceous cell	0
osteoarthritis/pododermatitis	0
pituitary hemorrhage/necrosis	0
pituitary tumor	17
polyarteritis	1
schwannoma	1
skin inflammation/necrosis	0
undetermined	7
undifferentiated carcinoma	0
urogenital inflammation/obstruction/calculi	0

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	0 mg/kg/week ISIS 301012	3 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 301012	25/20 mg/kg/week ISIS 301012
Number of Animals	60	60	60	60
Summary of Animal Disposition				
died prior to euthanasia	0	0	1	1
euthanized *in extremis*	32	37	38	31
found dead	6	8	6	13
terminal necropsy	22	15	15	15
Cause of Death				
adrenal gland angiectasis/cystic degeneration/necrosis	1	0	0	0
brain tumor	0	2	0	0
cardiomyopathy	1	1	0	1
chronic progressive nephropathy/uremia	0	0	1	12
fibrosarcoma/fibroma	0	1	4	2
fibrous histiocytoma	0	0	3	3
heart failure/atrial thrombus	0	0	0	3
heart tumor	0	0	0	0
hemorrhage	0	0	0	1
inflammation/septicemia	0	0	1	0
kidney tumor	0	0	0	0
liver inflammation/necrosis	0	0	1	0
lymphoid tumor	0	0	0	1

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	0 mg/kg/week ISIS 301012	3 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 301012	25/20 mg/kg/week ISIS 301012
Number of Animals	60	60	60	60
Cause of Death				
mammary tumor	14	16	16	12
pituitary tumor	21	20	15	8
schwannoma	0	1	0	0
skin inflammation/necrosis	1	0	0	1
skin tumor	0	0	0	0
thyroid tumor	0	0	1	0
undetermined	0	2	1	0
undetermined; this animal was brought to necropsy for a mass larger than 10 cm. the mass was not seen at necropsy.	0	1	0	0
urogenital inflammation/obstruction/calculi	0	1	0	0
uterus tumor	0	0	0	1
vagina tumor	0	0	1	0
zymbals gland tumor	0	0	1	0

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 **Summary of Probable Cause of Death - FEMALE**

Cause of Death	10 mg/kg/week ISIS 147768
Number of Animals	60
Summary of Animal Disposition	
died prior to euthanasia	1
euthanized *in extremis*	36
found dead	7
terminal necropsy	16
Cause of Death	
adrenal gland angiectasis/cystic degeneration/necrosis	0
brain tumor	0
cardiomyopathy	0
chronic progressive nephropathy/uremia	2
fibrosarcoma/fibroma	0
fibrous histiocytoma	0
heart failure/atrial thrombus	0
heart tumor	1
hemorrhage	0
inflammation/septicemia	0
kidney tumor	1
liver inflammation/necrosis	0
lymphoid tumor	0

(b) (4) Study Number 1213-003
2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats

Table 10 Summary of Probable Cause of Death - FEMALE

Cause of Death	10 mg/kg/week ISIS 147768
Number of Animals	60
Cause of Death	
mammary tumor	18
pituitary tumor	17
schwannoma	0
skin inflammation/necrosis	2
skin tumor	1
thyroid tumor	0
undetermined	0
undetermined; this animal was brought to necropsy for a mass larger than 10 cm. the mass was not seen at necropsy.	0
urogenital inflammation/obstruction/calculi	0
uterus tumor	1
vagina tumor	0
zymbals gland tumor	1

Clinical Signs

Signs consistent with reduced food consumption and body weight were apparent, especially at HD, as were signs of stress/poor condition.

A number of clinical signs showed a dose-related decreased incidence in the ISIS 301012 treatment groups. The majority of these are age-related findings (*e.g.,* various swellings, sparse and/or discolored hair, abrasions, scabbing, palmar/plantar ulcers, etc.), and the apparent test item-related decrease is an artifact of the early deaths/sacrifice of the 301012 treatment groups; these signs are not captured in the table below.

Clinical Signs -- Incidence/Animals Affected										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
Behavior/Activity										
Ataxia	0/0	8/6	2/2	2/2	2/2	2/2	6/3	1/1	5/5	4/2
Inappetence	5/4	8/5	3/3	13/11	2/1	n.e.				
Excretion										
Feces, few/absent	4/3	6/5	6/6	15/13	4/3	n.e.				
External Appearance										
Carriage, high	1/1	0/0	5/2	18/5	0/0	4/2	1/1	3/2	16/5	7/3
Lacrimation, Eye/left	n.e.					12/4	31/5	5/3	94/5	45/7
Lacrimation, Eye/right						6/4	9/4	90/5	135/9	83/4
Material around eyes, black Eye/left						6/4	18/8	5/2	25/6	16/7
Material around eyes, black Eye/right						11/5	18/8	11/3	26/7	22/6
Material around eyes, red Eye/left	30/10	43/11	18/8	70/9	83/11	54/12	50/8	10/7	141/13	44/4
Material around eyes, red Eye/right	94/12	8/6	144/15	114/11	50/10	50/15	17/10	109/10	189/18	60/4
Material around nose, brown	0/0	1/1	1/1	6/4	1/1	0/0	0/0	0/0	0/0	2/1
Posture hunched	23/8	41/12	61/17	71/32	61/13	15/9	34/14	19/12	52/19	29/9
Thin	27/13	47/18	99/28	209/42	74/19	85/14	52/13	109/21	272/34	83/14
Eye/Ocular										
Discolored, Pale, left	7/1	4/2	8/5	32/7	39/5	5/2	3/2	1/1	7/4	18/6

Discolored, Pale, right	10/2	4/2	13/6	34/9	39/5	4/2	2/2	10/1	7/4	4/3
Eyelid partially/completely closed, left	n.e.					15/6	30/8	4/3	22/11	22/7
Eyelid partially/completely closed, right						16/7	28/8	15/6	22/11	20/5
Pelage/Skin										
Hair discolored, brown, ventral surface	16/6	29/9	42/9	94/11	21/6	n.e.				
Hair discolored, tan, ventral surface	102/11	101/14	174/22	224/35	136/22	n.e.				
Hair sparse, ventral surface	2/1	20/1	30/2	122/14	11/2	n.e.				
Nodule, 1-5 mm, dorsal surface	n.e.					0/0	10/1	7/1	10/2	28/2
Piloerection	10/4	4/4	24/9	52/21	6/4	2/2	3/2	3/2	6/4	1/1
Scabbed area, dorsal surface	0/0	24/3	2/1	49/5	0/0	5/1	8/2	10/4	80/10	91/5
Scabbed area, thoracic region	n.e.					0/0	2/1	3/2	44/12	0/0
Skin, cold to touch	2/2	6/4	6/6	10/10	4/4	0/0	3/3	2/2	5/5	0/0
Skin, discolored, brown, tail	188/10	366/20	665/36	389/34	258/18	n.e.				
Skin, discolored, pale, entire body	3/2	4/4	3/3	37/15	16/4	3/3	1/1	2/2	18/8	6/3

n.e. = no effect
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Mass Findings

Aside from masses on the dorsal surface (captured in table, below) there were no clear associations between test item and the incidence of masses.

Dorsal Surface Mass Findings -- Incidence/Animals Affected										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
Mass 1										
Small (1-1.9 cm)	5/1	8/1	4/2	0/0	0/0	0/0	0/0	6/2	1/1	0/0
Medium (2-3.9 cm)	0/0	29/2	7/5	3/2	0/0	0/0	0/0	5/3	12/3	0/0
Large (≥ 4 cm)	0/0	0/0	12/4	8/2	0/0	0/0	0/0	15/3	18/4	0/0
> 10 cm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/2	0/0
Mass 2										
Small (1-1.9 cm)	0/0	0/0	0/0	0/0	0/0	0/0	2/1	8/3	2/2	0/0
Medium (2-3.9 cm)	0/0	0/0	0/0	0/0	0/0	0/0	1/1	13/5	2/1	0/0
Large (≥ 4 cm)	0/0	0/0	0/0	0/0	0/0	0/0	3/1	13/2	5/1	0/0
Mass 3										
Medium (2-3.9 cm)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1	0/0

The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Body Weights

Treatment-related decreases in mean body weight were noted in both sexes administered ISIS 301012 and ISIS 147768 when compared with controls over the course of the study, and exhibited a dose response pattern of effect for ISIS 301012. The effect becomes most apparent only after ~13 weeks of dosing, and may be related to deteriorating renal function.

Percent Differences in Mean Body Weight (g) from Control – Males					
Interval	Dose Level				
	0*	3 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 301012	30/25/20 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 147768
Week 4	398.9	0.88	1.13	0.70	↓0.23
Week 13	560.1	1.29	1.32	↓1.05	↓1.93
Week 26	657.8	↓0.23	↓1.00	↓5.85[b]	↓4.30[a]
Week 52	759.4	↓0.54	↓5.18[a]	↓20.74[b]	↓6.72[b]
Week 64	808.0	↓2.10	↓9.36[b]	↓27.49[b]	↓8.94[b]
Week 76	813.9	↓1.07	↓11.91[b]	NA	↓7.95[b]
Week 88	802.4	↓0.99	↓18.07[b]	NA	↓10.41[b]
Week 104	760.3	↓1.70	NA	NA	↓11.84[b]
* Control value is expressed in grams. [a]Significantly different from control; ($p<0.05$) [b]Significantly different from control; ($p<0.01$)					

Percent Differences in Mean Body Weight (g) from Control – Females					
Interval	Dose Level				
	0*	3 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 301012	25/20 mg/kg/week ISIS 301012	10 mg/kg/week ISIS 147768
Week 4	251.5	1.19	1.19	2.15	3.02
Week 13	311.5	0.77	↓1.28	↓2.86	2.18
Week 26	352.0	0.28	↓2.36	↓9.20[b]	0.31
Week 52	425.5	0.49	↓4.44	↓18.28[b]	↓0.07
Week 64	457.8	↓0.63	↓4.76	↓23.37[b]	↓2.88
Week 76	488.2	↓1.41	↓7.95	↓27.76[b]	↓6.43
Week 88	498.7	↓2.19	↓13.68[b]	↓31.26[b]	↓4.53
Week 104	492.0	NA	NA	NA	↓10.81[a]
* Control value is expressed in grams. [a]Significantly different from control; ($p<0.05$) [b]Significantly different from control; ($p<0.01$)					

Figure 2 Mean Body Weight Values - MALE

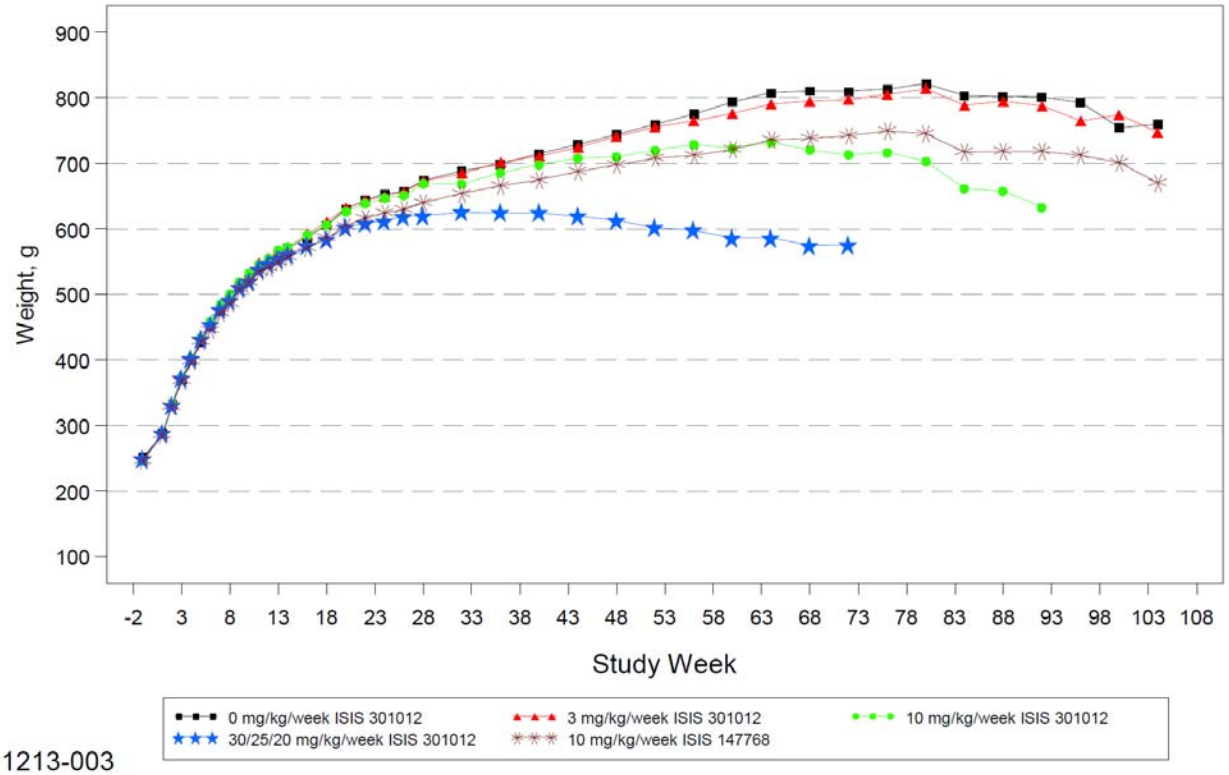


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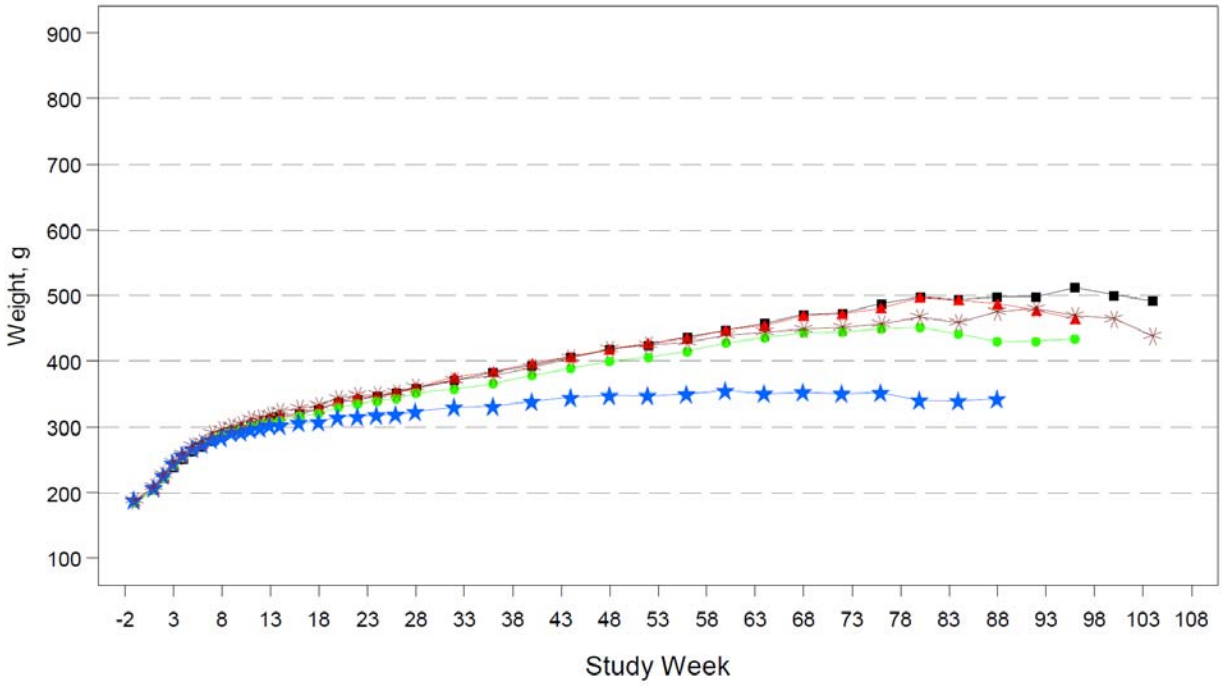
Figure 2A Mean Body Weight Values - FEMALE

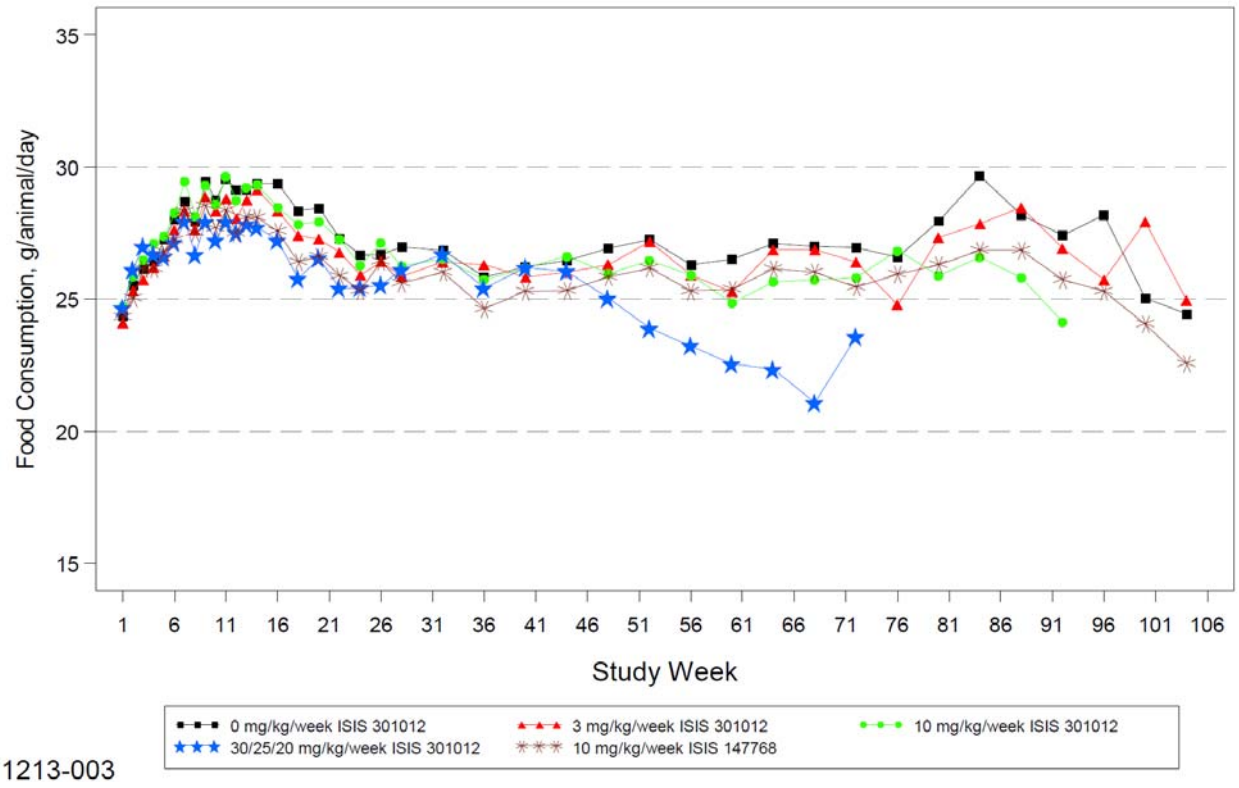


1213-003

Feed Consumption

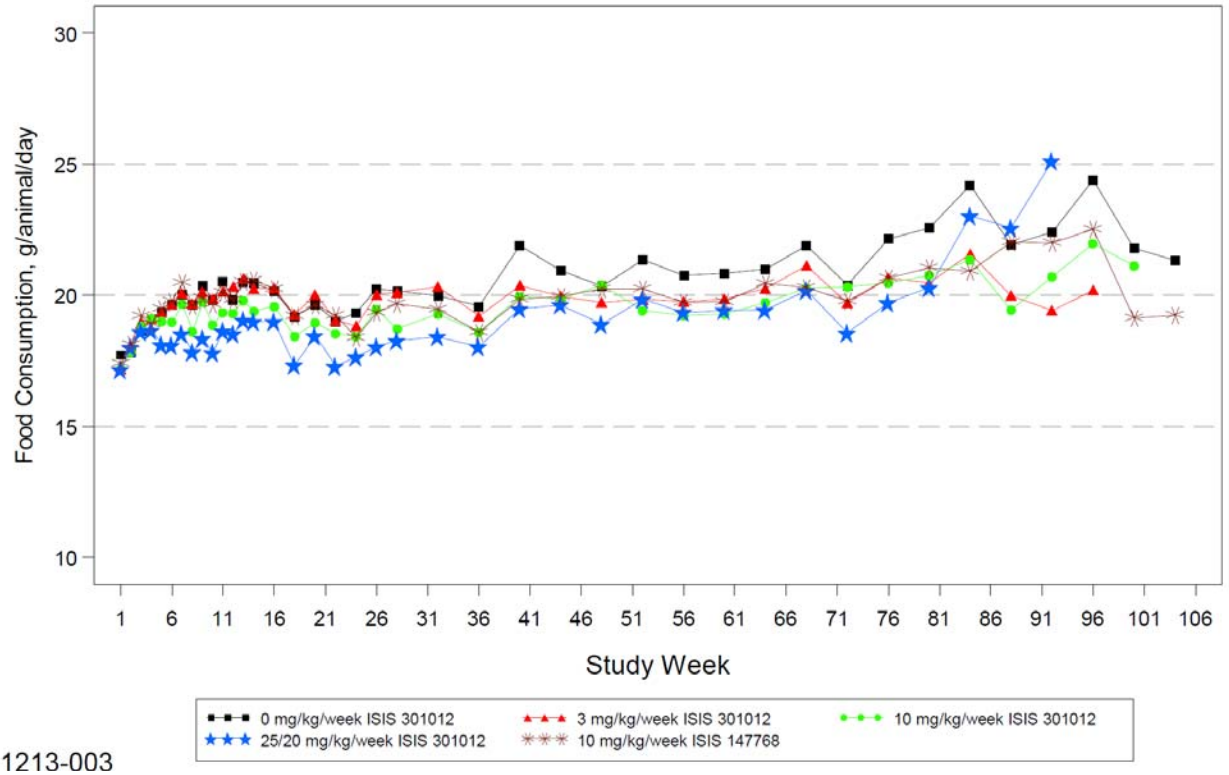
Slight treatment-related decreases in FC were noted during the study when compared to controls, correlating with (and generally preceding) the dose-dependent mean body weight decreases noted above. Statistical significance was variably noted among the treated groups, and was most often reached at the high dose of ISIS 301012 in both sexes.

Figure 3 Mean Food Consumption Values - MALE



1213-003

Figure 3A Mean Food Consumption Values - FEMALE



1213-003

Ophthalmoscopy

No treatment related effect.

Hematology

Parameters Evaluated		
Hematology	**Clinical Chemistry**	
Leukocyte count (total and differential)	Asparate aminotransferase	Albumin
Erythrocyte count	Alanine aminotransferase	Globulin and A/G (albumin/globulin) ratio (calculated)
Platelet count	Total cholesterol*	Glucose
Hemoglobin	Triglycerides*	Creatinine
Hematocrit	High density lipoprotein (HDL)*	Urea nitrogen
Mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated)	Low density lipoprotein (LDL)*	Alkaline phosphatase
	Very low density lipoprotein (VLDL)*	Calcium
	Total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dl)	Total protein
* The analyses were done in only Group 1, 4, and 5 animals.		

RBCs are ↓ at doses ≥ 10 mg/kg in ♂s and 20 mg/kg in ♀s (s.s. at HD in both sexes). This correlates with the dose groups showing the most severe renal toxicity, and may be secondary to impaired erythropoietin secretion from kidneys. Neutrophils are dose-dependently ↓ (s.s. at all doses in ♀s), as are eosinophils (s.s. in ♂s at doses ≥ 10 mg/kg). The basis for the changes in these immune cells is unclear.

Coagulation parameters were not measured.

Hematology -- % Change from Control										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
Erythrocytes ($10^6\mu L$)	7.737	n.e.	↓16	**↓24***	n.e.	6.696	↑9	n.e.	**↓17***	n.e.
Hemoglobin (g/dL)	12.91	n.e.	↓14	**↓21***	n.e.	12.13	↑8	↑9	↓8	n.e.
Hematocrit (%)	40.65	n.e.	↓12	**↓21***	n.e.	37.76	↑9	↑8	↓8	n.e.
MCV (fL)	52.59	n.e.	↑6	**↑5**	n.e.	56.50	n.e.	↑6	↑17	n.e.
MCH (pg)	16.70	n.e.	n.e.	n.e.	n.e.	18.15	n.e.	↑6	**↑15****	n.e.
Neutrophils ($10^3/\mu L$)	5.570	↓14	↓19	↓25	**↓42***	6.296	**↓43***	**↓62****	**↓44***	↓20
Eosinophils ($10^3/\mu L$)	0.160	↓35	**↓54***	**↓58***	↓31	0.095	↓20	↓26	↓34	↓38
Basophils ($10^3/\mu L$)	0.064	↑183	↑23	n.e.	n.e.	0.046	n.e.	↑9	**↑76***	↓28

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
n.e. = no effect
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Clinical Chemistry

BUN and creatinine were dose-dependently ↑ in ♂s (s.s. at HD). MD♀ also had a s.s. ↑ in BUN. These findings are consistent with impaired renal function. ISIS 301012 was also associated with ↑ in all assessed lipid parameters. Other apparent effects are difficult to interpret, due to the different days of terminal sacrifice for the different dose groups. ↑ alkaline phosphatase at HD is consistent with secondary hyperparathyroidism and ↑ bone turnover.

Electrolytes were not assessed.

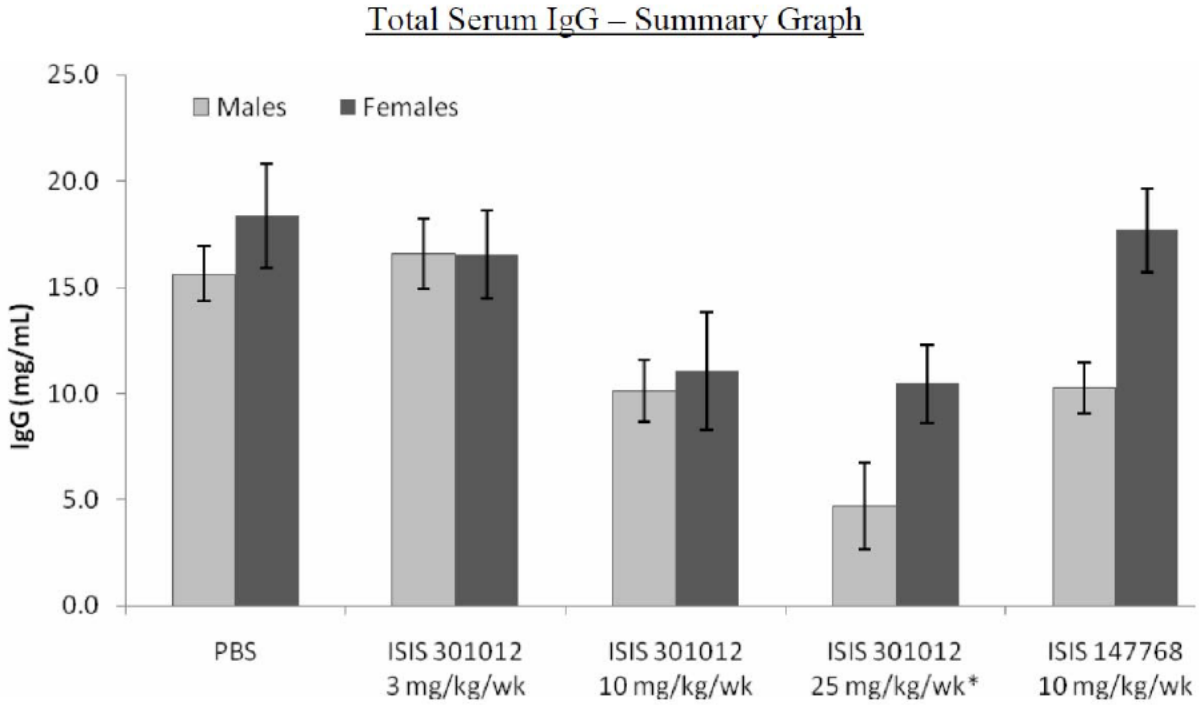
Clinical Chemistry -- % Change from Control										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
AlkPhos (U/L)	63.1	↓7	↑21	↑28	↓20	29.0	↑14	↓23	↑38	↑54
AST (U/L)	97.9	↓17	**↓39****	**↓60****	↓15	92.7	↑7	↓29	↓36	↑19
BUN (mg/dL)	15.1	↑7	↑94	**↑244***	↓13	12.9	↑5	**↑46***	↑22	n.e.
Creatinine (mg/dL)	0.43	↑9	↑109	**↑149***	↓9	0.39	n.e.	↑21	n.e.	n.e.
Total Protein (g/dL)	6.34	n.e.	n.e.	**↓10****	↑5	7.05	n.e.	n.e.	n.e.	n.e.
Albumin (g/dL)	2.54	n.e.	**↓12***	**↓23****	↑7	3.10	↑13	n.e.	**↓18***	n.e.
A:G Ratio	0.67	n.e.	↓15	**↓22****	n.e.	0.79	↑19	n.e.	↓22	n.e.
Triglyceride (mg/dL)	57.8	-	-	**↑1079****	↑37	63.5	-	-	**↑393****	n.e.
Cholesterol (mg/dL)	108.2	-	-	**↑179****	n.e.	107.6	-	-	**↑140****	↑9
HDL-C (mg/dL)	65.4	-	-	**↑90****	↑11	72.2	-	-	**↑136****	↑10
LDL-C (mg/dL)	9.2	-	-	↑255	↓27	5.5	-	-	↑176	↓16

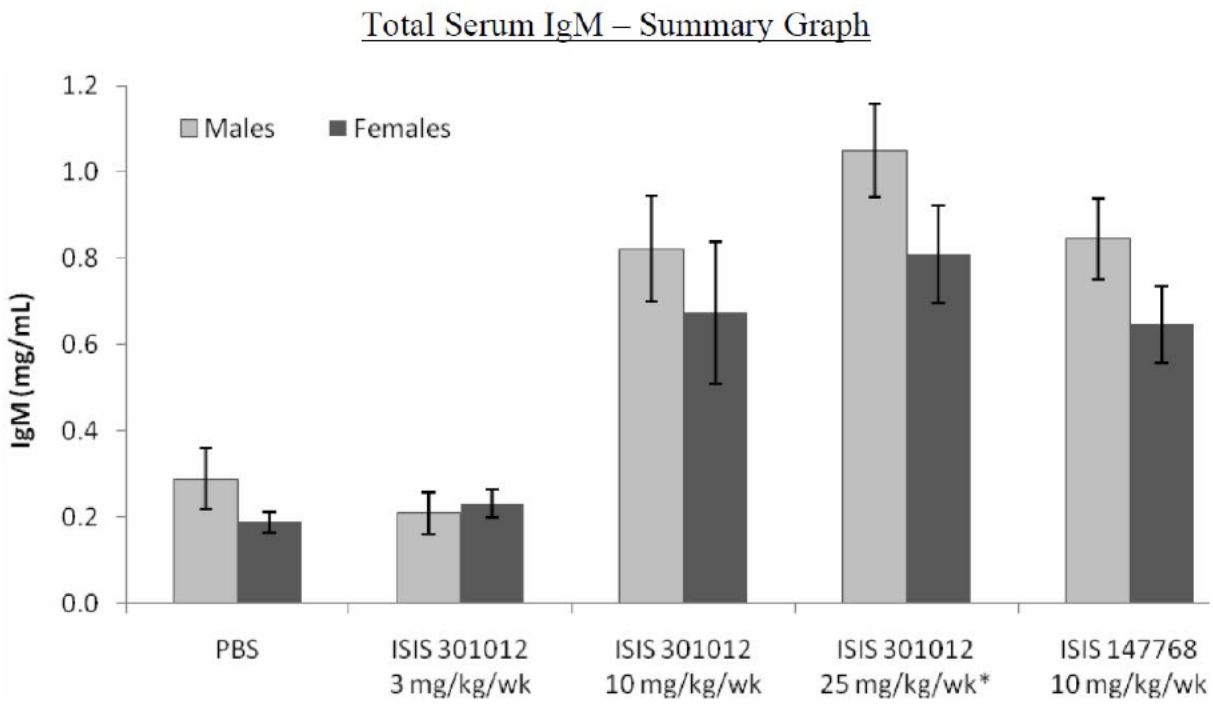
VLDL (mg/dL)	11.7	-	-	↑**1066****	↑35	12.5	-	-	↑**402****	n.e.

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
n.e. = no effect
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Immunoglobulin

At terminal sacrifice there were significant dose-dependent decreases in IgG and increases in IgM at doses ≥ 10 mg/kg/wk of either ISIS 301012 or ISIS 147768 (rat surrogate). ♂s were more sensitive to this effect than ♀s. This suggests than both antisense oligonucleotides may have had an inhibitory effect on class switching in the rat.





Cytokine/Chemokine Analysis

Blood samples for cytokine/chemokine analysis were collected from the first 10 animals/sex/group in Groups 1 to 4 prior to terminal necropsy via the vena cava after carbon dioxide inhalation. A multiplex ELISA approach (b) (4) *was used to measure the levels of IL-1α, IL-1β, IL-6, IL-10, TNF-α, MCP-1, MIP-1α, MIP-1β, and IFN-γ.*

Insufficient information was provided in the study report to fully assess the extent to which the assay system is suitable for the intended analysis. The assay is not validated. There is extremely large intragroup variability that limits the interpretability of intergroup changes. Many apparent increases (*e.g,* IL-1α, IL-1β, TNFα and IFNγ) are not fully dose-related, and are being driven by one or two animals in a dose group with exceptionally high levels. Nonetheless there is a subset of cytokines/chemokines that appear to show modest, dose-dependent increases; these include IL-10, MIP-1α, MIP-1β and MCP-1. Immune stimulation is a known attribute of phosphorothioate oligonucleotides.

Cytokine/Chemokine Levels at Terminal Sacrifice – Mean (SD)					
Sex		Male + Female			
Dose mg/kg/wk		0	3	10	20*
IL-1α	(pg/mL)	6.7 ± 4.0	81 ± 210	168 ± 259	20 ± 18
	fold change	-	12x	25x	3x
IL-1β	(pg/mL)	20 ± ?	58 ± 48	156 ± 208	61 ± 55
	fold change	-	3x	8x	3x
IL-6	(pg/mL)	508 ± ?	777 ± 1086	NA	NA

	fold change	-	1.5x	-	-
IL-10	(pg/mL)	12 ± 14	18 ± 20	30 ± 29	34 ± 50
	fold change	-	1.6x	2.6x	2.9x
TNF-α	(pg/mL)	177 ± 164	170 ± 170	158 ± 166	289 ± 512
	fold change	-	0.96x	0.89x	1.6x
IFN-γ	(pg/mL)	132 ± 178	3394 ± 6573	25,929 ± 79,116	258 ± 316
	fold change	-	26x	196x	2.0x
MIP-1α	(pg/mL)	10 ± 5	14 ± 5	17 ± 10	24 ± 13
	fold change	-	1.3x	1.7x	2.4x
MCP-1	(pg/mL)	1662 ± 530	2472 ± 1776	2988 ± 1915	4336 ± 2387
	fold change	-	1.5x	1.8x	2.6x
MIP-1β	(pg/mL)	9.1 ± 10	11 ± 8	28 ± 23	66 ± 84
	fold change	-	1.2x	3.1x	7.3x

*♂: 35↓25↓20, ♀: 25↓20
NA: not applicable (all individual values below the lower limit of quantification)

Urinalysis

Based on previous toxicity studies that identified the kidney as a target organ for toxicity, C & HD animals were subjected to periodic urinalysis. HD ISIS 301012 clearly has adverse effects on renal function in both sexes. Proteinuria is seen in both sexes at the earliest timepoint assessed (4 months). At 12 months, both sexes also show ↓ 24-hour creatinine, and males begin to show s.s. effects on urine volume and specific gravity.

Table 8 **Summary of Urinalysis Values - MALE**

Endpoint	Study Interval (Month)	0 mg/kg/week ISIS 301012 Mean	SD	N	30/25/20 mg/kg/week ISIS 301012 Mean	SD	N
Volume	4	25.3	32.70	10	24.4	21.84	60
mL	5	20.3	9.80	10	23.5	10.64	60
	6	15.1	3.20	10	19.0	6.96	59
	8	16.3	6.62	10	21.3	10.50	59
	12	14.4	2.87	10	33.2[b]	13.87	52
	14	16.6	4.89	10	33.9[b]	15.96	38
	17	21.6	5.27	10	38.6[a]	18.24	16
	20	31.2	7.63	10	NA	NA	0
Specific Gravity	4	1.0514	0.01792	10	1.0445	0.01567	60
	5	1.0470	0.01259	10	1.0426	0.01318	60
	6	1.0529	0.00731	10	1.0508	0.00989	59
	8	1.0537	0.01175	10	1.0523	0.01227	59
	12	1.0551	0.00530	10	1.0412[b]	0.01617	52
	14	1.0540	0.00667	10	1.0436	0.02089	38
	17	1.0490	0.00750	10	1.0347[b]	0.01466	16
	20	1.0412	0.00776	10	NA	NA	0
pH	4	7.55	0.284	10	7.48	0.356	60
	5	7.35	0.337	10	7.33	0.314	60
	6	7.40	0.316	10	7.39	0.323	59
	8	7.55	0.284	10	7.42	0.345	59
	12	7.80	0.258	10	7.26[b]	0.336	52
	14	7.45	0.369	10	6.97[b]	0.434	38
	17	7.60	0.211	10	6.78[b]	0.446	16
	20	7.40	0.316	10	NA	NA	0

N - Number of measures used to calculate mean
SD - Standard Deviation
NA - Not Applicable/Not Available

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Table 8 **Summary of Urinalysis Values - FEMALE**

Endpoint	Study Interval (Month)	0 mg/kg/week ISIS 301012 Mean	SD	N	25/20 mg/kg/week ISIS 301012 Mean	SD	N
Volume mL	4	12.9	11.65	10	17.2	19.52	60
	5	13.7	5.79	10	18.6	20.51	59
	6	13.7	4.52	10	14.0	4.87	60
	8	12.0	5.68	10	12.8	4.73	59
	12	19.9	5.08	10	19.7	8.29	55
	14	22.1	9.07	10	22.0	9.24	53
	17	31.3	18.74	10	31.0	12.62	42
	20	37.0	14.74	10	29.9	11.88	21
Specific Gravity	4	1.0509	0.01724	10	1.0409	0.01628	60
	5	1.0442	0.01269	10	1.0384	0.01228	59
	6	1.0454	0.00950	10	1.0433	0.00871	60
	8	1.0489	0.01117	10	1.0459	0.00973	59
	12	1.0323	0.00742	10	1.0370	0.01086	55
	14	1.0338	0.00932	10	1.0403	0.01300	53
	17	1.0289	0.00633	10	1.0332	0.00975	42
	20	1.0292	0.00625	10	1.0386[a]	0.01115	21
pH	4	7.20	0.422	10	7.33	0.328	60
	5	7.10	0.316	10	7.27	0.386	59
	6	7.25	0.354	10	7.17	0.314	60
	8	7.15	0.474	10	7.09	0.341	59
	12	7.45	0.284	10	7.27	0.331	55
	14	7.10	0.316	10	6.99	0.332	53
	17	7.15	0.242	10	6.96	0.433	42
	20	7.20	0.258	10	7.00	0.387	21

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)

Table 9 **Summary of Urine Chemistry Values - MALE**

Endpoint	Study Interval (Month)	0 mg/kg/week ISIS 301012 Mean	SD	N	30/25/20 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	4	135.20	47.988	10	115.65	42.936	60
	5	121.36	33.854	10	103.73	31.921	60
	6	145.90	15.110	10	120.47[b]	27.044	59
	8	148.03	29.127	10	112.71[b]	39.844	59
	12	148.75	15.071	10	67.74[b]	34.061	52
	14	138.60	20.998	10	67.39[b]	43.712	38
	17	135.40	33.073	10	57.38[b]	26.407	16
	20	84.30	18.951	10	NA	NA	0
Creatinine mg/24 hr	4	21.300	3.2177	10	21.395	3.9597	60
	5	21.850	3.5362	10	21.475	3.6684	60
	6	21.660	2.7228	10	21.268	2.6879	59
	8	22.390	3.0020	10	20.554	3.1224	59
	12	21.170	3.4702	10	18.587[a]	3.6460	52
	14	22.240	3.5406	10	17.079[b]	4.4060	38
	17	27.720	3.9538	10	18.425[b]	5.1877	16
	20	25.070	2.2196	10	NA	NA	0
Quantitative Urine Protein mg/dL	4	187.3	92.25	10	634.5[b]	277.22	60
	5	219.4	221.37	10	825.3[b]	489.87	60
	6	217.9	178.06	10	1519.1[b]	991.70	59
	8	341.7	362.07	10	3418.2[b]	1845.59	59
	12	490.9	498.14	10	4428.4[b]	2295.60	52
	14	498.5	505.73	10	4818.4[b]	2579.28	38
	17	442.3	309.97	10	3389.6[b]	1799.22	16
	20	793.3	1101.17	10	NA	NA	0

N - Number of measures used to calculate mean
SD - Standard Deviation
NA - Not Applicable/Not Available

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Table 9 **Summary of Urine Chemistry Values - MALE**

Endpoint	Study Interval (Month)	0 mg/kg/week ISIS 301012 Mean	SD	N	30/25/20 mg/kg/week ISIS 301012 Mean	SD	N
Protein/Creatinine Ratio	4	1.36	0.462	10	5.45[b]	1.294	60
	5	1.63	1.007	10	8.19[b]	4.222	60
	6	1.43	1.007	10	13.41[b]	11.286	59
	8	2.22	1.992	10	35.81[b]	23.541	59
	12	3.42	3.575	10	68.39[b]	23.824	52
	14	4.29	5.974	10	76.81[b]	20.397	38
	17	3.85	3.698	10	64.65[b]	30.017	16
	20	11.86	20.354	10	NA	NA	0
Quantitative Urine Protein mg/24 hr	4	28.4	6.72	10	116.9[b]	43.00	60
	5	33.1	12.22	10	172.1[b]	100.81	60
	6	30.2	18.80	10	286.8[b]	247.40	59
	8	46.0	28.81	10	736.4[b]	500.99	59
	12	67.7	65.94	10	1262.7[b]	487.83	52
	14	99.6	149.17	10	1277.8[b]	372.83	38
	17	104.9	91.15	10	1147.4[b]	493.98	16
	20	276.0	440.35	10	NA	NA	0

N - Number of measures used to calculate mean
SD - Standard Deviation
NA - Not Applicable/Not Available
[b]Significantly different from control; ($p<0.01$)

Table 9 **Summary of Urine Chemistry Values - FEMALE**

Endpoint	Study Interval (Month)	0 mg/kg/week ISIS 301012 Mean	SD	N	25/20 mg/kg/week ISIS 301012 Mean	SD	N
Creatinine mg/dL	4	105.90	37.758	10	85.00	34.472	59
	5	85.24	28.824	10	71.33	25.039	59
	6	93.00	17.448	10	76.58[a]	19.260	60
	8	98.73	24.639	10	87.44	25.365	59
	12	66.12	18.131	10	61.40	24.082	55
	14	66.70	21.484	10	56.35	24.631	53
	17	64.80	21.054	10	46.26[b]	20.235	42
	20	49.80	19.561	10	41.00	18.199	21
Creatinine mg/24 hr	4	10.160	2.9710	10	10.685	2.6186	59
	5	10.370	1.7082	10	9.578	2.5580	59
	6	12.070	2.0451	10	10.045[b]	1.8785	60
	8	10.610	2.3468	10	10.183	1.8360	59
	12	12.420	0.9784	10	10.595[b]	2.1757	55
	14	13.280	2.4845	10	10.660[b]	2.0393	53
	17	17.510	4.3603	10	12.636[b]	2.8447	42
	20	16.250	2.4401	10	10.433[b]	1.9027	21
Quantitative Urine Protein mg/dL	4	89.9	57.14	10	247.3[b]	132.70	59
	5	68.5	44.94	10	291.2[b]	142.20	59
	6	81.6	49.30	10	446.4[b]	441.87	60
	8	103.0	80.21	10	865.8[b]	1030.83	59
	12	86.2	47.11	10	1499.8[b]	1828.79	55
	14	57.5	21.66	10	2225.3[b]	2012.37	53
	17	60.2	32.19	10	2290.4[b]	1795.91	42
	20	353.3	787.43	10	3021.8[b]	1642.91	21

N - Number of measures used to calculate mean
SD - Standard Deviation

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Table 9 **Summary of Urine Chemistry Values - FEMALE**

Endpoint	Study Interval (Month)	0 mg/kg/week ISIS 301012 Mean	SD	N	25/20 mg/kg/week ISIS 301012 Mean	SD	N
Protein/Creatinine Ratio	4	0.86	0.465	10	2.84[b]	0.798	59
	5	0.78	0.385	10	4.24[b]	2.324	59
	6	0.86	0.462	10	6.14[b]	7.083	60
	8	1.03	0.643	10	11.76[b]	18.826	59
	12	1.39	0.901	10	30.12[b]	45.763	55
	14	0.96	0.536	10	47.13[b]	47.360	53
	17	1.15	0.968	10	57.94[b]	45.305	42
	20	9.56	21.569	10	84.85[b]	44.421	21
Quantitative Urine Protein mg/24 hr	4	8.2	4.00	10	30.1[b]	10.37	59
	5	7.8	3.53	10	41.7[b]	32.83	59
	6	10.2	5.51	10	62.0[b]	80.49	60
	8	10.1	5.63	10	125.5[b]	228.52	59
	12	17.2	10.93	10	309.6[b]	427.20	55
	14	13.4	8.58	10	504.8[b]	533.00	53
	17	21.5	21.23	10	705.0[b]	563.24	42
	20	147.0	311.59	10	853.1[b]	434.58	21

N - Number of measures used to calculate mean
SD - Standard Deviation
[b]Significantly different from control; ($p<0.01$)

Gross Pathology

See table below. For discussion of these findings, please refer to the Non-neoplastic Histopathology section.

Necropsy -- Incidence (Mean Severity)										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
Animal/whole body										
Body fat depleted	6 (3.2)	3 (2.7)	5 (2.8)	9 (3.0)	2 (2.5)	5 (2.8)	5 (3.0)	6 (2.7)	10 (2.8)	2 (2.5)
Aorta										
Dilatation	0 -	0 -	1 (3.0)	6 (2.8)	0 -	0 -	0 -	0 -	0 -	0 -
Cavity, abdominal										
Fluid, clear	0 -	0 -	0 -	1 (4.0)	0 -	0 -	0 -	0 -	1 (3.0)	0 -
Fluid, red	0 -	0 -	2 (2.5)	1 (2.0)	0 -	0 -	0 -	1 (2.0)	0 -	0 -
Cavity, thoracic										
Fluid, clear	0 -	0 -	1 (3.0)	2 (3.0)	0 -	0 -	0 -	1 (3.0)	1 (2.0)	0 -
Fluid, red	0 -	1 (2.0)	0 -	4 (2.8)	0 -	0 -	0 -	0 -	4 (3.0)	0 -
Epididymides										
Small	0 -	0 -	0 -	7 (2.4)	0 -					
Foot/feet										
Ulcer, plantar/palmar	49 (2.7)	44 (2.5)	21 (2.4)	19 (2.2)	34 (2.5)	42 (2.5)	33 (2.2)	30 (2.3)	21 (2.1)	32 (2.4)
Heart										
Discoloration, tan	0 -	0 -	0 -	1 (3.0)	1 (3.0)	0 -	1 (3.0)	0 -	2 (2.0)	0 -
Enlarged	0 -	1 (3.0)	2 (2.5)	0 -	0 -	0 -	0 -	0 -	0 -	0 -
Focus/foci, tan	0 -	1 (2.0)	1 (2.0)	1 (2.0)	0 -	0 -	0 -	0 -	0 -	0 -
Focus/foci, white	0 -	0 -	0 -	0 -	1 (2.0)	0 -	0 -	0 -	0 -	0 -
Kidneys										
Cyst	1 (1.0)	2 (2.0)	12 (2.5)	12 (2.1)	4 (2.0)	0 -	1 (4.0)	11 (2.3)	19 (2.2)	0 -
Dilatation, pelvic	1 (2.0)	1 (2.0)	1 (2.0)	2 (3.0)	0 -	0 -	1 (4.0)	0 -	0 -	0 -
Discoloration, tan	0 -	0 -	3 (3.0)	5 (2.8)	2 (2.5)	0 -	0 -	1 (2.0)	6 (2.3)	2 (3.0)
Enlarged	0 -	1 (2.0)	15 (2.8)	29 (3.1)	1 (2.0)	0 -	0 -	1 (3.0)	4 (2.2)	0 -
Irregular surface	1 (1.0)	1 (2.0)	5 (2.4)	8 (2.4)	1 (2.0)	0 -	0 -	2 (2.0)	5 (2.4)	0 -
Liver										
Discoloration, tan	0 -	0 -	1 (3.0)	4 (2.5)	1 (2.0)	0 -	0 -	0 -	3 (2.7)	1 (2.0)
Discoloration, white	0 -	0 -	0 -	1 (3.0)	0 -	0 -	0 -	0 -	0 -	0 -
Enlarged	2 (2.0)	1 (3.0)	2 (2.5)	6 (2.3)	2 (3.0)	2 (2.0)	0 -	0 -	2 (2.0)	0 -
Irregular surface	0 -	0 -	0 -	0 -	3 (2.6)	0 -	1 (2.0)	1 (3.0)	0 -	1 (2.0)
Mammary gland										
Swollen/thickened						18	12	11	12	18

						(2.6)	(2.7)	(2.4)	(2.3)	(2.6)
Pituitary gland										
Enlarged	22 (3.5)	31 (3.2)	14 (3.1)	3 (2.7)	22 (3.5)	45 (3.0)	44 (2.9)	38 (2.6)	24 (2.5)	47 (2.9)
Seminal vesicles										
Small	2 (2.5)	3 (2.0)	6 (3.2)	10 (3.1)	4 (2.5)					
Spleen										
Enlarged	1 (4.0)	1 (2.0)	9 (2.3)	6 (2.5)	10 (2.6)	5 (2.4)	3 (3.0)	10 (2.5)	7 (2.3)	5 (3.0)
Testes										
Small	2 (2.0)	3 (2.0)	9 (2.4)	14 (2.6)	3 (2.3)					
Thymus										
Small	7 (3.0)	3 (2.7)	4 (3.5)	10 (3.5)	6 (2.5)	4 (3.2)	7 (3.6)	7 (3.3)	12 (3.1)	8 (3.1)

Green text depicts findings that exhibited an inverse relationship to test item.
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Organ Weights

Test article-related effects on organ weight were seen in the kidney, liver, spleen and thymus.

Many organs exhibited a slight decrease in absolute weight and a marked increase in mass as a percentage of body weight. This pattern is most likely related to the dose-related decrease in body weight that was seen in both sexes, is judged to be toxicologically insignificant, and is not captured in the table below.

Organ Weights -- Percent Change from Control										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
Body Weight	753	n.e.	**↓16****	**↓27****	**↓11****	484	**↓14****	**↓18****	**↓32****	**↓13***
Brain										
Absolute (g)	2.221	n.e.	n.e.	**↓5****		2.042	n.e.	n.e.	n.e.	n.e.
% Body Weight	0.300	n.e.	**↑17****	**↑32****		0.431	**↑19****	**↑19****	**↑41****	↑15
Kidneys										
Absolute (g)	5.730	↑11	**↑80****	**↑96****	n.e.	3.555	n.e.	↑14	**↑36****	n.e.
% Body Weight	0.769	↑14	**↑117****	**↑167**	**↑11***	0.739	**↑15***	**↑40***	**↑104****	**↑17***
Brain Weight Ratio	2.587	↑11	**↑81****	**↑105****	n.e.	1.746	n.e.	↑16	**↑41****	n.e.
Liver										
Absolute (g)	22.814	n.e.	**↑25****	**↑38****	n.e.	16.827	**↓23****	↓8	↑13	↓6
% Body Weight	3.042	↑8	**↑50****	**↑90****	↑16	3.472	↓9	↑14	**↑69****	↑8
Brain Weight Ratio	10.307	↑5	**↑27****	**↑44****	n.e.	8.251	**↓24***	↓6	↑17	↓6
Spleen										
Absolute (g)	1.386	↑13	**↑66****	**↑57****	**↑52****	1.126	↓21	n.e.	↑12	n.e.
% Body Weight	0.186	↑17	**↑96****	**↑114****	**↑69****	0.232	↓7	↑28	**↑67****	↑17
Brain Weight Ratio	0.627	↑13	**↑68****	**↑64****	**↑52****	0.549	↓22	n.e.	↑17	n.e.
Thymus										
Absolute (g)	0.411	↓27	↓36	**↓65****	n.e.	0.265	↓25	↓17	↓27	↓21
% Body Weight	0.054	↓22	↓23	**↓50***	↑7	0.054	↓11	n.e.	↑11	↓5

Brain Weight Ratio	0.189	↓27	↓36	**↓63****	↓5	0.130	↓26	↓15	↓25	↓20

Bold indicates statistically significantly different from group 1
*p<0.05, **p<0.01
n.e. = no effect (effect of less than 5%)
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Histopathology

Peer Review

No peer review was conducted.

Neoplastic

Neoplasms – Incidence										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Day of Terminal Sacrifice	**731**	**731**	**666**	**517**	**731**	**731**	**681**	**697**	**641**	**731**
Adipose tissue, brown										
hibernoma (B, P)	-	-	-	-	1	-	-	-	-	-
hibernoma (M, P)	1	-	1	1	1	-	-	-	-	-
Adrenal glands										
adenoma, cortical (B, P)	2	1	-	-	1	2	-	1	-	3
pheochromocytoma (B, P)	10	6	8	3	9	2	3	3	1	4
pheochromocytoma, complex (B, P)	-	-	-	-	1	1	-	-	-	-
pheochromocytoma (M, P)	-	2	3	-	-	-	-	-	-	-
Brain										
astrocytoma (M, P)	1	-	-	1	1	-	1	-	-	-
carcinoma, pars distalis (M, S)	-	-	-	-	1	6	-	3	1	-
Mixed glioma (M, P)	-	-	-	-	-	-	1	-	-	-
Cavity, abdominal										
carcinoma, tubular cell (M, S)	-	-	-	-	1	-	-	-	-	-
fibroma (B, P)	1	-	-	-	-	-	-	-	-	-
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
liposarcoma (M, P)	-	-	-	-	1	-	-	-	-	-
schwannoma (M, P)	-	-	-	-	1	-	-	-	-	-
Cavity, thoracic										
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
Eyes										
adenocarcinoma (M, S)	-	-	-	-	1	-	-	-	-	-
Harderian glands										
adenocarcinoma (M, P)	-	-	-	-	1	-	-	-	-	-
Heart										
adenocarcinoma (M, S)	-	-	-	-	-	1	-	-	-	-
carcinoma, tubular cell (M, S)	-	-	-	-	1	-	-	-	-	-
carcinoma, undifferentiated (M, S)	-	-	-	1	-	-	-	-	-	-
schwannoma (M, P)	1	-	-	-	-	1	1	-	-	1
Kidneys										
adenoma, tubular cell (B, P)	-	-	1	-	-	-	1	-	1	-
carcinoma, transitional cell (M, P)	1	-	-	-	-	-	-	-	-	-

carcinoma, tubular cell (M, P)	1	-	-	-	2	-	-	2	1	1
liposarcoma (M, P)	-	1	-	-	-	-	-	-	1	-
renal mesenchymal tumor, (M, P)	-	-	-	-	1	-	-	-	-	-
tubular cell adenoma + carcinoma	1	-	1	-	2	x	x	x	x	x
Large intestine, cecum										
Leiomyoma (B, P)	-	1	-	-	-	-	-	-	-	-
Large intestine, rectum										
fibrosarcoma (M, S)	-	1	-	-		-	-	-	-	-
Larynx										
carcinoma, c-cell (M, S)	-	-	-	-	-	-	-	1	-	-
Liver										
adenoma, hepatocellular (B. P)	-	1	2	1	1	-	1	-	-	-
carcinoma, hepatocellular (M, P)	-	1	-	-	1	-	-	-	-	-
carcinoma, islet cell (M, S)	-	-	-	-	-	1	-	-	-	-
cholangiocarcinoma (M, P)	-	1	-	-	-	-	-	-	-	-
hepatocellular adenoma + carcinoma	-	2	2	1	2	x	x	x	x	x
Lung										
adenocarcinoma (M, S)	-	-	-	-	-	-	1	1	1	-
carcinoma, bronchiolar alveolar (M, P)	1	-	-	-	-	-	-	-	-	-
carcinoma, c-cell (M, S)	-	-	-	-	-	-	-	1	-	-
carcinoma, tubular cell (M, S)	-	-	-	-	1	-	-	-	-	-
carcinoma, undifferentiated (M, S)	-	-	-	1	-	-	-	-	-	-
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
hemangiosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
hibernoma (M, S)	1	-	-	-	-	-	-	-	-	-
pheochromocytoma (M, S)	-	-	2	-	-	-	-	-	-	-
adenomas + carcinomas, bronchiolar/alveolar										
Lymph node, axillary										
adenocarcinoma (M, S)	-	-	-	-	-	-	-	-	1	-
fibrous histiocytoma (M, S)	-	-	1	-	-	-	-	-	-	-
Lymph node, cervical										
carcinoma, c-cell (M, S)	-	-	-	-	-	-	-	1	-	-
Lymph node, iliac										
fibrous histiocytoma (M, S)	-	-	1	-	-	-	-	-	-	-
Lymph node, inguinal										
adenocarcinoma (M, S)	-	-	-	-	-	-	-	-	-	1
fibrous histiocytoma (M, S)	-	-	1	-	-	-	-	-	-	-
Lymph node, mandibular										
carcinoma, squamous cell (M, S)	-	-	-	-	-	-	-	1	-	-
Lymph node, mediastinal										
carcinoma, c-cell (M, S)	-	-	-	-	-	-	-	1	-	-
carcinoma, tubular cell (M,S)	-	-	-	-	1	-	-	-	-	-
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
Lymph node, mesenteric										
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
hemangioma (B, P)	-	-	-	-	1	-	-	-	-	-
hemangiosarcoma (M, P)	1	-	-	-	1	-	-	1	-	-
Lymph node, renal										
carcinoma, tubular cell (M,S)	-	-	-	-	1	-	-	-	-	-
Mammary gland										

adenocarcinoma (M, P)	-	-	-	-	-	31	29	28	15	25
adenoma, benign (B, P)	-	-	-	-	-	2	1	2	1	1
fibroadenoma (B, P)	2	-	-	1	-	33	21	23	19	22
fibrosarcoma (M, P)	-	-	-	-	-	1	-	-	-	-
fibroadenoma + fibrosarcoma	x	x	x	x	x	34	21	23	19	22
Multicentric neoplasm										
leukemia, large granular lymphocyte (M)	-	-	-	-	2	-	-	1	-	-
lymphoma (M)	2	1	1	-	-	-	-	1	1	-
sarcoma, histiocytic (M)	-	1	-	-	2	-	-	-	-	-
Nerve, trigeminal										
carcinoma, pars distalis (M, S)	-	-	-	-	-	-	-	-	1	-
Nose										
astrocytoma (M, S)	-	-	-	-	-	-	1	-	-	-
papilloma (B, P)	1	-	-	-	-	-	-	-	-	-
Oropharyngeal tissues										
carcinoma, sebaceous cell (M, P)	-	1	-	-	-	-	-	-	-	-
Ovaries										
granulosa cell tumor (B, P)						-	-	-	1	1
granulosa cell tumor (M, P)						-	-	-	1	-
Oviducts										
leiomyoma (B, P)						-	-	-	1	-
Pancreas										
adenoma, acinar cell (B, P)	-	-	-	-	-	-	-	-	1	-
adenoma, islet cell (B, P)	5	5	1	-	2	-	3	2	-	1
carcinoma, islet cell (M, P)	2	2	2	-	3	2	-	1	-	1
carcinoma, tubular cell (M, S)	-	-	-	-	1	-	-	-	-	-
Parathyroid glands										
adenoma (B, P)	2	2	2	-	1	-	1	-	-	1
carcinoma (M, S)	-	-	-	-	-	-	1	-	-	1
Pituitary gland										
adenoma, pars distalis (B, P)	36	47	29	13	36	43	51	45	27	50
adenoma, pars intermedia (B, P)	-	-	1	-	-	-	-	-	-	-
astrocytoma (M, S)	-	-	-	-	-	-	1	-	-	-
carcinoma, pars distalis (M, P)	-	-	-	-	1	6	-	3	2	-
anterior lobe, adenomas + carcinomas	36	47	30	13	36	49	51	48	29	50
Preputial glands										
carcinoma, squamous cell (M, P)	1	-	-	-	-					
papilloma, squamous cell (B, P)	-	-	-	1	-					
Seminal vesicles										
adenoma (B, P)	1	-	-	-	-					
Skeletal muscle										
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
hemangiosarcoma (M, P)	-	1	-	-	-	-	-	-	-	-
schwannoma (M, S)	-	-	-	-	1	-	-	-	-	-
Skin										
adenoma, basal cell (B, P)	-	-	1	-	-	-	-	-	-	1
adenoma, sebaceous cell (B, P)	-	-	-	-	1	-	-	-	-	-
carcinoma, sebaceous cell (M, P)	-	-	-	-	-	-	-	-	-	1
keratoacanthoma (B, P)	7	3	4	-	-	-	1	1	-	-
papilloma, squamous cell (B, P)	2	3	-	-	-	-	-	-	1	1

squamous cell papilloma + carcinoma + keratoacanthoma	9	6	4	-	-	-	1	1	1	1
Skin, subcutis										
carcinoma, undifferentiated (M, P)	-	-	-	1	-	-	-	-	-	-
fibroma (B, P)	1	4	1	-	1	1	1	2	1	-
fibrosarcoma (M, P)	-	1	1	1	2	-	1	4	5	1
fibrous histiocytoma (M, P)	-	1	3	3	1	-	-	3	4	-
hemangiosarcoma (M, P)	1	-	-	-	-	-	-	-	-	-
lipoma (B, P)	1	1	-	-	-	2	-	-	-	-
rhabdomyosarcoma (M, P)	-	-	-	-	-	-	-	1	-	-
schwannoma (M, P)	-	1	1	-	-	1	1	-	-	-
Small intestine, duodenum										
adenocarcinoma (M, P)	-	1	-	-	-	-	-	-	-	-
Small intestine, jejunum										
adenocarcinoma (M, P)	-	1	-	-	-	-	-	-	-	-
Spleen										
hemangiosarcoma (M, P)	1	-	-	-	-	-	-	-	-	-
leiomyosarcoma (M, P)	-	1	-	-	-	-	-	-	-	1
liposarcoma (M, P)	-	-	-	-	1	-	-	-	-	-
Stomach, glandular										
fibrosarcoma (M, S)	-	1	-	-	-	-	-	-	-	-
Stomach, nonglandular										
papilloma, squamous cell (B, P)	-	-	-	-	-	-	-	-	1	1
Tail										
papilloma, squamous cell (B, P)	-	1	1	-	-	-	-	-	-	-
Testes										
adenoma, interstitial cell (B, P)	2	-	2	-	1					
Thyroid gland										
adenoma, c-cell (B, P)	8	6	3	-	9	2	4	6	3	6
adenoma, follicular cell (B, P)	1	1	1	-	1	-	-	-	-	1
carcinoma, c-cell (M, P)	-	-	-	-	2	-	1	3	-	1
carcinoma, follicular cell (M, P)	-	2	2	1	-	-	-	-	-	1
adenomas + carcinomas, c-cell	8	6	3	-	11	2	5	9	3	7
adenomas + carcinomas, follicular cell	1	3	3	1	1	-	-	-	-	2
Urinary bladder										
carcinoma, transitional cell (M, P)	-	-	-	1	-	-	-	-	-	-
schwannoma (M, S)	-	-	-	-	-	1	-	-	-	-
Uterus with cervix										
granular cell tumor (B, P)						2	1	3	8	1
polyp, glandular (B, P)						-	-	1	-	-
polyp, stromal (B, P)						7	2	4	2	2
sarcoma, stromal (M, P)						-	-	-	-	2
schwannoma (M, P)						1	-	-	-	-
stromal polyp + sarcoma						7	2	4	2	4
Vagina										
granular cell tumor (B, P)						2	1	3	2	3
leiomyoma (B, P)						-	-	-	1	-
Zymbal's gland										
carcinoma, sebaceous cell (M, P)	-	-	1	-	-	-	-	1	1	1
carcinoma, squamous cell (M, P)	-	-	-	-	-	-	-	1	-	-

"-" = not observed in dose group
B = benign, M = malignant, P = primary, S = secondary

x = not calculated per current table for "Combining Tumors for Statistical Analysis"
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Sponsor's statistical analysis:

Males

No statistically significant increases in any type of neoplasm in male rats with the Fisher Exact test for pair-wise comparisons, the Cochran-Armitage Trend test, the Peto test, or the Onset Rate test. The Poly-3 pair-wise or trend analysis gave the following positive results:

malignant fibrous histiocytoma:

LD: 1/60, n.s.s.
MD: 3/60 or 5.00% overall rate, 9.36% adjusted rate
HD: 3/60 or 5.00% overall rate, 18.46% adjusted rate
C: 0/60
HC: 0-2%, mean incidence of 0.4% (rare tumor)

-- Sponsor concluded this tumor was related to the method of administration, rather than the test item per se.

thyroid follicular cell carcinoma:

LD: 2/60, n.s.s.
MD: 2/60 or 3.33% overall rate, 6.36% adjusted rate
HD: 1/60, n.s.s.
C: 0/60
HC: 0-2.9%, mean incidence of 0.5% (rare tumor)

-- Sponsor concluded this tumor was incidental based on lack of dose dependence, absence of comparable effect in ♀s, and marginal numerical increase over HC (historical control).

large granular lymphocyte leukemia:

LD: 0/60
MD: 0/60
HD: 0/60
C: 0/60
Surrogate: 2/60 or 3.33% overall rate, 4.49% adjusted rate
HC: 0-1.4%, mean incidence of 0.5% (rare tumor)

-- Sponsor concluded this tumor was incidental and not test article-related as the overall rate was only slightly greater than the historical control range, the concurrent control was at the bottom of the historical control range, and there was not a similar effect in the females..

Females

No statistically significant increases in any type of neoplasm in male rats with the Fisher Exact test for pair-wise comparisons, the Cochran-Armitage Trend test, the Peto test, or the Onset Rate test or the Poly-3 trend test. The Poly-3 pair-wise analysis gave the following positive results:

malignant fibrous histiocytoma:

LD: 0/60
MD: 3/60 or 5.00% overall rate, 7.91% adjusted rate
HD: 4/60 or 6.67% overall rate, 13.58% adjusted rate
C: 0/60
HC: 0-3.3%, mean incidence of 0.2% (rare tumor)

-- Sponsor concluded this tumor was related to the method of administration, rather than the test item per se.

fibrosarcoma

LD: 1/60, n.s.s.
MD: 4/5, n.s.s.
HD: 5/60 or 8.33% overall rate, 17.08% adjusted rate
C: 0/60
HC: 0-4.6%, mean incidence of 1.1% (common tumor)

-- Sponsor concluded this tumor was related to the method of administration, rather than the test item per se.

adenoma, pars distalis, pituitary

LD: 51/60 or 85.00% overall rate, 93.75% adjusted rate
MD: 45/60, n.s.s.
HD: 27/60, n.s.s.
C: 43/60 or 71.67% overall rate, 78.41% adjusted rate
HC: 60.0 to 88.3%, mean incidence of 76.7% (common tumor)

-- Sponsor concluded this tumor was incidental and not test article-related as the overall rate was within the historical control range, the increase was not dose related, and the combined incidence of adenoma and carcinoma of the pars distalis of the pituitary was not statistically significant as compared to the control group.

granular cell tumors, uterus

LD: 1/60, n.s.s.
MD: 3/60, n.s.s.
HD: 8/60 or 13.33% overall rate, 26.46% adjusted rate
C: 2/60 or 3.33% overall rate, 4.81% adjusted rate
HC: 0-10%, mean incidence of 1.6% (common tumor)

-- Sponsor concluded this tumor was incidental and not test article-related as the overall rate was only slightly greater than the (b) (4) *historical control range, the concurrent control group was on the low side of the historical control range, and the overall incidence was within the range of that reported for nine control groups from 104 week studies with* (b) (4) *Sprague-Dawley rats.*

Statistically Significantly Increased Tumors										
Sex	Male					Female				
Dose Group	1	2	3	4	5	1	2	3	4	5
Day of Terminal Sacrifice	731	731	666	517	731	731	681	697	641	731
Skin										
fibrosarcoma (M, P)	-	1	1	1	2	-	1	4	5*	1
fibrous histiocytoma (M, P)	-	1	3*	3*	1	-	-	3*	4*	-
Thyroid										
carcinoma, follicular cell (M, P)	-	2	2*	1	-	-	-	-	-	1
Uterus										
granular cell tumor (B, P)						2	1	3	8*	1

Non Neoplastic

Test item has multiple effects on a number of different organs. Perhaps most striking are the adverse effects of the test item on the kidney, which the Sponsor has characterized as a worsening of chronic progressive nephropathy (CPN). See Sponsor's table below. These renal histopathology findings correlate with increased blood urea, increased kidney weight, various gross pathology findings noted above. Interestingly, this renal effect appears to be much greater for the clinical candidate than it is for the murine surrogate.

Many of the observed histopathology findings are likely to be secondary to the drug-induced renal failure and/or the resultant uremia: ↑ renal cyst; renal thrombus; vascular and parenchymal mineralization in multiple tissues; parathyroid hyperplasia + bone fibrous osteodystrophy and hyperostosis (secondary hyperparathyroidism); polyarteritis in multiple tissues; brain/spinal cord edema and necrosis (uremic encephalopathy); reduced size/function of male reproductive tissues (uremic hypogonadism); uremic pneumonitis; ↑ erythrocytosis/erythrophagocytosis in lymph nodes and spleen; pancreatic edema; generalized lymphoid depletion (poor condition/stress); adipose depletion (poor condition, ↓FC, ↓BW), uremic gastropathy. The above findings were generally more severe or had a greater incidence in ♂s, which had a greater severity of CPN.

The relationship of other findings to the renal toxicity is less clear: centrilobular hepatocellular vacuolation/necrosis; chronic-passive congestion of the lungs; ↑ hyperplasia of squamous cells of the tongue.

Lymphohistiocytic infiltrates in many tissues, sinus histiocytosis in lymph nodes, and basophilic granules in Kupffer cells of the liver and in the cytoplasm of kidney tubule epithelial cells were common findings in rats from all treated groups with a clear dose response relationship for the number of tissues affected and/or the severity of the infiltrates. Per the Sponsor, in all cases, these findings represent uptake of the oligonucleotide test article into affected cells.

A number of findings exhibited an inverse relationship to test item dose. These are spontaneous findings whose incidence/severity generally increases with age. Therefore, the apparent decrease in the incidence/severity of these findings (depicted by green text in reviewer's table below) is considered an artifact of early death/sacrifice in the ISIS 301012-treated animals.

Test Article-related Microscopic Observations - Terminal
Male

Group	1	2	3	4	5
Number Examined	60	60	60	60	60
kidneys					
nephropathy, chronic progressive	54	51	58	59	55
-minimal	32	21	8	1	30
-mild	15	17	4	1	13
-moderate	3	9	4	5	9
-severe	4	4	42	52	3

1 – 0 mg/kg/week ISIS 301012
2 – 3 mg/kg/week ISIS 301012
3 – 10 mg/kg/week ISIS 301012
4 – 30/25/20 mg/kg/week ISIS 301012
5 – 10 mg/kg/week 147768

Test Article-related Microscopic Observations - Terminal
Female

Group	1	2	3	4	5
Number Examined	60	60	60	60	60
kidneys					
nephropathy, chronic progressive	37	27	50	57	38
-minimal	34	25	27	12	33
-mild	2	2	8	8	3
-moderate	1	0	6	14	0
-severe	0	0	9	23	2

1 – 0 mg/kg/week ISIS 301012
2 – 3 mg/kg/week ISIS 301012
3 – 10 mg/kg/week ISIS 301012
4 – 25/20 mg/kg/week ISIS 301012
5 – 10 mg/kg/week 147768

Non-neoplastic Histopathology Findings -- Incidence (Mean Severity)										
Sex	Male					Female				
Dose Group	1	2	3	4	5	1	2	3	4	5
Day of Terminal Sacrifice	731	731	666	517	731	731	681	697	641	731
Adrenal glands										
Angiectasis/cystic degeneration, focal, cortical	20/60 (1.4)	20/60 (1.4)	13/60 (1.7)	12/60 (1.5)	14/60 (1.8)	58/60 (2.1)	58/60 (2.2)	55/60 (2.1)	47/60 (2.3)	52/60 (2.1)
Atrophy, cortical	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (4.0)	1/60 (3.0)	4/60 (2.8)	4/60 (3.2)	1/60 (3.0)
Bacterial colonies	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	1/60 (1.0)	1/60 (1.0)	0/60 -
Congestion	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (3.0)	0/60 -
Hyperplasia, focal cortical	19/60 (1.2)	20/60 (1.1)	9/60 (1.3)	4/60 (1.2)	5/60 (1.4)	14/60 (1.5)	8/60 (1.4)	8/60 (1.1)	4/60 (1.2)	8/60 (1.1)
Necrosis	1/60 (4.0)	1/60 (1.0)	1/60 (1.0)	4/60 (1.5)	1/60 (4.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Aorta										
Mineralization	1/60 (3.0)	0/60 -	14/59 (1.8)	32/60 (2.9)	1/60 (3.0)	0/60 -	0/60 -	1/60 (3.0)	13/60 (2.7)	1/60 (3.0)
Bone, femur										
Fibrous osteodystrophy	0/60 -	0/60 -	21/60 (1.4)	26/60 (1.2)	0/60 -	0/60 -	0/60 -	1/60 (1.0)	10/60 (1.2)	1/60 (1.0)
Hyperostosis	0/60 -	0/60 -	0/60 -	6/60 (2.0)	1/60 (1.0)	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (2.0)
Bone, sternum										
Fibrous osteodystrophy	0/60 -	0/60 -	20/60 (1.1)	18/60 (1.1)	0/60 -	0/59 -	0/60 -	1/60 (1.0)	9/60 (1.1)	0/60 -
Hyperostosis	0/60 -	0/60 -	1/60 (1.0)	10/60 (3.0)	1/60 (1.0)	0/59 -	0/60 -	0/60 -	3/60 (2.0)	1/60 (2.0)
Brain										
Compression, ventral (pituitary tumor)	14/60 (2.9)	24/60 (2.9)	9/60 (2.7)	3/60 (2.3)	19/60 (2.8)	26/60 (2.8)	26/60 (2.6)	16/60 (2.8)	8/60 (2.9)	25/60 (2.4)
Degeneration/necrosis, neuronal	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (3.0)	0/60 -
Edema	0/60 -	0/60 -	1/60 (2.0)	2/60 (2.5)	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (2.0)	0/60 -
Gliosis, reactive	0/60 -	0/60 -	0/60 -	1/60 (1.0)	1/60 (2.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Necrosis, focal	1/60 (3.0)	0/60 -	3/60 (3.0)	10/60 (2.0)	0/60 -	0/60 -	0/60 -	1/60 (1.0)	4/60 (2.8)	0/60 -
Thrombus	0/60 -	0/60 -	0/60 -	1/60 (2.0)	0/60 -	1/60 (1.0)	0/60 -	1/60 (1.0)	0/60 -	0/60 -
Coagulating glands										
Depletion, secretory	5/60 (2.8)	7/60 (3.9)	18/60 (3.8)	30/60 (3.7)	3/60 (3.7)					
Epididymides										
Oligospermia, germ cell debris, bilateral	2/60 (2.5)	3/60 (4.0)	16/60 (3.5)	24/60 (3.7)	1/60 (3.0)					
Heart										
Cardiomyopathy	58/60 (1.2)	56/60 (1.1)	58/60 (1.5)	59/60 (1.6)	58/60 (1.1)	55/60 (1.1)	48/60 (1.0)	50/60 (1.0)	53/60 (1.2)	55/60 (1.0)
Endocarditis, valvular vegetative	0/60 -	1/60 (4.0)	1/60 (4.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Hypertrophy/hyperplasia, mesothelial cell	0/60 -	0/60 -	1/60 (2.0)	1/60 (2.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Mineralization, myofiber	1/60 (1.0)	0/60 -	5/60 (2.8)	16/60 (1.7)	0/60 -	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -

Mineralization, vascular	1/60 (2.0)	0/60 -	11/60 (1.7)	27/60 (1.9)	1/60 (1.0)	0/60 -	1/60 (1.0)	2/60 (1.0)	9/60 (1.6)	0/60 -
Kidneys										
Basophilic granules,. tubular cell	0/60 -	59/60 (1.9)	59/60 (2.7)	60/60 (3.0)	59/60 (2.0)	0/60 -	60/60 (1.7)	60/60 (2.1)	60/60 (2.6)	60/60 (1.7)
Cyst	4/60 (1.5)	3/60 (1.7)	9/60 (2.1)	10/60 (2.0)	3/60 (1.0)	2/60 (1.0)	4/60 (1.8)	10/60 (1.7)	19/60 (1.9)	0/60 -
Mineralization, pelvic	19/60 (1.1)	6/60 (1.0)	5/60 (1.0)	0/60 -	11/60 (1.0)	55/60 (1.0)	49/60 (1.1)	46/60 (1.0)	19/60 (1.0)	50/60 (1.0)
Mineralization, vascular	0/60 -	0/60 -	7/60 (1.3)	21/60 (1.3)	1/60 (1.0)	0/60 -	0/60 -	1/60 (2.0)	6/60 (1.3)	1/60 (2.0)
Nephropathy, chronic, progressive	54/60 (1.6)	51/60 (1.9)	58/60 (3.4)	59/60 (3.8)	55/60 (1.7)	37/60 (1.1)	27/60 (1.1)	50/60 (1.9)	57/60 (2.8)	38/60 (1.2)
Pyelitis	16/60 (1.0)	13/60 (1.1)	5/60 (1.4)	1/60 (3.0)	15/60 (1.5)	8/60 (1.2)	8/60 (1.0)	3/60 (1.3)	5/60 (1.2)	9/60 (1.1)
Thrombus	0/60 -	0/60 -	9/60 (1.8)	11/60 (2.0)	1/60 (1.0)	0/60 -	0/60 -	1/60 (3.0)	5/60 (2.2)	1/60 (2.0)
Lacrimal glands, exorbital										
Mineralization, vascular	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Large intestine, cecum										
Mineralization, vascular	0/60 -	0/60 -	1/60 (1.0)	3/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Large intestine, colon										
Mineralization	0/60 -	0/60 -	0/60 -	1/60 (2.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Mineralization, vascular	0/60 -	0/60 -	2/60 (1.0)	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Large intestine, rectum										
Mineralization, vascular	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Larynx										
Erosion/ulcer	0/59 -	0/60 -	3/60 (1.7)	2/60 (1.5)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Inflammation, acute	1/59 (1.0)	3/60 (1.0)	3/60 (1.3)	4/60 (1.2)	0/60 -	0/60 -	3/60 (1.0)	0/60 -	3/60 (1.0)	0/60 -
Mineralization	0/59 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Liver										
Basophilic granules, Kupffer cell	0/60 -	55/60 (1.0)	58/60 (1.8)	59/60 (2.5)	57/60 (2.0)	0/60 -	58/60 (1.0)	59/60 (1.8)	60/60 (2.2)	58/60 (1.7)
Focus of cellular alteration, basophilic	8/60 (1.1)	11/60 (1.4)	7/60 (1.0)	0/60 -	7/60 (1.0)	23/60 (1.1)	13/60 (1.0)	14/60 (1.0)	5/60 (1.0)	6/60 (1.0)
Hyperplasia, bile duct	35/60 (1.0)	38/60 (1.1)	26/60 (1.0)	17/60 (1.0)	43/60 (1.1)	22/60 (1.0)	34/60 (1.1)	30/60 (1.0)	18/60 (1.0)	25/60 (1.0)
Infiltration, mononuclear cell	14/60 (1.2)	4/60 (1.0)	1/60 (1.0)	5/60 (1.0)	4/60 (1.0)	10/60 (1.0)	6/60 (1.0)	5/60 (1.0)	6/60 (1.0)	12/60 (1.0)
Necrosis, hepatocytes, centrilobular	0/60 -	0/60 -	1/60 (1.0)	1/60 (3.0)	3/60 (2.3)	0/60 -	0/60 -	2/60 (2.5)	4/60 (2.0)	1/60 (2.0)
Vacuolation, centrilobular	2/60 (1.0)	1/60 (1.0)	7/60 (1.4)	12/60 (2.1)	1/60 (2.0)	1/60 (1.0)	1/60 (2.0)	4/60 (1.8)	17/60 (1.5)	4/60 (1.8)
Vacuolation, diffuse	0/60 -	0/60 -	4/60 (1.8)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (1.0)
Vacuolation, periportal	14/60 (1.3)	8/60 (1.4)	4/60 (1.5)	2/60 (1.0)	5/60 (1.6)	16/60 (1.3)	12/60 (1.5)	8/60 (1.2)	2/60 (1.5)	8/60 (1.5)
Lung										
Congestion, chronic passive	0/60 -	0/60 -	1/60 (2.0)	2/60 (2.0)	2/60 (1.5)	0/60 -	0/60 -	0/60 -	3/60 (2.7)	0/60 -
Hemorrhage	0/60 -	0/60 -	0/60 -	3/60 (1.0)	2/60 (1.5)	1/60 (1.0)	1/60 (2.0)	0/60 -	1/60 (3.0)	0/60 -

Histiocytosis, alveolar	27/60 (1.0)	20/60 (1.0)	16/60 (1.0)	10/60 (1.0)	22/60 (1.0)	26/60 (1.1)	17/60 (1.0)	21/60 (1.1)	20/60 (1.1)	27/60 (1.1)
Pneumonitis, uremic	1/60 (1.0)	0/60 -	4/60 (2.2)	15/60 (2.3)	0/60 -	0/60 -	0/60 -	0/60 -	2/60 (2.5)	0/60 -
Thrombus	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Lymph node, mesenteric										
Erythrocytosis/ erythrophagocytosis, sinus	1/60 (3.0)	4/60 (1.0)	11/59 (1.1)	4/58 (1.0)	5/60 (1.8)	1/60 (2.0)	3/60 (1.0)	14/60 (1.6)	16/60 (1.3)	5/60 (1.0)
Thrombus	0/60 -	0/60 -	0/59 -	1/58 (2.0)	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (1.0)	0/60 -
Mesentery/peritoneum										
Mineralization, vascular	0/1 -	0/1 -	2/12 (3.0)	1/6 (3.0)	0/2 -	0/0 -	0/0 -	0/2 -	2/4 (1.5)	0/1 -
Nose, level a										
Fibrous osteodystrophy	0/60 -	0/60 -	13/60 (1.2)	20/60 (1.1)	0/60 -	0/60 -	0/60 -	1/60 (1.0)	1/60 (1.0)	0/60 -
Metaplasia, squamous	3/60 (1.0)	2/60 (1.5)	2/60 (1.0)	0/60 -	2/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Nose, level b										
Fibrous osteodystrophy	0/60 -	0/60 -	14/60 (1.6)	23/60 (1.2)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Inflammation, acute	4/60 (1.5)	4/60 (1.0)	5/60 (1.2)	0/60 -	1/60 (1.0)	1/60 (2.0)	1/60 (1.0)	1/60 (1.0)	0/60 -	1/60 (1.0)
Metaplasia, squamous	2/60 (1.0)	2/60 (1.5)	4/60 (1.2)	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Nose, level c										
Fibrous osteodystrophy	0/60 -	0/60 -	15/60 (1.5)	25/60 (1.2)	0/60 -	0/60 -	0/60 -	0/60 -	5/60 (1.0)	0/60 -
Inflammation, acute	3/60 (1.5)	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Nose, level d										
Fibrous osteodystrophy	0/60 -	0/60 -	16/60 (1.5)	27/60 (1.3)	0/60 -	0/60 -	0/60 -	0/60 -	6/60 (1.0)	0/60 -
Inflammation, acute	1/60 (2.0)	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Odontitis/periodonitis	5/60 (2.0)	0/60 -	1/60 (1.0)	1/60 (2.0)	1/60 (2.0)	1/60 (1.0)	1/60 (2.0)	0/60 -	0/60 -	3/60 (2.0)
Ovaries										
Cyst						18/60 (1.5)	11/60 (1.2)	8/60 (1.5)	9/60 (1.6)	11/60 (1.6)
Pancreas										
Edema	0/60 -	0/60 -	1/60 (1.0)	3/60 (2.0)	1/60 (2.0)	0/60 -	0/60 -	1/59 (2.0)	2/60 (2.0)	0/60 -
Fibrosis	4/60 (1.2)	3/60 (1.0)	0/60 -	0/60 -	4/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)
Mineralization, vascular	0/60 -	0/60 -	3/60 (2.0)	6/60 (1.0)	0/60 -	0/60 -	0/60 -	1/59 (1.0)	3/60 (1.3)	1/60 (1.0)
Parathyroid glands										
Hyperplasia, diffuse	0/53 -	0/49 -	16/51 (2.1)	24/54 (2.0)	0/47 -	0/51 -	0/51 -	0/49 -	5/51 (1.8)	0/48 -
Hyperplasia, focal	5/53 (1.6)	2/49 (1.0)	0/51 -	0/54 -	1/47 (2.0)	0/51 -	1/51 (1.0)	1/49 (1.0)	1/51 (1.0)	1/48 (2.0)
Peyers patch										
Depletion lymphoid,. generalized	0/59 -	0/60 -	3/55 (2.7)	4/53 (2.8)	0/58 -	0/57 -	0/60 -	0/60 -	3/59 (2.3)	0/60 -
Pituitary gland										
Hyperplasia, focal, pars distalis	11/60 (1.4)	3/60 (2.0)	9/60 (1.7)	8/60 (1.6)	9/60 (1.8)	1/60 (2.0)	2/60 (2.0)	8/60 (1.6)	11/60 (1.6)	4/60 (1.8)

Prostate gland										
Inflammation, acute	2/60 (1.5)	5/60 (2.6)	7/60 (1.7)	8/60 (1.4)	3/60 (2.3)					
Salivary gland, sublingual										
Mineralization, vascular	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/59 -	0/60 -	0/60 -	0/60 -	0/60 -
Seminal vesicles										
Depletion, secretory	5/60 (3.4)	7/60 (3.9)	19/60 (3.8)	31/60 (3.7)	4/60 (3.2)					
Inflammation, acute	1/60 (2.0)	2/60 (3.5)	0/60 -	4/60 (1.8)	2/60 (2.5)					
Mineralization, vascular	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -					
Skeletal muscle										
Mineralization, myofiber	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Skin										
Depletion, adipose	1/60 (4.0)	5/60 (3.2)	18/60 (3.8)	26/60 (3.9)	3/60 (3.0)	4/60 (3.0)	3/60 (3.3)	7/60 (3.1)	12/60 (3.7)	3/60 (4.0)
Edema	0/60 -	0/60 -	0/60 -	2/60 (2.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Mineralization	0/60 -	0/60 -	0/60 -	1/60 (3.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Small intestine, duodenum										
Erosion/ulcer	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (2.0)	0/60 -
Small intestine, ileum										
Mineralization, vascular	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Spinal cord, cervical										
Degeneration, axonal/myelin	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Necrosis, focal	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Spleen										
Hematopoiesis, extramedullary, increased	18/60 (1.1)	25/60 (1.1)	19/60 (1.1)	22/60 (1.4)	20/60 (1.4)	38/60 (1.3)	26/60 (1.4)	41/60 (1.3)	40/59 (1.4)	30/60 (1.2)
Stomach, glandular										
Gastropathy, uremic	1/60 (2.0)	0/60 -	10/60 (1.9)	26/60 (1.9)	2/60 (1.0)	0/60 -	0/60 -	1/60 (2.0)	10/60 (1.8)	1/60 (2.0)
Stomach, Nonglandular										
Edema	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (2.0)	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (2.0)
Gastropathy, uremic	0/60 -	0/60 -	3/60 (1.0)	6/60 (1.7)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Testes										
Degeneration/atrophy, seminiferous tubules, bilateral	3/60 (2.3)	2/60 (4.0)	18/60 (3.2)	26/60 (3.0)	2/60 (1.0)					
Mineralization, vascular	0/60 -	0/60 -	0/60 -	1/60 (2.0)	0/60 -					
Thymus										
Depletion, lymphoid, generalized	56/57 (3.5)	58/58 (3.4)	54/56 (3.6)	57/57 (3.8)	55/57 (3.5)	56/56 (3.3)	60/60 (3.4)	58/59 (3.4)	56/58 (3.8)	58/59 (3.3)
Hyperplasia, epithelial cell	8/60 (1.0)	5/60 (1.2)	1/60 (2.0)	0/60 -	3/60 (1.0)	37/59 (1.1)	22/60 (1.1)	32/59 (1.2)	32/58 (1.3)	21/59 (1.0)
Thyroid										
Hyperplasia, c-cell, focal	6/60 (1.3)	7/60 (1.1)	3/60 (1.0)	1/60 (2.0)	3/60 (1.0)	7/60 (1.7)	8/60 (1.4)	2/60 (1.5)	1/60 (1.0)	6/60 (1.2)
Tongue										

Erosion/ulcer	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (2.0)	1/60 (2.0)	2/60 (2.0)	0/60 -
Hyperplasia, squamous cell	0/60 -	1/60 (2.0)	1/60 (2.0)	2/60 (2.5)	0/60 -	1/60 (3.0)	2/60 (2.5)	2/60 (2.5)	6/60 (2.5)	1/60 (3.0)
Mineralization, vascular	1/60 (1.0)	0/60 -	4/60 (1.8)	18/60 (1.4)	0/60 -	0/60 -	0/60 -	1/60 (1.0)	7/60 (1.1)	1/60 (2.0)
Uterus w/ cervix										
Dilatation, gland/lumen						17/60 (1.5)	18/60 (1.7)	11/60 (1.7)	9/60 (1.4)	16/60 (1.9)

Green text depicts findings that exhibited an inverse relationship to test item.
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Polyarteritis -- Incidence (Mean Severity)										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Adrenal gland	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Aorta	0/60 -	0/60 -	0/59 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Brain	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -
Coagulating glands	0/60 -	0/60 -	2/60 (1.5)	5/60 (1.0)	0/60 -					
Epididymides	1/60 (2.0)	1/60 (1.0)	13/60 (1.5)	23/60 (1.4)	2/60 (1.5)					
Harderian glands	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Heart	0/60 -	0/60 -	1/60 (2.0)	3/60 (1.0)	1/60 (3.0)	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -
Kidney	0/60 -	0/60 -	1/60 (2.0)	0/60 -	1/60 (3.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Large intestine, cecum	2/60 (2.0)	1/60 (1.0)	2/60 (1.5)	15/60 (1.6)	2/60 (1.0)	0/60 -	1/60 (2.0)	0/60 -	2/60 (1.0)	0/60 -
Large intestine, colon	1/60 (1.0)	0/60 -	4/60 (1.5)	13/60 (1.5)	0/60 -	0/60 -	0/60 -	0/60 -	3/60 (1.0)	0/60 -
Large intestine, rectum	0/60 -	0/60 -	6/60 (1.0)	14/60 (1.1)	1/60 (2.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Larynx	0/59 -	0/60 -	0/59 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Liver	0/60 -	0/60 -	2/60 (1.0)	3/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	1/60 (1.0)
Lymph node, mesenteric	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Mesentery/peritoneum	1/1 (1.0)	0/1 -	11/12 (2.6)	6/6 (3.2)	1/2 (4.0)	0/0 -	0/0 -	1/2 (3.0)	3/4 (2.3)	0/1 -
Pancreas	2/60 (2.5)	0/60 -	12/60 (2.2)	25/60 (2.0)	2/60 (2.5)	0/60 -	0/60 -	0/60 -	10/60 (1.6)	0/60 -
Preputial glands	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -					
Prostate gland	0/60 -	0/60 -	2/60 (1.5)	4/60 (1.0)	0/60 -					
Seminal vesicles	0/60 -	0/60 -	3/60 (1.3)	7/60 (1.0)	0/60 -					
Small intestine, duodenum	0/60 -	0/60 -	0/60 -	5/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (2.0)	0/60 -
Small intestine, ileum	0/60	0/60	2/60	8/60	0/60	0/60	0/60	0/60	2/60	0/60

	-	-	(1.0)	(1.0)	-	-	-	-	(1.5)	-
Small intestine, jejunum	0/60 -	0/60 -	0/60 -	4/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Spleen	0/60 -	0/60 -	1/60 (1.0)	5/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/59 (1.0)	0/60 -
Stomach, glandular	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Stomach, nonglandular	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Testes	3/60 (1.3)	1/60 (1.0)	22/60 (1.9)	25/60 (1.4)	2/60 (1.0)					
Thymus	0/60 -	0/60 -	1/60 (1.0)	1/60 (1.0)	0/60 -	0/59 -	0/60 -	0/59 -	1/58 (1.0)	0/59 -
Tongue	0/60 -	0/60 -	4/60 (1.0)	5/60 (1.2)	0/60 -	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -
Ureters	0/60 -	0/59 -	1/60 (1.0)	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Urinary bladder	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Uterus w/ cervix						0/60 -	0/60 -	1/60 (1.0)	2/60 (1.0)	0/60 -

Green text depicts findings that exhibited an inverse relationship to test item.
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Lymphohistiocytic Infiltrates – Incidence (Mean Severity of Affected Animals)										
Sex	**Male**					**Female**				
Dose Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Brain	0/60 -	0/60 -	18/60 (1.0)	45/60 (1.0)	14/60 (1.0)	0/60 -	0/60 -	18/60 (1.0)	33/60 (1.0)	12/60 (1.0)
Cavity, thoracic	0/1 -	0/1 -	0/1 -	4/6 (2.0)	0/2 -	0/0 -	0/0 -	0/1 -	0/1 -	0/0 -
Coagulating glands	0/60 -	0/60 -	33/60 (1.0)	48/60 (1.0)	31/60 (1.0)					
Epididymides	0/60 -	9/60 (1.0)	53/60 (1.0)	56/60 (1.4)	44/60 (1.0)					
Eyes	0/60 -	0/60 -	21/60 (1.0)	35/60 (1.0)	29/60 (1.0)	0/60 -	0/60 -	2/60 (1.0)	10/60 (1.0)	8/60 (1.0)
Harderian glands	0/60 -	0/60 -	0/60 -	4/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Heart	0/60 -	6/60 (1.0)	54/60 (1.2)	59/60 (1.8)	40/60 (1.0)	0/60 -	5/60 (1.0)	45/60 (1.0)	58/60 (1.3)	29/60 (1.0)
Injection site 1	0/60 -	10/60 (1.0)	13/60 (1.0)	19/60 (1.1)	7/60 (1.0)	0/60 -	8/60 (1.0)	14/60 (1.0)	34/60 (1.1)	7/60 (1.0)
Injection site 2	0/60 -	16/60 (1.0)	10/60 (1.0)	22/60 (1.0)	13/60 (1.0)	0/60 -	10/60 (1.0)	24/60 (1.0)	30/60 (1.0)	6/60 (1.0)
Injection site 3	0/60 -	1/60 (1.0)	4/60 (1.0)	9/60 (1.0)	4/60 (1.0)	0/60 -	2/60 (1.0)	15/60 (1.0)	20/60 (1.0)	8/60 (1.0)
Injection site 4	0/60 -	6/60 (1.0)	9/60 (1.0)	13/60 (1.0)	3/60 (1.0)	0/60 -	9/60 (1.0)	19/60 (1.1)	30/60 (1.0)	4/60 (1.0)
Joint, tibiofemoral	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Kidney	0/60 -	0/60 -	22/60 (1.0)	51/60 (1.1)	0/60 -	0/60 -	0/60 -	4/60 (1.0)	23/60 (1.0)	3/60 (1.0)
Large intestine, rectum	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Larynx	0/59 -	0/60 -	11/60 (1.0)	27/60 (1.0)	25/60 (1.0)	0/60 -	1/60 (1.0)	9/60 (1.0)	10/57 (1.0)	6/60 (1.0)
Lung	0/60 -	0/60 -	6/60 (1.0)	31/60 (1.0)	1/60 (1.0)	0/60 -	0/60 -	0/60 -	4/60 (1.0)	0/60 -

Lymph node, axillary (histiocytosis, sinus)	0/5 -	0/7 -	1/6 (1.0)	2/2 (1.0)	1/3 (1.0)	0/29 -	0/20 -	12/24 (1.0)	13/18 (1.1)	4/19 (1.8)
Lymph node, inguinal (histiocytosis, sinus)	0/6 -	0/5 -	1/6 (1.0)	1/3 (1.0)	0/1 -	0/20 -	0/15 -	3/17 (1.0)	6/14 (1.0)	2/20 (1.0)
Lymph node, mandibular (histiocytosis, sinus)	0/60 -	3/60 (1.0)	44/60 (1.1)	51/59 (1.2)	47/60 (1.0)	0/60 -	1/60 (1.0)	42/60 (1.0)	48/60 (1.1)	35/60 (1.0)
Lymph node, mesenteric (histiocytosis, sinus)	0/60 -	6/60 (1.0)	37/59 (1.2)	53/58 (1.8)	45/60 (1.2)	0/60 -	2/60 (1.0)	42/60 (1.2)	49/60 (1.6)	56/60 (1.4)
Pancreas	1/60 (1.0)	0/60 -	29/60 (1.0)	54/60 (1.3)	31/60 (1.0)	0/60 -	0/60 -	36/59 (1.0)	49/60 (1.0)	19/60 (1.0)
Pharynx	0/60 -	0/59 -	0/60 -	5/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Preputial glands	0/60 -	0/60 -	0/60 -	4/60 (1.0)	0/60 -					
Prostate gland	0/60 -	0/60 -	1/60 (1.0)	19/60 (1.0)	3/60 (1.3)					
Seminal vesicles	0/60 -	0/60 -	35/60 (1.0)	50/60 (1.0)	35/60 (1.0)					
Skin	0/60 -	1/60 (1.0)	5/60 (1.0)	8/60 (1.0)	0/60 -	0/60 -	0/60 -	2/60 (1.0)	9/60 (1.0)	1/60 (1.0)
Small intestine, duodenum	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Small intestine, ileum	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Small intestine, jejunum	0/60 -	0/60 -	0/60 -	2/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Spleen	0/60 -	0/60 -	0/60 -	28/60 (1.6)	0/60 -	0/60 -	0/60 -	0/60 -	2/59 (1.0)	0/60 -
Stomach, glandular	0/60 -	0/60 -	0/60 -	5/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -	0/60 -
Testes	0/60 -	2/60 (1.0)	46/60 (1.0)	57/60 (1.3)	56/60 (1.0)					
Tongue	0/60 -	0/60 -	0/60 -	10/60 (1.0)	0/60 -	0/60 -	0/60 -	0/60 -	1/60 (1.0)	0/60 -
Urinary bladder	0/60 -	0/60 -	2/60 (1.0)	12/60 (1.0)	0/60 -	0/60 -	0/60 -	2/60 (1.0)	12/60 (1.0)	4/60 (1.0)
Uterus w/ cervix						0/60 -	0/60 -	2/60 (1.0)	13/60 (1.0)	0/60 -
Vagina						0/60 -	0/60 -	0/60 -	10/60 (1.0)	1/60 (1.0)

Green text depicts findings that exhibited an inverse relationship to test item.
The column for group 5 is slightly shaded to indicate that it should be analyzed separately from groups 1-4.

Toxicokinetics

Oligonucleotides are rapidly removed from circulation, and therefore plasma oligo levels are not a meaningful means of assessing exposure. Sponsor collected samples from the liver (target organ), kidney and spleen, which were flash frozen. The oligonucleotide concentration in these organs was subsequently determined by a validated method. ISIS 301012 was recoverable in all three tissues assessed, and concentrations exhibited a generally dose-dependent increase, with the highest levels appearing in the kidney, followed by the liver and then the spleen.

Pharmacodynamic Analysis

In order to confirm that the mouse surrogate (SIS 147764) was having the anticipated pharmacological effect during the course of the study, Sponsor collected liver samples (15/sex) from ISIS 147764-treated rats and control rats and analyzed them for apoB mRNA level. A n.s.s. 36% decrease in hepatic apoB mRNA was observed, compared to control.

Dosing Solution Analysis

Stability analyses conducted by ISIS Pharmaceuticals showed that the test article solutions provided for this study remained stable throughout their period of use at the range of concentrations utilized in this study.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: COMBINED FERTILITY AND DEVELOPMENTAL TOXICITY STUDY OF ISIS 301012 AND ISIS 147764 ADMINISTERED BY SUBCUTANEOUS INJECTION IN MICE

Study no.:	301012-AS07 ((b) (4) # ADQ00013)
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 December 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-001, 92.8% ISIS 147764, RP147764-001, ?

Key Study Findings

- Neither mipomersen or the mouse surrogate had any effect on fertility or embryofetal development.

Methods

Doses (ISIS 301012):	0, 3, 10, 25 mg/kg/dose (0, 10.5, 35, 87.5 mg/kg/week)
Doses (ISIS 147764; mouse surrogate):	25 mg/kg/dose (87.5 mg/kg/week)
Frequency of dosing:	every other day
Dose volume:	10 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	Phosphate buffered saline, pH 7.4
Species/Strain:	Crl:CD-1 (ICR) BR mice
Number/Sex/Group:	25
Satellite groups:	none
Study design:	♂s: alternate day dosing initiated 28 days before cohabitation period (maximum of 16 days) and continuing through the day before sacrifice. ♀s: alternate day dosing initiated 15 days before cohabitation, through cohabitation to day 16 of presumed gestation (GD16).
Deviation from study protocol:	None material to the interpretation of the study

Observations and Results

Mortality

No test item-related deaths.

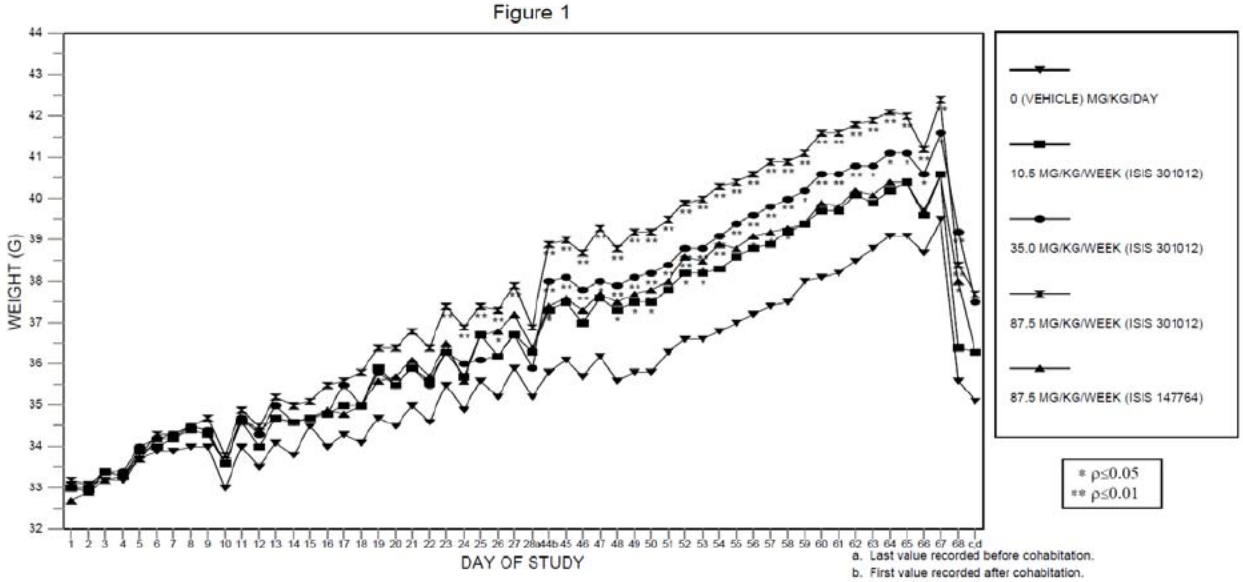
Clinical Signs

No test article-related effects in either sex.

Body Weight

S.s. d.d. ↑ in body weight gain in both sexes, most pronounced in ♂s.

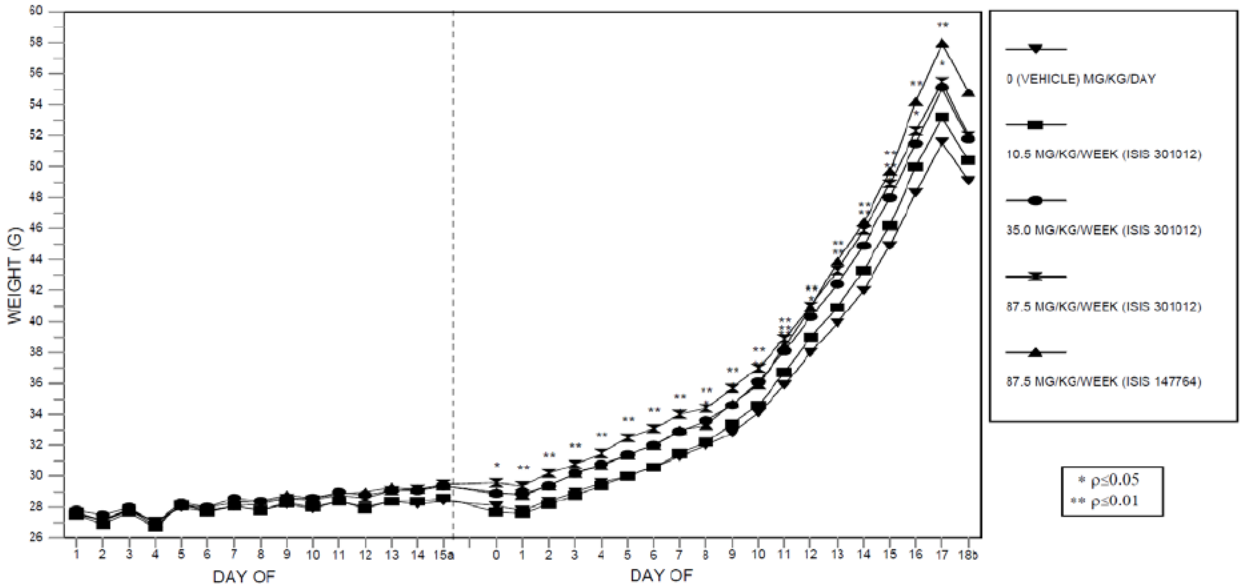
BODY WEIGHTS
MALE MICE
Figure 1



a. Last value recorded before cohabitation.
b. First value recorded after cohabitation.
c. Terminal body weight.
d. Mice were fasted on the day before sacrifice.

BODY WEIGHTS
FEMALE MICE
Figure 2

BEST AVAILABLE COPY



a. Last value recorded before cohabitation.
b. Mice were fasted on the day before sacrifice.

Feed Consumption

Not measured.

Toxicokinetics

Due to rapid distribution of mipomersen out of the circulation and into tissues, the tissue levels of mipomersen in the maternal kidney and liver were used as a proxy of systemic exposure. Distribution to the placenta, fetal kidney and fetal liver was also assessed.

Dose-dependent systemic exposure of the dams is confirmed by dose-proportional increases in mipomersen level in the maternal liver at all doses (see Sponsor's table below). Exposure level is highest in kidney and lowest in placenta. At high dose, relative maternal tissue levels were 416>252>23 μg/g for kidney, liver and placenta respectively. Mipomersen metabolites were seen in all examined maternal tissues, generally representing < 20% of total oligo. Notably, the largest mipomersen metabolite seen was N-6 (14-mer) and the smallest is N-15 (5-mer), consistent with endonuclease cleavage, followed by exonuclease cleavage of the newly exposed non-MOE protected 3'- and 5- termini.

Consistent with the absence of any clear test item-related effects on embryofetal development, intact mipomersen was not detected in fetal liver or fetal kidney. 1 of 5 MD fetal kidneys analyzed did show low levels (3.47 μg/g) of an N-12 metabolite of mipomersen, but no parent oligo or any other metabolites (see Sponsor's table below). However none of the other MD fetal kidneys, nor any of the HD fetal kidneys had any measurable level of oligos. Similarly, 1/3 LD fetal liver samples had low levels (1.70 μg/g) of an N-11 metabolite. Again, no parent compound, nor any other metabolite was seen in this sample, and no positive signals (parent or metabolite) were seen in any of the other LD, MD or HD fetal liver samples. Sponsor suggests that these are artifactual results resulting from interfering peaks arising from the analyte matrix, and this reviewer agrees that this is the most likely explanation.

Table 1. ISIS 301012 and total oligonucleotide concentrations (µg/g) in maternal and fetal samples collected 2 days after the last dose (Day 18 of presumed gestation).

Organ	Group	Dose (mg/kg/week)	Dose (mg/kg/dose)	N	ISIS 301012	Total Oligonucleotide	%Intact
Fetal Kidney	2	10.5	3	5	BLQ	BLQ	NA
	3	35.0	10	5	BLQ	0.694 ± 1.55 [c]	0
	4	87.5	25	5	BLQ	BLQ	NA
Fetal Liver	2	10.5	3	3[a]	BLQ	0.565 ± 0.979 [c]	0
	3	35.0	10	5	BLQ	BLQ	NA
	4	87.5	25	4[b]	BLQ	BLQ	NA
Placenta	2	10.5	3	5	2.75 ± 1.95	2.75 ± 1.95	100 ± 0
	3	35.0	10	5	9.12 ± 1.47	10.6 ± 3.5	89.6 ± 14.4
	4	87.5	25	5	22.9 ± 2.4	26.0 ± 7.7	91.9 ± 14.9
Kidney	2	10.5	3	5	153 ± 47	163 ± 53	94.8 ± 3.3
	3	35.0	10	5	393 ± 187	410 ± 205	97.0 ± 4.3
	4	87.5	25	5	416 ± 127	420 ± 131	99.2 ± 1.2
Liver	2	10.5	3	5	47.2 ± 7.0	86.0 ± 14.7	55.5 ± 9.3
	3	35.0	10	5	112 ± 37	112 ± 37	100 ± 0
	4	87.5	25	5	252 ± 80	269 ± 96	95.2 ± 4.5

Data is represented as average ± standard deviation.

N Number of observations

BLQ Below the limit of quantitation (< 1.52 µg/g). BLQ value was treated as "0" in the calculation of descriptive statistics.

NA Not applicable

[a] Two samples cannot be quantitated interference on electropherogrms.

[b] One sample cannot be quantitated interference on electropherogrms.

[c] Only 1 of the samples had measurable levels, others were BLQ.

Appendix 1. ISIS 301012, Metabolite, and Total Oligonucleotide Concentrations (µg/g) in Individual Tissues Samples Collected 2 Days following Last Dose on Day 18 of Presumed Gestation for Study 301012-AS07.

Organ	Group	Dose mg/kg	Animal No	ISIS 301012	N+1	N-1	N-2	N-3	N-4	N-5	N-6	N-7	N-8	N-9	N-10	N-11	N-12	N-13	N-14	N-15	Total	%Intact
Fetal Kidney	2	3	2906	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	2	3	2912	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	2	3	2919	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	2	3	2920	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	2	3	2922	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	3	10	2928	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	3	10	2929	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	3	10	2931	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	3.47	BLQ	BLQ	BLQ	3.47	0.00
Fetal Kidney	3	10	2937	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	3	10	2943	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	4	25	2952	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	4	25	2958	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	4	25	2960	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	4	25	2962	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Kidney	4	25	2964	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	2	3	2912	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	2	3	2919	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	1.70	BLQ	BLQ	BLQ	BLQ	1.70	0.00
Fetal Liver	2	3	2920	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	3	10	2928	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	3	10	2929	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	3	10	2931	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	3	10	2937	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	3	10	2943	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	4	25	2952	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	4	25	2960	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	4	25	2962	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA
Fetal Liver	4	25	2964	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	NA

Dosing Solution Analysis

Formulation is a solution, so no assessment of homogeneity was required or conducted. Mipomersen was reportedly stable under the conditions employed in this study.

Necropsy

♂: No effect on reproductive organ weight or gross lesions.

♀: No treatment-related gross lesions were observed.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

♂: No significant effect on sperm count or motility, nor any effect on mating or any fertility parameter.

♀: No effect on estrous cyclicity nor any other mating or fertility parameter. No Caesarean-sectioning or litter parameters were affected by ISIS 301012 or ISIS 147764 at any tested dose. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percentage of resorbed conceptuses, and percentage of live male fetuses were comparable among the five dose groups and did not significantly differ. All placentas appeared normal.

Offspring (Malformations, Variations, etc.)

There was no change in the overall rate of malformations with treatment. All malformations observed lacked dose-dependence and/or were within the historical background rate for the testing facility.

TABLE C12 (PAGE 1): FETAL ALTERATIONS – SUMMARY – F1 GENERATION LITTERS/FETUSES

DOSE GROUP		I	II	III	IV	V
TEST ARTICLE		VEHICLE	ISIS 301012	ISIS 301012	ISIS 301012	ISIS 147764
DOSE (MG/KG/WEEK)a		0	10.5	35.0	87.5	87.5
LITTERS EVALUATED	N	19b	18b	24b	23c	19b,c
FETUSES EVALUATED	N	212	207	277	272	237
LIVE	N	212	206	277	271	235
DEAD	N	0	1d	0	1d	2d
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	16(84.2)	13(72.2)	19(79.2)	18(78.3)	17(89.5)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	47(22.2)	32(15.5)	49(17.7)	55(20.3)	43(18.3)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	25.8 ± 24.2	15.3 ± 15.6	17.0 ± 14.6	20.2 ± 17.9	19.1 ± 13.4

a. Once every other day dosing. Weekly dose (mg/kg/week) will be calculated 3.5 times of each dose (mg/kg) from day 1 of study through day 16 of presumed gestation.
b. Excludes litters that the dam had no confirmed mating date.
c. Excludes litters that the dam had no confirmed mating date and delivered.
d. Dead fetuses were excluded from summarization and statistical analyses. Observations for these conceptuses are cited on Table C25.

9.2 Embryonic Fetal Development

Study title: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF ISIS 301012 AND ISIS 233183 IN RABBITS

Study no.:	301012-AS08 ((b) (4) # ADQ00016)
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	20 April 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-002, 93.6 ISIS 233183, ?, ? (rabbit surrogate, RbS)

Key Study Findings

- It is concluded that mipomersen does not cross the placenta as it was not detectable in fetal liver or kidney.
- Mipomersen was not associated with any adverse effects on maintenance of pregnancy nor embryofetal development in the rabbit.
- ISIS 233183 (rabbit surrogate) at 52.5 mg/kg/week was associated with reduced food consumption and weight gain a resulted in spontaneous abortion in 1 doe. A s.s. reduction in fetal body weight and in ossification sites in thoracic vertebrae

was also seen with the rabbit surrogate. This effect of ISIS 233183 does not appear to arise from its pharmacological activity, since there was no effect on LDL-C in this dose group.

Methods

Doses ISIS 301012:	0, 2.5, 5, 15 mg/kg QOD (0, 8.75, 17.5, and 52.5 mg/kg/week)
Doses ISIS 233183:	15 mg/kg QOD (52.5 mg/kg/week)
Frequency of dosing:	every other day
Dose volume:	0.25 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	Phosphate buffered saline, pH 7.4
Species/Strain:	New Zealand White Rabbits (timed-mated)
Number/Sex/Group:	20
Satellite groups (TK):	3 (LD, MD & HD only)
Study design:	Timed-mated does were dosed from presumptive gestation day (GD) 6 through GD 18. Does necropsied on GD 29 and uterine contents examined. The number of corpora lutea, implantation sites, early/late resorptions and live/dead fetuses were recorded. The fetuses were weighed, examined for gross external alterations and individually identified with a tag noting study number, litter number and uterine distribution. Live fetuses were sacrificed and examined internally to identify sex. All fetuses were examined for skeletal alterations after staining with alizarin red S. Portions of maternal liver and kidney were retained from three rabbits per dose group on DG 20 (TK group) and five rabbits per dose group on DG 29 for toxicokinetic evaluation. The placentas, fetal livers and fetal kidneys (bilateral) were collected from all fetuses from three litters per dose group on DG 20 and five fetuses from five litters per dose group on DG 29. Individual fetal tissues were pooled per litter to provide adequate samples for analysis.
Deviation from study protocol:	None.

Observations and Results

Mortality & Abortions

No treatment-related mortality.

No mipomersen-treated animals aborted. On doe treated with the rabbit surrogate aborted on GD 27 and was sacrificed. This doe lost 220 g of bodyweight from GD 6-26, had ungroomed coat (GD 15-18), scant feces (GD 22-26) and consumed little feed (2 to 74 g) from GD 16-26.

Clinical Signs

No effect of mipomersen treatment on clinical signs; however the rabbit surrogate was associated with a s.s. ↑ in the number of animals affected and frequency of the finding of scant feces, and was also associated with a n.s.s. ↑ in number of animals and frequency of ungroomed coat.

TABLE 1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY

DOSE GROUP	I	II	III	IV	V
DOSE (MG/KG/WEEK)a	0	8.75	17.5	52.5	52.5
ISIS TEST ARTICLE	PBS b	301012 c	301012 c	301012 c	233183 d
MAXIMUM POSSIBLE INCIDENCE	465/ 20	480/ 20e	480/ 20e	480/ 20e	478/ 20
MORIBUND SACRIFICED	1f	0	0	0	0
ABORTED AND SACRIFICED	0	0	0	0	1g
SCANT FECES	2/ 2f	3/ 1	5/ 2	6/ 3	33/ 9**g
UNGROOMED COAT	3/ 2	6/ 4	8/ 4	10/ 2	30/ 7g

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RABBITS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RABBITS)/NUMBER OF RABBITS EXAMINED PER GROUP ON DAYS 6 THROUGH 29 OF PRESUMED GESTATION.
N/N - TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RABBITS WITH OBSERVATION.
a. Dose administration occurred every other day beginning on day 6 of presumed gestation and continuing through day 18 of presumed gestation.
b. Phosphate buffered saline.
c. Human apoB-100 antisense inhibitor.
d. Rabbit-specific apoB-100 antisense inhibitor.
e. Excludes values for does that were assigned to toxicokinetic evaluation.
f. Doe 873 was moribund sacrificed on day 14 of gestation.
g. Doe 952 aborted and was sacrificed on day 27 of gestation.
** Significantly different from the control group value (p≤0.01).

Body Weight

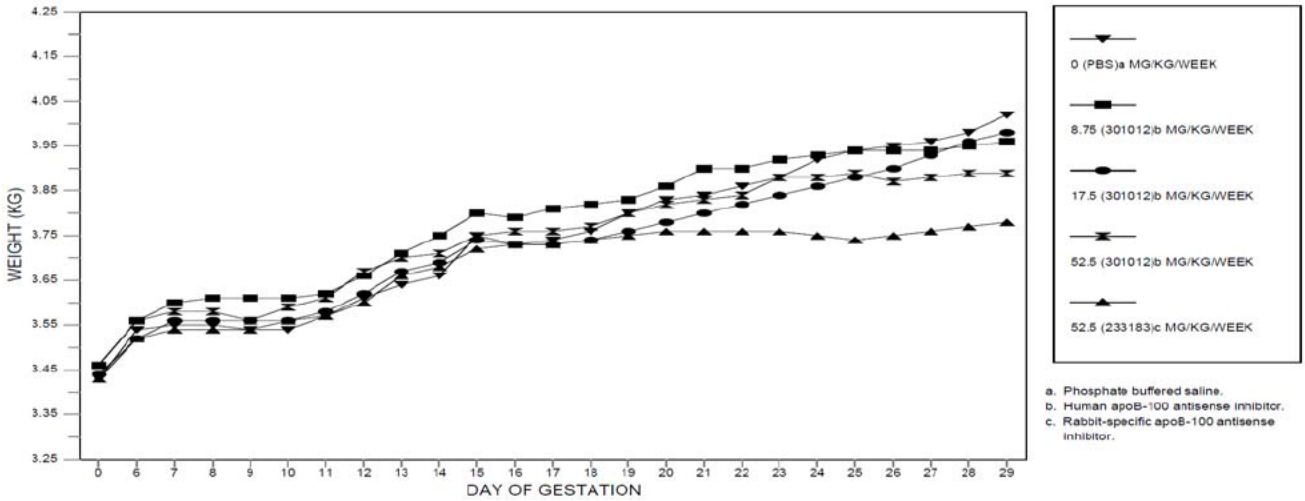
No s.s. effect on bodyweight was seen with mipomersen treatment, although there was a slight trend towards lower bodyweight beginning around GD26.

The doe treated with the rabbit surrogate experienced a s.s. reduction in weight gain beginning around GD 19, experiencing a 36% reduction in weight gain, compared with C between GD 6-29.

The effects on bodyweight gain are correlated with reduced feed consumption.

MATERNAL BODY WEIGHTS

Figure 1



Feed Consumption

FC was s.s. ↓ at HD in the period between GD24 to GD29, and in the rabbit surrogate-treated does in all time periods assessed after GD 15.

TABLE 5 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/WEEK)a		0	8.75	17.5	52.5	52.5
ISIS TEST ARTICLE		PBS b	301012 c	301012 c	301012 c	233183 d
RABBITS TESTED	N	20	23	23	23	20
PREGNANT	N	19	23	23	22	20
INCLUDED IN ANALYSES	N	19	20e	20e	19e	20
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 6 - 9	MEAN±S.D.	169.2 ± 22.4	176.1 ± 8.2	174.9 ± 13.2	175.8 ± 12.2	169.5 ± 23.3
DAYS 9 - 12	MEAN±S.D.	168.9 ± 24.0	172.4 ± 14.1	170.4 ± 18.2 [19]f	171.8 ± 18.0	161.1 ± 36.3
DAYS 12 - 15	MEAN±S.D.	166.4 ± 23.7 [18]g	171.3 ± 12.7	163.9 ± 24.3	156.5 ± 39.8	158.3 ± 26.4
DAYS 15 - 19	MEAN±S.D.	177.1 ± 8.6 [17]f,g	176.1 ± 9.4	175.6 ± 10.7 [19]f	171.2 ± 18.3	153.5 ± 31.2*
DAYS 6 - 19	MEAN±S.D.	171.4 ± 17.6 [17]f,g	174.2 ± 8.5	171.4 ± 14.7 [19]g	169.0 ± 19.5	160.1 ± 21.8
DAYS 19 - 24	MEAN±S.D.	160.8 ± 29.8 [18]g	158.4 ± 23.2 [19]f	152.3 ± 29.3	149.3 ± 30.3	95.9 ± 44.8** [19]g
DAYS 24 - 29	MEAN±S.D.	128.1 ± 38.9 [17]f,g	101.9 ± 49.9	119.8 ± 30.2	91.0 ± 54.3*	76.3 ± 48.6** [19]g
DAYS 19 - 29	MEAN±S.D.	144.1 ± 25.4 [17]f,g	130.4 ± 31.4	136.2 ± 26.6	120.9 ± 35.4*	89.1 ± 31.6** [19]g
DAYS 6 - 29	MEAN±S.D.	159.0 ± 19.2 [17]f,g	155.3 ± 16.3	156.2 ± 17.3	148.3 ± 23.6	129.3 ± 17.8** [19]g

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred every other day beginning on day 6 of gestation and continuing through day 18 of gestation.
b. Phosphate buffered saline.
c. Human apoB-100 antisense inhibitor.
d. Rabbit-specific apoB-100 antisense inhibitor.
e. Excludes values for does that were assigned to toxicokinetic evaluation.
f. Excludes values that were associated with spillage or wet/soiled feed.
g. Excludes values for does that aborted or were moribund sacrificed.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

Pharmacodynamic Marker

LDL/VLDL was measured, and showed no decrease with mipomersen or the rabbit surrogate (ISIS 233183).

C = 3.9 LD = 3.9 MD= 3.0 HD = 4.8 RbS = 5.7

Toxicokinetics

Due to rapid distribution of mipomersen out of the circulation and into tissues, the tissue levels of mipomersen in the maternal kidney and liver were used as a proxy of systemic exposure. Distribution to the placenta, fetal kidney and fetal liver was also assessed.

Dose-dependent systemic exposure is confirmed by dose-related (although hypoproportional) increases in mipomersen level in the maternal liver and kidney at all doses (see Sponsor's table below). Exposure level is highest in kidney and lowest in

placenta. At high dose on GD20, relative maternal tissue levels were 2336>194>15 μg/g for kidney, liver and placenta respectively. Mipomersen metabolites were seen in all examined maternal tissues, generally representing as much as 46% of total oligo in the liver and < 20% in the kidney. Notably, the largest mipomersen metabolite seen was N-6 (14-mer) and the smallest is N-15 (5-mer), consistent with endonuclease cleavage, followed by exonuclease cleavage of the newly exposed non-MOE protected 3'- and 5- termini.

Consistent with the absence of any clear test item-related effects on embryofetal development, intact mipomersen was not detected in fetal liver or fetal kidney. 1 of 5 GD29 MD fetal kidneys analyzed did show low levels (1.53 μg/g) of an N-15 metabolite of mipomersen, but no parent oligo or any other metabolites. No other MD fetal kidneys (including those from GD20), nor any of the HD fetal kidneys had any measurable level of oligos. Sponsor suggests that these are artifactual results resulting from interfering peaks arising from the analyte matrix, and this reviewer agrees that this is the most likely explanation.

Table 1. ISIS 301012 and total oligonucleotide concentrations (µg/g) in maternal and fetal samples for Study 301012-AS08

Organ	Group	Dose (mg/kg/week)	Dose (mg/kg)	N	ISIS 301012	Total Oligonucleotide	%Intact
Day 20 of presumed gestation (2 days after the last dose):							
Fetal Kidney	2	8.75	2.5	3	BLQ	BLQ	NA
	3	17.5	5	3	BLQ	BLQ	NA
	4	52.5	15	3	BLQ	BLQ	NA
Fetal Liver	2	8.75	2.5	3	BLQ	BLQ	NA
	3	17.5	5	3	BLQ	BLQ	NA
	4	52.5	15	3	BLQ	BLQ	NA
Placenta	2	8.75	2.5	3	0.00	2.97 ± 5.14	0.00
	3	17.5	5	3	4.28 ± 2.32	4.81 ± 2.49	91.0 ± 15.6
	4	52.5	15	3	15.1 ± 4.5	15.1 ± 4.5	100 ± 0
Kidney	2	8.75	2.5	3	767 ± 190	912 ± 284	86.0 ± 15.2
	3	17.5	5	3	1239 ± 496	1547 ± 636	80.2 ± 2.6
	4	52.5	15	3	2336 ± 607	2596 ± 549	89.3 ± 6.6
Liver	2	8.75	2.5	3	28.5 ± 13.2	32.2 ± 15.6	90.9 ± 11.9
	3	17.5	5	3	99.2 ± 8.0	178 ± 27	56.2 ± 4.8
	4	52.5	15	3	194 ± 19	254 ± 43	78.7 ± 20.8
Day 29 of presumed gestation (11 days after the last dose):							
Fetal Kidney	2	8.75	2.5	5	BLQ	BLQ	NA
	3	17.5	5	5	BLQ	0.306 ± 0.685 [b]	0.00
	4	52.5	15	5	BLQ	BLQ	NA
Fetal Liver	2	8.75	2.5	5	BLQ	BLQ	NA
	3	17.5	5	5	BLQ	BLQ	NA
	4	52.5	15	4 [a]	BLQ	BLQ	NA
Placenta	2	8.75	2.5	5	0.43 ± 0.96 [b]	0.43 ± 0.96 [b]	100
	3	17.5	5	5	0.52 ± 1.16 [b]	0.52 ± 1.16 [b]	100
	4	52.5	15	5	6.93 ± 2.60	6.93 ±2.60	100 ± 0
Kidney	2	8.75	2.5	5	447 ± 189	539 ±148	80.1 ± 23.7
	3	17.5	5	5	756 ± 233	930 ±247	80.3 ± 5.1
	4	52.5	15	5	2152 ± 512	2596 ±800	84.2 ± 8.6
Liver	2	8.75	2.5	5	22.4 ± 8.6	34.7 ±11.1	63.4 ± 12.2
	3	17.5	5	5	62.2 ± 15.1	105 ±42	62.7 ± 13.3
	4	52.5	15	5	178 ± 44	336 ±94	54.0 ± 7.4

Data is represented as average ± standard deviation.

N Number of observations

BLQ Below the limit of quantitation (< 1.52 µg/g). BLQ value was treated as "0" in the calculation of descriptive statistics.

NA Not applicable

[a] One sample cannot be quantitated due to contamination at sample preparation.

[b] Only 1 of the samples had measurable levels, others were BLQ.

Dosing Solution Analysis

Formulations of mipomersen and the rabbit surrogate are true solutions, so no homogeneity assessment was required or conducted. Sponsor's table below shows that these oligonucleotides were stable over the course of the study.

ISIS 301012 Analysis

Lot Number	Labeled Concentration (mg/mL)	Assay Value (% Label claim)*		% Intact**
		Beginning-of-Study	End-of-Study	
RP301012-016	10	[redacted]		(b) (4)
RP301012-020	20			
RP301012-021	60			
RP301012-022	120			
RP301012-024	10			

ISIS 233183 Analysis

Lot Number	Labeled Concentration (mg/mL)	Assay Value (% Label claim)*		% Intact**
		Beginning-of-Study	End-of-Study	
RP233183-002	60	[redacted]		(b) (4)
RP233183-003	120			

*Laboratory Notebook Reference: Q0857 pg 44, 126, Q1110 pg 24; Q1060, pg 76 and SDB Q1060-3

**% intact at the end of study in comparison with assay value at the beginning of the study (equals End-of-Study value divided by Beginning-of-Study Value x 100).

Necropsy

No effect of mipomersen. The rabbit surrogate (RbS) was associated with a s.s. ↑ in the finding of bilateral pale kidneys in 8/20 does. The RbS does also had a s.s. ↑ in liver weight.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

ISIS 233183 (rabbit surrogate) caused a s.s. ~14% decrease in mean fetal body weight. No other C-section or litter parameters were affected by treatment with mipomersen or the rabbit surrogate.

Offspring (Malformations, Variations, etc.)

There were no treatment related malformations or variations with mipomersen or the surrogate.

The rabbit surrogate was associated with a s.s. ↓ in the # of ossification sites per fetus per litter in the thoracic vertebrae --12.39 vs. 12.69 (outside the HC range of 12.46-12.75) -- but not in any other skeletal site.

9.3 Prenatal and Postnatal Development

Study title: Study of Pre- and Postnatal Development in the Rat

Study no.:	GT-348-TX-6 (b) (4) # 8200712)
Study report location:	4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 May 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301013, CA301012-12, 88.3% ISIS 147768, RA147786-001C, ?

Key Study Findings

- Dams have d.d. ↓ in reticulocytes, ALT & AST (s.s. in MD, HD & RS), d.d. ↑ in creatinine (s.s. at HD & RS), d.d. ↑ in liver (s.s. at HD) and spleen (s.s. in MD, HD & RS) weights.
- Dams have a dose-dependent ↓ in food consumption during the lactation period (s.s. at HD)
- ISIS 301012 is present in rat milk
- Slight reduction in % surviving to weaning at HD
- Dose related ↓ in pup bodyweights (♂ & ♀)during lactation period
- Impaired visual placing response and grip strength in MD, HD & RS groups
- ♂s have d.d. reduced food consumption and bodyweight gain post weaning
- Delay in sexual maturation in RS♂s and in HD♀s.

Methods

Doses (ISIS 301012):	0, 2, 10, 20 mg/kg/dose (0, 7, 35 & 70 mg/kg/week)
Doses (ISIS 147768):	10 mg/kg/dose (35 mg/kg/week), rat surrogate (RS)
Frequency of dosing:	Twice per week
Dose volume:	5 mL/kg
Route of administration:	SC Injection (rotated between 4 sites)
Formulation/Vehicle:	Phosphate buffered saline, pH 7.4
Species/Strain:	Mated ♀ Crl:CD(SD) rats
Number/Sex/Group:	F_0: 25 ♀/group F_1: 20/sex/group
Satellite groups:	None
Study design:	Test article was administered from gestation day 6 to post partum Day (PPD) 21, inclusive. Dams were allowed to litter and rear offspring to weaning. On PP Day 4 litters were culled to a maximum of eight pups. In addition to standard assessments of growth, survival and physical development, offspring were subjected to the following assessments: pinna unfolding, incisor eruption, eye opening, surface righting reflex (PPD 1), air righting reflex (PPD 17), grip strength (PPD 21), pupillary reflex (PPD 21), auditory response (PPD 21), visual placing response (PPD 21), learning ability (water maze) PPW 5 & 6, and motor activity (PPW 4). 20/sex selected to form F1 mating generation. ~12 weeks post weaning these animals were paired for mating. Mated F_1 ♀s were sacrificed on gestation Day 13 and the uterine contents examined.
Deviation from study protocol:	None material to the interpretation of the study

Observations and Results (Optional Table)

F_0 Dams

Survival:	No unscheduled deaths
Clinical signs:	↑ incidence (animals & # of days) of stained fur dorsally and on the head
Body weight:	No effect of test item during gestation or lactation
Feed consumption:	No effect of test item during gestation. During lactation there is a slight, dose-related in food consumption (see Sponsor's figure, below).
Hematology:	D.d. (s.s. at ≥ MD) ↓ in reticulocytes (0.93x to .79x C)
Clinical Chemistry:	D.d. (s.s. at ≥ MD) ↓ in ALT & AST (0.92x to 0.82x C) D.d. (s.s. at HD & RS) ↑ in CRE (↑10% at HD)
Uterine content:	1 RS♀ had total embryo/fetal loss Total litter loss in 1, 0, 1, 2, 1 (C, LD, MD, HD, RS) litters
Necropsy observation:	Kidney pale: 0, 0, 1, 1, 4 (C, LD, MD, HD, RS) Liver large: 0, 1, 1, 5, 1 Liver mottled: 0, 0, 0, 0, 2 Urinary bladder distended: 0, 0, 1, 1, 4 D.d. ↑ in Liver weight s.s. at HD D.d. s.s. ↑ spleen weight
Toxicokinetics:	D.d. hypoproportional ↑ in ISIS 301012 in maternal kidney, liver and spleen (see Sponsor's table below).
Dosing Solution Analysis	Ranged between 98 to 104% of nominal
Other:	Milk was collected from lactating rats 24 h after that final dose and the level of ISIS 301012 measured (study GT-348-TX-11). ISIS 301012 was transferred to rat milk in a dose proportional manner (see Sponsor's figure below)

Figure 2
Group mean food intake – P generation

Test article	Control		ISIS 301012		ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/occasion)	0	2	10	20	10
Level (mg/kg/week)	0	7	35	70	35

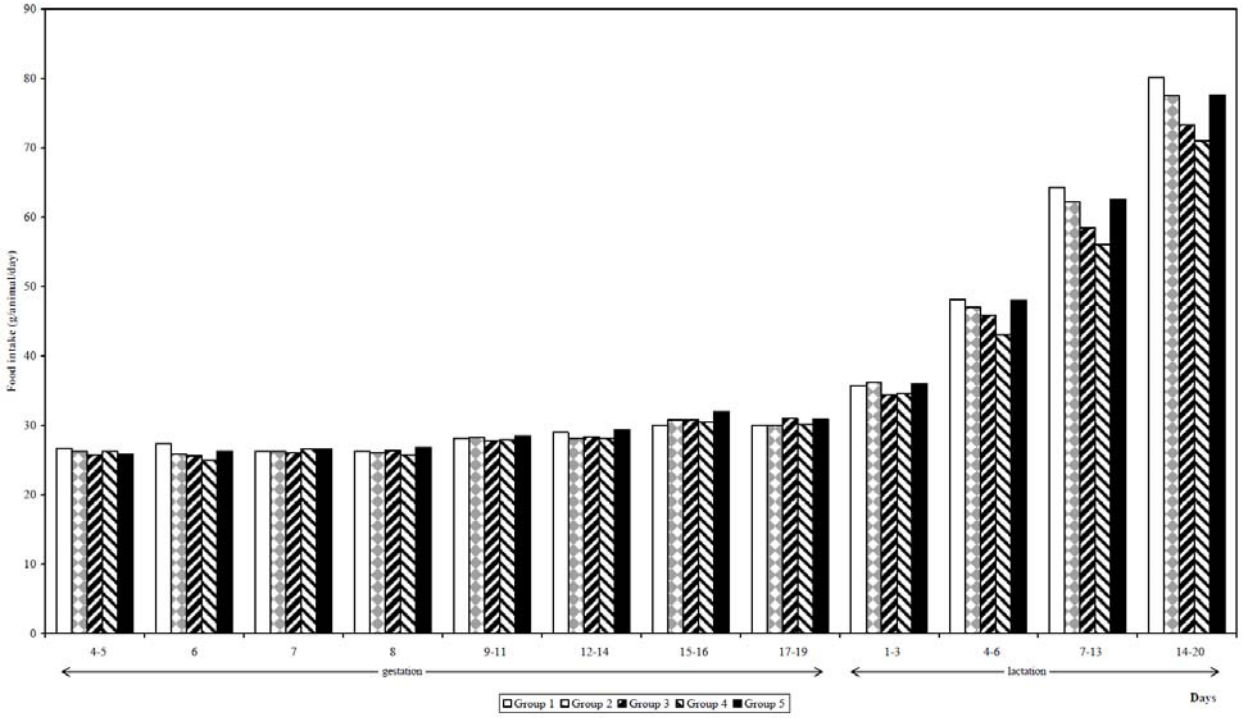


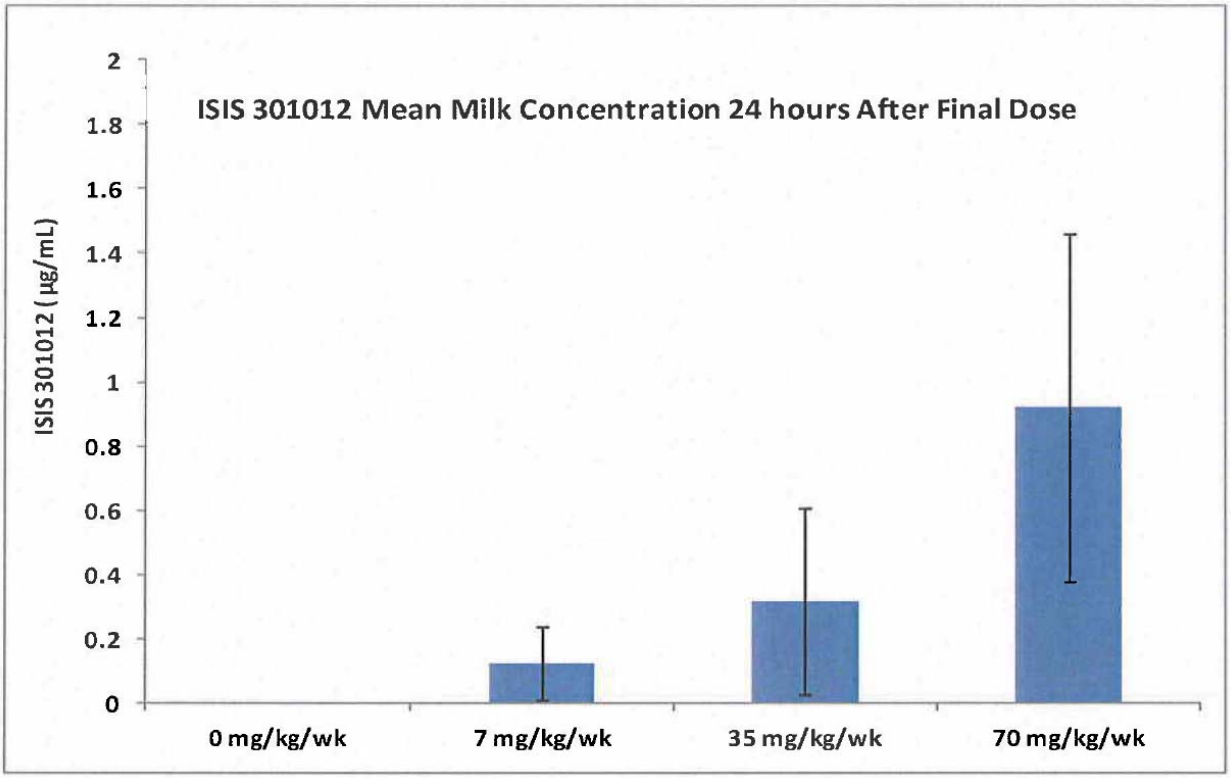
Table 1. ISIS 301012 and total oligonucleotide concentrations (µg/g) in maternal samples collected 24 hours after the last dose (Day 21 post-partum)[a]

Organ	Group	Total Weekly SC Dose (mg/kg/week)	SC Dose (mg/kg/dose)	N	ISIS 301012 (µg/g)	Total Oligonucleotide (µg/g)	%Intact
Kidney	2	7	2	10	929 ± 109	1007 ± 109	92.3 ± 4.7
	3	35	10	10	2153 ± 384	2250 ± 497	96.6 ± 6.3
	4	70	20	10	2012 ± 246	2132 ± 422	95.6 ± 8.4
Liver	2	7	2	10	109 ± 20	121 ± 26	91.6 ± 9.5
	3	35	10	10	511 ± 67	511 ± 67	100 ± 0
	4	70	20	10	756 ± 183	821 ± 282	94.9 ± 10.7
Spleen	2	7	2	10	44 ± 11	46 ± 15	97.1 ± 6.2
	3	35	10	10	215 ± 33	227 ± 35	94.8 ± 4.7
	4	70	20	10	318 ± 39	327 ± 46	97.3 ± 3.9

[a] Dose was scheduled to be administered on alternate days from Day 6 of gestation to Day 21 post *partum* (or Day 26 after mating for females that do not litter) via subcutaneous injection. Some animals gave birth on Day 23 of gestation; therefore, at time of necropsy on Day 22 *post partum*, they had had their last dose on Day 20 *post partum*, so 48 hr had passed since last dose

N Number of observations; SC Subcutaneous

Data are represented as average ± standard deviation



F_1 Generation

Survival:	HD had a n.s.s. ↓ in viability index between PPD 4-7 (98.6 vs. 100) and between PPD 7-14 (99.2 vs. 100). After weaning there were no unscheduled deaths.
Clinical signs:	No effect of test item.
Body weight:	Comparable at birth and start of lactation, but by LD7 there were d.d. s.s. ↓ in mean pup weight in MD, HD & RS, such that by LD21 HD pups were ~10% lighter than C pups. After weaning ♂s continued to show reduced bodyweight gain (0.98x, 0.94x, 0.91x, 0.93x of C at LD, MD, HD, RS, respectively). ♀ weight gain was comparable to C after weaning through mating and gestation.
Feed consumption:	Males had reduced food consumption, generally consistent with reduced body weight gain. No effect on FC in ♀.
Physical development:	No effect of test item on Pinna unfolding, incisor eruption, eye opening, S.s. delay in balano-preputial separation in RS♂ (PPD50 vs. 47 in C [see Sponsor's table below]). Delay in vaginal opening in treated ♀s (s.s. at HD; PPD37 vs. 35) [see Sponsor's table below].
Neurological assessment:	No effect of test item on surface righting, auditory response, motor activity or learning and memory. S.s. impairment in visual placing response and grip strength at MD, HD & RS (see Sponsor's table below).
Reproduction:	No effect on time to mating. ↓ % of pregnant ♀s (85% at HD vs. 95% in C). No effect on intrauterine parameters.
Other:	

Table 15
Summary of physical development data - F_1 generation

Test article	Control		ISIS 301012		ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/occasion)	0	2	10	20	10
Level (mg/kg/week)	0	7	35	70	35

15.1 Balano-preputial separation

Group and sex	Number of animals with complete development on Day *post partum*														Mean day *post partum* for complete development	Mean body weight (g) on day of complete development
	40	41	42	43	44	45	46	47	48	49	50	51	52	53		
1M				1		4	2	2	4	3		3			47	251.9
2M				1		2	2	4	4	2	1	3	1		48	266.8
3M						3	2	2	4	3	3	3			48	253.6
4M						3	4	1	4	2	4	2			48	242.5
5M						3	1		2	1	3	4	4	2	50**	270.8*
Statistics															J,W	J,W

J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon (Group 1 v Groups 2, 3, 4)
W = Wilcoxon rank sum test (Group 1 v Group 5)

* P<0.05
** P<0.01
*** P<0.001

Table 15
Summary of physical development data - F_1 generation

Test article	Control		ISIS 301012		ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/occasion)	0	2	10	20	10
Level (mg/kg/week)	0	7	35	70	35

15.2 Vaginal opening

Group and sex	Number of animals with complete development on Day *post partum*														Mean day *post partum* for complete development	Mean weight (g) on day of complete development
	30	31	32	33	34	35	36	37	38	39	40	41	42	43		
1F				1	8	3	3	3	1				1		35	122.8
2F					1	5	8	3	1		1		1		36*	133.0**
3F			1		2	5	5	2	2	3					36	126.2
4F					2	2	3	5	2	4	1			1	37**	130.5
5F				1	2	6	4	3	2	1	1				36	126.7
Statistics															J,W	J,W

J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon (Group 1 v Groups 2, 3, 4)
W = Wilcoxon rank sum test (Group 1 v Group 5)

* P<0.05
** P<0.01
*** P<0.001

7.5 Functional tests – mean scores – females with live pups at Day 21 *post partum*

Day *post partum*			Group 1	Group 2	Group 3	Group 4	Group 5	Statistics
1	Surface righting:	male	1.2	1.2	1.1	1.0	1.1	W–
		female	0.7	0.8	0.8	0.9	0.9	W–
		combined	1.0	1.0	1.0	0.9	1.0	W–
17	Air righting:	male	2.0	1.8	1.9	1.6*	1.9	F–,f–
		female	1.8	1.7	1.8	1.7	1.8	F–,f–
		combined	1.9	1.8	1.9	1.7	1.9	F–,f–
21	Auditory response:	male	2.0	2.0	2.0	2.0	1.9	X
		female	2.0	2.0	2.0	2.0	1.9	X
		combined	2.0	2.0	2.0	2.0	1.9	X
21	Pupillary reflex:	male	0.3	0.2	0.0*	0.2	0.3	W–
		female	0.3	0.2	0.2	0.3	0.6	W–
		combined	0.3	0.2	0.1	0.2	0.5	W–
21	Visual placing:	male	1.6	1.4*	1.5	1.2**	1.5	W–
		female	1.6	1.3	1.4	1.1***	1.3	W–
		combined	1.7	1.4	1.5*	1.1***	1.4*	W–
21	Grip strength:	male	1.5	1.4	1.4	1.3	1.3*	W–
		female	1.6	1.5	1.4*	1.3*	1.2***	W–
		combined	1.5	1.5	1.4	1.3*	1.2**	W–

F– = Cochran-Armitage and Fisher's Exact (lower tail, Group 1 v Groups 2, 3, 4)
f– = Fisher's Exact (lower tail, Group 1 v Group 5)
W– = Wilcoxon rank sum test (lower tail, Group 1 v Groups 2, 3, 4, 5)
X = not analysed

* P<0.05
** P<0.01
*** P<0.001

F_2 Generation

Survival:	Not Examined
Body weight:	
External evaluation:	
Male/Female ratio:	
Other:	

10 Special Toxicology Studies

Study title: ISIS 301012: 10 Week Toxicity Study in the Juvenile Rat with a 4 Week Recovery Period

Study no.:	GT-349-TX-5 (b) (4) # 8200717)
Study report location:	4.2.3.5.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	29 May 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA301012-012, 88.3% ISIS 147768, RP147768-002, ?

Key Study Findings

- Mipomersen caused ↓ FC (all doses) and BW gain (≥ 10 mkw) both sexes, as did 10 mkw rat surrogate. The resultant differences in bodyweight (and food consumption) persisted throughout the treatment-free period.

- Despite effects on FC & BW gain there was no effect on long bone growth, and no biologically meaningful effects on sexual maturation. There was no effect on performance in the functional observational battery.
- Hematological perturbations, primarily at 50 mkw. Minimal ↓ in measures of RBC mass and reticulocyte count (Hct was normal following recovery period). Minimal to slight ↓ in lymphocytes at all doses (recoverable). Minimal ↑ in neutrophils at 50 mkw (recoverable). At recovery sacrifice ↑ mean platelet volume and platelet distribution width (but no change in platelet count), suggesting increased platelet turnover.
- ↑ weight (% of BW) of spleen, liver and kidney at doses ≥ 10 mkw. ↓ weight (% of BW) of heart at 50 mkw. Organ weight effects demonstrated only partial reversibility over the recovery period.
- Pale liver ≥ 10 mkw; large spleen, pale kidney and small thymus at 50 mkw. No clear recovery.
- Depletion of marrow fat at 50 mkw (not reversible over the recovery period) is likely a consequence of reduced food consumption.
- Foamy macrophages (minimal-moderate) in multiple tissues (liver, spleen, lymph nodes, adrenals, ovary) (partially reversible over the recovery period).
- Lymphoid hyperplasia in the spleen (minimal-slight) (not reversible over the recovery period).
- Thymic atrophy (minimal-slight) at 50 mkw in both sexes (reversible).
- Injection site reactions (cellulitis [minimal] and myositis/myopathy [minimal-slight]) at all doses (partially reversible).

Methods

Doses ISIS 301012:	0, 3, 10 & 50 mg/kg
Doses ISIS 147768:	10 mg/kg (rat surrogate, RS)
Frequency of dosing:	Weekly
Route of administration:	SC injection
Dose volume:	5mL/kg
Formulation/Vehicle:	phosphate buffered saline, pH 7.4
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10
Age:	22 days old at initiation of dosing
Satellite groups:	10 recovery (C, HD & RS only) 27 TK (LD, MD & HD only)
Unique study design:	None of note
Deviation from study protocol:	None affecting study interpretation.

Observations and Results

Mortality

No unscheduled deaths.

Clinical Signs

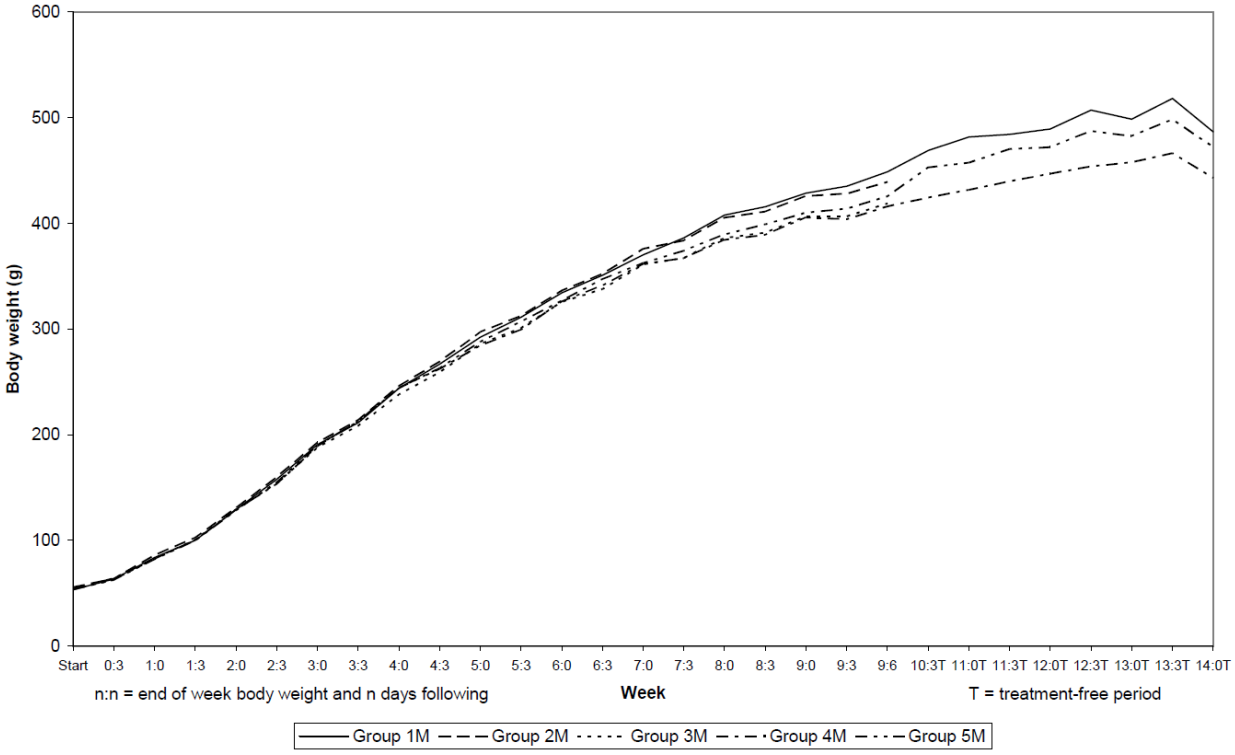
HD ♂&♀: ↑ of sores/lesion, staining of fur, thinning fur. All signs showed improvement during the recovery period.

Body Weights

Beginning in Week 5 body weight gain was reduced in MD, HD & RS groups for both sexes, which correlated with reduced food consumption. After cessation of dosing body weight gain was somewhat more comparable between C & treated animals; however, there was no 'catch-up' bodyweight gain.

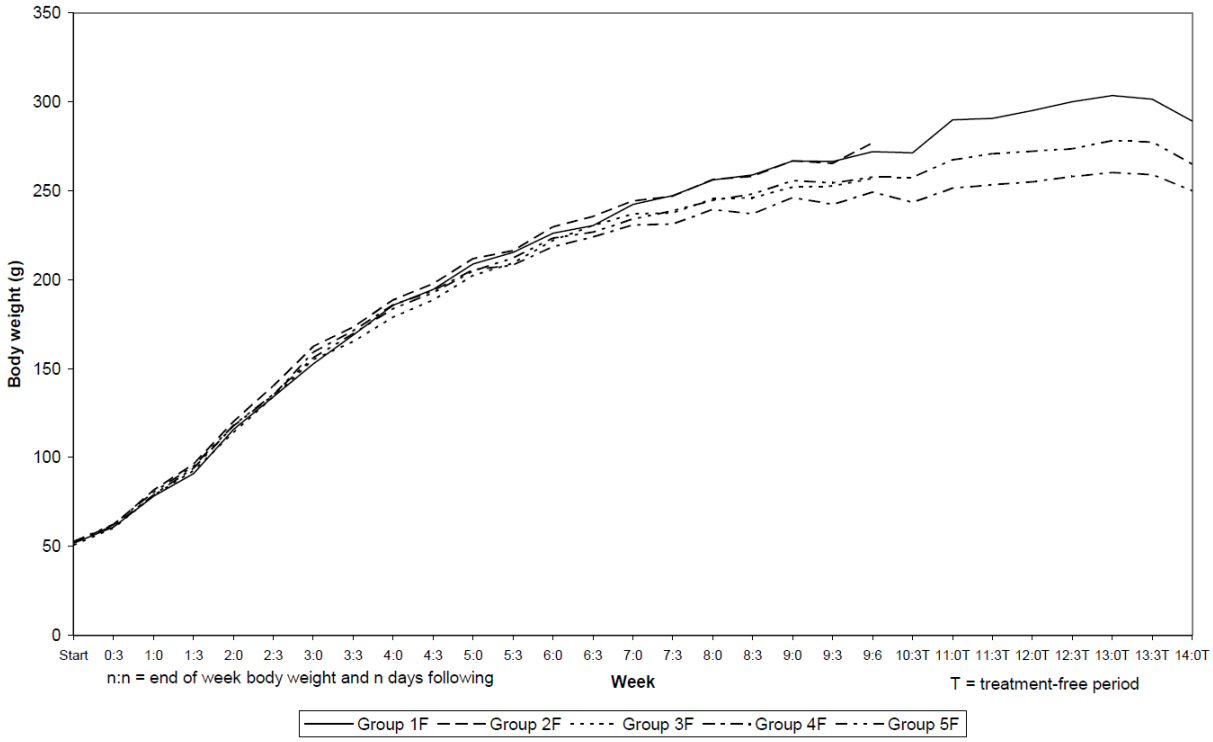
Group mean body weight change
1.1 Males

Test article	Control	ISIS 301012			ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/week)	0	3	10	50	10



Group mean body weight change
1.2 Females

Test article	Control	ISIS 301012			ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/week)	0	3	10	50	10

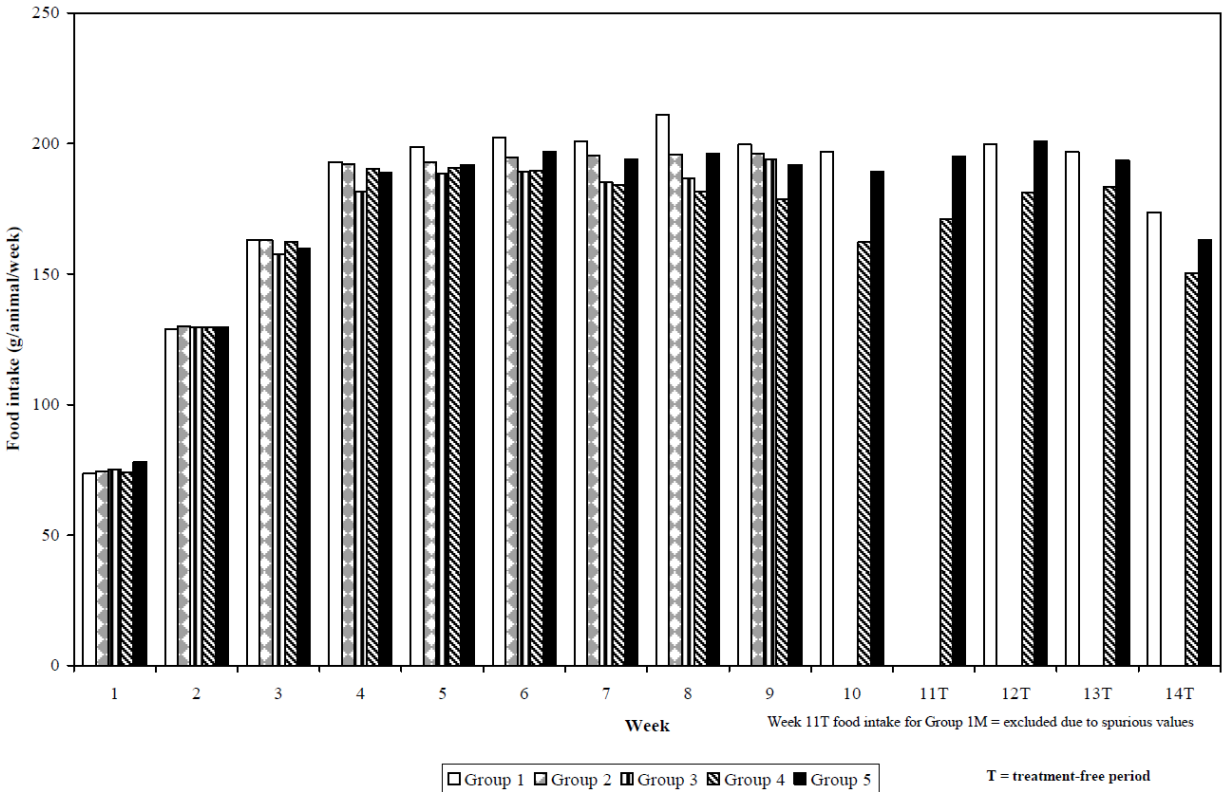


Feed Consumption

Mipomersen-treated and surrogate-treated rats consumed less food during the treatment period than controls. This deficit continued into the treatment-free period; however this may reflect the lower body weight (and therefore lower caloric requirement) of the previously mipomersen/surrogate-treated rats. Sponsor did not conduct an analysis of food consumption per unit mass of body weight.

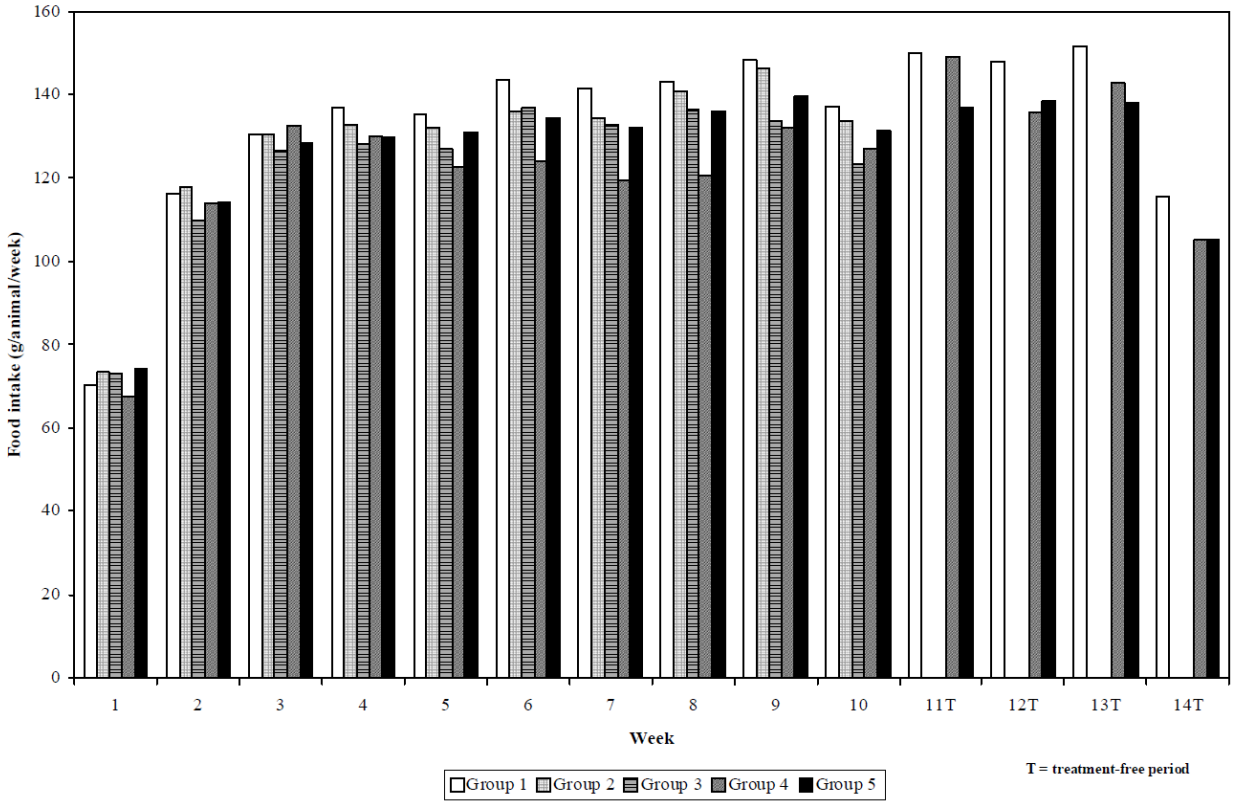
Group mean food intake
2.1 Males

Test article	Control	ISIS 301012		ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/week)	0	3	10	50	10



Group mean food intake
2.2 Females

Test article	Control	ISIS 301012		ISIS 147768
Group	1	2	3	4	5
Level (mg/kg/week)	0	3	10	50	10



Physical Development

Despite the effect on FC and BW gain, there was no discernable effect on long bone growth of either mipomersen or the rat surrogate.

No effect on ♂ sexual maturation.

S.s. effect of rat surrogate to accelerate sexual maturation in ♀s (PND 38.9 vs. 39.6). Also MD achieved sexual maturation at a s.s. lighter weight (125.1 vs. 136.9 g).

Functional Observational Battery

No effect of either mipomersen or the rat surrogate.

Hematology

Dose-related slight ↓ in reticulocyte number was seen with both oligos. Multiple RBC parameters were likewise slightly decreased. These changes were frequently s.s. at HD. Hematocrit, but not hemoglobin or reticulocyte count, were comparable to control following the recovery period.

WBCs were down in both sexes, and appeared to reflect a dose-related ↓ in lymphocytes. ♂s also had an ↑ in neutrophils at HD. S.s. at HD and/or RS. These effects were reversible.

Although no effect on platelet count was apparent, there was a slight s.s. ↑ in platelet volume and ↑ in platelet distribution width following the recovery period (but not at the end of dosing), suggesting that there was increased turnover of platelets with a greater proportion of larger, recently generated platelets.

Hematology Group Means											
Sex		**Male**					**Female**				
ISIS 301012 m/k/w		**0**	**3**	**10**	**50**	**-**	**0**	**3**	**10**	**50**	**-**
ISIS 147768 m/k/w		**-**	**-**	**-**	**-**	**10**	**-**	**-**	**-**	**-**	**10**
Hemoglobin(g/dL)	Term	15.5	15.6	15.5	**14.6*** **	15.6	14.8	14.8	14.9	**13.8*** **	14.9
	Reco	15.4			**14.8***	15.1	15.1			**14.3****	15.3
RBC (10^{12}/L)	Term	8.44	8.28	8.20	8.12	8.54	7.86	7.99	8.06	7.62	8.13
	Reco	9.10			8.97	8.86	8.43			8.31	8.50
Hematocrit (%)	Term	45.3	44.4	44.2	**42.1*** **	44.9	42.1	41.3	41.5	**38.6*** **	42.1
	Reco	46.8			45.6	46.1	44.5			43.0	45.4
Reticulocytes (10^9/L)	Term	220.7	205.4	198.1	**165.9*** **	**175.8****	182.9	184.6	170.4	141.3	194.4
	Reco	259.6			**192.8*** **	238.9	155.3			**121.2****	149.4
MCV (fL)	Term	53.7	53.6	53.9	**51.8***	52.6	53.6	51.7	51.6	**50.7****	51.8
	Reco	51.5			50.8	52.0	52.9			51.8	53.5
MCHC (g/dL)	Term	34.2	**35.1*** **	**35.0*** **	**34.7***	**34.8****	35.1	**35.9****	**35.9****	**35.8***	35.5
	Reco	32.8			32.5	32.7	33.8			33.3	33.6
HDW (g/dL)	Term	3.13	3.14	3.05	3.17	3.16	2.74	2.89	2.85	**3.08****	2.80
	Reco	2.97			2.81	2.89	2.44			2.52	2.49
MPV (fL)	Term	7.7	7.6	7.5	7.3	**7.2****	7.5	7.4	7.4	7.2	**7.1***
	Reco	8.5			**9.4*** **	**9.2*** **	8.5			**9.3*** **	**9.3*** **
Platelet Dist. Width (%)	Term	53.2	49.9	50.4	50.1	**47.4***	50.3	47.3	47.5	47.6	47.7
	Reco	45.6			**48.6****	45.9	42.4			**47.1*** **	**45.8****
WBC (10^9/L)	Term	14.9	14.9	13.1	12.7	**10.2*** **	12.4	**8.8***	9.8	**8.5***	**7.6***
	Reco	14.3			12.1	10.4	8.9			7.7	6.2
Neutrophils (10^9/L)	Term	2.2	2.9	2.7	**5.5*** **	2.0	1.4	1.2	1.4	1.8	1.6
	Reco	2.6			1.8	1.7	1.5			1.2	0.7
Lymphocytes (10^9/L)	Term	12.1	11.4	9.8	**6.4*** **	**7.6*** **	10.4	**7.2***	7.9	**6.2****	**5.7****
	Reco	11.1			9.8	8.2	7.0			6.3	5.2

Term = terminal, Reco = recovery
Bold indicates s.s. Δ from C
*p<0.05, **p<0.01, ***p<0.001

Clinical Chemistry

No toxicologically significant test item-related effects.

Urinalysis

Unremarkable.

Gross Pathology

Effects seen primarily in organs with greatest exposure to mipomersen -- kidney, liver and spleen and the immune system (lymph nodes). How an injection lesion should come to located in the cecum was not addressed in the report.

Gross Pathology											
Sex		Male					Female				
ISIS 301012 mg/kg/week		0	3	10	50	-	0	3	10	50	-
ISIS 147768 mg/kg/week		-	-	-	-	10	-	-	-	-	10
Liver, Large	Term	5	5	5	6	4	-	-	-	1	-
	Reco	2			3	3	-			1	-
Liver, Pale	Term	-	-	1	1	2	-	-	1	1	1
	Reco	-			-	1	-			-	-
Spleen, Large	Term	-	-	-	6	2	-	-	-	3	-
	Reco	-			-	-	-			1	-
Ileum, Red	Term	-	-	-	-	-	-	-	1	1	-
	Reco	-			-	-	-			-	-
Cecum, Injection Lesion	Term	-	2	2	1	1	-	-	2	1	
	Reco	-			2	-	1			3	1
Kidney, Pale	Term	-	1	2	3	2	-	-	1	6	1
	Reco	2			2	4	-			3	1
Kidney, Depressed Focus	Term	-	-	-	-	-	-	-	-	1	1
	Reco	1			1	2	-			-	-
Kidney, Pelvic Dilatation	Term	1	-	-	1	-	-	-	-	-	-
	Reco	-			-	1	-		-	-	1
Urinary Bladder, Distension	Term	-	-	-	1	-	-	-	-	-	-
	Reco	-			-	1	-			1	-
Thymus, Small	Term	-	-	-	2	-	-	-	-	4	-
	Reco	-			-	-	-			1	-
Poplitieal Lymph Node, Large	Term	-	-	-	1	-	-	-	-	1	1
	Reco										
Scruff, Red	Term	-	-	1	1	2	-	-	-	1	-
	Reco	-			-	-	-			-	-
Scruff, Sore	Term	-	-	-	-	-	-	-	-	1	-
	Reco	-			-	-	-			-	-
Lung, Pale Focus	Term	-	-	-	-	-	-	-	-	-	-
	Reco	-			-	1	1			-	2

Term = terminal, Reco = recovery, "-" no finding

Organ Weights

Spleen (BW adjusted): d.d. ↑ in ♂&♀, s.s. at MD (♀ only), HD and RS. Max ↑ (at HD) = 52-76%
Liver (BW adjusted): ♀s only had a d.d. ↑ in liver weight (s.s. at MD & HD). Max ↑ (at HD) = 29%
Kidneys (BW adjusted): s.s. ↑ (↑ 10%) in HD♀
Heart (BW adjusted): 8-9% ↓ in both sexes at HD (s.s. in HD♀)
These effects were only partially reversed during the recovery period.

Histopathology

Adequate Battery

Yes

Peer Review

No

Histological Findings

Most findings are consistent with those seen in adult rats: injection site reactions, foamy macrophages in multiple tissues, basophilic granules (accumulated drug) in the kidney, inflammatory cell foci, lymphoid hyperplasia.

Findings that appear to be more pronounced in (or confined to) juvenile animals include: depletion of marrow fat at HD, minimal focal nephropathy (♀s, all doses), minimal-slight tubular vacuolation (HD♀s) and minimal-slight thymic atrophy (HD). The depletion in marrow fat is likely secondary to reduced food consumption in the treated animals. Except for thymic atrophy, these findings were not clearly reversible following the recovery period.

Group incidence of marrow fat: femoral and sternal marrow – terminal kill

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/week)	0	3*	10*	50*	10#	0	3*	10*	50*	10#
Femur+marrow	No. examined:	10	10	10	10	10	10	10	10	10	10
marrow fat	Grade -	0	0	0	2	0	2	2	1	8	1
	1	2	3	3	8	4	8	8	9	2	8
	2	8	7	7	0	6	0	0	0	0	1
Sternum+marrow	No. examined:	10	10	10	10	10	10	10	10	10	10
marrow fat	Grade -	2	2	2	10	3	6	4	4	8	2
	1	7	8	8	0	7	4	6	6	2	7
	2	1	0	0	0	0	0	0	0	0	1

Key
* = ISIS 301012 Human-specific oligonucleotide
# = ISIS 147768 Rat-specific oligonucleotide
"-" = finding not present, 1 = minimal, 2 = slight

Group incidence of marrow fat: femoral and sternal marrow – treatment free kill

		Males					Females				
		1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Tissue and finding	Level (mg/kg/week)	0	3*	10*	50*	10#	0	3*	10*	50*	10#
Femur+marrow	No. examined:	10	-	-	10	10	10	-	-	10	10
marrow fat	Grade -	0	-	-	0	0	0	-	-	1	1
	1	2	-	-	7	2	7	-	-	9	8
	2	7	-	-	3	7	3	-	-	0	1
	3	1	-	-	0	1	0	-	-	0	0
Sternum+marrow	No. examined:	10	-	-	10	10	10	-	-	10	10
marrow fat	Grade -	3	-	-	8	2	1	-	-	5	1
	1	7	-	-	2	8	8	-	-	5	7
	2	0	-	-	0	0	1	-	-	0	2

Key
* = ISIS 301012 Human-specific oligonucleotide
# = ISIS 147768 Rat-specific oligonucleotide
"-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate

Histopathology -- Incidence (mean severity)											
Sex		Male					Female				
ISIS 301012 m/k/w		0	3	10	50	-	0	3	10	50	-
ISIS 147768 m/k/w		-	-	-	-	10	-	-	-	-	10
Liver Foamy macrophages	Term	0/10 -	0/10 -	0/10 -	5/10 (1.0)	0/10 -	0/10 -	0/10 -	5/10 (1.0)	10/10 (1.9)	0/10 -
	Reco	0/10 -			10/10 (1.0)	0/10 -	0/10 -			9/10 (1.4)	0/10 -
Liver Caudate lobe necrosis	Term	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -
	Reco	0/10 -			1/10 (3.0)	0/10 -	0/10 -			0/10 -	0/10 -
Spleen Agonal congestion/hemorrhage	Term	0/10 -	0/10 -	0/10 -	2/10 (P)	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -
	Reco	0/10 -			0/10 -	0/10 -	0/10 -			0/10 -	0/10 -
Spleen Lymphoid Hyperplasia	Term	2/10 (1.0)	3/10 (1.0)	8/10 (1.1)	8/10 (1.5)	4/10 (1.5)	0/10 -	3/10 (1.0)	9/10 (1.6)	8/10 (1.4)	7/10 (1.1)
	Reco	0/10 -			8/10 (1.2)	4/10 (1.0)	0/10 -			7/10 (1.7)	6/10 (1.2)
Spleen Foamy macrophages	Term	0/10 -	0/10 -	0/10 -	6/10 (1.0)	2/10 (1.0)	0/10 -	0/10 -	0/10 -	8/10 (1.2)	3/10 (1.0)
	Reco	0/10 -			1/10 (1.0)	0/10 -	0/10 -			0/10 -	0/10 -
Lymph Node (mesenteric) Foamy macrophages	Term	0/10 -	0/10 -	0/10 -	10/10 (2.1)	0/10 -	0/10 -	0/10 -	3/10 (1.0)	10/10 (2.1)	0/10 -
	Reco	0/10 -			10/10 (1.9)	0/10 -	0/10 -			10/10 (1.6)	0/10 -
Lymph Node (mandibular) Foamy macrophages	Term	0/10 -	0/10 -	0/10 -	10/10 (2.0)	0/10 -	0/10 -	0/10 -	1/10 (1.0)	10/10 (1.8)	0/10 -
	Reco	0/10 -			10/10 (1.7)	0/10 -	0/10 -			10/10 (1.7)	0/10 -
Adrenal Foamy macrophages	Term	0/10 -	0/10 -	0/10 -	10/10 (1.3)	0/10 -	0/10 -	0/10 -	0/10 -	9/10 (1.2)	0/10 -
	Reco	0/10 -			8/10 (1.2)	0/10 -	0/10 -			8/10 (1.0)	0/10 -
Kidney Inflammatory cell foci	Term	0/10 -	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -	0/10 -	1/10 (1.0)	1/10 (1.0)
	Reco	1/10 (1.0)			2/10 (1.0)	0/10 -	0/10 -			0/10 -	0/10 -
Kidney Focal nephropathy	Term	3/10 (1.3)	4/10 (1.0)	3/10 (1.0)	1/10 (1.0)	1/10 (1.0)	0/10 -	2/10 (1.0)	2/10 (1.0)	2/10 (1.0)	1/10 (1.0)
	Reco	4/10 (1.0)			2/10 (1.0)	4/10 (1.0)	2/10 (1.0)			4/10 (1.0)	0/10 -
Kidney Hyaline droplets	Term	9/10 (1.2)	2/10 (1.5)	5/10 (1.0)	1/10 (1.0)	9/10 (1.3)	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -
	Reco	9/10 (1.7)			1/10 (1.0)	10/10 (1.3)	0/10 -			0/10 -	0/10 -
Kidney Basophilic granules	Term	0/10 -	6/10 (1.0)	9/10 (1.4)	10/10 (2.1)	10/10 (1.2)	0/10 -	9/10 (1.3)	10/10 (2.5)	10/10 (2.8)	10/10 (2.4)
	Reco	0/10 -			10/10 (1.9)	8/10 (1.0)	0/10 -			10/10 (2.3)	10/10 (1.8)
Kidney Tubular vacuolation	Term	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	6/10 (1.3)	0/10 -
	Reco	0/10 -			0/10 -	0/10 -	0/10 -			8/10 (1.5)	1/10 (1.0)
Urinary Bladder Distension	Term	0/10 -	0/10 -	0/10 -	1/10 (P)	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -

	Reco	0/10 -			0/10 -	1/10 (P)	0/10 -			1/10 (P)	0/10 -
Ovary **Foamy macrophages**	Term						0/10 -	0/10 -	0/10 -	5/10 (1.0)	0/10 -
	Reco						0/10 -			1/10 (1.0)	0/10 -
Thymus **Atrophy**	Term	0/10 -	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -	0/10 -	3/10 (1.3)	0/10 -
	Reco	0/10 -			0/10 -	0/10 -	0/10 -			0/10 -	0/10 -
Injection site (scruff) **Cellulitis (infilt. mixed inflamm. cells)**	Term	0/10 -	7/10 (1.1)	6/10 (1.2)	4/10 (1.5)	8/10 (1.4)	0/10 -	5/10 (1.0)	3/10 (1.0)	3/10 (1.3)	4/10 (1.0)
	Reco	0/10 -			2/10 (1.0)	0/10 -	0/10 -			1/10 (1.0)	1/10 (1.0)
Injection site (scruff) **Acanthosis**	Term	0/10 -	0/10 -	0/10 -	1/10 (1.0)	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -	0/10 -
	Reco	0/10 -			0/10 -	0/10 -	0/10 -			1/10 (1.0)	0/10 -
Injection site (scruff) **Myositis/myopathy**	Term	0/10 -	2/10 (1.0)	2/10 (1.0)	2/10 (1.0)	1/10 (1.0)	0/10 -	0/10 -	1/10 (1.0)	1/10 (1.0)	0/10 -
	Reco	0/10 -			0/10 -	0/10 -	0/10 -			0/10 -	1/10 (1.0)

Term = terminal, Reco = recovery, "-" not examined/calculated, "P" = present

Toxicokinetics

Dose-related, hyperproportional ↑s in AUC exposure was apparent. There were no sex-based differences in exposure. Generally comparable plasma Cmax were observed between PND 22 and 91 for all dose groups, indicating little or no plasma accumulation. However, plasma AUC on PND 91 was 2 to 3 fold higher than PND 22, indicative of slower plasma clearance with repeat administration. As in adults, mipomersen is rapidly cleared from plasma and distributed to tissues, with a rank order of kidney>liver>spleen (not shown). Generally greater than 95% of detected oligonucleotide is intact in all tissues.

Table 2 Pooled Plasma pharmacokinetic parameters in rats following subcutaneous administration(s) of ISIS 301012

Group	Dose	Post Natal Day	Gender	C_{max} (µg/mL)	T_{max} (hr)	$AUC_{0\rightarrow24}$ hr (µg*hr/mL)
2	3 mg/kg	22	F	4.14	0.25	7.76
			M	4.49	0.5	8.12
		91	F	4.16	1	14.6
			M	3.07	0.5	21.8
3	10 mg/kg	22	F	17.9	0.5	40.1
			M	19.3	0.5	36.6
		91	F	16.9	0.5	91.1
			M	15.0	0.5	89.9
4	50 mg/kg	22	F	79.4	0.5	305
			M	85.5	0.5	270
		91	F	107	1	681
			M	82.4	1	801

Dosing Solution Analysis

Formulation is a true solution, so a homogeneity analysis was not conducted. Solutions were stable throughout the study period.

Study title: Repeat-Dose Subcutaneous Injection Toxicity Study with ISIS 301012 Manufactured by (b) (4) with Process-Related Impurities in Mice

Study no.:	301012-AS18 ((b) (4) study no. IG06084)
Study report location:	eCTD 4.2.3.7.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3 October 2006
GLP compliance:	Yes, but with exceptions: 1. Analysis of the test article and test article formulations were conducted in compliance with applicable FDA cGMP regulations. 2. Toxicokinetic evaluation (tissue analysis and TK summary) of ISIS 30 10 12 in tissue.
QA statement:	Yes
Drug, lot #, and % purity:	ISIS 301012, CA-301012-001, 92.8% (groups 2 & 3) ISIS 301012, ARM-301012-13, 90.0% (groups 4-9)

Key Study Findings

- There was no appreciable difference after 5 weeks of dosing in the toxicity profile of ISIS 301012 prepared with either the [redacted] (b) (4).
- There was no toxicologically significant differences after 5 weeks of dosing in the toxicity profile of ISIS 301012 with our without either cocktail of impurities.
- The NOAEL is considered to be 25 mg/kg BIW (50 mg/kg/week).

Methods

Doses:	See below
Duration of dosing:	5 weeks
Frequency of dosing:	twice weekly
Route of administration:	SC injection
Dose volume:	10 mL/kg
Formulation/Vehicle:	phosphate buffered saline
Species/Strain:	Cr1jBgi:CD 1 (ICR) mice
Number/Sex/Group:	6/s/g
Age:	6 weeks
Weight:	♂: 28.8 to 34.1 g ♀: 23.3 to 29.3 g
Satellite groups:	none
Unique study design:	Intent is to compare ISIS 301012 prepared by two different methods of synthesis, as well as toxicologic profile of process-related impurities. See Sponsor's table below for additional details.
Deviation from study protocol:	None material to the interpretation of the study

Table 20: Group Designation and Dose Levels

Group	Test Article	No. of Animals[a]		Dose Level		ISIS 301012 Conc.	Dose Volume
		Male	Female	(mg/kg/day)	(mg/kg/wk)	(mg/mL)	(mL/kg)
1	Vehicle Control (PBS)	6	6	0	0	0	10
2	ISIS 301012/ (b) (4)	6	6	5	10	0.5	10
3	ISIS 301012/ (b) (4)	6	6	25	50	2.5	10
4	ISIS 301012/ (b) (4)	6	6	5	10	0.5	10
5	ISIS 301012/ (b) (4)	6	6	25	50	2.5	10
6	ISIS 301012/ Impurities (b) (4)	6	6	5	10	0.5	10
7	ISIS 301012/ Impurities (b) (4)	6	6	25	50	2.5	10
8	ISIS 301012/ Impurities (b) (4)	6	6	5	10	0.5	10
9	ISIS 301012/ Impurities (b) (4)	6	6	25	50	2.5	10

[a] Twice weekly subcutaneous dosing for 10 doses with necropsies occurring on Day 35, 48±2 hours after the last dose.
[b] Animals in Groups 2 and 3 received ISIS 301012 manufactured by (b) (4)
[c] Animals in Groups 4 and 5 received ISIS 301012 manufactured by
[d] Animals in Groups 6 and 7 received ISIS 301012 manufactured by (b) (4)
[e] Animals in Groups 8 and 9 received ISIS 301012 manufactured by (b) (4) (b) (4)

Observations and Results

Mortality

No early deaths.

Clinical Signs

Limited to infrequent findings of skin ulceration at injection sites and associated alopecia. No clear relationship to either [redacted] (b) (4) or process impurities.

Body Weights

No effect.

Feed Consumption

No effect.

Hematology

Monocytes ↑ in ♂s with API from both types [redacted] (b) (4). No effect of impurities. No effect in ♀s.

Neutrophils n.s.s. ↓ in ♀s with API from both types [redacted] (b) (4); however [redacted] (b) (4) lot has moderately stronger effect. Addition of impurity cocktail 2 appeared to augment this effect. No effect on neutrophils of [redacted] (b) (4) or impurities in ♂s.

The effects observed were generally slight to mild and not of toxicological concern.

Clinical Chemistry

Albumin s.s. ↓ in HD♂ with impurity cocktail 2 (3.28 vs. 3.56 g/dL). Given the small magnitude of the decrease, this change is not considered to be toxicologically significant.

Urinalysis

No conducted.

Gross Pathology

No clear effect of any of the test items.

Organ Weights

Brain, kidneys, liver with gall bladder, ovaries, spleen, testes and thymus were assessed.

Unremarkable.

Histopathology

Adequate Battery

Limited panel: Kidney, Urinary bladder, Liver, Heart, Spleen, Thymus, Pancreas, Lung, Cecum, Colon, Mesenteric lymph node, Femur, Marrow (femur), Injection site, GALT, Testes, Ovaries, Uterus/Cervix, Abnormal lesions.

Only the 50 mg/kg/week dose groups and control group tissues were examined histopathologically.

The tissue panel and group sampling are deemed adequate for the intended purpose of the study.

Peer Review

No.

Histological Findings

Primary findings included changes indicative of a proinflammatory effect: increase in lymphohistiocytic cell infiltrates in the liver, extramedullary hematopoiesis in the spleen, sinus histiocytosis in the lymph nodes, and subcutaneous edema or granuloma in the subcutaneous injection site skin in the dose groups receiving 50 mg/kg/week ISIS 301012. Because of the general mildness (minimal to slight) of the effects, these are not considered adverse.

Table 2. Summary of Incidence of Microscopic Findings (N=6)

Organ / Findings	Control		Group3		Group5		Group7		Group9	
	M	F	M	F	M	F	M	F	M	F
Liver										
Lymphohistiocytic cell infiltrates	-	-	-	1	-	1	-	1	1	-
Granuloma	-	1	-	-	-	-	-	-	-	-
Spleen										
Hemopoiesis	-	2	2	2	2	2	4	2	2	2
Mesenteric lymph node										
Sinus histiocytosis	1	1	2	1	1	1	1	1	4	-

-: no finding

2 G5♂s had minimal cardiomyopathy. This finding is considered to be incidental since it was not seen in groups 7 and 9, which received the same dose and source of mipomersen as group 5.

No clear differential effects of the (b) (4) or the impurities was apparent.

Special Evaluation

None.

Toxicokinetics

Levels of ISIS 301012 in the kidney were measured. There was no effect of either (b) (4) or impurities on drug exposure.

Table 1. Summary of Kidney Concentrations of ISIS 301012 and Total Measured Oligonucleotide (µg/g) in Mice Collected 2 Days (on Day 35) following Five Weeks of Subcutaneous Treatment with ISIS 301012

Dose (mg/kg)	Dose (mg/kg/week)	Group	Synthesis	ISIS 301012 Conc. (µg/g)	
				ISIS 301012	Total Oligo.
5	10	2	(b) (4)	173 ± 56	201 ± 57
		4		113 ± 31	123 ± 31
		6		110 ± 25	130 ± 41
		8		131 ± 22	150 ± 32
25	50	3		220 ± 41	263 ± 40
		5		221 ± 40	255 ± 47
		7		157 ± 39	191 ± 40
		9		213 ± 37	248 ± 42

Data presented are mean ± standard deviation (n=5 or 6).

Study title: 5 Week Subcutaneous (Impurities Qualification) Toxicity Study in the Mouse

Study no.:	GT-348-TX-7 (UNF0008)
Study report location:	EDR, eCTD 4.2.3.7.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12-03-2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See below

Key Study Findings

- At the concentrations tested, none of the six mixtures of impurities examined had any material effect on the toxicity profile of mipomersen.
- The NOAEL is considered to be 25 mg/kg BIW (50 mg/kg/week)

Methods

Doses:	5 or 25 mg/kg ± different impurity mixes (see below)
Frequency of dosing:	Twice weekly
Duration of Dosing:	5 weeks
Route of administration:	SC injection
Dose volume:	5 mL/kg
Formulation/Vehicle:	Soln. in PBS
Species/Strain:	CD-1 mice
Number/Sex/Group:	6/s/g
Age:	6 weeks at initiation of dosing
Weight:	♂: 28 to 40 g ♀: 22 to 32 g
Satellite groups:	None
Unique study design:	Intent is to compare the toxicity profile of two different lots of ISIS 301012 ± various cocktails of impurities. See Sponsor's tables below for additional details.
Deviation from study protocol:	None material to study interpretation

Group number	Colour code	Number of animals		Animal identification numbers		Compound	Dose level (mg/kg)
		Males	Females	Males	Females		
1	White	6	6	1 - 6	103 - 108	Vehicle (Control)	Control
2	Pink	6	6	7 - 12	109 - 114	ISIS 301012 Lot 2	5
3	Pink/Black	6	6	13 - 18	115 - 120	ISIS 301012 Lot 2	25
4	Gold	6	6	19 - 24	121 - 126	ISIS 301012 Lot 17	5
5	Gold/Black	6	6	25 - 30	127 - 132	ISIS 301012 Lot 17	25
6	Green	6	6	31 - 36	133 - 138	ISIS 301012 Impurity (b) (4)	5
7	Green/ Black	6	6	37 - 42	139 - 144	ISIS 301012 Impurity (b) (4)	25
8	Yellow	6	6	43 - 48	145 - 150	ISIS 301012 Impurity (b) (4)	5
9	Yellow/ Black	6	6	49 - 54	151 - 156	ISIS 301012 Impurity (b) (4)	25
10	Purple	6	6	55 - 60	157 - 162	ISIS 301012 Impurity (b) (4)	5
11	Purple/ Black	6	6	61 - 66	163 - 168	ISIS 301012 Impurity (b) (4)	25
12	Red	6	6	67 - 72	169 - 174	ISIS 301012 Impurity (b) (4)	5
13	Red/Black	6	6	73 - 78	175 - 180	ISIS 301012 Impurity (b) (4)	25
14	Blue	6	6	79 - 84	181 - 186	ISIS 301012 Impurity (b) (4)	5
15	Blue/Black	6	6	85 - 90	187 - 192	ISIS 301012 Impurity (b) (4)	25
16	Dark Green	6	6	91 - 96	193 - 198	ISIS 301012 Impurity (b) (4)	5
17	Dark Green/ Black	6	6	97 - 102	199 - 204	ISIS 301012 Impurity (b) (4)	25

Impurity (b) (4)	
Species	% Composition
(b) (4)	

Impurity (b) (4)	
Species	% Composition
(b) (4)	

Impurity (b) (4)	
Species	% Composition
(b) (4)	

Impurity (b) (4)	
Species	% Composition
(b) (4)	

Impurity (b) (4)	
Species	% Composition
(b) (4)	

Impurity (b) (4)	
Species	% Composition
(b) (4)	

Observations and Results

Mortality

Male 11, group 2 (5 mg/kg drug lot 2, no added impurities) was found dead on Day 24. Was noted to have rapid breathing beginning Day 15, hunched posture beginning Day 22 and abnormal gait and piloerection beginning Day 23. Necropsy revealed a mass on the left auricle of the heart. This death is not considered to be related to test article.

Male 99, group 17 (25 mg/kg drug lot 2 + impurity (b) (4) escaped from its cage, had a convulsion and died on Day 12. This death is not considered to be related to test article.

Female 134, group 6 (5 mg/kg drug lot 2 + impurity (b) (4)) was euthanized on Day 22. This animal was observed to be twitching and unable to right itself. There were no findings at necropsy. As there were no comparable observations in other animals in this dose group nor in the higher dose group (group 7), this death is not considered to be related to test article.

Clinical Signs

No test item-related signs were noted.

Body Weights

No test item-related signs were noted.

Feed Consumption

No test item-related signs were noted.

Ophthalmoscopy

Not assessed.

Hematology

No toxicologically significant differences between the various dose groups.

Clinical Chemistry

No toxicologically significant differences between the various dose groups.

Urinalysis

Not assessed.

Gross Pathology

Injection site discoloration (reddening) was seen, but no differences between the various dose groups (aside from C, which did not show this finding).

Organ Weights

No toxicologically significant differences between the various dose groups.

Histopathology

Adequate Battery

Yes

Peer Review

No

Histological Findings

1 G2 male (early decedent) had a (moderate) atrial thrombus.

Chronic tenosynovitis (slight to moderate) was seen in 1/6 ♂s in G6, G8, G15.

Dose-related injection site reactions (subcutaneous fibroplasia ± hemorrhage occasionally with inflammation) was seen in some animals in most dose groups in both sexes. There was no apparent difference between mipomersen with or without impurities.

No test item (or impurity) related findings were seen in the kidney in either sex. Oddly, the presence of basophilic granules was not reported in the proximal tubule epithelial cells.

There were occasional liver findings in the treated groups, including: minimal-moderate centrilobular hepatocyte degeneration, minimal-slight occasional degenerate hepatocytes, minimal-moderate erythrocytes in hepatocytes, and minimal-slight focal hepatocyte degeneration/inflammation. However, there was no clear dose-relationship, nor any clear effect of any of the impurity cocktails. It is peculiar that there was no mention of basophilic granules in the Kupffer cells.

Extramedullary hematopoiesis was seen in the spleen of both sexes (even C), severity was ↑ at HD in ♂s, but there was no effect of impurities.

Special Evaluation

None.

Toxicokinetics

Plasma samples were not collected. Concentrations of ISIS 301012 in kidney samples collected at necropsy were dose-dependent but hypoproportional to dose. Concentrations of ISIS 301012 in liver samples collected at necropsy were dose-dependent and essentially dose-proportional.

Dosing Solution Analysis

Dosing formulations were true solutions, so no homogeneity assessment was conducted. Reported concentrations were similar at the start and end of the dosing period.

IMMUNOTOXICOLOGY

Title: Effects of Three Oligonucleotides in a Mouse Mast Cell Degranulation Assay

Study no.: GT-348-TX-8

Study design: This study was designed to address whether mipomersen, in the range of 300 ng/mL to 300 μg/mL, could directly stimulate macrophage degranulation, as read out by histamine release. Cultured murine MC/9 cells (a mast cell line) were used for this assessment. Phorbol myristate acetate (PMA) and A23187 (a calcium ionophore) were used as in combination as a positive control. Triton X-100 (a detergent that solubilizes the plasma membrane) served as a maximum release control.

Results: No dose of mipomersen appreciably stimulated histamine release under the conditions of the assay.

Histamine Release from MC/9 Mast Cells by Mipomersen

Treatment*	Concentration	Histamine Release (ng/mL)
Medium (spontaneous release)	NA	20.4
PMA plus A23187	10 nM/1 μM	61.6
Triton X-100 (maximum release)	0.2%	92.7
ISIS 301012 (Mipomersen)	300 ng/mL	16.7
	3 μg/mL	20.9
	30 μg/mL	22.5
	300 μg/mL	19.8

* All incubations were for 30 min at 37°C. The mean results from triplicate wells are shown.

Conclusion: The assay found no evidence that mipomersen at concentrations up to 300 μg/mL could directly stimulate mast cell degranulation. This assay does not however address whether mipomersen could indirectly stimulate mast cell degranulation (*e.g.,* via complement).

Title: Evaluation of ISIS 301012 in the Mouse Influenza Host Resistance Model

Study no.: 301012-AS20

Study design: The mouse influenza host resistance model was used to assess whether mipomersen or ISIS 147764, a mouse surrogate, adversely affects the ability of female balb/c mice to mount a protective immune response. Mice were treated biweekly with 10, 25 or 50 mg/kg (20, 50, or 100 mg/kg/week) Mipomersen or 50 mg/kg BIW ISIS

147764 for 4 weeks prior to challenge with influenza virus and for 3 weeks after infection. A separate group of animals were treated with 20 mg/kg/day dexamethasone (positive control) for 3 weeks prior to infection and 3 weeks after infection. Another group of animals received no treatment prior to or after influenza infection (negative controls). In this model, immunotoxicity is defined as impaired clearance of the infectious agent, influenza virus, from the lung. Additional endpoints examined included anti-influenza IgG levels in the lungs. Other assessments conducted outside of the scope of the immunotoxicity endpoints are not discussed, but have been reviewed.

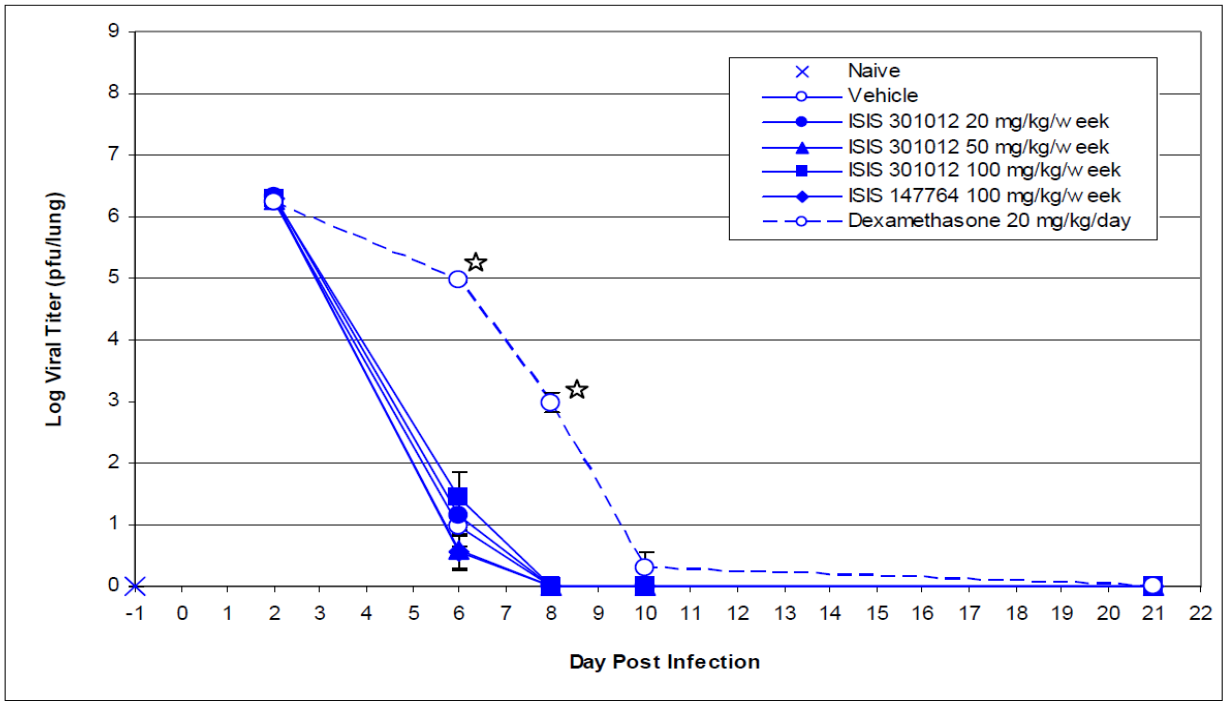
Results: While all doses of mipomersen and the mouse surrogate did s.s. ↓ the levels of influenza-specific IgG in lung homogenates (Sponsor's Table 5), the oligonucleotides did not impair clearance of virus from the lungs (Sponsor's Figure 4a).

Table 5: Influenza-specific IgG in Lung Homogenate

Day	Naïve		Vehicle		ISIS 301012 20 mg/kg/week		ISIS 301012 50 mg/kg/week		ISIS 301012 100 mg/kg/week		ISIS 147764 100 mg/kg/week		DEX 20 mg/kg/day	
	ng/ml	SE	ng/ml	SE	ng/ml	SE	ng/ml	SE	ng/ml	SE	ng/ml	SE	ng/ml	SE
-1	0	0												
2			0	0	0	0	0	0	0	0	0	0	0	0
10			852	47	***677***	20	***690***	35	***504***	19	***611***	17	***244***	26
21			5282	269	4993	160	4703	295	***3921***	212	4573	252	***1110***	163

Values represent group means in ng/mL.
SE = standard error of the mean
DEX = Dexamethasone
Bold italics = Different from vehicle by a statistically significant margin ($p < 0.05$)

Figure 4a: Log Viral Titer (PFU/lung)



Each point represents treatment-group mean ± SE.
* Different from vehicle by a statistically significant margin ($p < 0.05$).

Conclusion: Neither mipomersen or the mouse surrogate exhibited functional immunosuppression in this assay. There were however subclinical reductions in the lung levels of anti-influenza IgG, suggesting that mipomersen has the potential for exacerbating immunosuppression, albeit at relatively high doses.

11 Integrated Summary and Safety Evaluation

Mipomersen is a phosphorothioate antisense oligonucleotide (PS ASO) that inhibits the synthesis of human apoB_{100}, the intended consequence of which is a reduction in the levels of VLDL-C, LDL-C and Lp(a) in patients with familial homozygous hypercholesterolemia. The expectation being that these changes would have a favorable effect on cardiovascular health in these individuals. Sponsor's analysis suggests that mipomersen is selective for apoB, and that neither mipomersen nor its metabolites are likely to materially affect the expression level of other proteins.

Proof of concept studies in multiple species have established that antisense oligonucleotide inhibition of apoB_{100} synthesis effectively reduces the levels of circulating apoB-containing lipoproteins (*e.g.* VLDL, LDL and Lp(a)). Moreover, in rodent models of atherosclerosis (the LDLr- KO Mouse, the Human ApoB transgenic/LDLr-KO Mouse, and the ApoE-KO Mouse), ASO inhibition of apoB_{100} synthesis significantly decreased aortic plaque burden. Although these are imperfect models of human atherosclerotic disease (*e.g.,* they do not accurately model plaque rupture and myocardial infarction), the results are consistent with the potential for favorable cardiovascular effects in the HoFH population.

The toxicity profile of mipomersen (and species-specific surrogate oligonucleotides) has been studied in the mouse, rat, monkey and rabbit. The effects seen are largely consistent with what would be expected, based on the literature, for a phosphorothioate oligonucleotide[2]. In general, the nonclinical program has not identified any toxicity specifically associated with reduction in apoB_{100} expression (via use of species-specific surrogates) that was not also seen with mipomersen (which is only partially active in monkeys and not active in rodents or rabbits).

The observed systemic toxicities were generally dose-dependent, and primarily involved the immune system (discussed below), the hematopoietic system (↓ RBC mass, ↑ extramedullary hematopoiesis), the coagulative pathways (transient ↑ in APTT, ↓ in platelets in monkeys), the vasculature (discussed below) and the organs exhibiting the highest concentrations of drug -- the kidney and the liver (discussed below).

Immunological Effects

Based on the literature, PS ASOs are expected to induce proinflammatory effects, primarily through activation of cells of the innate immune system, especially

[2] Henry, S.P., *et al.,* Toxicologic Properties of 2'-*O*-Methoxyethyl Chimeric Antisense Inhibitors in Animals and Man, in *Antisense Drug Technology,* Crooke, S.T., ed., CRC Press, Boca Raton, 2008.

macrophages and dendritic cells, which subsequently produce proinflammatory cytokines and chemokines. Proinflammatory effects were generally dose-related, appearing at all doses, and were seen in all nonclinical species, and included 1) evidence of an acute phase response, 2) changes in lymphoid organ weight, 3) infiltration of histiocytes ± lymphocytes into myriad tissues, 4) activation of histiocytes (hypertrophy, and/or hyperplasia), 5) occasional infiltration of other leukocytes (*i.e.,* neutrophils or eosinophils) in to multiple tissues, 6) lymphoid hyperplasia, 7) changes in circulating levels of leukocytes, 8) elevation of certain cytokines/chemokines, and 9) injection site inflammation.

PS ASOs are also known to activate complement, primarily through the alternative pathway by inhibiting factor H, a negatively regulator of the alternative pathway. Complement activation was apparent at SC doses of ≥ 20 mg/kg in the monkey (and at an IV dose of 12 mg/kg) , as evidenced by transient rises in the level of the complement factor B split product, Bb, and the depletion of C3 with repeated dosing. The literature indicates that activation of complement by PS ASOs results from plasma concentrations exceeding a certain threshold, and are therefore C_{max}-driven. The monkey toxicity studies with mipomersen tend to support this hypothesis.

An *in vitro* assessment revealed no potential for direct activation of murine Mast cells; however, it should be noted that this study does not address the potential for indirect activation of Mast cells, for example via complement activation. An influenza host resistance assay in mice found no clinically meaningful effect on the ability to control and clear the infection, although there was a slight to moderate decrease in the magnitude of anti-influenza IgG response at all doses (20-100 mg/kg/week) of mipomersen and with the mouse surrogate. While these data indicate that it is unlikely that mipomersen has high potential for inducing immunosuppression by itself, they also suggest that mipomersen has the potential for augmenting immunosuppression, albeit at high relative doses.

Cardiovascular Effects

Perhaps the most significant inflammatory effect of mipomersen observed in the nonclinical studies, especially given the intended patient population for this product, are inflammatory vascular changes. Polyarteritis was seen in multiple tissues in rats in the 2-year carcinogenicity study in ♂s at doses ≥ 10 mg/kg/week and in ♀s at 20 mg/kg/week; however, these findings may be of limited clinical relevance, as it seems likely that these changes were secondary to a marked exacerbation of spontaneous chronic progressive nephropathy (w/ uremia) that was elicited by mipomersen in this study.

In the mouse 2-year study there is a suggestion that mipomersen (and the mouse surrogate) increased the incidence of cardiac thrombus. However attribution to the test item is confounded by the fact that this is a common finding in aged mice of this strain, and the highest incidence in each sex lies just outside of the highest incidence in the historical background database. It is notable though that a different PS ASO (ISIS 2302) also exhibited an apparent increase in the incidence of cardiac thrombus in a

mouse 2-year study. Given the absence of a mipomersen-related increase in incidence of thrombotic events in other tissues, nor an increase in thrombotic events in other species, perhaps these data are best interpreted as indicating a propensity to worsen or accelerate formation of spontaneous lesions in the mouse. Another cardiovascular finding in the mouse that may be related to mipomersen exposure is slight-moderate atrial/ventricular dilatation in ~3% of females at doses $\geq$ 20 mg/kg, which was correlated with a slight worsening of severity and incidence of cardiomyopathy. These effects were also apparent with the mouse surrogate (ISIS 147764). Atrial/ventricular dilatation was not reported in the historical background database.

In the 1-year monkey study, 2 monkeys at 30 mg/kg/week (animal #s 5007 and 5603 had findings that were initially characterized as extensive vasculitis and/or perivasculitis, with intimal hyperplasia (and rare medial hyperplasia). Upon subsequent peer review by a vascular pathology expert (Dr. Kerns) and an expert in the pathology of macaques (Dr. Palate), these lesions were re-characterized as intimal thickening/hyperplasia with lymphocytic infiltration, occasionally in combination with eosinophils. Dr. Palate also noted that the endothelium in some arteries from these animals appeared reactive (with rounded nuclei) and appeared focally denuded. Moreover, these areas of damaged endothelial cells were sporadically associated with light eosinophilic material suggestive of early fibrin accumulation in the lumen.

The precise etiology of these lesions is unclear. Sponsor posits that a deficiency in complement C3 (which is consumed during complement activation) interferes with the ability of complement to clear immune complexes from the vasculature, which is one of the recognized functions of basal complement activity. This deficiency, especially in the face of an immune response to a pathogenic agent (resulting in high levels of antigen-antibody complexes), could result in vascular lesions. In support of such an etiology, it is notable that both monkeys #5007 and 5603 were diagnosed with bacterial infections (Campylobacter and Shigella, respectively) around study day 310 (while no other assessed HD monkey had similar findings. Animal #5007 had a very low C3% (18% vs. 58% for the rest of the dose cohort); however, animal #5603 had a C3% that was comparable to the rest of the dose cohort. It is also notable that these two animals had among the greatest reductions in platelets, and also the greatest percentage of platelets with upregulated activation markers (CD62P). This is relevant to the discussion, since it is recognized that activated complement can cause consumption of platelets by inducing platelet activation and subsequent removal in the spleen.

It is also notable that the HD monkey (#5506) that exhibited the most profound decrease in platelets was ultimately sacrificed moribund. In addition to thrombocytopenia, this animal also had severe anemia (RBCs ~13% of baseline), and schistocytic RBCs 2 days prior to sacrifice, consistent with microangiopathic hemolytic anemia (although microangiopathy was not noted in the histopathology report). The postmortem was notable for widespread multifocal hemorrhage (with thrombocytopenia presumably being contributory), and multifocal necrosis (possibly secondary to local tissue anoxia associated with anemia and altered tissue perfusion).

Renal & Hepatic Effects

The kidney is generally the site of highest tissue exposures, especially in the renal cortex proximal tubules. All species show a dose-related finding of the presence of basophilic granules in the cytoplasm of kidney tubule epithelial cells. These granules are thought to represent drug taken up by the cells. The liver is generally the organ with the second highest tissue concentration (except for mice where liver concentrations typically exceed kidney concentrations), and all species have dose-related basophilic granules in the Kupffer cells. Again, these granules are thought to reflect mipomersen taken up by the cells. Steady state tissues levels are reached by ~26 weeks. A comparison between kidney and liver tissue concentrations of mipomersen in mice, rat and monkeys (and the toxicities accompanying these exposures) is provided by Sponsor's tables below.

Table 24: Liver/Kidney Toxicity and Exposure Correlation Following Mipomersen Administration

6-Months in Mice (301012-AS14)			
Dose, Route Summary of Liver and Kidney Effects		Concentration (µg/g)[a] Total Oligonucleotide	
		Liver	Kidney
10 mg/kg/week, s.c.: No toxicologically significant findings. Minimal increases in organ weights (~1.1 to 1.2-fold over control); Basophilic granules	6-mo	85.3 ± 23.2	84.4 ± 36.8
25 mg/kg/week, s.c.: Minimal increases in organ weights (1.2-fold over control); Basophilic granules	6-mo	278 ± 79	182 ± 65
75 mg/kg/week, s.c. :Mild increases in AST and ALT (~1.6 to 3.7-fold over control); Basophilic granules	6-mo	908 ± 239	236 ± 104

Values are mean ± standard deviation
[a] Mouse tissue concentrations were measured at 48 hours after the last injection (n = 6)

5-Months in Rats (301012-AS21)			
Dose, Route Summary of Liver and Kidney Effects		Concentration (µg/g)[a] Total Oligonucleotide	
		Liver	Kidney
3 mg/kg/week, s.c.: No toxicologically significant findings; Basophilic granules	5-mo	114 ± 34.7	537 ± 89.3
10 mg/kg/week, s.c.: Increases in liver weight (1.1-fold over control); Significant increases in quantitative urine protein and P/C ratio; Basophilic granules	5-mo	306 ± 98.8	875 ± 277
30 mg/kg/week, s.c.: Increases in liver weight (1.2 to 1.3-fold over control); Mild increases in AST and ALT (~1.8 to 2.4-fold over control) in females; Significant increases in quantitative urine protein and P/C ratio; Increased incidence of chronic progressive nephropathy in males; Basophilic granules	5-mo	746 ± 170	1354 ± 784
50 mg/kg/week, s.c. : Increases in liver weight (1.4 to 1.5-fold over control); Mild increases in AST and ALT (~2.2 to 3-fold over control) in females; Significant increases in quantitative urine protein and P/C ratio; Increased incidence of chronic progressive nephropathy; Basophilic granules.	5-mo	941 ± 345	1714 ± 660

Values are mean ± standard deviation
[a] Rat tissue concentrations were measured at 48 hours after the last injection (n = 10)

Up to 1 year in Monkeys (301012-AS15)			
Dose, Route Summary of Liver and Kidney Effects		Concentration (µg/g)[a] Total Oligonucleotide	
		Liver	Kidney[b]
1 & 3 mg/kg/week, s.c.: No toxicologically significant findings. Basophilic granules, Kupffer cell hyperplasia/hypertrophy.	6-mo	376 ± 140	504 ± 295
	12-mo	298 ± 42	474 ± 130
10 mg/kg/week, s.c.: No toxicologically significant findings. Basophilic granules, Kupffer cell hyperplasia/ hypertrophy. One of 6 monkeys - renal tubular epithelial cell degeneration and vacuolation only at 12-mo.[c]	6-mo	742 ± 182	1477 ± 288
	12-mo	638 ± 177	1081 ± 643
30 mg/kg/week, s.c.: Minimal tubular epithelial cell degeneration (4 of 6 monkeys) and minimal to mild vacuolation (6 of 6 monkeys) only at 1-year time point (not at 6-month). Kupffer cell hyperplasia/ hypertrophy	6-mo	1088 ± 578	2041 ± 334
	12-mo	1112 ± 370	2271 ± 421

Values are mean ± standard deviation
[a] Monkey tissue concentrations at 48 hours after the last infusion or injection (n = 4 or 6)
[b] Kidney = kidney cortex for monkey
[c] Monkey No. 4503 (10 mg/kg/week) had 2-fold higher kidney concentration (2046 µg/g) than its group mean value

KIDNEY

At 5 to 6 months with a comparable dose of 25-30 mg/kg/week, the highest kidney concentration of mipomersen was seen in the monkey (~2000 μg/g), the lowest was seen in mice (182 μg/g), and the rat was intermediate (~1350 μg/g).

There was no indication of mipomersen-related renal toxicity in mice, but, as noted above, mice had the lowest levels of mipomersen in the kidney of any of the assessed species (~10-fold lower than the monkey).

In rats, doses ≥ 10 mg/kg/week were associated with a profound worsening of chronic progressive nephropathy (CPN) in males and a worsening and increase in incidence of CPN in females. This was associated with proteinuria, increased blood urea nitrogen, and increased deaths due to CPN/uremia in both sexes in the 2-year rat study. Given the apparent etiology of the toxicity as an exacerbation of an underlying condition, the clinical significance of this finding is unclear, but does suggest caution in administering to patients with underlying kidney disease.

In monkeys, SC doses ≥ 30 mg/kg/week were associated with minimal-moderate multifocal cytoplasmic vacuolation and minimal-slight degeneration of the tubular epithelium. Sponsor considers that the vacuoles are an artifact of fixation, arising from fluid influx into oligonucleotide-laden (hydroscopic) phagolysosomes, resulting in washing out of the drug and swelling of the phagolysosomes, and notes that basophilic material was visible in some of the vacuoles; however this etiology has not been rigorously established. 12 mg/kg q4d (21 mg/kg/week) administered intravenously for 13 weeks was additionally associated with minimal-moderate tubular epithelial cell regeneration (see Sponsor's table below). Sponsor's table fails to capture that this dose was also associated with minimal-moderate intratubular hemorrhage in 4/6 monkeys, accompanied by hematuria; this finding was not seen in the concurrent 20 mg/kg q4d (35 mg/kg/week) SC dose group. It is interesting that while the 12 mg/kg q4d IV dose was associated with greater renal toxicity and a higher C_{max} than the 20 mg/kg q4d SC dose used in the same study, it did not result in a higher AUC, nor a higher local concentration in the kidney, suggesting that the increased degree of renal toxicity may be related to complement activation, which was much greater in the monkeys treated with 12 mg/kg q4d IV than in the monkeys treated with the 20 mg/kg q4d SC dose. Minimal multifocal tubular hemorrhage (with hematuria) was also seen in the 52-week study in 1/6 monkeys dosed at 30 mg/kg qw, a dose that was also associated with marked complement activation.

On the basis of the monkey findings at 30 mg/kg qw of minimal tubular epithelial cell degeneration in 4/6 monkeys and minimal-mild tubular vacuolation in 6/6 monkeys, the NOAEL is considered to be 10 mg/kg/week SC for kidney toxicity. Note that the 1/6 monkeys in the 1-year study at 10 mg/kg/week with similar findings had kidney levels of drug twice that of its cohort and similar to the 30 mg/kg/week cohort, and was not considered to be representative of the 10 mg/kg/week cohort.

Table 25: Comparison of Histologic Changes in the Kidneys of Monkeys Treated with 301012 for up to 1 year (301012-AS02 and 301012-AS15)

Parameter	Week 13[a]		Week 26[b]		Week 52[b]	
(Kidney Cortex)	21 (IV)[c,d]	35 (SC)[d]	10 (SC)[d]	30 (SC)[d]	10 (SC)[d]	30 (SC)[d]
Tissue Concentration (μg/g)[a]	1425 ± 903	1700 ± 628	1248 ± 256	1821 ± 403	985 ± 565	1898 ± 342
Basophilic Granules	6 of 6 (mild to marked)	6 of 6 (mod. to mark.)	4 of 4 (mild to mod.)	4 of 4 (mild to mod.)	6 of 6 (mild)	6 of 6 (mild to mod.)
Tubular Vacuolation[e]	2 of 6 (mild to mod.)	4 of 6 (min. to mod.)	None	None	1 of 6 (mod.)	6 of 6 (min. to mild)
Tubular Epithelial Cell Degeneration[f]	1 of 6 (minimal)	None	None	None	1 of 6 (mild)	4 of 6 (min.)
Tubular Epithelial Cell Regeneration[g]	5 of 6 (min. to mod.)	None	None	None	None	None

[a] Presented as mean with standard deviation; Study source: 301012-AS02
[b] Presented as mean with standard deviation; Study source: 301012-AS15
[c] n = 2 (two unscheduled necropsies during the study due to septicaemia resulting from chronic i.v. catheterisation)
[d] Dose (mg/kg/week)
[e] Minimal microvesiculation (multiple small vacuoles) was present in 2 of 6 control monkeys at Week 13
[f] Minimal tubular epithelial cell degeneration was observed in 1 of 4 control monkeys at recovery time point
[g] Minimal regeneration was present in 1 of 4 and 3 of 6 control monkeys at 4 and 13 weeks time point, respectively.

LIVER

Liver concentrations of mipomersen follow the same general pattern as the kidney, with the lowest level in mice (~280 μg/g), the highest in monkeys (~1090 μg/g) and an intermediate level in rats (~750 μg/g) following a comparable exposure of 25-30 mg/kg/week for 5-6 months. Unlike in the kidney (where oligonucleotide remains largely confined to the proximal tubule), oligonucleotide in the liver is distributed to all cell types. The relative cellular distribution of PS ASOs is reported to be similar across the evaluated species. The highest levels of mipomersen are seen in Kupffer cells, which concentrate the oligo in the lysosomes, giving rise to basophilic granules.

Liver findings in mice following 3-6 month included basophilic granules accumulation in Kupffer cells at all doses and increased liver weight at doses ≥ 25 mg/kg/week. Higher doses (≥ 44 mg/kg/week) were also associated with elevations in ALT, AST & ALP and decreases in albumin. Treatment with the mouse surrogate, ISIS 147764, at a dose of

75 mg/kg/week for 6 months was associated with hepatocyte Karyomegaly, and occasionally with single cell necrosis. After 2 years of dosing, the findings also include an increase in the incidence of basophilic foci of cellular alteration in males at all doses ≥ 5 mg/kg/week and eosinophilic foci of cellular alteration in females at doses of 60 mg/kg/week. There was also an increase in the incidence and severity of minimal-moderate single cell necrosis at all doses of mipomersen (≥ 5 mg/kg/week) in both sexes. An increased incidence and/or severity of extramedullary hematopoiesis was seen at 60 mg/kg/week in both sexes. Counterintuitively, given the proinflammatory action of PS ASOs, the incidence of mononuclear cell infiltration was decreased in females at doses ≥ 5 mg/kg/week, and in males at 60 mg/kg/week of mipomersen, but were unaffected by 60 mg/kg/week of ISIS 147764 (mouse surrogate). The mouse surrogate was however associated with the foci of cellular alteration and increase in single cell necrosis noted above. Foci of cellular alteration often occur as a precursor to neoplastic changes. Indeed the Executive CAC found that 60 mg/kg/week of mipomersen was associated with an increased incidence of hepatocellular adenoma and hepatocellular adenoma + carcinoma in females. They also found that 60 mg/kg/week of the surrogate (ISIS 147764) was associated with an increased incidence of hepatocellular adenoma or carcinoma (combined) in both sexes. The neoplastic effects of mipomersen are considered more fully below.

In the rat, treatment with mipomersen for 5 months at doses ≥ 10 mg/kg/week was associated with increased liver weight in both sexes, and all doses were associated with accumulation of basophilic granules in the Kupffer cells. There was no increase in inflammatory infiltrates noted, and, as in mice, the incidence of monocyte cell infiltration was actually decreased at mipomersen ≥ 10 mg/kg/week in females and ≥ 30 mg/kg/week in males. There were no changes in serum transaminases in males, but females had elevated AST and ALT at doses ≥ 30 mg/kg/week. Cholesterol (total, HDL & LDL) was increased at 50 mg/kg/week. VLDL and triglyceride were decreased at mipomersen ≥ 3 mg/kg/week in males and ≥ 30 mg/kg/week in females. In the 2-year study, liver weights were increased at doses ≥ 10 mg/kg/week, and accumulation of basophilic granules in Kupffer cells was seen at all doses. As for the 5-month study, there was no increase in inflammatory infiltrates, and the incidence of monocyte cell infiltration was decreased by mipomersen at all doses (≥ 3 mg/kg/week). Doses ≥ 10 mg/kg (mipomersen or rat surrogate) were associated with an increased incidence of centrilobular vacuolation and necrosis. Both sexes saw decreases in AST (but no change in ALT) and albumin at ≥ 10 mg/kg/week mipomersen (but not the rat surrogate). There were marked increases in triglycerides and cholesterol (total, HDL, LDL and VLDL) at 20 mg/kg/week, but not with the surrogate.

Mipomersen treatment in monkeys for 1 year was likewise associated with accumulation of basophilic granules in Kupffer cells at all doses and increases in liver weight at 30 mg/kg/week. Doses ≥ 3 mg/kg/week were also associated with diffuse hypertrophy/hyperplasia of Kupffer cells (a finding that was not seen in rodents). Unlike rodents there were no changes in serum transaminases and no effect on serum lipid parameters. There was a reversible decline in albumin levels at 30 mg/kg/week.

Steatosis was not apparent with mipomersen or species-specific surrogates in any species in any study, including a 22-week study in mice rendered hypercholesterolemic by consumption of a high fat diet and treated with a mouse surrogate (ISIS 147764) or a 5-week study in monkeys rendered hypercholesterolemic by feeding a high fat diet and treated with a monkey surrogate (ISIS 326358).

Based on the foregoing, the NOAEL for liver toxicity is considered to be < 5 mg/kg/week in mice on the basis of the increased incidence/severity of single cell (hepatocyte) necrosis at all doses examined; 3 mg/kg/week in the rat on the basis of increased incidence/severity of centrilobular vacuolation and necrosis; and 30 mg/kg/week in the monkey (the highest dose tested).

REPRODUCTIVE TOXICITY

Mipomersen (and ISIS 147764) was without effect on fertility, maintenance of pregnancy or embryofetal development in mice at dose up to 87.5 mg/kg/week. Likewise mipomersen and a rabbit surrogate (ISIS 233183) did not affect effect embryofetal development in the rabbit at doses up to 52.5 mg/kg/week. The rabbit surrogate (but not mipomersen) was associated with reduced bodyweight in the dams and early delivery in 1 dam. Mipomersen does not appear to cross the placenta, and it was undetectable in fetal liver or kidney in either the combined fertility/embryofetal development study in the mouse or the embryofetal development study in the rabbit.

The NOAEL for fertility and embryofetal development was > 87.5 mg/kg/week in mice and > 52.5 mg/kg/week in rabbits.

The pre- and post-natal study in rats found that mipomersen caused a decrease in food consumption in dams during lactation (all doses, s.s. at 70 mg/kg/week). Mipomersen was without effect on birth weight of the pups, but by LD 7 maternal doses of $\geq$ 35 mg/kg/week were associated with reduced pup bodyweight, and reduced survival at 70 mg/kg/week. Mipomersen treatment of the dams was associated with a slight, statistically significant delay in vaginal opening at 70 mg/kg/week. There was no effect of mipomersen on surface righting, auditory response, motor activity or learning and memory. There were a statistically significant impairment in visual placing response and grip strength at mipomersen doses $\geq$ 35 mg/kg/week (and with the rat surrogate). These deficits may relate the decreased body mass in these cohorts at the time of assessment. Notably, mipomersen is present in milk; however, absorption of PS ASOs from the oral route is considered to be negligible (~5% bioavailable).

The NOAEL for pre- and post-natal development in the rat is considered to be 7 mg/kg/week based on reduced bodyweight gain and impaired performance in the visual placing response and grip strength.

JUVENILE TOXICITY

In general, the toxicity profile in the juvenile rats resembles that in adult rats. There were decreases in food consumption and bodyweight gain (which did not reverse in a 4-week recovery period), but no meaningful effects on long bone growth, performance in the functional observational battery, or sexual maturation. Decreases in RBC mass and reticulocyte number are similar to those seen in adults, and hematocrit was normal following the recovery period. As in adults there are increases in spleen, kidney and liver weight, and accumulation of foamy macrophages in these tissues, and other signs of immune stimulation (*i.e.,* lymphoid hyperplasia in the spleen. Injection site reactions were also apparent. These effects were partially reversible following the recovery period. Reactions that seemed novel or exaggerated compared to adults was a reduction in fat tissue in the bone marrow (not reversible), and thymic atrophy (reversible) at 50 mg/kg/week, which were not reported in adults. The reduction in marrow fat is likely to be secondary to the reduced food consumption, and therefore caloric reserves, in the treated animals. Also, females had minimal-slight tubular vacuolation at 50 mg/kg/week (not reversible). The NOAEL in juvenile rats was considered to be 10 mg/kg/week.

GENETIC TOXICITY

Mipomersen was assessed for genotoxicity in a full battery of studies: 1) Ames assay, 2) mouse lymphoma assay and 3) *in vivo* micronucleus assay. All three assays were considered to be valid and to have yielded negative results.

CARCINOGENICITY

The carcinogenic potential of mipomersen or species-specific PS ASO targeting of $ApoB_{100}$ was assessed in 2-year bioassays conducted in the mouse and the rat. The Executive Carcinogenicity Assessment Committee (ExecCAC) met on 31 July 2012 to examine the results of these studies. The ExecCAC concluded that mipomersen was associated with neoplasms in both species, as follows (excerpted from the ExecCAC minutes:

Mouse:

- The Committee agreed that the study was adequate, noting prior FDA concurrence with the doses as well as with Sponsor's proposal for early termination of dosing and early sacrifice in the high-dose group of both sexes and in mid-dose females.
- The Committee concurred that the following neoplasms were drug related:
  - Hepatocellular adenomas and combined hepatocellular adenomas or carcinomas in females administered 60 mg/kg/week mipomersen.
  - Hepatocellular adenomas or carcinomas, combined, in both sexes administered 60 mg/kg/week ISIS 147764 (mouse surrogate).
  - Fibrosarcoma of the skin/subcutis in males administered 60 mg/kg/week mipomersen.
  - Hemangiosarcomas in mice given 60 mg/kg/week mipomersen.

Rat:

- The Committee agreed that the study was adequate despite the absence of prior FDA concurrence with doses and a high intercurrent mortality rate in the clinical candidate treatment groups.
- The Committee concurred that the following neoplasms were drug related:
  - Fibrous histiocytoma of the skin/subcutis in males and females at ≥ 10 mg/kg/week.
  - Fibrosarcoma of the skin/subcutis in females at ≥ 10 mg/kg/week.
  - Combined fibroma/fibrosarcoma/fibrous histiocytoma of the skin/subcutis in females at ≥ 10 mg/kg/week.

It was noted that the above neoplasms of the skin/subcutis were not associated with the injection sites, and are therefore unlikely to be a consequence of the method of administration.

It is notable that another phosphorothioate antisense oligonucleotide (to this reviewer's knowledge the only other PS ASO with a carcinogenicity study to be reviewed by FDA) also caused fibrohistiocytic tumors (malignant fibrous histiocytoma, histiocytic sarcoma), as well as hemangiosarcoma in mice. This suggests that such tumors may be a class effect of phosphorothioate oligonucleotides.

Table 4: Results of significant positive dose-tumor trend tests for mice

Tumors	Group 1*	Group 2*	Group 3	Group 4	Group 5	p-values
Male						
Number examined	60	55	60	60	60	
Hemolymphoreticular System/Hemangiosarcoma	1	1	2	5	16 **< 0.0001[a]**	**< 0.0001[†]**
Hemolymphoreticular System/Sarcoma, histiocytic	0	1	9 **0.0004[a]**	11 **0.0001[a]**	13 **< 0.0001[a]**	**< 0.0001[†]** **0.0002[‡]**
Skin, Subcutis/Histiocytoma, fibrous, malignant	0	0	0	1	3 **0.0064[a]**	**0.0051[†]**
Female						
Number Examined	55	55	60	60	60	
Hemolymphoreticular System/Hemangiosarcoma	1	2	1	5	9 **< 0.0001[a]**	**< 0.0001[†]**
Hemolymphoreticular System/Sarcoma, histiocytic	1	3	8	6	26 **0.0001[a]**	**< 0.0001[†]**
Uterus, Cervix/Polyp	0	0	0	2 **0.0155[a**]**	0	**0.0083[‡*]**

Source data: dataset received on 12/1/2005, analysis data M1M64905 and M1F64905.
[†]: p-values of trend tests for dose groups 1-5.
[‡]: p-values of trend tests for dose goups 1-4.
[a]: p-values for pairwise comparison between the controls 1 & 2 vs. each individual treated dose group.
[*]: Statistical significance at 0.025 level. [**] : Statistical significance at 0.05 level.

Groups 1 and 2 = controls 1 and 2, Group 3 = 2 mg/kg, Group 4 = 5 mg/kg, Group 5 = 15 mg/kg.

TABULAR SUMMARY

Tabular Summary of Notable Findings and Clinical Safety Margins				
Finding	**Species**	**Sex**	**NOAEL**	**Safety Margin[a]**
Immune System				
Proinflammatory Effects	Mouse	M/F	<2 mg/kg qw	<0.6x
	Rat	M/F	<3 mg/kg qw	<1x
	Monkey	M/F	<1 mg/kg qw	<0.3x
Injection Site Reactions	Mouse	M/F	20 mg/kg qw	6x
	Rat	M/F	10 mg/kg qw	3x
	Monkey	M/F	<1 mg/kg qw	<0.3x
Complement Activation	Monkey	M/F	10 mg/kg qw	3x
Acute Phase Response	Monkey	M/F	10 mg/kg qw	3x
Impaired Antibody Response to Influenza[b]	Mouse	M/F	25 mg/kg biw	8x
Cardiovascular System				
Polyarteritis[c]	Rat	M/F	3/10 mg/kg qw	1-3x
Cardiac Thrombus (↑ incidence)[d]	Mouse	M/F	5 mg/kg qw	1.5x
Atrial/Ventricular Dilatation	Mouse	F	5 mg/kg qw	1.5x
Intimal hyperplasia + mixed cell infiltration + (rarely) focally denuded endothelium + (rarely) fibrin in lumen	Monkey	M/F	10 mg/kg qw	3x
Microangiopathic hemolytic anemia?[e]	Monkey	F	10 mg/kg qw	3x
Hematopoietic				
Thrombocytopenia	Monkey	M/F	10 mg/kg qw	3x
Kidney				
Multifocal Tubular Hemorrhage + Hematuria[f]	Monkey	M	10 mg/kg qw	3x
Multifocal cytoplasmic vacuolation (minimal-moderate)[g,h]	Monkey	M/F	10 mg/kg qw	1x
Degeneration of tubular epithelium (minimal-slight)[h]	Monkey	M/F	10 mg/kg qw	1x
Liver				
Isolated hepatocyte necrosis	Mouse	M/F	<5 mg/kg qw	<0.1x
Centrilobular hepatocyte necrosis	Rat	M/F	3 mg/kg qw	0.1x
Transaminase elevation	Mouse	M/F	25 mg/kg qw	0.6x
	Rat	F	30 mg/kg qw	1.5x
Kupffer cell hypertrophy/hyperplasia	Monkey	M/F	<3 mg/kg qw	<0.3x
Decreased serum albumin	Mouse	M/F	10 mg/kg qw	0.2x
	Rat	M/F	10 mg/kg qw	0.5x
	Monkey	M/F	10 mg/kg qw	1x
Pre- and Post-natal Development				
↓ Food Consumption in Dams	Rat	F	<7 mg/kg qw	<0.3x
↓ Weight Gain in Suckling Pups + Impaired Visual Placing Response + ↓ Grip Strength	Rat	M/F	7 mg/kg qw	0.3x
↓ Survival of Suckling Pups	Rat	M/F	35 mg/kg qw	1.7x
Juvenile Toxicity				
↓ Marrow Fat + Thymic Atrophy	Rat	M/F	10 mg/kg qw	0.5x
Kidney Tubular Vacuolation (minimal-slight)	Rat	F	10 mg/kg qw	0.5x
Lactation				
Mipomersen Present in Milk[i]	Rat	F	<7 mg/kg qw	<0.3x
Carcinogenicity				
Liver adenomas ± carcinomas	Mouse	F	20 mg/kg qw	0.5x
Fibrosarcoma (skin, subcutis)	Mouse	M	20 mg/kg qw	0.5x

	Rat	F	3 mg/kg qw	0.3x
Combined Fibroma/Fibrosarcoma/Fibrous Histiocytoma (Skin/Subcutis)	Rat	F	3 mg/kg qw	0.3x
Malignant Fibrous Histiocytoma (skin, subcutis)	Rat	M/F	3 mg/kg qw	0.3x
Hemangiosarcoma	Mouse	M/F	20 mg/kg qw	0.5x

a -- Based on NOEL/NOAEL dose using body mass scaling for toxicity judged to arise from intra-vasculature/ C_{max}-related exposures, and body surface area scaling for all others. Comparisons are to a clinical dose of 200 mg qw = 3.3 mg/kg/week = 110 mg/m^2.

b -- Although the titer of anti-influenza antibodies were dose-dependently ↓, there was no effect on viral clearance

c -- May not be clinically relevant (secondary to chronic progressive nephropathy/uremia)

d -- May not be clinically relevant, since this is an exacerbation of a spontaneous lesion in this species

e -- A single monkey at 30 mkw had thrombocytopenia, anemia, and schistocytes

f -- Possibly secondary to complement activation

g -- Sponsor proposes that the vacuoles are not indicative of cellular injury, but of drug uptake

h -- 1/6 monkeys in the 10 mkw dose group had min. epithelial degeneration and mod. cytoplasmic vacuolation; however drug concentrations in the liver (2046 μg/g) were 2x the group mean (~1000 μg/g), and more similar to the group mean of the 30 mkw dose group (~2200 μg/g)

i -- May not be clinically relevant, since bioavailability from the oral route is estimated to be ~5%

SAFETY EVALUATION

Mipomersen clearly has the potential to cause adverse events in the liver, kidney, cardiovascular and immune systems in animals at what the Agency considers to be clinically relevant doses. It is notable thought that most of these adverse effects are clinically monitorable. Indeed, Dr. Criag's review of the clinical data shows that AEs related to the proinflammatory effects of mipomersen (*e.g.,* injection site reactions and flu-like symptoms) were detected and were a common cause of discontinuation in the clinical trials, as were elevated liver transaminases.

Potentially more problematic are the adverse cardiovascular effects seen in mice during the 2-year (lifetime) bioassay and in the 1-year monkey study. These findings warrant further consideration both from the standpoint that they would not be easily clinically monitorable, and that these effects (should they become manifest in the clinic) could potentially counteract the presumed benefit of the lowering of LDL-C and Lp(a). As discussed above, the clinical relevance of an increase in the incidence/severity of cardiac thrombosis (a background lesion) in a lifetime study in the mouse is unclear. Uncertainty of clinical relevance also extends to vascular lesions seen in the 1-year monkey study, since the affected monkeys clearly experienced complement activation and significant chronic reductions in C3 complement levels (not seen in the clinic), which likely contributed to the vascular lesions. It is also bears keeping in mind that these animals did not have elevated LDL-C levels, and therefore could not derive any (presumed) benefit from the pharmacologic activity of mipomersen (or the species-specific surrogate). Moreover, antisense inhibition of apoB synthesis was associated with significant decreases in plaque burden in mouse models of atherosclerosis. It is not possible to tell from the aggregate of nonclinical data whether, on balance, the presumed beneficial effects on cardiovascular health of lowering LDL-C and Lp(a) will

outweigh the possible adverse effects suggested by the toxicology data in normolipidemic animals, but such an outcome is at least plausible.

The other concern raised by the nonclinical program is the tumorigenic potential of mipomersen. The hepatocellular adenomas are of particular note as they are occurring at a clinically relevant exposure in the target tissue of intended pharmacology. It is also notable that the pharmacologically active surrogate oligonucleotide caused ~2x the tumor incidence of mipomersen. On the other hand, this is not a malignant neoplasm, and conversion of hepatocellular adenoma to hepatocellular carcinoma in humans is reportedly rare. The clinical significance of the remaining tumors is doubtful. Mice are reportedly susceptible to formation of hemangiosarcoma under conditions of hypoxia and macrophage activation, both of which were apparent in the 2-year study. With regard to the observed fibrohistiocytic tumors (fibrosarcomas, malignant fibrous histiocytoma) of the skin/subcutis, rodents are reportedly highly susceptible to these tumors with chronic irritation/inflammation of the subcutis, which was observed in the 2-year studies. Overall, it is judged that the possible tumor risk attending mipomersen treatment in humans can be adequately addressed via labeling

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/s/

RONALD L WANGE
11/30/2012

KAREN L DAVIS BRUNO
12/03/2012
concur with recommendation see Supervisory memo

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 203568 **Applicant: Genzyme** **Stamp Date: 03/29/2012**

Drug Name: Mipomersen **NDA/BLA Type: NDA**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	Only doses used in animals are presented. Human dose multiples are not presented. *NB* - mg/kg dose extrapolations may be reasonable for 2-MOE-substituted phosphorothioate oligos.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Impurity qualifying studies were conducted in mice, which evaluated the effected of concentrated process impurities, in order to support process changes in manufacturing.
11	Has the applicant addressed any abuse potential issues in the submission?		X	However, there is no basis for supposing that mipomersen has any abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ___Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

RONALD L WANGE
05/14/2012
Fileable.

KAREN L DAVIS BRUNO
05/14/2012