PHM

DIVISION OF ANTI INFLAMMATORY, ANALGESIC AND OPHTHALMOLOGIC DRUG PRODUCTS

PHARMACOLOGY AND TOXICOLOGY REVIEW

NDA:	**20-961**
DRUG:	**Fomivirsen Sodium; Vitravene™**
CODE NAME:	**ISIS 2922**
SPONSOR:	Isis Pharmaceuticals, Inc. 2292 Faraday Ave. Carlsbad, CA 92008
SUBMISSION DATE:	April 06, 1998
TYPE OF SUBMISSION:	Original [505 (b)(1)]
DATE COMPLETED:	August 4, 1998
REVIEWER:	W. C. Josie Yang, Ph.D.
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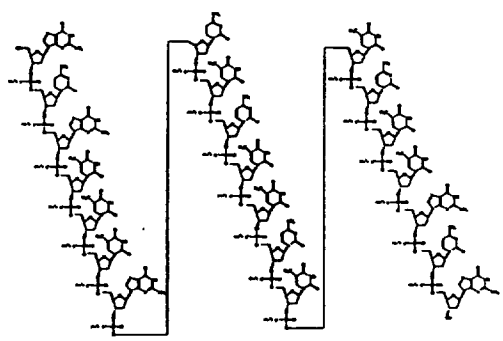
DATE ASSIGNED TO REVIEWER:	April 15, 1998
SAFETY REVIEW DATE:	N/A
DRUG CATEGORY:	Phosphorothioate Oliogonucleotide
FORMULA:	5′-GCG TTT GTC CTT CTT CTT GCG-3′; $C_{204}H_{234}H_{63}O_{114}P_{20}S_{20}Na_{20}$; MW=7112

[redacted]



CAS Nº:	160369-77-7
INDICATION:	Cytomegalovirus Retinitis (CMVR) in AIDS patients
DOSAGE FORM:	Injectable, 6.6 mg/ml
ROUTE OF ADMINISTRATION:	Intravitreal Injection
RELATED INDs:	[redacted]

TABLE OF CONTENTS

NDA 20-961

1. PHARMACOLOGY 2

1.1 OVERVIEW 2
1.2 MECHANISM-RELATED PHARMACOLOGY 2
1.2.1 antiviral activity of fomivirsen and ganciclovir against CMV (AD169) 2
1.2.2 Assays In Primary Human Retinal Pigment Epithelial (RPE) Cells and Human Fibroblast Cell Line (MRC-5) 3
1.2.3 Antiviral activity of fomivirsen against HCMV clinical isolates 3
1.3 SAFETY PHARMACOLOGY 4
1.4 REFERENCES 4

2. ADME 5

2.1 OCULAR EXPOSURE 5
2.1.1 Rabbit study 5
2.1.2 Monkey Studies 7

3. TOXICOLOGY 10

3.1 SINGLE-DOSE OCULAR TOXICITY STUDIES 10
3.1.1 Rabbit Studies 10
3.2 REPEATED DOSE OCULAR TOLERABILITY TOXICITY STUDIES 16
3.2.1 Monkey Studies 16
3.3 ACUTE TOXICITY STUDY - SINGLE-DOSE INTRAVENOUS 23
3.4 MULTIPLE-DOSE INTRAVENOUS TOXICITY STUDY 23
3.5 REPRODUCTIVE TOXICITY STUDIES 26
3.6 CARCINOGEICITY STUDIES 26
3.7 GENETIC TOXICITY STUDIES 26

4. LABELING REVIEW: 29

5. OVERALL SUMMARY AND EVALUATION: 30

5.1 PHARMACOLOGY 30
5.2 ADME 30
5.2.1 Ocular Exposure 30
5.2.2 Metabolism and elimination from the eye 31
5.2.3 Systemic Exposure 31
5.3 TOXICOLOGY 31
5.3.1 Ocular Toxicity 31
5.3.2 Systemic toxicity 33
5.3.3 Reproductive Toxicology 33
5.3.4 Genetic Toxicology 33
5.3.5 Carcinogenicity 33

6. CONCLUSION: 34

7. RECOMMENDATION: 35

1. PHARMACOLOGY

1.1 OVERVIEW

Fomivirsen is a phosphorothioated oligodeoxyribonucleotide, 21 nucleotides in length and complementary to mRNA of human cytomegalovirus (HCMV) immediately early transcriptional unit region 2 (IE2). Fomivirsen was designed to limit virus replication through an antisense mechanism of action. It has been reported that IE2 encodes for proteins that regulate viral gene expression and are crucial for the production of infectious CMV. Hybridization of an oligonucleotide with a target mRNA can lead to inhibition of specific protein production through a number of mechanisms that include translation arrest and degradation of target mRNA by RNase H cleavage. Due to lack of animal models for HCMV infection, the anti-CMV activity of fomivirsen has been demonstrated in *in vitro* tissue culture settings.

1.2 MECHANISM-RELATED PHARMACOLOGY

Fomivirsen has been shown to have potent antiviral activity in several cell culture systems.

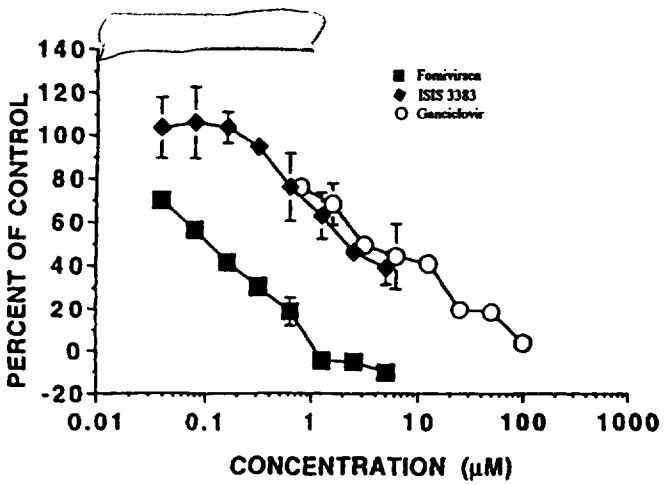
1.2.1 ANTIVIRAL ACTIVITY OF FOMIVIRSEN AND GANCICLOVIR AGAINST CMV (AD169)

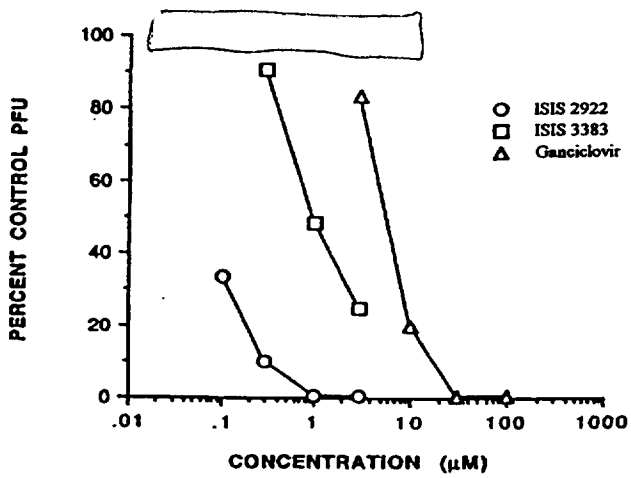
1.2.1.1 Assays Using Primary Human Dermal Fibroblasts

The anti-viral activity of fomivirsen against HCMV AD169, a laboratory strain, was assessed in primary human dermal fibroblasts.

Fomivirsen was also more potent than a control oligonucleotide, ISIS 3383[1]. ISIS 3383 is a random-sequence phosphorothioate oligonucleotide with no homology to the HCMV genome.

10-fold less potent than fomivirsen as shown in the following figures. Inhibition of viral replication at high concentrations





[1] Sequence for ISIS 3383: 5'TGG GCA CGT GCC TGA CAC GGC-3'

of control oligonucleotide implied that sequence-independent effects might play a significant role in the antiviral mechanism. Antiviral activity of non-complementary phosphorothioate oligonucleotides has been reported for herpes simplex virus (HSV)[2,3,4]. The inhibition of HSV replication by non-complementary phosphorothioate oligonucleotides was through direct inhibition of viral DNA polymerase or interaction with virus particles[2,3,4]. The anti-CMV activity and the reduction of CMV IE protein levels observed in cells treated with relatively high concentrations of non-complementary phosphorothioate oligonucleotides in this study were proposed to occur through a mechanism by which oligonucleotide interaction with CMV virions consequently prevents viral absorption or uncoating. A sequence-independent inhibition of virion adsorption to host cells by fomivirsen and other phosphorothioate oligonucleotides was demonstrated[5]. Therefore, it is possible that the antiviral activity of fomivirsen observed is the results of a combination of sequence-dependent and sequence-independent effects via interfering specifically with viral replication processes.

1.2.2 ASSAYS IN PRIMARY HUMAN RETINAL PIGMENT EPITHELIAL (RPE) CELLS AND HUMAN FIBROBLAST CELL LINE (MRC-5)

It has been hypothesized that the cells infected with HCMV in AIDS patients with chorioretinitis be retinal origin. In order to evaluate the ability of fomivirsen to inhibit CMV replication in retinal cells, primary human retinal pigment epithelial (RPE) cells were used as indicator cells. Dose-dependent inhibitory effects on HCMV AD169-induced plaque formation were observed. The EC_{50} values for ISIS 2922 and ganciclovir are presented in the following table.

Indicator Cells	Assay Type	EC_{50}	
		ISIS 2922	Ganciclovir
RPE		0.03	0.24
		0.05	0.4
MRC-5		0.2	ND
		0.25	2.0

ND = Not Determined; CPE = Cytophatic Effect.

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It appeared that ISIS 2922 and ganciclovir were more effectively prohibiting HCMV AD 169 replication in the RPE cells than in MRC-5 cells.

1.2.3 ANTIVIRAL ACTIVITY OF FOMIVIRSEN AGAINST HCMV CLINICAL ISOLATES

The antiviral activity of fomivirsen was also assessed against clinical isolates in a assay in normal human diploid fibroblast (NHDF) at UCSF, in MRC-5 cells and in RPE cells. The EC_{50} values for ISIS 2922, ganciclovir, foscarnet, and cidofovir against HCMV clinical isolates are shown in the following table. Only one of the 21 clinical CMV isolates evaluated required a concentration of fomivirsen >1 μM for 50% inhibition of CMV formation. Fomivirsen was able to inhibit replication of HCMV isolates cells that were resistant to either ganciclovir, forscarnet or cidofovir in NHDF cell system.

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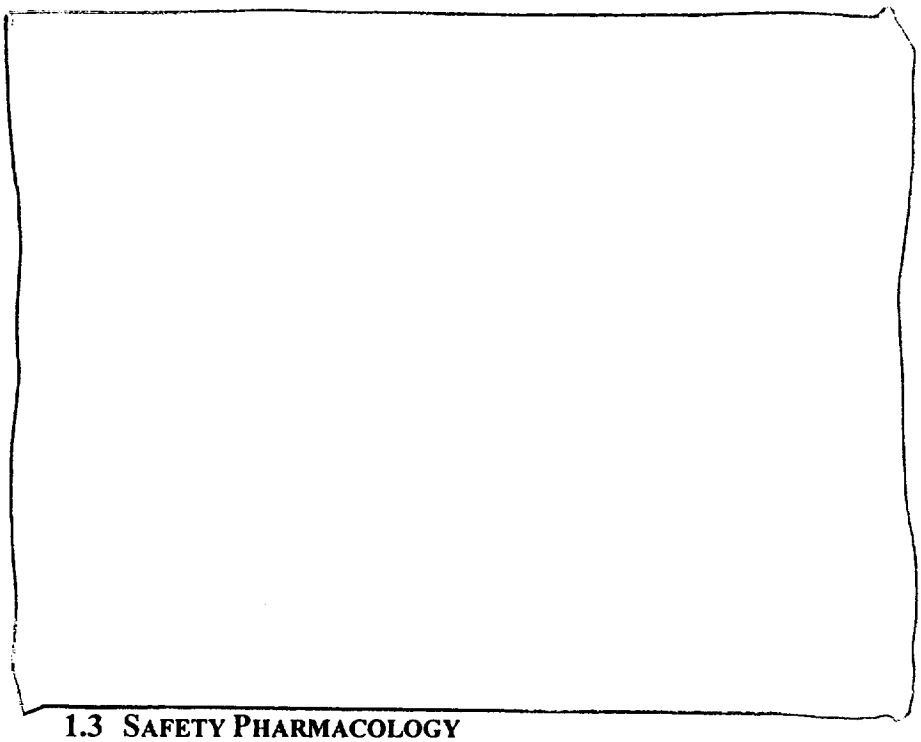
2 Gao W-Y, Hanes RN, Vasquez-Padua MA, Stein CA, Cohen JS, Cheng Y. Inhibition of herpes simplex virus type 2 growth by phosphorothioate oligodeoxynucleotides. *Antimicrobial Agents and Chemotherapy.* 1990a;34(5):808-812.

3 Gao W-Y, Stein CA, Cohen JS, Dutschman GE, Cheng Y-C. Effect of phosphorothioate homo-oligodeoxynucleotides on herpes simplex virus type 2-induced DNA polymerase. *Journal of Biological Chemistry.* 1989;264(19):11521-11526.

4 Gao W-Y, Jaroszewski JW, Cohen JS, Cheng Y-C. Mechanisms of inhibition of herpes simplex virus type 2 growth by 28-mer phosphorothioate oligodeoxycytidine. *The Journal of Biological Chemistry.* 1990b;265(33):20172-20178.

5 Anderson KP, Fox MC, Brown-Driver V, Martin MJ, Azad RF. Inhibition of human cytomegalovirus immediate-early gene expression by an antisense oligonucleotide complementary to immediate-early RNA. *Antimicrobial Agents and Chemotherapy.* 1996;40(9).



1.3 SAFETY PHARMACOLOGY

There were no safety pharmacology studies conducted with ISIS2922.

1.4 REFERENCES

The following pharmacology study reports were submitted in this NDA.

1. ISIS 2922-IP01 Inhibition of Human Cytomegalovirus Replication by the Phosphorothioate Oligonucleotide, ISIS 2922 (Vol. 03, p. 002).
2. ISIS 2922-IP02, In Vitro Pharmacology of ISIS 2922 (Vol. 03, p.069)
3. ISIS 2922-IP03, ISIS 2922 Antiviral Activity When Used in Combination with Approved Antiviral Compounds (Vol. 03, p.106).
4. ISIS 2922-EP04, Inhibition of Replication of Clinical and Drug-Resistant Isolates of Human Cytomegalovirus by ISIS 2922 (Vol. 03, p.180).
5. ISIS 2922-IP05, Selection for Human Cytomegalovirus with Reduced Sensitivity to ISIS 2922 Treatment in Cell Culture (Vol. 03, p.217).
6. ISIS 2922-IP06, A Human Cytomegalovirus Mutant with Sequence Dependent Resistance to the Phosphorothioate Oligonucleotide Fomivirsen (Vol. 03, p.227).
7. ISIS 13312-IP01, Inhibition of Human Cytomegalovirus Replication by 21 Methoxyethoxy-Modified Phosphorothioate Oligonucleotide, ISIS 13312, in Human Retinal Pigment Epithelial Cells (Vol. 03, p. 245).
8. ISIS 13312-IP02, Inhibition of Laboratory Clinical, and Drug-Resistant Isolates of Human Cytomegalovirus by the Antisense Phosphorothioate Oligonucleotide Inhibitors, ISIS 2922 and ISIS 13312 (Vol. 03, p.275).

2. ADME

2.1 OCULAR EXPOSURE

2.1.1 RABBIT STUDY

2.1.1.1 ISIS 2922-APK01, Biodistribution and Clearance Study of ^{14}C-Radiolabeled ISIS 2922 Following Intravitreous Administration to New Zealand White Rabbits (Vol. 10, p. 003)

Study Nº: 2-P32
Report Nº: ISIS 2922-APK01
Study Aims: To determine the ocular distribution and kinetics of radiolabeled fomivirsen (^{14}C-ISIS 2922) following intravitreal administration and to determine if fomivirsen or metabolites entered systemic circulation in NZW rabbits.
Compound: ^{14}C-ISIS 2922 (Lot Nº ISI-0026, 8.43 μCi/ml; 12.5 μCi/mg), 0.66 mg/ml
Dose and Route: 66 μg in 100 μl, intravitreal injection
Animal: 30 % NZW-SPF rabbits 3-5 months of age
Study Site:
Study Date: 3/30/93 -10/6/93
In Life Observation: 4/13/93 - 5/3/93
GLP/QAC Compliance: Yes
Study Design: A single dose of ^{14}C-ISIS 2922, 66 μg/100 μl, was given to rabbits (left eye only) by intravitreal injection. Fomivirsen used in this study was radiolabeled with ^{14}C on the C-2 position of thymidine resulting in label on 10 of the 21 nucleotides. Blood, urine, feces, and tissue samples were collected according to the schedule as shown in the following table. Vehicle control or sham-injection was not conducted. Animals were observed for clinical signs and mortality. Body weights were measured prior to treatment and before necropsy.

Group	Nº of Rabbits	Compound	Dose (μg)	Route	Blood Sampling	Urine/Feces Sampling	Tissue Sampling
1	2	^{14}C-ISIS 2922	66	intravitreal	0 min, 4 hr	-	4 hr
2	3	^{14}C-ISIS 2922	66	Intravitreal	10 min, 24 hr	-	24 hr
3	2	^{14}C-ISIS 2922	66	Intravitreal	20 min, 72 hr	-	72 hr
4	2	^{14}C-ISIS 2922	66	Intravitreal	40 min, 120 hr	-	120 hr
5	2	^{14}C-ISIS 2922	66	Intravitreal	60 min, 168 hr	-	168 hr
6	3	^{14}C-ISIS 2922	66	Intravitreal	2 hr, 240 hr	-	240 hr
7	2	^{14}C-ISIS 2922	66	Intravitreal	8 hr, 288 hr	-	288 hr
8	2	^{14}C-ISIS 2922	66	Intravitreal	48 hr, 336 hr	-	336 hr
9	2	^{14}C-ISIS 2922	66	Intravitreal	96 hr, 384 hr	-	384 hr
10	3	^{14}C-ISIS 2922	66	Intravitreal	144 hr, 432 hr	-	432 hr
11	3	^{14}C-ISIS 2922	66	Intravitreal	480 hr	per 24 hr over a 20-day period	480 hr

The following tissues were collected and weighed: adrenal glands; brain; eye (treated) - vitreous humor, aqueous humor, retina (separated from choroid), iris , and optic nerve up to the optic chiasm; fat (mesenteric); large intestine; small intestine; kidneys; liver; lungs; lymph nodes - mandibular and mesenteric; skeletal muscle; skin; spleen; testes. All samples (blood, urine, feces, and tissues) were processed for analysis of ^{14}C radioactivity by a scintillation counter.

Results:

- Clinical Signs and Body Weight - Treatment-related symptoms were observed. These observations included mild to moderate erythema in the left eye with swelling and occasional squinting and usually disappeared by Day 3 after intravitreal injection. No treatment-related body weight changes were recorded.
- Tissue Distribution of Radioactivity - Due to extremely low recoveries of radioactivity from one each animal @ Groups 5 and 8 (10 and 3.9%, respectively) as a result of dosing error, data were excluded.

Ocular Tissues: Following intravitreal injection of ^{14}C-labeled fomivirsen, highest levels of radioactivity in the vitreous humor were detected at the 1st time point examined (4 hr), and steadily decreased with a $T_{½}$ value of 77.5 hr (first-order kinetics). Radioactivity appeared in the aqueous humor within 4 hours, but the level was about 7-16% of that in the vitreous humor. Radioactivity was detected in retina within 4 hours post injection and the concentrations increased between 72 to 120 hr with a C_{max} of 86-96 µg equivalents/g, and declined thereafter. The levels of radioactivity in the iris were comparable to the retina. Contrarily, the amount of radioactivity in the optic nerve was very low. The calculated $T_{½}$ of radioactivity in the retina, vitreous humor, and aqueous humor are shown in the following table.

	Retina	Aqueous Humor	Vitreous Humor
$T_{½}$ (hr)	164.11 ± 8.69	83.22 ± 8.00	77.57 ± 3.85

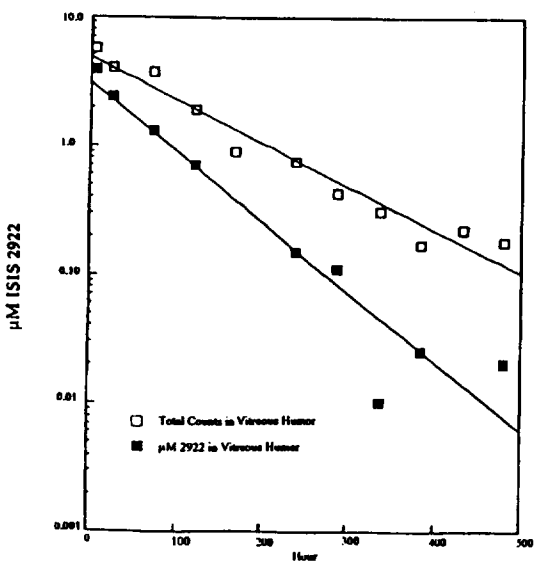
Blood and Plasma Levels: Low or undetectable levels of radioactivity could be measured at various time points.

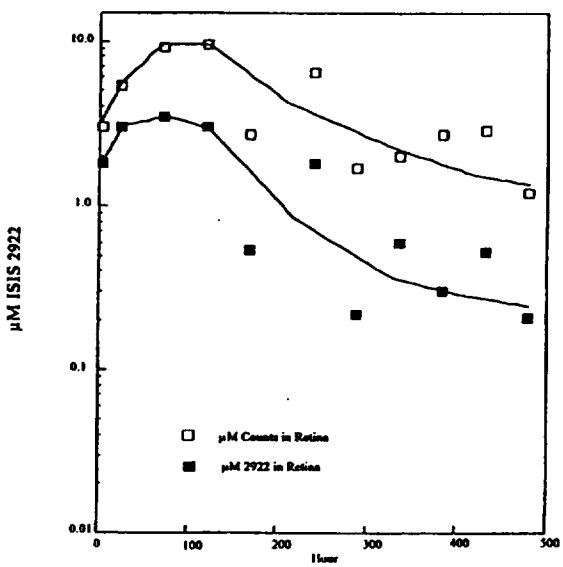
Other Tissues: The levels of radioactivity in internal organs and tissues were generally very low (~2 to 3% of the total dose at 72 hr) with the highest concentrations detected in kidneys and liver, at the earlier time points. Accumulation of radioactivity in kidney and liver is consistent with the observations of other phosphorothioate oligonucleotides and metabolites resulting from intravenous injection. **Extremely and unrealistically high concentrations of radioactivity were noted in skin samples with values of 129-445% of the total dose at 4 hr postdose, an indicative of sloppy laboratory handling procedures.**

Urine and Feces: Over the 20-day collection period, the accumulative amount of the dose excreted in the urine was approximately 16.7% and in feces was only 3.2%.

2.1.1.2 ISIS 2922-APK03, Determination of Levels of Intact ISIS 2922 in Vitreous Humor and Retinal Extracts After a Single Intravitreal Injection in New Zealand White Rabbits and Cynomolgus Monkeys (Vol. 10, p. 176)

Ocular tissues obtained from the previous study (ISIS 2922-APK01) were analyzed to determine the percentage of intact fomivirsen (i.e., full-length oligonucleotide) and to determine the extent of protein binding in a few vitreous samples [redacted] to separate most metabolites from the parent compound and [redacted] to separate oligonucleotides according to their chain length. The concentrations of full length ISIS 2922 and total radioactivity in the vitreous humor and retina at various time points are depicted in the following two figures. At 4 hours postdosing, the measured concentration of intact fomivirsen in vitreous humor was 3.9 μM. The rate of decline in the concentration of intact ISIS 2922

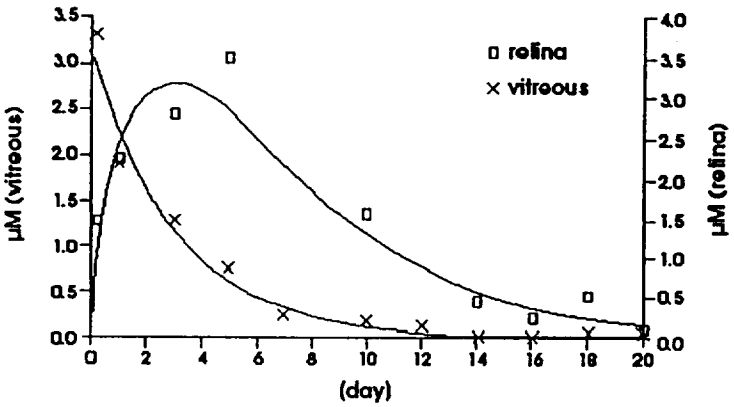




was first-order kinetics with a half-life of ~60 hours (2.5 days). Clearance half-lives for vitreous (60 hr) and retina (96 hr) were determined from slope of log-linear plots. On Day 10, the level of intact ISIS 2922 in

vitreous humor was 0.2 μM, representing ~20% of the total radioactivity remaining in the vitreous. Results from size exclusion chromatography showed that approximately **40% of the ^{14}C-ISIS 2922 was protein-bound.**

Intact fomivirsen and total radioactivity accumulated in the retina during the first 72hr post injection with a C_{max} value of 3.5 μM and a $T_{½}$ value of ~96 hr. By Day 10, ~28% (1.8 μM) of the total oligonucleotide remaining in retina was still intact. Retinal concentrations at Day 10 were about 10x greater than those in the vitreous humor. Ocular kinetics of fomivirsen following intravitreal injection of 66 mg (4.6 μM) was shown in the following figure.



2.1.2 MONKEY STUDIES

2.1.2.1 ISIS 2922-APK02 Tissue Distribution of Radioactivity of ^{14}C-ISIS 2922 Following a Single Intravitreal Injection to Cynomolgus Monkeys

Study Nº: [redacted]/6490-101
Report Nº: ISIS 2922-APKS02
Study Aims: To access the distribution of ^{14}C-ISIS 2922 in the eyes of cynomolgus monkeys following a single intravitreal injection.
Compound: ^{14}C-ISIS 2922 (Lot Nº ISI-0035, 11.3 μCi/mg, 0.33 mg/ml, 82% purity)
Dose and Route: 51 μg (0.5 μCi), equivalent to 4.8 μM, in 100 μl, intravitreal injection
Animal: 3 ♂ mature cynomolgus monkeys [redacted] weighing 4.1-4.8 kg
Study Site: [redacted]
In Life Observation: 5/7/93 -5/28/93
GLP/QAC Compliance: Yes
Study Design: Three monkeys were given a single dose of ^{14}C-ISIS 2922, 51 μg/100 μl, by intravitreal injection into each eye. Animals (1/time point) were sacrificed on Days 7, 14, or 21 postdose. Blood samples were collected into heparinized tubes. Left and right eyes tissues (aqueous humor, vitreous humor, and retina) were removed and weighed. Samples collected from the left eye and aliquots of plasma samples were shipped to the sponsor for the determination of drug product and its metabolites. The remaining samples were analyzed for the radioactivity by a liquid scintillation counter.

Results:

- Radioactivity in Ocular Tissues - The total amount of radioactivity in eye tissues at Day 7 postdose was ~6.5% of the dose and decreased to ~0.6% at Day 21. The radioactivity distribution in eye tissues at various sampling time points is presented in the following table.

Eye Tissues	Sampling Day	Radioactivity/Eye							
		% Dose		μg		μg equiv./g		μM	
		OD	OS	OD	OS	OD	OS	OD	OS
Vitreous Humor	7	3.87	2.77	2.01	1.35	1.10	0.706	0.154	0.099
	14	0.20	0.11	0.10	0.06	0.075	0.044	0.010	0.006
	21	0.06	0.11	0.03	0.06	0.02	0.038	0.003	0.006
Aqueous Humor	7	0.17	NA	0.09	NA	0.049	NA	0.070	NA
	14	0.01	NA	0.01	NA	0.041	NA	0.010	NA
	21	ND	NA	ND	NA	ND	NA	ND	NA
Retina	7	2.5	NA	1.29	NA	7.13	NA	0.999	NA
	14	0.79	NA	0.42	NA	2.33	NA	0.331	NA
	21	0.52	NA	0.27	NA	1.44	NA	0.204	NA

NA = not available; ND = Not detectable.

- Radioactivity in Plasma - No radioactivity (background range) could be detected in the plasma samples.

2.1.2.2 ISIS 2922-APK04, Pharmacokinetic Study in Cynomolgus Monkeys with ISIS 2922 Administered by Intravitreal Injection (Vol. 10, p. 257)

Study Nº: [redacted]6490-103
Report Nº: ISIS 2922-APKS04
Study Aims: To evaluate vitreal and retinal PK following a single intravitreal injection of ISIS 2922 to cynomolgus monkeys and to determine the potential for accumulation in the ocular tissues following multiple doses of administration.
Compound: ISIS 2922 (Lot Nº: ISI-0150 & ISI-0153, 2.3 mg/ml; ISI-0149 & ISI-0154, 1.15 mg/ml; ISI-0148 & ISI-0155, 0.23 mg/ml)
Dose and Route: 11, 57 and 115 μg (equivalent to 1, 5, or 10 μM calculated based on a volume of 1.5 ml vitreous) in 50 μl, intravitreal injection
Animal: ♂ mature cynomolgus monkeys [redacted] weighing 2.1-5.1 kg
Study Site: [redacted]
Study Date: 6/13/95 - 2/2/96
In Life Observation: 6/21/95 -8/1/93
GLP/QAC Compliance: Yes
Study Design: Monkeys received intravitreal injections of ISIS 2922 at doses of 11, 57 or 115 μg in 50 μl and were sacrificed according to the schedule listed in the following table.

Group	Dosing Days	Dose (μg)	Nº of Animals	Sacrifice Interval (Day)
1	1, 15, 29	115	2	31, 42
2	1, 8	115	2	17, 29
3[a]	1	115	6	3, 8, 15
4	1, 8, 15, 29	57	2	31, 42
5	1, 15, 29	57	2	31, 42
6	1, 8, 15	57	2	17, 29
7	1, 15	57	2	17, 29
8[a]	1	57	6	3, 8, 15
9	1, 8, 15, 29	11	2	31, 42
10	1, 15, 29	11	2	31, 42
11	1, 8, 15	11	2	17, 29
12	1, 15	11	2	17, 29
13	1	11	6	3[a], 6[b], 8[a], 15

[a] Two animals were sacrificed at each time point; [b] One animal was sacrificed following development of bilateral uveitis.

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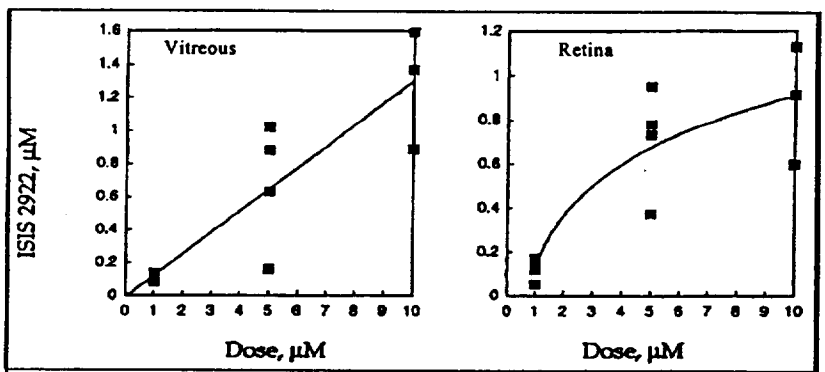
One monkey was sacrificed at each time point unless otherwise specified. At scheduled sacrifices, retina and vitreous humor from each eye were collected, weighed, and quick-frozen in N_2. Tissues samples were then shipped to the sponsor for analysis of drug levels. Vitreous and retinal concentrations of parent drug and oligonucleotide metabolites were determined using capillary gel electrophoresis (CGE).

The following observations were performed during the study:

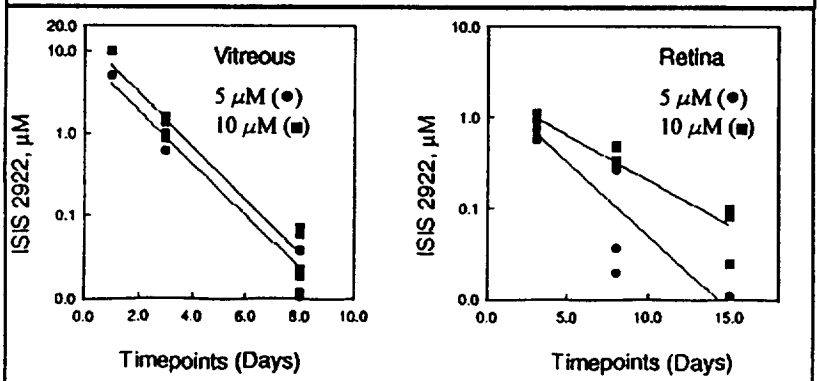
- Mortality and Clinical Signs - 2x/day.
- Body weights - 1x/week pretreatment, Day 1, and 1x/week thereafter, and on the day of sacrifice.
- Food Consumption - 1x/day by visual inspection.
- Ophthalmic Examinations [redacted] Days 1, 3, 8, 15, 17, 29, 31, and 42.
- Termination - Days 3, 8, and 15 for Groups 3, 8, and 13 (2/time point); Days 17 and 29 for Groups 2, 6, 7, 11, and 12; Days 31 and 42 for Groups 1, 4, 5, 9, and 10. No necropsies were performed.

Results:

- Mortality and Clinical Signs - One animal in Group 13 (11 μg) had cloudy eyes (bilateral uveitis) and was sacrificed on Day 6 for humane reasons. Dilated or constricted pupils, opacity, squinting, spots on the iris or cloudy eyes were observed in some animals given 11 or 57 μg/eye during the week after 1st injection.
- Body Weights and Food Consumption - No effects of treatment on either body weights or food consumption were seen.
- Ophthalmological Examinations - Cyclitis, characterized by inflammatory cells in the anterior chamber, flare in the anterior chamber, fibrin blocking the pupillary space, and a mitotic pupil, and anterior uveitis were identified during the week after 1st treatment in some animals @ 11 or 57 μg/eye groups (14% and 17% of treated eyes, respectively). In the more severe cases, the ocular alterations were extended to posterior chamber including inflammatory cells and fibrin deposits in the vitreous, obscuring the visualization of fundus. Chorioretinal scars with characteristics of depigmentation of pigment epithelium and choroid (1 OD on Day 42), and bilateral retinal cell and choroidal pigment cell epitheliopathy with active retinal vasculitis (2 OU on Day 31) were noted in some Group 1 (115 μg x 3) animals. Cellular infiltration around retinal vessels, retinal edema and retinal vasculitis were also noted in some animals (1 OD @ 57 μg x 2; 1 OS @ 57 μg x 3; 1 OD and 1 OU @ 11 μg x 3).
- PK - The levels of ISIS 2922 and its metabolites in the vitreous and retina were determined by a capillary gel electrophoresis method. Retinal concentrations increased with dose, but not dose-proportional. In contrast, the concentrations observed in the vitreous were dose linear two days after dosing. The concentrations of fomivirsen in vitreous and retina 2 days post-dosing (top panels) and kinetics of fomivirsen (bottom panels) in vitreous and retina following a single dose were depicted in the following figures.



The Concentrations of Intact ISIS 2922 in Vitreous and Retinal 2 Days after a Single Intravitreal Injection

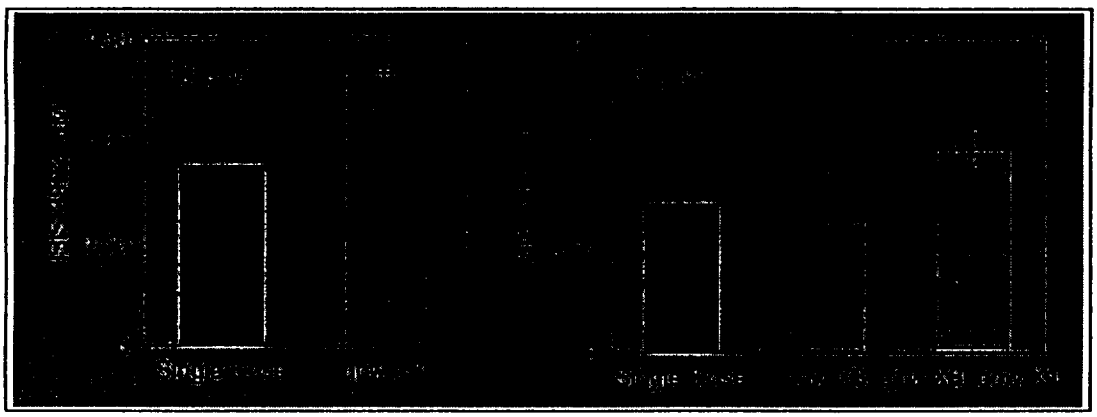


Kinetics of Intact ISIS 2922 in Vitreous and Retina

The estimated $T_{1/2}$ values for ISIS 2922 in vitreous and retina are shown in the following table.

Dose (μg)	$T_{1/2}$ (hr)	
	Vitreous Humor	Retina
115	22.2	78
57	22.6	45

There was no accumulation of ISIS 2922 in the vitreous as similar concentrations were obtained after either multiple- or single-dose injections. Contrarily, the concentration of oligonucleotide in retina after multiple doses, as displayed in the following figure, was higher than after a single dose, an indicative of accumulation. Retina samples were collected either 2 days after a single dose, 2 days after three doses administered every-other-week (qow x 3), or 2 days after three weekly doses and one dose administered every-other-week (q1w x 3, qow x 1).



3. TOXICOLOGY

3.1 SINGLE-DOSE OCULAR TOXICITY STUDIES

3.1.1 RABBIT STUDIES

3.1.1.1 ISIS 2922-AS04, Pilot Study of the Ocular Tolerance of ISIS 2922 Administered to Rabbits by Intravitreal Injection (Vol. 4, p. 004) (Non-GLP)

Study Nº: ISIS 2922-AS04
Report Nº: ISIS 2922-AS04
Study Aims: To examine ocular tolerability of ISIS 2922 by intravitreal injection to rabbits.
Compound:
- Experiment 1 - ISIS 2922 (Lot Nº: NGMP-0222-2922, ~70% purity of full-length) in Balance Salt Solution (BSS): 1, 4, and 16 mg/ml (Lot Nº: DC347-94-1, DC347-94-4, and DC347-94-16)
- Experiment 2 - ISIS 2922 (Lot Nº: NGMP-0245-2922, 95% purity of full-length) in sterile saline: 4 mg/ml (Lot Nº: Q0160-1-4) and ISIS 2922 (Lot Nº: NGMP-0222-2922, ~70% purity of full-length) in Balance Salt Solution (BSS): 4 mg/ml (Lot Nº: DC347-94-4)
- Experiment 3 - ISIS 2922 (Lot Nº: NGMP-0247-2922 & NGMP-0255-2922, 85 and 96% purity of full-length, respectively) in saline: 0.1, 0.33, and 1 mg/ml

Dose and Route: 1, 4, 16 (Exp. 1) or 4 (Exp. 2), or 0.01, 0.033, 0.1 (Exp. 3) mg in 0.1 ml, intravitreal injection
Animal: New Zealand white rabbits
Study Site:
Study Date: Not indicated
GLP/QAC Compliance: No

Study Design: No vehicle control groups were included in this study. Groups of rabbits were given intravitreal injection of 0.1 ml of various concentrations of ISIS 2922 (see table) either in BSS or saline to both eyes. Indirect ophthalmoscopic examinations were conducted 2x/week and animals were observed for ≥2 weeks. All animals were sacrificed and eyes were sectioned for the microscopic evaluation.

Experiment	ISIS 2922 Lot Nº	Concentrations (mg/ml)	Vehicle	Vol. of Injection	Nº Animal
1	NGMP-0222-2922	1, 4, 16	BSS	0.1 ml/eye	2/dose
2	NGMP-0245-2922	4	Saline	0.1 ml/eye	2
	NGMP-0222-2922	4	BSS	0.1 ml/eye	2
3	NGMP-0247-2922	0.1, 0.33, 1	Saline	0.1 ml/eye	3-4/dose
	NGMP-0255-2922	0.1, 0.33, 1	Saline	0.1 ml/eye	3-4/dose

APPEARS THIS WAY ON ORIGINAL

Results:

- Exp. 1 - Dose-dependent ocular changes including inflammation (vitritis), hazy vitreous, pale regions of the fundus (around the medullary ray, optic nerve), inflammation in the anterior chamber (high dose group only) and retinal damage were identified by ophthalmoscopic examinations within 7-10 days post injection. Some animals received a 2nd injection 2 weeks post 1st dosing. All animals were sacrificed 3 weeks after 1st injection. Microscopic evaluation was performed on eyes obtained from low dose group (1 mg/ml) and revealed inflammatory cells in the vitreous (PMNs, macrophages and lymphocytes) and moderate damage to the outer retinal photoreceptor cell layer.
- Exp. 2 - Similar ophthalmoscopic and microscopic findings seen in the Exp. 1 were characterized. However, less severe ocular changes were seen in eyes receiving the preparation of ISIS 2922 with higher purity.
- Exp. 3 - Findings with characteristics of inflammation in the vitreous, around optic disk, and medullary ray were revealed by ophthalmoscopic examination. These changes were dose-dependent. Milder responses were recognized when higher purity of preparations were used. Microscopic findings seen in the high dose group 2 weeks post dosing included inflammatory cell infiltrations in the retina around the retinal vessels, medullary ray, optic nerve, and vitreous humor. Mild to minimal inflammatory infiltration in vitreous but not in the retina was identified in eyes receiving 3 μM (0.033 μg) of ISIS 2922 (Lot Nº 0255 with 95% purity) or very mild microscopic alterations were seen in eyes received 1 μM (0.01 mg) (Lot Nº 0247 with 84% purity). All the microscopic findings seen at 2 weeks after dosing were not identified in animals that were scarified at 6 weeks post-dose.

Therefore, based upon provided information it could be concluded that (1) the impurity in the oligonucleotide preparations potentiated pathological changes in the eyes elicited by ISIS 2922 and (2) ocular pathological alterations were reversible.

3.1.1.2 ISIS 2922-AS06, Ocular Toxicity Study of ISIS 2922 after a Single Intravitreal Injection in Rabbits (Vol. 4, p. 018) (GLP)

Study Nº: 16490-111
Report Nº: ISIS 2922-AS06
Study Aims: To access the toxicity of ISIS 2922 following a single intravitreal injection to the eyes (with pigmented retinas) of rabbits.
Compound: ISIS 2922 (Lot Nº ISI-0118), 6.6 mg/ml
Dose and Route: 0, 16.5, 33, 82.5, 165, and 300 μg in 50 μl, intravitreal injection
Vehicle Control: Bicarbonate buffered, pH 8.8 (Lot Nº ISI-0028)
Animal: ♂ Hax:(DB)SPF rabbits [redacted] 13 weeks old, weighing 1322 to 1604 g, 6/group
Study Site: [redacted]
Study Date: 8/17/1995 - 2/5/1996
In Life Observation: 8/24/95 - 9/7/95
GLP/QAC Compliance: Yes
Study Design: Animals grouping and dose assignment are shown in the following table.

Group	Dose Level *(μM)	Total Dose (μg)	Dose Vol. (μl)	Nº of Animals	Day of Sacrifice
1	0	0	50	6	Day 8 and 15
2	2	16.5	50	6	Day 8 and 15
3	4	33	50	6	Day 8 and 15
4	10	82.5	50	6	Day 8 and 15
5	20	165	50	6	Day 8 and 15
6	40	300	50	6	Day 8 and 15

* Estimate based on a mean of 1.1 ml vitreal vol.

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weights - Days -7, 1, 8, and 15.
- Food Consumption - 1x/day
- Ophthalmology - Days 1 (predose), 8 and 15.
- Necropsy - Days 8 and 15, 3/group. Both eyes from each animal were collected and preserved in 2.0% paraformaldehyde/2.5% glutaldehyde. The following tissues were examined microscopically: anterior and posterior ocular segments (a narrow section through entire globe), ciliary body, peripheral retina, retina/optic nerve streak, and lesions in the posterior segment.

Results:

- Mortality and Clinical Signs - No deaths occurred. Swollen conjunctivae and red sclera were observed in all ISIS 2922-treated groups.
- Body Weights - Comparable cumulative body weight gain was noted for control and ISIS 2922-treated groups.
- Food Consumption - Occasionally, reduced food consumption was noted in control and ISIS 2922 treated animals.
- Ophthalmology - Incidence of ophthalmic alterations revealed by ophthalmoscopic and slitlamp examinations is shown in the following table.

Findings	1 (Control)		2 (2 μM)		3 (4 μM)		4 (10 μM)		5 (20 μM)		6 (40 μM)	
	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15
Indirect Examination												
Vitreous Floaters			7/12		12/12	3/6	12/12	5/6	12/12	6/6	12/12	5/6
Vitreous Flare			7/12		12/12		12/12		12/12		12/12	
Cloudy Vitreous							12/12	6/6	11/12	6/6	12/12	5/6
Vitreous Hemorrhage												1/6
Retina Inflammation, Cellular Infiltrate			1/12						2/12		4/12	1/6
Retina Edema										1/6		
Retinal Detachment										1/6		
Slitlamp Examination												
Anterior Chambers - Aqueous Flare							3/12					
Inflammatory Cells							1/12					
Iris Inflammation							2/12		4/12		5/12	
Iris Posterior Synechia									1/12			

- Gross and Histopathology - No gross lesions were identified during necropsy. Major microscopic changes characterized in ISIS-treated eyes on Day 8 sacrifice included histiocytic infiltrates (consisting of small lymphocytes and macrophages) in ciliary body, retina, vitreous, and choroid; minimal to moderately-severe hyperplasia of the ciliary body; minimal→severe degeneration of the ciliary body epithelium (except eyes treated with 2 μM); mild→moderate retinal degeneration [1/6 eyes @ 20 μM (165 μg) and 3/6 eyes @ 40 μM (330 μg)]. These changes were dose-dependent. Posterior synechia of the iris to the lens was observed in one Group 5 animal. One high dose animal had minimal clumping of the pigment epithelium layer in the right central retina.

  On Day 15, similar histopathological findings were noted in animals treated with ≥4 μM of ISIS 2922 and the incidence and severity of pathological changes in the vitreous and ciliary body were reduced. However, the incidence of retinal degeneration had increased (2/6 eyes @ 20 μM and 5/6 @ 40 μM). Detached retina was also identified in one each of Group 5 (OU) and Group 6 (OD). Moderately→severe hemorrhage

present in the vitreous, clumping of the pigment epithelium layer, and moderately→severe degeneration of the rod and cones layer of the retina were also identified in some animals given 40 μM. These changes correlated with the findings revealed by ophthalmic examinations.

Therefore, intravitreal injection of fomivirsen caused apparent inflammatory response in rabbit eyes that was initially evident in the ciliary body. At doses ≥165 μg (equivalent to vitreal concentrations of ≥20 μM), the severity of inflammation involved other ocular structures including the retina. The pathological changes in the retina included retinal degeneration, retinal detachment, hemorrhage, and clumping of retinal pigment epithelium.

3.1.1.3 ISIS 2922-AS07, Ocular Toxicity Study of ISIS 2922 after a Single Intravitreal Injection in Rabbits with Subconjunctival Injections of Triamcinolone (Vol. 4, p. 212)

Study Nº: [redacted]6490-116
Report Nº: ISIS 2922-AS07
Study Aims: To assess the toxicity of ISIS 2922 following intravitreal injection to the rabbits with pigmented retina with concomitant administration of prophylactic steroid (triamcinolone)
Compound: ISIS 2922 (Lot Nº ISI-0118), 6.6 mg/ml; Triamcinolone
Dose and Route: 16.5, 33, 82.5, 165, or 333 μg/0.5 ml/eye, intravitreal injection; Triamcinolone: 10 mg/eye, subconjunctival application
Vehicle Control: Bicarbonate buffered saline, pH 8.8 (Lot Nº ISI-0028 and ISI-0028a)
Animal: ♂ Hax:(DB)SPF rabbits [redacted] 13 weeks old, weighing 1254 to 1557 g, 6/group
Study Site: [redacted]
Study Date: 11/17/95 - 03/20/96
In-Life: 11/30/95 -12/14/95
GLP/QAC Compliance: Yes
Study Design: Groups of rabbits were given intravitreous injection with 50 μl of ISIS 2922 or vehicle, carbonate buffered saline, to both eyes on Day 1. Each eye also received a subconjunctival application of triamcinolone (10 mg/eye) on Days 1 and 7 post ISIS 2922 treatment.

Group	Dose Level *(μM)	Total Dose (μg)	Dose Vol. (μl)	Triamcinolone (10 mg/eye)	Nº of Animals
1	0	0	50	Days 1, 8	3
2	2	16.5	50	Days 1, 8	3
3	4	33	50	Days 1, 8	3
4	10	82.5	50	Days 1, 8	3
5	20	165	50	Days 1, 8	3
6	40	300	50	Days 1, 8	3

* Estimate based on a mean of 1.1 ml vitreal vol.

APPEARS THIS WAY ON ORIGINAL

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weights - Days -7, -1, 1, 8, and 15.
- Food Consumption - 1x/day by visual inspection.
- Ophthalmology [redacted] - Days 1 (predose), 8 and 15.
- Necropsy - Day 15. Both eyes from each animal were collected and preserved in 2.0% paraformaldehyde/2.5% glutaldehyde. The following tissues were examined microscopically: anterior and posterior ocular segments (a narrow section through entire globe), ciliary body, peripheral retina, retina/optic nerve streak, and lesions in the posterior segment.

Results:

- Mortality and Clinical Signs - No deaths occurred. Red conjunctivae and red sclerae were observed in some animals in fomivirsen-treated or control groups.
- Body Weights - Comparable cumulative body weight gain was noted for control and ISIS 2922-treated groups.

- Food Consumption - Occasionally, reduced food consumption was noted in control and ISIS 2922 treated animals.
- Ophthalmology [redacted] Incidence of ophthalmic findings is summarized in the following table.

Findings	Group											
	1 (Control)		2 (2 μM)		3 (4 μM)		4 (10 μM)		5 (20 μM)		6 (40 μM)	
	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15	Day 8	Day 15
Indirect Examination												
Vitreous Floaters			2/6[a]		1/6			2/6		3/6		4/6
Vitreous Inflammation											2/6	
Vitreous Flare							2/6		5/6		6/6	
Cloudy Vitreous							6/6	4/6		5/6	6/6	6/6
Slitlamp Examination												
Vitreous Floaters												
Anterior Chambers - Aqueous Flare			2/6							1/6		
Corneal Edema										1/6		

[a] Number of eyes affected/number of eyes examined.

No ocular lesions were noted for animals in the control group on Days 1, 8 and 15. Dose-dependent inflammation of ciliary body (cyclitis) with floaters and vitreous flare were identified in all treated animals on Day 8. By Day 15, vitreous floaters and cloudy vitreous were the remaining identifiable ocular alterations.

- Necropsy - There were no treatment-related gross abnormalities noted at necropsy. Minimal→slight lymphohistiocytic infiltrates in the superficial regions of the retina were observed in all ISIS 2922 treated but not control animals during microscopic examination. In addition, other histopathological changes identified in eyes treated with ≥10 μM (82.5 μg) of ISIS 2922 were (1) edema, epithelial degeneration, epithelial hyperplasia, and lymphohistiocytic infiltrates in the ciliary body, (2) lymphohistiocytic infiltrates in the vitreous, (3) hemorrhage in the retina and vitreous, (4) pigment clumping in the retina, and (5) proteinaceous material. No retinal degeneration, edema and detachment were observed in any dose group. The incidence of microscopic findings for both eyes is listed in the following table.

Microscopic Findings		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Ciliary Body	Degeneration, epithelium	-	-	-	2/6 m→s	2/6 m	2/6 m→s
	Edema	-	-	-	2/6 m→s	1/6 s	4/6 m→s
	Hyperplasia, epithelium	-	-	-	3/6 m	4/6 m	4/6 m→s
	Infiltrates, lymphohistiocytic	-	-	-	3/6 m	4/6 m	4/6 m
Retina	Hemorrhage	-	-	-	-	-	1/6 m
	Infiltrate, lymphohistiocytic	-	5/6 m	4/6 m	6/6 m	5/6 m→s	6/6 m
	Pigment clumping	-	-	-	-	1/6 m	2/6 m
	Vacuolation	-	-	-	-	-	1/6 m
Vitreous	Hemorrhage	-	-	-	-	1/6 m	-
	Infiltrate, lymphohistiocytic	-	-	-	1/6 m	3/6 m→s	4/6 m

m= Minimal, severity score of 1; s = Slight, severity score of 2.

Ocular changes (microscopic and ophthalmic examinations) observed in this study were less severe than those seen in the previous study (ISIS 2922-AS07). **Therefore, subconjunctival application of triamcinolone (10 mg/eye on Days 1 and 8) could alleviate but not prevent the toxicity induced by intravitreal injection of ISIS 2922.**

3.1.1.4 ISIS 13312-AS01, Comparison of Ocular Toxicity and Kinetics between ISIS 2922 and ISIS 13312 After a Single Intravitreal Injection in Rabbits (Vol. 5, p. 001)

Study Nº: [redacted]6409-128
Report Nº: ISIS 13312-AS01
Study Aims: To evaluate comparative toxicity and ocular kinetics of ISIS 2922 and ISIS 13312 following a single intravitreal injection in rabbits.

Compound: ISIS 2922 (Lot Nº ISI-0079, 6.6 mg/ml) and ISIS 13312[6] (Lot Nº ISI-0242, 7.2 mg/ml)
Dose : ISIS 2922: 33 and 82.5 µg/50 µl/eye; ISIS 13312: 36 and 90 µg/50 µl/eye
Route: Single intravitreal injection
Vehicle Control: Bicarbonate-buffered saline, pH 8.8 (Lot Nº ISI-0028)
Animal: ♂, DB)SPF rabbits 12-17 weeks of age, weighing 1491 - 1925 g
Study Site:
Study Date: 04/15/96-09/11/97
In-Life Observation: 04/25-06/07/96
GLP/QAC Compliance: Yes
Study Design:

Group	Compound	Dose Level (µM)		Total Dose/Eye (µg)		Nº of Animals
		OD	OS	OD	OS	
1	ISIS 2292	10	4	82.5	33	15
2	ISIS 13312	10	4	90	36	15
3	ISIS 13312	0 (Vehicle)	10	0	90	12

APPEARS THIS WAY ON ORIGINAL

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weights - Days -7, -1, 1, and 1x/week thereafter.
- Ophthalmology - Days -7 (predose), 3, 8, 15, 29, and 44.
- Necropsy - 3/groups on Days 3 (Groups 1 & 3), 8, 15, 29, and 44. Both eyes from Group 3 animals were collected and preserved. Anterior and posterior ocular segment and globes were evaluated microscopically.
- PK - Vitreous humor and retina from each eye of Groups 1 & 2 animals were collected, weighed and quickly frozen in liquid N_2. Samples were shipped to the sponsor for analysis of residual oligonucleotide levels by a capillary electrophoresis method. The lower limits of detection and quantitation for ISIS 2922 extracted from retina were 0.01 and 0.05 μM, respectively. The lower limits of detection and quantitation for ISIS 13312 in retina and vitreous were 0.01 and 0.05 μM, respectively.

Results:

- Mortality and Clinical Signs - No death occurred. Swollen conjunctivae, red, conjunctivae, injected sclerae, and red sclerae were noted in the eyes soon after receiving ISIS 2292, ISIS-13312 or vehicle control injection.
- Body Weights - No remarkable changes attributable to the treatment were seen.
- Ophthalmology - No noticeable changes could be identified by examination in vehicle or ISIS 13312 treated eyes. Signs of slight cloudy vitreous were observed in several animals treated with either 33 or 82.5 µg of ISIS 2922 on Days 3 (8/15 OD & 8/15 OS), 8 (11/12 OD & 11/12 OS), and 15 (9/9 OD & 5/9 OS). Vitreous floaters containing organized fibrin as results of ↑ protein contents in the vitreous secondary to mild cyclitis were also identified in ISIS 2922 treated animals on Days 8 (6/12 OD & 8/12 OS), 15 (9/9 OD & 7/9 OS), 29 (4/6 OD), and 44 (1/3 OD). Apparently, cyclitis persisted longer in the eyes treated with 82.5 (or 10 µM) of ISIS 2922. Flare (↑ protein), neovascularization of the iris, inflammation of irides and mild posterior synechia were also revealed by indirect biomicroscopic examination in Group 1 animals that were treated with ISIS 2922 on Day 3 but not Days 8 or 15. A mild decrease in IOP was recorded on Days 3 and 8 in Group 1 animals. The reduction in IOP was not noted in Groups 2 & 3 animals.
- Gross and Microscopic Pathology - No macroscopic changes were noted in any animal during necropsy. No microscopic changes in the eyes of Group 3 animals were noted.
- PK - Dose-dependent increases in the concentrations of ISIS 2922 and ISIS 13312 in vitreous were noted. The levels of ISIS 2922 and 13312 in vitreous and retina are shown in the following table. **Toxicokinetics**

[6] ISIS 13312 (5'-GCG TTT GCT CTT CTT CTT GCG-3') has the same sequences as ISIS 2922, but contains 2'-methoxyethoxy modifications on residues 1-6 and 16-20.

of retinal samples from eyes treated with ISIS 2922 collected on Days 3, 8, and 15 were not available due to sample loss during laboratory accident as indicated by the sponsor.

Sampling Day	Dose							
	4 μM				10 μM			
	Vitreous		Retina		Vitreous		Retina	
	ISIS 2922 Concentrations (μM)							
	Parent	Total	Parent	Total	Parent	Total	Parent	Total
3	0.87 ± 0.84	1.9 ± 1.9	SL[b]	SL	5.2 ± 3.6	9.9 ± 6.3	SL	SL
8	0.11 ± 0.06	0.27 ± 0.08	SL	SL	0.87 ± 0.29	3.4 ± 1.7	SL	SL
15	<0.01	<0.01	SL	SL	<0.11	<0.36	SL	SL
29	BLD[a]	BLD	BLD	BLD	BLD	BLD	0.10 ± 0.03	0.40 ± 0.10
44	BLD	BLD	BLD	BLD	BLD	BLD	<0.13	<0.13
	ISIS 13312 Concentrations (μM)							
3	-	1.2 ± 0.4	-	3.9 ± 0.8	-	2.8 ± 1.6	-	6.2 ± 0.3
8	-	0.14 ± 0.10	-	2.9 ± 0.1	-	<0.17	-	4.3 ± 1.5
15	-	BLD	-	1.9 ± 0.8	-	<0.11	-	4.2 ± 0.3
29	-	BLD	-	6.4 ± 0.8	-	BLD	-	9.3 ± 1.4
44	-	BLD	-	5.1 ± 3.0	-	BLD	-	9.5 ± 3.6

[a] BLD = below detection limit; [b] SL = sample lost

3.2 REPEATED DOSE OCULAR TOLERABILITY TOXICITY STUDIES

3.2.1 MONKEY STUDIES

3.2.1.1 ISIS 2922-AS01, 5-Week Toxicity Study of ISIS 2922 Administered by Intravitreal Injection to Cynomolgus Monkeys (Vol. 6, p.001)

Study Nº: 6490-100
Report Nº: ISIS 2922-AS01
Study Aims: To evaluate local and systemic toxicity of ISIS 2922 when administered biweekly (left eye) and weekly (right eye) by intravitreal injection to cynomolgus monkeys for a total of 2 and 4 injections, respectively.
Compound: ISIS 2922, 0.033, 0.1, and 0.33 mg/ml (Lot Nº: ISI-0029 to ISI-0031)
Dose and Route: 0, 3.3, 10, and 33 μg/0.1 ml/intravitreal injection.
Vehicle Control: Carbonate buffered 0.9% NaCl (Lot Nº: ISI-0028)
Animal: ♂ & ♀ young adult/adult cynomolgus monkeys 2.3-4.0 kg.
Study Site:
Study Date (In-Life): 5/07/93 to 7/12/93
GLP Compliance: Yes
Study Design: Groups of 3-5 monkeys received intravitreal injections of 100 μl of ISIS 2922 or vehicle control. The doses and dosing frequency are listed in the following table. All animals sacrificed during Week 5 except 2/sex from Groups 1 & 4 that were allowed to have a 5-week of recovery period.

Group	ISIS 2922 Dose/Injection (μg)	Dose Vol. (ml)	Dosing Frequency (Days)		Nº Animal	
			Right Eye	Left Eye	♂	♀
1 (control)	0	0.1	1, 8, 15, & 22	1 & 15	5	5
2	3.3	0.1	1, 8, 15, & 22	1 & 15	3	3
3	10	0.1	1, 8, 15, & 22	1 & 15	3	3
4	33	0.1	1, 8, 15, & 22	1 & 15	5	5

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weight - 1x/pretest, day 1 and 1x/week thereafter.
- Food Consumption - 1x/day (qualitative inspection).
- ECG - 1x/pretest and day 28 on anesthetized animals with Leads I, II, III, aVR, aVL, and aVF.

- Ophthalmic Examination (Indirect Ophthalmoscope and Slitlamp Biomicroscope) - Days 1, 8, 15, and 28 before treatment and on Day 28 for all animals and Days 49 & 64 for recovery phase animals. Photographs of ocular abnormalities were taken on Days 15, 22, 28, 49, and 64. Electroretinograms (ERG) were done on Day 28 for all animals and on Day 64 for recovery phase animals. Samples of vitreous from both eyes of five selected animals were collected on Day 28 and cultured for evaluation of bacterial growth. The following aerobic organisms were routinely being examined *Actinobacillus, Bacillus, Corynebacterium, Dermatophilis, Erysipelothrix, Escherichia, Klebsiella, Moraxella, Pasteurella, Pseudomonas, Staphylococcus,* and *Streptococcus.*
- Clinical Pathology - Clinical chemistry and hematology analyses will be performed on all animals during Weeks -2 and 5. Urinalysis was done during Week -2 only. The following parameters were evaluated.

Hematology	
Red blood cell count; White blood cell count	Blood cell morphology
Hemoglobin	Differential blood cell count
Hematocrit	Nucleated red blood cell count
Mean corpuscular volume	Corrected white blood cell count
Mean corpuscular hemoglobin	Segmented neutrophil count; Band neutrophil count
Mean corpuscular hemoglobin concentration	Lymphocyte count
Platelet count	Monocyte count
Prothrombin time	Eosinophil count
Activated partial thromboplastin time (aPPT)	Basophil count
Clinical Chemistry	
Glucose	Gamma-glutamyl transferase
Urea nitrogen	Creatine kinase
Creatinine	Calcium
Total protein; Albumin; Globulin	Inorganic phosphorus
Cholesterol	Sodium
Aspartate aminotransferase (AST)	Chloride
Alanine aminotransferase (ALT)	Potassium
Triglycerides	
Urinalysis	
Volume; Specific gravity	Bilirubin
pH	Blood
Protein	Urobilinogen
Glucose	Physical appearance
Ketones	Microscopic examination of sediment

- Terminal Sacrifice and Necropsy - Weeks 5 (3/sex/group) &10 (recovery phase - Groups 1 & 4, 2/sex). The following organs (when present) were weighed and paired organs were weighed separately: Adrenals, Ovaries, Brain, Testes, Kidneys, Thyroids with Parathyroid, and Liver. The following (when present) or representative samples were preserved in 10% phosphate-buffered formalin, unless otherwise specified, and embedded in paraffin, sectioned, stained with haematoxylin and eosin.

Adrenals	Gallbladder	Mammary gland (females only)	Skin
Aorta	Heart	Ovaries	Spleen
Brain	Ileum	Pancreas	Stomach
Cecum	Jejunum	Pituitary	Testes
Cervix	Kidneys	Prostate	Thymus
Colon	Lesions	Rectum	Thyroids with parathyroid
Duodenum	Liver	Salivary gland (submandibular)	Trachea
Esophagus	Lungs	Sciatic nerve	Urinary bladder
Epididymides	Lymph nodes (mesenteric, anterior cervical, and inguinal)		Uterus
Eyes (preserved in Zenker's solution for scheduled sacrifice animals)		Skeletal muscle	Vagina
Femur with bone marrow (articular surface of the distal end)		Spinal cord (cervical, mid- thoracic, and lumbar)	

Eyes from all animals (both Weeks 5 and 10 sacrifice) were examined microscopically. Sections from each animal in the control and high-dose groups (Week 5 sacrifice only), and one Group 3 ♂ that was sacrificed during Week 4 as a result of endophthalmitis (right eye) were also examined. Bone marrow smears from the sternum of each animal at each scheduled sacrifice were prepared, stained with Wright's stain, and retained for possible examination.

Results: Due to acute uveitis, some animals were not dosed in OD either on Days 15 (1♂ @ 10 μg) or 22 (control: 1♀; 3.3 μg: 1♀; 10 μg: 1♂, 2♀; 33 μg: 3♂, 1♀).

- Mortality and Clinical Signs - One control ♂ had pneumonia on Day 1 and was treated with 2 mg/kg of gentamicin (im, bid) for 8 days and 40,000 IU of penicillin BP (im, sid) for 13 days. One Group 3 ♂ had endophthalmitis and was sacrificed on Day 28. Signs of ocular abnormalities, opaque eyes and constricted pupils, were noted for some animals in ISIS 2922-treated and control groups. These changes were seen primarily in the right eye, except in the control animals, and first appeared during Days 15 to 18. These ocular abnormalities generally resolved during the recovery period. There were no systemic effects observed.
- Body Weight - No test material-related effect on body weight was noted.
- Food Consumption - Low food consumption was observed intermittently in animals from all groups.
- ECG - Neither significant changes in P-R, QRS, and Q-T intervals nor arrhythmia nor conduction disturbances were observed.
- Ophthalmic Examination - On Day 15 ophthalmic examinations (before dosing), cyclitis (inflammation in the posterior segment - ciliary body and vitreous cavity) was noted in the right eye of one each Group 2 & 3 monkeys. On Day 22, ocular inflammation was identified in additional animals (Group1: 2♂ & 4♀; Group 2: 2♀; Group 3: 1♂ & 2♀; Group 4: 4♂ & 1♀). The incidence of ocular inflammation detected on or prior to Day 28 as shown in the following table.

Group	Dose μg/injection	Nº of Affected Eyes						
		Right Eye (OD)			Left Eye (OS)			Total
		♂	♀	♂ + ♀	♂	♀	♂ + ♀	
1	0	0/5	1/5	1/10	2/5	3/5	5/10	6/20
2	0.33	2/3	2/3	4/6	0/3	0/3	0/6	4/12
3	1.0	1/3	2/3	3/6	0/3	0/3	0/6	3/12
4	3.3	4/5	0/5	4/10	2/5	1/5	3/10	7/20

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Inflammation of the iris and the presence of protein and inflammatory cells the anterior chamber were observed in the more severe cases of cyclitis. In addition, blocking vision of the fundus by protein and fibrin in the pupillary space was also noted in the more severe cases. Data presented in the above table showed that ocular inflammation was sporadic and might be as consequence of intravitreal injection.

Results from ERG performed on Day 28 showed normal A-waves, an indicative of normal photoreceptor cells, in all monkeys and decreased or absent B-waves in some animals. These ERG abnormalities occurred only in animals with ocular inflammation as revealed by the indirect and biomicroscopic examinations. The abnormal observations in B-waves might indicate defected cells in the inner nuclear layers of retina or abnormalities in the conductivity as consequence of ocular inflammation.. **However, it is worthy to note that ERG B-waves can provide a measure of cone and rod system activity in the cynomolgus monkeys and humans[7]. Therefore, decreases or absence in B-waves might be as results of abnormal retinal neuron functions.**

Staphylococcus aureus (one each ♂ @ 10 and 33 μg) and *Staphylococcus spp.* (1♀ @ 10 μg) were isolated from vitreous of the right eye of some animals.

- Clinical Pathology - No apparent effect on clinical pathology results was identified.
- Gross and Histopathology - There were no treatment-related changes in absolute or relative organ weights. At the Week 5 sacrifice, lymphohistiocytic or suppurative (mainly neutrophilic infiltrate) inflammatory changes were identified in the various eye structures of animals that had ocular inflammation clinically. These histopathological changes were consistent with the ophthalmic findings. These changes were unlikely caused by ISIS 2922 treatment as similar findings were also identified in the control eyes.
- Assessment in Recovery Animals - Two monkeys/sex from the control and high-dose (33 μg) groups were allowed to have a 5-week recovery period. One monkey from each group had no ocular changes at the

[7] Electrical Phenomena in the Retina by EL Berson, in Adler's Physiology of the Eye, 9th Edition, WM Hart, Jr. (Editor), Mosby Year Book, 1992.

onset of and throughout the recovery period. All of the affected eyes showed improvement on subsequent ophthalmological examinations (Days 49 and 64). However, some changes (e.g., posterior synechia) persisted in some eyes. Normal ERG values were noted on Day 64 in all but one high dose ♂ that had a further decrease in the B-wave amplitude in the left eye. Histopathological examination of this eye showed retinal degeneration and multifocal areas of hemorrhage and lymphohistiocytic infiltrations. Moderate, focal lymphohistiocytic inflammation was identified in ½ ♂ (OD) @ 33 μg. Slight, focal lymphohistiocytic inflammation was observed in OS of 2/2 ♀ @ 0 and ½ ♀ @ 33 μg.

These findings from the recovery period indicated that no latent effects were caused by the test material, and majority of the preexisting ocular changes showed substantial improvement after discontinuing treatment.

3.2.1.2 ISIS 2922-AS03, 4-Week Toxicity Study of ISIS 2922 Administered by Intravitreal Injection to Cynomolgus Monkeys (Vol. 7, p. 001)

Study Nº: 6490-102
Report Nº: ISIS 2922-AS03
Study Aims: To evaluate local and systemic toxicity of ISIS 2922 when administrated by intravitreal injection to cynomolgus monkeys on a biweekly or weekly basis for a total of 2 or 4 injections, respectively.
Compound: ISIS 2922, 0.34, 0.68 and 1.37 mg/ml (Lot Nº: ISI-0039 to ISI-0041)
Dose and Route: 0, 17, 34 or 69 μg /0.05 ml/intravitreal injection.
Vehicle Control: Carbonate buffered 0.9% NaCl (Lot Nº: ISI-0028)
Animal: ♂ & ♀ young adult/adult cynomolgus monkeys 2.3-6.0 kg.
Study Site:
Study Date (In-Life): 6/18/93 to 7/13/93
GLP Compliance: Yes
Study Design: Fomivirsen in 50 μl was administered to two groups of cynomolgus monkeys (3 animals/sex) at doses of 17, 34, and 69 μg/eye. The estimated vitreal concentrations achieved by these doses were 2, 4, and 8 μM, respectively (assuming a vitreal volume of 1.2 ml). The dosing schedule for each group is listed as followings:

Group	Dose Vol.	ISIS 2922 Dose/Injection (μg)		Dosing Frequency (Days)		Nº Animal	
	(μl)	OD	OS	OD	OS	♂	♀
1 (control)	50	0	0	1, 7, 15, 22	1, 7, 15, 22	3	3
2	50	17	34	1, 7, 15, 22	1, 7, 15, 22	3	3
3	50	69	69	1, 7, 15, 22	1, 15	3	3

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weight - 1x/week before initiation of treatment, day 1 and 1x/week thereafter.
- Food Consumption - daily visual inspection (qualitatively).
- Ophthalmic Examination Days 1, 7, 15, and 22 before treatment. Photographs of ocular abnormalities were taken on Days 15 and 22. Electroretinograms (ERG) were done on Day 22. Samples of vitreous from eyes that showed clinical signs of uveitis, miosis, and cyclitis and from the contralateral (unaffected) eye were collected on Day 26 (prior to necropsy) and cultured for evaluation of bacterial growth.
- Clinical Pathology - Clinical chemistry and hematology analyses will be performed on all animals during pretreatment and week 4. The following parameters were evaluated.

Hematology		Clinical Chemistry	
Red blood cell count	Blood cell morphology	Glucose	Aspartate aminotransferase (AST)
White blood cell count	Differential blood cell count	Urea nitrogen	Alanine aminotransferase (ALT)
Hemoglobin	Nucleated red blood cell count	Creatinine	Gamma-glutamyl transferase
Hematocrit	Corrected white blood cell count	Total protein	Creatine kinase
Mean corpuscular volume	Segmented neutrophil count	Albumin	Calcium

Mean corpuscular hemoglobin	Band neutrophil count	Globulin	Inorganic phosphorus
Mean corpuscular hemoglobin concentration	Lymphocyte count	Cholesterol	Sodium
Platelet count	Monocyte count	Triglycerides	Chloride
Prothrombin time	Eosinophil count		Potassium
Activated partial thromboplastin time (aPPT)	Basophil count		

- Terminal Sacrifice and Necropsy - Weeks 4 (3-4 days after the last dosing). The following (when present) or representative samples were preserved in 10% phosphate-buffered formalin, unless otherwise specified, and embedded in paraffin, sectioned, stained with haematoxylin and eosin.

Adrenals*	Gallbladder	Mammary gland (females only)	Skin
Aorta	Heart	Ovaries*	Spleen
Brain*	Ileum	Pancreas	Stomach
Cecum	Jejunum	Pituitary	Testes*
Cervix	Kidneys*	Prostate	Thymus
Colon	Lesions	Rectum	Thyroids with parathyroid*
Duodenum	Liver*	Salivary gland (submandibular)	Trachea
Esophagus	Lungs	Sciatic nerve	Urinary bladder
Epididymides	Lymph nodes (mesenteric, anterior cervical, and inguinal)		Uterus
Eyes (preserved in Zenker's solution for scheduled sacrifice animals)		Skeletal muscle	Vagina
Femur with bone marrow (articular surface of the distal end)		Spinal cord (cervical, mid- thoracic, and lumbar)	

*Organs (when present) were weighed and paired organs were weighed separately.

Eyes from all animals (both Weeks 5 and 10 sacrifice) were examined microscopically. Sections from each animal in the control and high-dose groups (Week 5 sacrifice only), and one Group 3 ♂ that was sacrificed during Week 4 as results of endophthalmitis (right eye). Bone marrow smears from the sternum of each animal at each scheduled sacrifice were prepared, stained with Wright's stain, and retained for possible examination.

Results: Due to inflammation in the eye (OS or OD), some animals did not receive treatment on Days 15 (1 Group 1 ♀, OD) and /or 22 Day (1 Group 1 ♀, OD; 1 Group 2 ♀, OS; 1 Group 2 ♂, OD; 1 Group 3 ♂, OD).

- Mortality and Clinical Signs - No mortality was noted. Major ocular anomalies including opaque, constricted pupils, and clear or cloudy discharge were seen in some animals from each group (including controls). These observations appeared not to be drug-related.
- Body Weight - No treatment-related effects were observed.
- Food Consumption - Low food consumption was noted in some monkeys (Group 1: 1♂; Group 2: 2♀; Group 3: 1♂ & 1♀).
- Ophthalmic Examination [redacted] - Most often observed ocular changes were inflammation in the posterior segment, vitreous cavity and ciliary body (cyclitis). In the severe cases, inflammation of iris and presence of protein and inflammatory cells in the anterior chamber were identified. [redacted] examination revealed that the cells and flare migrated from the posterior chamber in to the anterior chamber through the pupillary opening. The incidence of cloudy eyes and ocular discharge detected on Day 22 are shown in the following table.

Group	Dose µg/injection		Nº of Affected Eyes					
			OD			OS		
	OD	OS	♂	♀	♂ + ♀	♂	♀	♂ + ♀
1	0	0	0/3	1/3	1/6	0/3	0/3	0/6
2	17	34	1/3	0/3	1/6	0/3	1/3	1/6
3	69	69	1/3	0/3	1/6	0/3	0/3	0/6

Results from ERG examination showed decreased or absent B-waves in animals with ocular inflammation revealed by indirect [redacted] evaluations. These observations might indicate defected cells in the inner nuclear layers of retina or abnormalities in the conductivity as consequence of ocular inflammation. No alterations in A-waves were noted an indicative of normal photoreceptor cells. These observations were consistent with the findings seen in the previous study report (see **3.2.1.1,** ISIS 2922-AS01).

No aerobic bacteria, including *Actinobacillus, Bacillus, Corynebacterium, Dermatophilis, Erysipelothrix, Escherichia, Klebsiella, Moraxella, Pasteurella, Pseudomonas, Staphylococcus, and Streptococcus,* were

isolated from vitreous samples from 5 unilaterally affected monkeys. Therefore, the ocular inflammation observed in these animals was not caused by aerobic bacterial infection.

- Clinical Pathology - No significant changes in hematological parameters were identified. Elevated ALT (SGPT) values (>2x of mean control value) was noted in 2♂ and 1♀ in Group 3 during Week 4 analysis indicating that liver function might be compromised as a result of drug treatment-induced systemic toxicity (?). Due to the equivocal liver enzyme findings, small numbers of animals used in each group, and no clinical chemistry analysis performed during pre-treatment, it would require further study to draw conclusions. The individual and mean ALT and AST values of each group during Week 4 evaluation are shown in the following table.

Parameters		Group 1		Group 2		Group 3	
		♂	♀	♂	♀	♂	♀
ALT	Individual	37, 36, 23	42, 32, 73	46, 47, 56	45, 55, 33	[illegible]9, 55	[illegible]4, 25, 46
	Mean	32 ± 7.8	49 ± 21.4	50 ± 5.5	44 ± 11	71 ± 16.6	68 ± 57.8
AST	Individual	27, 28, 26	31, 26, 25	24, 24, 32	30, 36, 29	25, 19, 22	35, 22, 26
	Mean	27 ± 4.6	27 ± 3.2	27 ± 4.6	32 ± 3.8	22 ± 3.0	28 ± 6.7

- Gross Pathology - Opaque cornea was identified in 1♂ each in Groups 1 & 3 and 1♀ in Group 2. Enlarged anterior cervical lymph node was identified in 1 Group 3 ♂ and 1 Group 1 ♀. Light focus or diffused rough surface in liver was also noted in 3 animals (1♂ each in Groups 1 & 2 and 1♀ in Group 2).
- Histopathology - Microscopic evaluation was limited to the eyes from each group. Incidence of microscopic changes (minimal→moderate) is listed in the following table. Apparently, higher accumulated events were noted in Group 3 (high dose).

Microscopic Findings in Eyes		Group 1		Group 2		Group 3	
		♂	♀	♂	♀	♂	♀
Hemorrhage	OD			1		1	
	OS	1					
Supprative Inflammation (presence of PMN and fibrinous material in the chambers of the eye)	OD			1		1	
	OS	1					
Lymphohistiocytic Inflammation (presence and perivascular accumulation of a mixture of lymphohistiocytic cells in various structure of the eye)	OD		1			1	2
	OS				1	2	2
Retinal Degeneration	OD					1	
	OS						

3.2.1.3 ISIS 2922-AS08, Ocular Toxicity and Systemic Exposure of ISIS 2922 Following Intravitreal Injection in Cynomolgus Monkeys (Vol. 8, p. 001)

Study Nº: [illegible]6490-124
Report Nº: ISIS 2922-AS08
Study Aims: To evaluate the effects of ISIS 2922 on ocular tissues in cynomolgus monkeys that have received prophylactic corticosteroid treatment and to determine the systemic exposure (i.e. plasma, kidney, liver, and uterus concentrations) following multiple intravitreal injection.
Compound: ISIS 2922, 2.3 mg/ml (Lot Nº: ISI-0215 to ISI-0041)
Dose and Route: 0 or 115μg /0.05 ml/intravitreal injection, 1x/2wk for a total of 8 doses.
Vehicle Control: Carbonate buffered 0.9% NaCl (Lot Nº: ISI-0028)
Animal: ♂ & ♀ young adult/adult cynomolgus monkeys [illegible] 2.3-4.8 kg.
Study Site: [illegible]
Study Date: 03/22/96 - 08/14/97
In-Life: 4/09/96 to 07/17/96
GLP Compliance: Yes
Study Design: Monkeys were given intravitreal injections of ISIS 2922, 0 or 115 μg (equivalent to a 10 μM intravitreal concentration of fomivirsen initially assuming a 1.5-ml vitreal volume), to both eyes on Days 1, 15, 28, 42, 56, 70, 83, and 93. Prophylactic steroid treatment (10 mg triamcinolone acetonide, USP) was given to Group 2 animals only by subconjunctival injection at the time of fomivirsen treatment. Due to

severe ocular toxicity, intravitreal injection of ISIS 2922 was discontinued for animals in group 3 that were not receiving subconjunctival steroid injections.

Group	Dose Vol. (μl)	Dose Level (μg)	Steroid Treatment[a]	N° Animal	Sacrifice (Study Day)
1 (control)	50	0	No	3♂, 3♀	3, 30, 98
2	50	115	Yes	9♀	3, 30, 98
3	50	115	No	3♂, 3♀	3, 30, 98

[a] A subconjunctival injection of triamcinolone (10 mg) was given as prophylactic steroid treatment.

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weight - 1x/week before initiation of treatment, Day 1 and 1x/week thereafter.
- Food Consumption - daily visual inspection (qualitatively).
- Ophthalmic Examination [redacted] and IOP Measurement - 1x/pre-test and Days 3, 8, 15, 22, 28, 30, 34, 42, 51, 56, 63, 70, 78, 83, 93, and 98. Photographs of ocular abnormalities were taken at the discretion of the ophthalmologist.
- Terminal Sacrifice and Necropsy - 1/sex from Groups 1 & 3 and 3 from Group 2 on Days 3 , 30, and 98. External body surface, all orifices and the eyes were examined grossly. Eyes from all animals were preserved in 2% paraformaldehyde/2.5% glutaldehyde. Globes were dissected. The anterior and posterior ocular segments were sectioned and examined microscopically.
- PK/TK - On Day1, blood samples were collected from 3 Group 2 monkeys that were scheduled for sacrifice on Day 3 at 0, 1, 3, 5, 7, and 24 hr post-doing. Approximately 1 g of liver, kidneys, uterus, and ovaries were collected from all Group 2 animals on Days 3, 30 and 98, and quick-frozen in liquid N_2. Analysis of plasma samples for fomivirsen and its oligonucleotide metabolites was performed with a validated [redacted] method.

Results:

- Mortality and Clinical Signs - No deaths occurred. No unusual clinical signs were observed in Groups 1 & 2 animals. Group 3 animals were sacrificed on Days 3 (1♂, 1♀), 5 (1♀), 16 (1♂), and 23 (1♀) due to severe ocular toxicity (characterized as swollen conjunctivae; dilated or constricted pupils; squinting; opaque, cloudy, or closed eyes; and periobital swelling) after 1st intravitreal injection of ISIS 2922. One remaining Group 3 (♂) monkey was kept in study without treatment and sacrificed on Day 98.
- Body Weight - No apparent effects on the body weight could be characterized.
- Food Consumption - Occasionally, low or no food consumption was noted in Groups 2 and 3.
- Ophthalmic Examination [redacted] and IOP Measurement - Group 2 (115 μg of fomivirsen + triamcinolone) or in control animals had no ocular alterations. A transient decrease in IOP was identified in 3/6 Group 2 animals. Group 3 monkeys had acute anterior uveitis and cyclitis characterized by aqueous flare, inflammatory cells in the anterior chamber, hemorrhage into anterior chamber, and neovascularization of the iris, anterior synechiae of both irides as revealed by ophthalmic examination on Days 5, 8, 15 and 22. Due to protein and cellular exudate in the pupil, the posterior segment was not visible. In addition, low IOP (3 mmHg vs 15 mmHg baseline) was identified on Days 8, 15, and 22 in OD of one Group 3 ♀ that was sacrificed on Day 23. One Group 3 ♂ that was sacrificed on Day 16 had increased IOP (46 mmHg vs 16 mmHg baseline) in both eyes. Posterior synechia of irides were identified in 4/5 eyes with cyclitis. Inflammatory vasculitis was also observed in a few eyes. The incidences of ophthalmic observations are presented in the following table.

Ophthalmic Observations	Control	115 μg ISIS 2922 + 10 mg Triamcinolone	115 μg ISIS 2922
No Visible Lesion	20/20	20/20	7/12
Cyclitis			5/12
Iris, neovascularization			2/12
Retina, edema/ infiltrate			2/12
Synechia			4/12
Hemorrhage			4/12
Hypopion			1/12

- Terminal Sacrifice and Necropsy -

  Group 1: No gross and microscopic pathological changes were identified.

  Group 2: Light-colored and red area on the sclera were observed during necropsy. Microscopic examination revealed scleral edema, scleral hemorrhage or scleral necrosis with acute or chronic active scleral inflammation (1/3 OD & 3/3 OS on Day 3; 2/3 OD & 1/3 OS on Day 30; 2/3 OD & 1/3 OS on Day 98). These pathological changes might be due to subconjunctival injection of cortical steroid or ISIS 2922 treatment induced toxicity as similar findings were observed in some Group 3 animals.

  Group 3: No macro- and micro-scopic changes were identified in the animals that were sacrificed on Days 3 (1♂ & 1♀) & 98 (1♂). One ♀ was sacrificed on Day 5 had bilateral ocular lesions. Acute inflammation with moderate→severe edema of the choroid and sclera were major histopathological findings. Acute inflammation involved the ciliary body, aqueous, retina, and vitreous of both eyes and the optic nerve streak of the left eye. Other microscopic findings included epithelial degeneration and moderate hyperplasia of the ciliary body; minimal→slight hemorrhage of the ciliary body, retina, and vitreous; minimal chronic inflammation of the sclera and ciliary body; moderately→severe proteinaceous exudate in the aqueous of the left eye; and moderate epithelial hyperplasia of the ciliary body and minimal proteinaceous exudate in the aqueous of the right eye. The male that was sacrificed on Day 16 had chronic inflammation involving ciliary body, retina, sclera, vitreous, and optic nerve streak; anterior synechiae and optic disk cupping of the of the left eye; and slight edema of the choroid and minimal clumping of the retinal pigment epithelium in the left eye. The female that was sacrificed on Day 23 had moderate to severe edema or proteinaceous exudate in the choroid, aqueous, and vitreous; detached retina with moderate degenerative changes; edema, hyperplasia, chronic inflammation, and epithelial degeneration in ciliary body; and minimal chronic active inflammation of the vitreous, moderately→severe chronic inflammation of the optic nerve streak, and minimal chronic inflammation of the sclera.

- PK/TK - No detectable levels of ISIS 2922 in the plasma samples were obtained. The levels of ISIS 2922 in the liver, kidney cortex, kidney medulla, uterus or ovary were either below the limit of detection or quantitation (0.35 μg/g of tissue) as determined by the method of CGE.

3.3 Acute Toxicity Study - Single-Dose Intravenous

LD_{50} in mice was estimated as part of range finding studies for the mouse micronucleus assay (see 3.7.4 for detail). Groups of 5/sex ICR mice were given a single dose of ISIS 2922 (600, 800, or 1,000 mg/kg) or vehicle control by tail vein injection. The mice were observed for a 7-day period for mortality, body weight, and clinical condition. The total incidence of mortality was 30%, 70% and 70% of treated mice at the 600, 800, and 1,000 mg/kg dose levels, respectively. Clinical evidence of toxicity included lethargy at all dose levels and irregular breathing at 800 and 1,000 mg/kg. Changes in body weight at any dose level were not noted. The LD_{50} value for fomivirsen was calculated to be 720 mg/kg.

3.4 Multiple-dose Intravenous Toxicity Study

3.4.1.1 ISIS 2922-AS02, 28-Day Intravenous Toxicology of ISIS 2922 in Mice (Vol. 9, p. 001)

Study Nº:	Nº 066865
Report Nº:	ISIS 2922-AS02
Study Aims:	To evaluate the intravenous toxicity of ISIS 2922 when administrated by caudal vein injection to (on alternate days) CD-1 mice for 28 days.
Compound:	ISIS 2922 (Lot Nº: ISI-0029 to ISI-0032)
Dose and Route:	0.2, 1.0, 5.0, and 50.0 mg/kg iv at an approximate rate of 2 ml/min, via the caudal vein for a total of 14 doses.
Vehicle Control:	Carbonate buffered saline (LotNº: ISI-0028), ~300 mOsm.
Animal:	CD-1 mice, 6-8 weeks of age, 20-30 g.
Study Site:	
Study Date:	5/11/93 to 6/09/93
GLP Compliance:	Not specified.
Study Design:	

Group	Dose (mg/kg)	Dose Vol. (ml/kg)	Nº Animals/Sex/Group Experiment 1	Experiment 2
1 (Placebo)	0	15	5/sex	5/sex
2	0.2	6	5/sex	5/sex
3	1.0	10	5/sex	5/sex
4	5.0	15	5/sex	5/sex
5	50.0	10	2♂, 3♀	3♂, 2♀

The following observations were conducted:

- Mortality and Clinical Signs - 2x/day.
- Body Weight and Food Consumption - Days 1 (prior to dosing), 8, 15, 22, 27 and 28.
- Clinical Pathology - Clinical pathology analyses will be performed on all surviving animals at termination of the study.

  Hematology: Analyses performed were: Erythrocyte count; Total leukocyte count; Differential leukocyte count; Hemoglobin; Hematocrit; Platelet Count; MCH; MCV; MCHC.

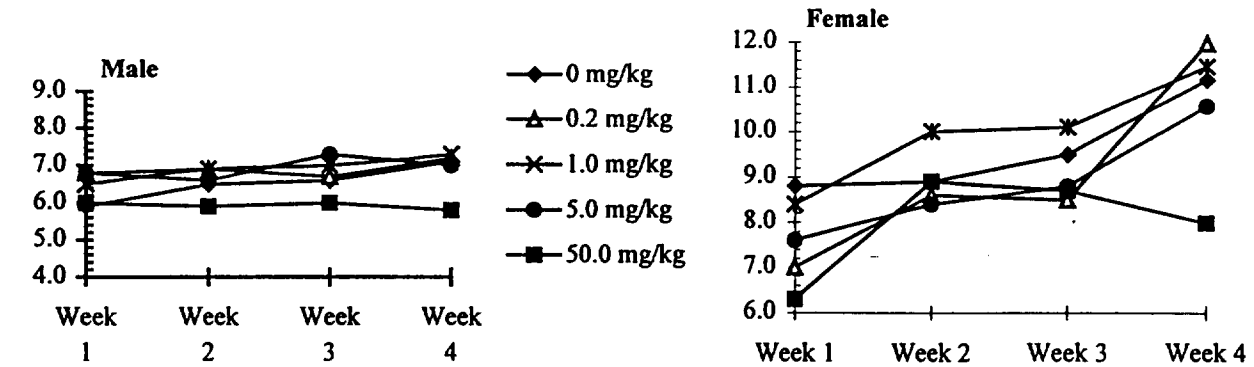
  Serum Chemistry: Analyses performed included: Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT); Total Protein; Albumin; Globulin (calculated); A/G Ratio (calculated); Blood Urea Nitrogen; Creatinine; Cholesterol; Triglyceride; Total Bilirubin; Glucose; Alkaline Phosphatase (ALP); Phosphorous; Sodium; Potassium; Chloride; Calcium.
- Terminal Sacrifice and Necropsy - All surviving animals were subjected to a necropsy on Day 28.

  Organ Weights: The following organs will be weighed: Adrenal glands (2); Kidneys (2); Liver; Testes (2)/Ovaries (2); Lungs; Spleen; and Heart. Paired tissues will be weighed together.

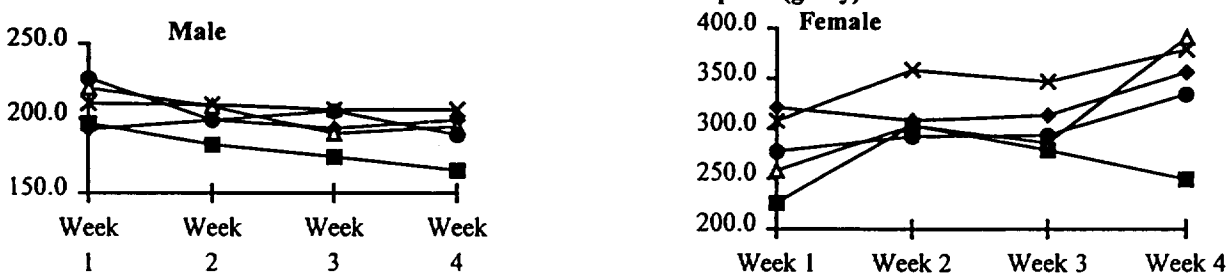
  Histopathology: The following organs and tissues were collected and preserved for all animals, including those that died or were sacrificed moribund, they were: adrenal glands (2); brain; cervical lymph nodes*; gastrointestinal tract (stomach, duodenum, jejunum, ileum, colon); heart with aorta; injection site*; kidneys* (2); liver*; lungs with mainstem bronchi; mesenteric lymph nodes*; pancreas; peripheral nerve; pituitary; prostate; salivary glands; skeletal muscle; spleen*; sternum with bone marrow*; testes/ovaries (2); thyroid with parathyroid glands; thymus; urinary bladder; uterus; and gross lesions. Tissues from the control and high dose groups, animals that died or were sacrificed moribund, and "target" tissues (indicated by an asterisk*) from all animals were examined microscopically.

Results:

- Mortality and Clinical Signs - One male at 5.0 mg/kg died on Day 19 and one male at 1.0 mg/kg, with clinical signs of slight yellow perineal staining on Days 10-15 and moderate yellow perineal staining, labored respiration and lethargy on Day 21, was sacrificed moribund on Day 22. No deaths occurred in the high dose group (50.0 mg/kg) or the lowest dose group (0.2 mg/kg). Other treatment caused clinical signs observed in the animals @ 50 mg/kg included hematoma-like sites around the caudal veins with or without erythema (slight→moderate) (2♂ and 4♀ between Days 21 and 27), and ↑ post-injection bleeding, and cold to touch with slight→moderate lethargy when fasted body weights were taken prior to necropsy. The observations of hematoma/erythema around injection sites and ↑ post-injection bleeding were consistent with hematological findings of ↓ platelet numbers and histopathological observations of megakaryocyte depletion.
- Body Weight and Food Consumption - No significant differences in mean body weight between ISIS 2922-treated and control animals were noted. Significant decreases in absolute (g/day; top panels) and relative (to body weight, g/kg/day; bottom panels) food consummations values were noted for animals @ 50 mg/kg as depicted in the following figures.



Absolute Food Consumption (g/day)



Relative Food Consumption (g/kg/day)

- Clinical Pathology - Significant decreases in platelet counts (↓ 60% and 71% in ♂ and ♀, respectively) and significant increases in relative (↑11x) as well as absolute monocyte counts (↑12-15x) were observed in the animals @ 50 mg/kg. Although highly variable data presented in the report, significantly elevated AST (♂: 2x; ♀: 5x) and ALT (♂: 4.3x; ♀:3.8x), an indication of hepatic abnomality, were identified in high dose animals. In addition, high dose animals had ↑ALP (♂: 1.7x; ♀: 1.3x) values and decreased cholesterol and triglyceride (♂ only).
- Gross and Histopathology -
  Unscheduled Deaths: Gross necropsy observations for the animal @ 5.0 mg/kg that died on Day 19 included dark red material which resembled a large hematoma near the atrium/aortic region of the heart and a small thymus. Histopathological lesions included mild→moderate inflammation of duodenum, kidney, lung and injection site, moderate congestion of duodenum and liver, a postmortem blood clot attached to the endocardium, urinary bladder concretion, mild focal hemorrhage at the injection site and moderate multifocal hemorrhage of the lung. Based on histopathological evaluation, the probable cause of death was systemic inflammation, likely due to infection. Gross necropsy revealed a distended urinary bladder for the animal @ 1.0 mg/kg that was sacrificed on Day 22. Microscopic examination showed mild→moderate nonspecific congestion in kidneys, liver, lymph nodes and spleen, moderate vacuolation of the urinary bladder epithelium and moderate focal hemorrhage at the injection sites.
  Scheduled Sacrifices: Increased relative liver was identified in animals @ 50 mg/kg with values 23-24%. Relative as well as absolute spleen weights were also significantly increased in animals @ 5 mg/kg (↑30-38 %) and 50.0 mg/kg (↑170-240 %). Treatment-related gross findings were enlarged spleens (♂ & ♀ @ 5.0 and 50.0 mg/kg) and purple hematoma-like areas around the injections sites (caudal veins) (♂ & ♀ @ 50.0 mg/kg). Chronic hepatocellular inflammation (dose-related) was identified in animals @ ≥1.0 mg/kg. Stromal hyperplasia in liver, kidney, lung, skeletal muscle, brain, heart, pancreas, salivary glands, glandular stomach, ileum, duodenum, thymus, urinary bladder, prostate and uterus, and megakaryocyte depletion in the bone marrow were observed in both males and females @ 50.0 mg/kg.

Therefore, intravenous injection of ISIS 2922 induced: (1) stromal hyperplasia and megakaryocyte depletion in the bone marrow of animals @ 50 mg/kg and (2) chronic inflammation (predominately monocytes, plasma cells, and lymphocytes) in the liver with dose-dependent severity of animals @ ≥1.0 mg/kg.

3.5 REPRODUCTIVE TOXICITY STUDIES

No studies were conducted. The sponsor agrees to initiate Seg I and II studies in the mice August, 1998.

3.6 CARCINOGEICITY STUDIES

No Studies were conducted.

3.7 GENETIC TOXICITY STUDIES

3.7.1.1 ISIS 2922-IS01, *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and *Escherichia coli* WP2 *uvr*A Reverse Mutation Assay with a Confirmatory Assay (Vol. 9, p. 181)

Study Nº:	Study No. TD158.501088
Report Nº:	ISIS 2922-IS01
Study Aims:	to evaluate the mutagenic potential of the test article (or its metabolites) by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at a selected locus of *E. coli* WP2 *uvr*A in the presence and absence of an S9 metabolic activation mix.
Compound:	ISIS2922 (Lot Nº: ISI-0033), 50 mg/ml
Dose:	6.7, 10, 33, 67, 100, 333, 663, 1000, 3333 and 5000 μg/plate
Vehicle Control:	Not indicated.
S9 Mix:	Rat liver S9 mix was prepared from the liver of male Sprague-Dawley rats that received ip injection of 500 mg/kg of Aroclor 1254 5 days prior to sacrifice.
Indicator Cells:	*Salmonella typhimurium*
Study Site:	
Study Date:	5/20/93 to 08/23/93
GLP/QAC Compliance:	Yes

Results: Data from two independent tests are summarized in the following table. Although slight increases in the revertants over the background control were noted when the test strain TA1538 incubated with ISIS 2922 in the absence of S9 mix, the increases were not dose-dependent and not statistically significant. Therefore, ISIS 2922 up to 5000 μg/plate, was not mutagenic in the presence or absence of S9 activation under the current testing condition.

3.7.1.2 ISIS 2922-IS02, *In Vitro* Mammalian Cytogenetic Test (Vol. 9, p. 245)

Study Nº:	Study Nº G95AP90.332
Report Nº:	ISIS 2922-IS02
Study Aims:	To determine the clastogenic potential of ISIS 2922 to induce chromosome aberration in Chinese hamster ovary (CHO) cells.
Compound:	ISIS2922 (Lot Nº: ISI-0128), 50 mg/ml
Dose:	Initial Test: 150, 500, 1500 μg/plate, and 5000; Independent Repeat Test: 625, 1250, 2500, and 5000 μg/plate; Confirmatory Repeated Test: 625, 1250, 2500, 3800, and 5000 μg/plate
Vehicle Control:	Bicarbonate buffered 0.9% saline, pH 8.7 (Lot Nº ISI-0129 & ISI-0028)
S9 Mix:	Rat liver S9 mix was prepared from the liver of male Sprague-Dawley rats that had ip injection of 500 mg/kg of Aroclor 1254 5 days prior to sacrifice.
Indicator Cells:	CHO-K1 cells (ATCC repository Nº CCL61)
Study Site:	
Study Date:	5/04/95 to 12/08/95
GLP/QAC Compliance:	Yes

Results: Data from initial experiment and two independent tests are summarized in the following table. **Significant and dose-dependent increases in structural chromosome aberrations were noted when cells were treated with ISIS 2922 (≥2500 μg/ml) for 44 hr without activation mix.** In the repeated comfirmatory experiment, a slight but not significant increase (~2x) in structural chromosome aberration was noted in cells treated with ISIS 2922 at 5000 μg/ml under the same condition.

[redacted]

3.7.1.3 ISIS 2922-IS03, L5178Y+/- Mouse Lymphoma Mutagenesis with a Confirmatory Assay (Vol. 9, p. 292)

Study Nº: [redacted] Study Nº G95AP90.702001
Report Nº: ISIS 2922-IS03
Study Aims: To determine the mutagenic potential of ISIS 2922 to induce forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells.
Compound: ISIS2922 (Lot Nº: ISI-0128), 50 mg/ml
Dose: 313, 625, 1250, 2500, and 5000 μg/plate
Vehicle Control: Bicarbonate buffered 0.9% saline, pH 8.7 (Lot Nº ISI-0129)
S9 Mix: Rat liver S9 mix was prepared from the liver of male Sprague-Dawley rats that had ip injection of 500 mg/kg of Aroclor 1254 5 days prior to sacrifice.
Indicator Cells: L5178Y/TK+/- cells, clone 3.7.2C
Positive Control:

Compound	S9	Concentration
Ethyl methanesulfonate	-	25 & 50 μl/ml
7,12-Dmethyl-benzanthracene (7,12-DMBA)	+	250 & 400 μg/ml

Study Site: [redacted]
Study Date: 5/05/95 to 8/22/95
GLP/QAC Compliance: Yes

Results: ISIS 2922, at concentrations up to 5000 μg/ml, did not cause mutation in L5178Y/TK+/- mouse lymphoma cells.

3.7.1.4 ISIS 2922-AS05, Micronucleus Cytogenetic Assay in Mice (Vol. 9, p. 328)

Study Nº: [redacted] Study Nº G95AP90.122
Report Nº: ISIS 2922-IS05
Study Aims: To determine the clastogenic potential of ISIS 2922 to induce micronucleated polychromatic erythrocytes in mouse bone marrow.

Compound: ISIS 2922 (Lot Nº: ISI-0128), 50 mg/ml
Dose: 0, 600, 800, or 1000 mg/kg iv for toxicity study and 145, 190, and 580 mg/kg for micronucleus assay
Vehicle Control: Bicarbonate buffered 0.9% saline, pH 8.7 (Lot Nº ISI-0129 & ISI-0028)
Positive Control: Cyclophosphamide (CP), 3 mg/ml, 60 mg/kg iv
Animals: ICR mice, 6-8 weeks of age
Toxicity Study - weighing 30-32.4 g for ♂ and 28.2-31.5 g for ♀, 5/sex/ dose group
Micronucleus Assay - weighing 26.7-34.4 g for ♂ and 23.5-30.3 g for ♀, 15-20/sex/dose group
Study Site:
Study Date: 4/20/95 to 8/28/95
GLP/QAC Compliance: Yes
Study Design:
Toxicity Study - Four groups of 5/sex mice were given a single iv injection of either vehicle, 600, 800, or 1000 mg/kg of ISIS 2922. Mice were monitored for 7 days for signs of toxicity and mortality. Body weights were recorded on Days 0, 1, 3, and 7.
Micronucleus Assay - The dose and group assignments are as followings:

Compound	Dose (mg/kg)	Nº of Mice/Dose	Nº of Mice Used for Bone Marrow Collection		
			24 hr	48 hr	72 hr
Vehicle Control	0	15/sex	5/sex	5/sex	5/sex
ISIS 2922	145	15/sex	5/sex	5/sex	5/sex
ISIS 2922	190	15/sex	5/sex	5/sex	5/sex
ISIS 2922	580	20/sex	5/sex	5/sex	5♂, 4♀
CP (Positive Control)	60	5/sex	5/sex		

Results:
Toxicity Study - The body weight changes and mortality data during 7-day observation are shown in the following table.

Parameters		Dose (mg/kg)							
		Vehicle Control		600		800		1000	
		♀	♂	♀	♂	♀	♂	♀	♂
Mortality		0/5	0/5	2/5	1/5	5/5	2/5	4/5	3/5
Body Weights (g)	Day 0	31.8	29.1	30.9	28.7	30.8	29.7	31.2	30.0
	Day 1	31.4	28.8	30.1	25.6	29.3	27.6	29.6	27.4
	Day 3	31.4	29.0	27.7	28.5	35.4	24.7	25.7	23.5
	Day 7	33.4	30.1	29.0	28.5	ND	27.9	30.9	23.1

Micronucleus Assay - A total of 6 high dose (580 mg/kg) ♀ were found dead within 2 days after dosing. Lethargy was noted in mice receiving 290 and 580 mg/kg. ISIS 2922 did not cause an increase in the incidence of micronucleated polychromatic erythrocytes. On contrast, cyclophosphamide, positive control, induced a significant increase in micronucleated polychromatic erythrocytes.

4. LABELING REVIEW:

5. OVERALL SUMMARY AND EVALUATION:

5.1 PHARMACOLOGY

ISIS 2922 (fomivirsen) was demonstrated to inhibit HCMV (clinical isolates or laboratory strain AD-169) replication in tissue culture systems as measured by plaque reduction, total viral yield, or immunoassays with ID_{50} values of ≤ 1 μM. It was shown to be effective in limiting viral replication of HCMV clinical isolates that were resistant to either ganciclovir, forscarnet or cidofovir in human diploid fibroblasts. Only one of the 21 clinical CMV isolates evaluated required a concentration of fomivirsen 2 μM to achieve 50% inhibition of CMV plaque formation.

5.2 ADME

5.2.1 OCULAR EXPOSURE

Ocular kinetic parameters of fomivirsen following intravitreal injection to rabbits and monkeys are summarized in the following table.

Species	Tissue	Dose (μg)	C_{max} (μM)	$T_{½}$ (hr)	T_{max} (hr)
Rabbit	Vitreous	66 (4.6 μM)	3.9	60	4
	Retina	66 (4.6 μM)	3.5	96	72
Monkey	Vitreous	11 (1 μM)	0.11	-	-
		57 (5 μM)	0.7	24	-
		115 (10 μM)	1.2	22	-
	Retina	11 (1 μM)	0.12	-	-
		57 (5 μM)	0.7	45	-
		115 (10 μM)	0.9	78	-

From studies with radiolabeled ISIS 2922, retina and iris were determined to be the primary sites of distribution of fomivirsen following intravitreal injection. Only 10% radioactivity of total dose could be measured in vitreous humor. Exposure to fomivirsen in optic nerve was very low (< 0.1% of administered dose).

5.2.2 METABOLISM AND ELIMINATION FROM THE EYE

Metabolism of fomivirsen was apparent in both vitreous and retina, and occurred predominantly through exonuclease-mediated cleavage of nucleotide residues from the parent oligonucleotide as analyzed by HPLC and phosphorimaging of sequencing gel electrophoresis methods.

5.2.3 SYSTEMIC EXPOSURE

Rabbits: Systemic exposure to fomivirsen following intravitreal administration was low. In rabbits treated with radiolabeled fomivirsen, it was possible to measure low levels (~2 to 3% of the total dose) of radioactivity in kidneys and liver. No measurable levels of radioactivity could be detected in the plasma samples.

Monkeys: In the monkey study, capillary gel electrophoresis was used to quantitate fomivirsen and its oligonucleotide metabolites. Fomivirsen was below limits of detection in monkeys (n=3) with the exception of one time point in one animal. No intact fomivirsen was observed in any of tissues (kidney, liver, ovary, and uterus) following 1, 3, or 8 doses of 10 μM (115 μg) fomivirsen administered every-other-week for three months. The quantitation limit was 0.35 μg/g tissue. A small peak with a migration pattern consistent with an oligonucleotide metabolite (19-nucleotide metabolite) was detected in plasma, liver, and kidney (1 of 3 animals each) after 8 doses, but the levels were below limits of quantitation.

5.3 TOXICOLOGY

5.3.1 OCULAR TOXICITY

- Rabbits - Two single-dose ocular toxicity studies in the rabbit were conducted. Dose-dependent ocular inflammation (cyclitis) was noted. In the severe cases, retina was involved. Treatment of triamicinolone reduced the ocular alterations caused by intravitreal injection of ISIS 2922. The incidence of ocular abnormalities in rabbits treated with fomivirsen or fomivirsen plus triamcinolone is presented in the following table.

ISIS 2922 Dose (μg)	Observations	Fomivirsen (ISIS 2922-AS06)	Fomivirsen +Triamcinolone[a] (ISIS 2922-AS07)
0	No visible lesion	-	-
16.5	Acute cyclitis	6/12 (mild)	2/6 (mild)
33	Acute cyclitis	12/12 (mod.)	1/6 (mild)
82.5	Acute cyclitis	12/12 (mod.)	6/6 (mild)
	Anterior chamber flare	3/12	-
165	Acute cyclitis	12/12 (severe)	6/6 (mod.)
	Iritis	4/12	-
	Posterior synechia	1/12	-
	Retinal inflammation	2/12	-
	Retinal detachment, exudative	1/12	-
300	Acute cyclitis	12/12 (severe)	6/6 (mod.)
	Iritis	3/12	-
	Posterior synechia	-	-
	Retinal inflammation	10/12	2/6
	Retinal detachment, exudative	1/12	-
	Retinal pigment epitheliopathy	1/12	-
	Hemorrhage	1/12	-

[a] Triamcinolone was administered as subconjunctival dose of 10 mg on Days 1 and 8.

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- Monkeys - Ocular findings from ocular toxicity and ocular PK studies are summarized in the following table.

Dose (μg)	Ophthalmic Findings[a]	5-Week (2922-AS01)	4-Week (2922-AS03)	3-Month (2922-AS08)	Ocular PK[c] (2922- APK04)
Control	No Visible Lesion	14/20	5/6		
	Cyclitis	6/20	1/6		
	Iris, neovascularization	5/20	1/6		
	Retina, edema/ infiltrate	4/20			
	Synechia	1/20			
3.3	No Visible Lesion	8/12			
	Cyclitis	4/12			
	Iris, neovascularization	2/12			
	Retina, edema/ infiltrate	1 12			
10 to 17		10 μg	17 μg		11 μg
	No Visible Lesion	9/12	5/6		23/28
	Cyclitis	3/12	1/6		5/28
	Iris, neovascularization	2/12	1/6		4/28
	Retina, edema/ infiltrate				4/28
33 to 57		33 μg	34 μg		57 μg
	No Visible Lesion	13/ 20	5/ 6		24/28
	Cyclitis	7/ 20	1/ 6		4/28
	Iris, neovascularization	5/ 20	1/ 6		3/28
	Retina, edema/ infiltrate	3/ 20			3/28
	Hypopion				2/28
69 to 115			69 μg	115 μg	115 μg
	No Visible Lesion		11/12	7/12	20/20
	Cyclitis		1/12	5/12	
	Iris, neovascularization		1/12	2/12	
	Retina, edema/ infiltrate			2/12	
	Synechia			4/12	
	Hemorrhage			4/12	
	Hypopion			1/12	

APPEARS THIS WAY ON ORIGINAL

[a] Incidence of ophthalmic findings were presented as the number of eyes/total treated eyes at each specified dose level.

[b] Ophthalmic findings presented are from eyes treated with fomivirsen only. There were no ocular abnormalities in eyes treated with fomivirsen and triamcinolone.

[c] Incidence of ophthalmic abnormalities represent findings after the first dose. Triamcinolone was administered starting with the second dose to control cyclitis.

5.3.2 *SYSTEMIC TOXICITY*

A summary of systemic toxicity of fomivirsen following a single or repeated intravenous administration is shown in the following table.

Species	Route	Regimen	Dose (mg/kg)	Findings
Mouse	iv	Single-dose	720	Calculated LD_{50} = 720 mg
	iv	q2d for 4 weeks	50	Splenomegaly (↑ 3 x), fibroblast proliferation; inflammatory mononuclear infiltrates in numerous tissue; moderate increases in liver weight (↑ 1.25 x); ↑ AST (♂: 2x; ♀: 5 x) and ALT (♂: 4.3x; ♀:3.8 x); monocytosis (↑ 11-15 x) and thrombocytopenia (↓ 60-70 %).
	iv	q2d for 4 weeks	5	Splenomegaly (1.3 x), mild cell infiltrates in liver
	iv	q2d for 4 weeks	1	Minimal cell infiltrate in liver; No-Observed-Adverse-Effect-Dose
	iv	q2d for 4 weeks	0.2	No-Observed-Effect-Dose

5.3.3 *REPRODUCTIVE TOXICOLOGY*

No information was provided.

APPEARS THIS WAY
ON ORIGINAL

5.3.4 *GENETIC TOXICOLOGY*

The mutagenic potentials of fomivirsen were evaluated in both *in vitro* and *in vivo* systems and results were summarized in the following table.

Assay System	Indicator Cells	ISIS 2922 Conc.	Findings	Report Nº
Ames	*Salmonella typhimurium:* TA98, TA100, TA1535, TA1537 and TA1538; *Escherichia coli* WP2 *uvr*A.	6.7 - 5000 μg/plate	Not mutagenic	ISIS 2922-IS01
Chromosome Aberration	CHO-K1 cells	150 - 5000 μg/plate	**Significant and dose-dependent increases in structural chromosome aberrations were noted when cells were treated with ISIS 2922 (≥2500 μg/ml) for 44 hr without activation mix.** In the repeated comfirmatory experiment, a slight but not significant increase (~2x) in structural chromosome was noted in cells treated with ISIS 2922 at 5000 μg/ml under the same condition.	ISIS 2922-IS02
Gene Mutation	L5178Y+/- Mouse Lymphoma	313 -5000 μg/plate	Not mutagenic	ISIS 2922-IS03
Micronucleus Assay	ICR mice -Bone Marrow Cells	0, 600, 800, or 1000 mg/kg iv	Not clastogenic	ISIS 2922-IS05

5.3.5 *CARCINOGENICITY*

No information was provided.

A waiver from performing carcinogenicity studies was requested by the sponsor. The sponsor stated that "lack of meaningful systemic exposure, additional systemic toxicity studies are neither relevant nor useful for assessing the safety profile of this compound".

Data obtained from one of the clinical study (Study Nº ISIS 2922-CS5) showed that concentrations of fomivirsen in vitreous following the 150 μg dose were 1.23 - 11.82 μM at at approximately one hour post-injection. Four of the five patients receiving a dose of 150 μg exhibited measurable drug concentrations in the vitreous humor, however, one patient had no detectable drug. At the 330 μg dose level, concentrations of intact fomivirsen approximately one hour after treatment for the two patients examined were 32.7 and 6.18 μM, respectively. The kinetics of fomivirsen in the vitreous and plasma for each individual patient are shown in the following table. As limited number of subjects enrolled and highly variable concentrations of fomivirsen in the vitreous were noted, conclusive information on the systemic exposure can not be derived from this study. More complete information would be needed to make any conclusions regarding systemic exposure of fomivirsen in the patients.

Patient No.	Vitreous Concentration (μM)					Plasma Conc. (ng/mL)	
	Time Point (Vitreous)	Fomivirsen[a] (intact 21-mer)	n-1[b]	n-2[b]	% Intact[c]	Time Point (Plasma)	Fomivirsen
150 μg - Day 1							
150 μg - Day 8							
150 μg - Day 15							
330 μg - Day 1							

nm = not measured due to no quantifiable parent compound or metabolites
bld = below the assay's limit of detection (LOD is 2 nM for plasma and 10 nM for vitreous)
blq = below the assay's limit of quantitation (LOQ is 10 nM for plasma and 25 nM for vitreous)
a = measured concentration of fomivirsen in vitreous
b = measured concentrations of fomivirsen metabolites shortened by 1 or 2 nucleotides
c = percentage of fomivirsen comprised to total measured oligonucleotide ([fomivirsen] /[Total oligonucleotide]*100)

However, due to the fact that fomivirsen is indicated for the local treatment of CMV retinitis in AIDS patients and the dosing schedule stated in the labeling[8], the review division would accept the request from the sponsor for a waiver of carcinogenicity studies on fomivirsen.

6. CONCLUSION:

It appeared that intravitreal injection of ISIS 2922 caused cyclitis in the animals, monkeys and rabbits, and humans. The observed ocular changes were dose-dependent. In the severe cases, retina was involved in the ocular alterations. The following table shows the incidence of ocular inflammation caused by intravitreal injection of ISIS 2922.

MONKEYS					HUMANS				
Dose (μg)	Dose (μM)	N° of Eyes	Ocular Inflammation (% eye)	Obscured Fundus (% eye)	Dose (μg)	Dose (μM)	N° of Eyes	Ocular Inflammation (% eye)	Obscured Fundus (% eye)
10-17	1	40	19	>90	75	2.5	18	11	0
33-57	5	54	22	>90	165	5	118	14	23
69-115	10	44	14	>90	330	10	296	34	12

The following information needed to be conveyed to the sponsor.

1. The whole submission is in such poor quality, it would require extra time to complete review process. The sponsor is encouraged to improve the quality and oganization of subsequent submissions.
2. A lot of conflicting information was presented in the NDA. Obviously, the quality of jobs performed by the QAC unit is in question. Perhaps, the DSI should be notified.
3. For all of individual study reports included in the electronic submission are in image format that do not facilitate reviewing process.

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8 *Newly Diagnosed Disease* : Three consecutive, weekly intravitreal injections of 165 μg (0.025 mL) per eye should be administered as the Induction portion of the dosing regimen. Thereafter, one 165 μg intravitreal injection every 2 weeks should be administered as the maintenance regimen.

Previously Treated Disease: One intravitreal injection of 330 μg (0.05 mL) per eye every other week for two doses should be administered as the Induction portion of the dosing regimen. For Maintenance, an intravitreal injection of one 330 μg dose should be administered once every 4 weeks.

4. The legend for Fig. 1 in Study Report ISIS2922-APK03 (Sec. 05, Vol. 010, p.196) is different from that stated in the figure title. In the legend, the sponsor stated that "the amount of total radioactivity, expressed as μM equivalents, and intact ISIS 2922 ....in the **retina** .....". However, the title of the figure indicated that concentration of the total radioactivity and intact ISIS 2922 in **vitreous** after a single intravitreal injection. The sponsor need to provide explanations for this kind of conflicting information reported in the study.
5. Study Report Nº ISIS 2922-APK04, treatment with corticosteroid was neither mentioned in the report prepared by the contract laboratory nor stated in the study protocol. However, in the overall summary (Sec 05, Vol. 002, p. 05) for PK/ADME section, the sponsor stated "After treatment with corticosteroids, cyclitis was limit to one animal.....". **Discordant reports require further clarification from the sponsor.**
6. Discrepancy statements for the sequence modification on ISIS 13312 (Sec. 05, Vol. 003, p.253 and Sec. 05, Vol. 005, p. 012) were noted. On p.253 of Sec. 05, Vol. 003, the sponsor stated that ISIS 13312 contained 2'-methoxyethoxy substituents on the seven nucleotides on the 5'-end (1-7) and six nucleotides on the 3'-end (15-20) of the oligonucleotide, ISIS 2922. Yet, on p. 012 of Sec. 05, Vol. 005, "ISIS 13312 (5'-GCG TTT GCT CTT CTT CTT GCG-3') has the same sequences as ISIS 2922, but contains 2'-methoxyethoxy modifications on residues 1-6 and 16-20" was stated. Therefor, the sponsor should provide the information on ISIS 13312 containing correct numbers of 2'-methoxyethoxy substituents.
7. There was no IOP information provided in the single intravitreal injection ocular toxicity study reports (ISIS 2922-AS06 and ISIS 2922-AS07). Therefore, data used to plot figure 5 on p. 064 of Sec 05, Vol. 002 needed to provided to the agency.
8. Ocular alterations (cyclitis, retina involvement) caused by intravitreal injection of ISIS 2922 observed in the rabbit and monkey were also reported in the human trial (Sec. 05, Vol. 002, p.061). In addition, higher incidence was seen in the patients treated with 330 μg of fomivirsen. Apparently, treatment with fomivirsen may potentiate the ocular pathological changes caused by CMV. Therefore, it may be appropiate to carefully evaluate risk/benefit ratios in the clinical settings.
9. No reproductive toxicity studies were conducted. The sponsor planned to initiate Segment I and II studies in August or September of 1998.

7. RECOMMENDATION:

1. Approval of Vitravene™ (fomivirsen sodium) is recommended.

4. Additional experiments in chromosome aberration are required if the sponsor intends to modify the labeling - "Carcinogenesis, Mutagenesis, Impairment of Fertility" section.

/S/ 8/4/98
W.C. Josie Yang, Ph.D.

/S/ 8/5/98
Andrea Weir, Ph.D.

Concur by team leader: Yes ☒ No ☐