BPH

Clinical Pharmacology/Biopharmaceutics Review

NDA:	20-961	SUBMISSION DATE: 04/09/98
PRODUCT:	Vitravene™ (6.6 mg/ml) (Fomiversen sodium) ISIS 2922	
SPONSOR:	Isis Pharmaceuticals, Inc. Carlsbad, CA	REVIEWER: Veneeta Tandon, Ph.D.

Review of a NDA

I. Background

Vitravene™ (fomivirsen sodium injection) is a sterile, aqueous, preservative-free, bicarbonate-buffered solution for intravitreal injection. It is indicated for the local treatment of cytomegalovirus (CMV) retinitis in patients with acquired immunodefieciency syndrome (AIDS). Fomivirsen sodium is a phosphorothioate oligonucleotide, twenty-one nucleotides in length, with the following sequence:

5'-GCG TTT GCT CTT CTT CTT GCG-3'

where, G = guanosine, C= cytidine and T = thymidine linkage.
Fomivirsen inhibits human cytomegalovirus (HCMV) replication through an antisense mechanism. The nucleotide sequence of fomivirsen is complementary to a sequence in mRNA transcripts of the major immediate early region 2 (IE2) of HCMV. This region of mRNA encodes several proteins responsible for regulation of viral gene expression that are essential for production of infectious CMV. Binding of fomivirsen to the target mRNA, results in inhibition of IE2 protein synthesis, subsequently inhibiting virus replication. Its sequence appears to be unique for CMV, as it is not complementary to any other known mRNA sequence in humans. Fomivirsen inhibits CMV replication *in vitro* over a range of concentrations from 0.1 to 1 μM (EC_{50}).

Dosage and Administration

Newly Diagnosed Disease
Three consecutive, weekly intravitreal injections of 165 μg (0.025mL) per eye should be administered as the induction portion of the dosing regimen. Thereafter, one 165μg intravitreal injection every 2 weeks should be administered as the maintenance regimen.
Previously Treated Disease
One intravitreal injection of 330 μg (0.05mL) 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.

II. Pharmacokinetics Study Overview

This NDA consists of one single dose human pharmacokinetic study in AIDS patients with CMV retinitis. An interim report of the study has been submitted for review. 10 patients have completed the study out of the proposed 28. There are insufficient number of subjects enrolled for Day 3, 8 and 15 for the 150 μg dose group and none for the 300 μg dose group. With this limited information it is not appropriate to draw any conclusions from the study. Details of the study design, proposed plan and the limited data available to this date is described below in study ISIS 2922-CS5.

Study # ISIS 2922-CS5

An open label pharmacokinetic study of intravitreal ISIS 2922 in AIDS patients with cytomegalovirus retinitis (CMVR)

Study Objective

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The objective of this study was to evaluate the intravitreal pharmacokinetics and plasma exposure of fomivirsen following a single 150 μg or 330 μg intravitreal injection.

Assay validation

The analytical validation report for the determination of fomivirsen in the vitreous humor and the plasma has not been submitted along with the study. The only information provided is the limit of quantitation and the validation range.

Study Design

This was a multicenter, open-label, non-randomized study designed to evaluate the ocular

and systemic pharmacokinetics of fomivirsen following a single intravitreal injection of 150 μg or 330 μg, given prior to Vitrasert® implantation. A single vitreous sample (taken at the time of Vitrasert® implantation) and a plasma sample was obtained from each patient. This application consists of an interim report from 10 patients out of a total of 28 patients proposed. The propsed number of patients and the completed number of patients for the dose group of 150 μg or 330 μg is given below for the different Regimens.

- Day 1, Regimen 1(1 hour)

single vitreous and plasma sample taken one hour after a single fomivirsen injection (proposed number of patients=5+5, completed=5+2).

- Day 3, Regimen 3(48 hours)

(proposed number of patients=3+3, completed=0+0)

- Day 8, Regimen 8 (168 hours)

(proposed number of patients=3+3, completed=1+0)

- Day 15, Regimen 15 (336 hours)

(proposed number of patients=3+3, completed=1+0)

All patients were to have a follow-up visit two weeks after their vitreous and plasma sampling procedures.

Treatment assignment was based upon the degree of retinal involvement with CMVR: If < 25%, assigned to 150 μg; if ≥ 25%, assigned to 330 μg. Assignment to sampling times was based upon the convenience of the investigator and patient.

The pharmacokinetic parameters proposed to be calculated were as follows:

- Maximum vitreous and plasma concentrations (C_{max})
- Vitreal concentration half-life ($t_{1/2}$)
- Area under the vitreal concentration-time curve (AUC)
- Mean residence time (MRT) = ratio of area under the first moment concentration – time curve(AUMC):AUC
- Vitreal clearance (CL) = ratio of dose:AUC

However, none of these have been calculated due to the limited data.

Vitreal volume was calculated using biometry A-scan ultrasound measurements. Vitreal volume was determined using the equation for volume of a sphere and the combined distance of lens thickness and lens-to-sclera distance to estimate the radius of vitreal chamber.

Concentration of fomivirsen in the vitreous was converted to amount of fomivirsen in the vitreal compartment by multiplying vitreal concentration in μM (μmoles/L) by volume (L). This estimate of amount of fomivirsen normalizes the observed concentrations by the volume. Another method for normalizing the observed concentration to the measured volume was also performed. For this method, the measured concentration of fomivirsen in the vitreous was normalized to the mean

volume, by multiplying each concentration (μM) by the ratio of the measured vitreal volume( ) to the mean vitreal volume (mean of the 5 eyes treated with 150 μg fomivirsen and sampled at the 1 hour time point). The purpose for normalizing the fomivirsen concentration in vitreous by volume is to remove variability directly associated with different vitreal volumes found between the eyes.

Demographics

Of the 10 patients in this study, eight were males and two were females. The mean age for the study population was 39.3(± 8.8) years. Of the 10 patients studied, three (30%) were Caucasian, two (20%) were Black, one (10%) was Asian, and four (40%) were other. The table describing the demographics is attached in the Appendix on page 9.

Pharmacokinetic results

Drug concentrations in the vitreous humor

Day 1

Concentrations of fomivirsen in vitreous following the 150 μg dose ranged from 1.23 μM to 11.82 μM 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.

Day 8

Samples were taken at day 7 instead of day 8. The concentration of fomivirsen measured in vitreous at 7 days, for one eye treated with 150 μg of fomivirsen, was 0.055 μM. The half life of fomivirsen in the vitreous humor of the monkey was found to be 22 hours. Assuming it to be 24 hours the predicted concentration range on day 8 would be from 0.0096 -0.0923 μM. The concentration obatined in the one treated eye appears to fall in this range. With the limited amount of data provided the vitreal half cannot be calculated precisely. However, with one subject, no conclusion can be made and no credibility can be given to the single data.

Day 15

Samples were taken on day 13 and on day 18 instead of day 15. By day 13 (12 days after fomivirsen injection) the vitreal concentration of fomivirsen was 0.037 μM in the one sample analyzed, and by day 18, drug concentrations of oligonucleotide were no longer quantifiable (below the assay's limit of quantitation; LOQ).

At the 330 μg dose level, concentrations of intact fomivirsen approximately 1 hour after treatment for the two patients examined were 32.7 μM and 6.18 μM, respectively. There is a very high variability seen here.

The vitreal concentrations of fomivirsen as a function of time are summarized in Table 1.

Table 1: Vitreous and plasma concentration of fomivirsen and its metabolites.

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 [redacted] for plasma and 10 nM for vitreous)
blq = below the assay's limit of quantitation (LOQ is [redacted] 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)

A-scan ultrasound data for measuring the vitreal volume is attached in the Appendix on page 9. Actual estimated vitreal volumes ranges from [redacted] for eyes evaluated in Regimen 1. The mean and standard deviation for the estimated vitreal volume for the entire population of eyes evaluated in the study was 5.2 ± 1.3 ml. Normalization of the observed vitreal concentrations of fomivirsen to amount of fomivirsen in the vitreous using A-scan resulted in only a slight decrease in the variability between patients (page 10). This was also confirmed by normalization of the observed concentration to the mean volume. This suggests that the volume differences between the eyes do not contribute much to the observed variability.

Metabolism

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Metabolism of fomivirsen is the result of exonuclease mediated cleavage of the oligonuleotide to yield an oligonucleotide shortened by one nucleotide.
Metabolism was apparent at 1 to 2 hours after injection of fomivirsen in the vitreous to a small degree (See % intact in the Table above and n-1 and n-2). At 7 days post-injection, considerable metabolism was apparent with measurable concentrations of oligonucleotide shortened by one and two nucleotides (n-1 and n-2). % intact Fomivirsen comprised approximately 41% of the total measurable oligonucleotide at the 7 day time point. However, the validation for the assessment of the metabolites in the vitreous humor has also not been submitted.

Metabolism could not be reliably assessed at the later time points (12 and 17 days after injection) since the concentration of fomivirsen was at or below the assays limit of quantitation.

Drug Concentrations in Plasma

Fomivirsen was not detectable in plasma collected at any evaluated time point (See Table 1). There were also no detectable levels of related oligonucleotide metabolites in plasma.

Observations from the study

- From the limited number of subjects, no age, gender or race related differences were observed.
- Large variability was observed in the data. One patient had no observable concentration at the 1 hr 50 minutes time point from the 150 μg dose group and one subject had extremely high concentration (32.7 μM) as compared to other subjects in the 330 μg dose group . With the limited data provided no significant conclusion can be drawn from the study. This variability could be attributed to various factors, such as, lack of mixing of the oligonucleotide in the sampling compartment, improper sampling of the biological fluid, improper dosing, insufficient migration from injection site, relationship of the injection site from the sampling site, physiologically different rates of clearance/outflow between individuals.
- No pharmacokinetic parameters was calculated because of the limited data. The half life in human vitreous humor is not known.

III. Deficiencies

1. This is an interim report of the study, only 10 subjects have completed the study out of the 28 planned. 5 have completed Regimen 1, no subjects have been enrolled for Regimen 3 as yet, and only one subject out of 3 have completed Regimen 8, and 2 have completed Regimen 15 for 150 μg dose group. Two have completed Regimen 1 and, none have completed the study for Regimen 3, 8 and 15 for the 330 μg dose group.
2. Out of these limited patients sampling schedule has not been followed, sample is taken on day 7 instead of day 8 from the only subject analyzed for Regimen 8, and on day 12 and 17 for the Regimen 15. This further complicates the comparison between the two subjects in the Regimen 15 for 150 μg dose group.
3. Assumption has been made that 1-2 hour time point post dosing on Day 1 reflected the maximum concentration in the vitreous humor, this may not be true. In rabbits the maximum concentration was observed at 4 hours post dosing.
4. A published article on the quantitation of phosphorothioate oligonucleotide in human plasma has been submitted along with the study. However, the assay validation is more pertinent to ISIS 2302 as compared to ISIS 2922 (fomivirsen), the compound of

interest. Analytical validation report the quantitation of fomivirsen in the vitreous humor has also not been provided. The only information provided is the limit of quantitation and detection, which is not sufficient to determine the accuracy and precision of the concentrations obtained. The method employed is the same for plasma as well as the vitreous humor samples, however, the sensitivity of the assay world vary. The high variability observed in the data could also be attributed to the accuracy and precision of the assay and the methodology itself.

5. The reliability in the assessment of the metabolites of the oligonucleotide shortened by one or two nucleotides is not well defined. It is unclear whether the limit of detection of the metabolites is the same as that of fomivirsen.
6. The single dose pharmacokinetic study is done using 150 μg as the dose, where as the indicated dose in the label is 165 μg. Although, not so drastically different, the pharmacokinetic study is always desirable using the actual recommended dose/dosage regimen.

IV. Recommendation

With the interim report on one pharmacokinetic study performed for this application, and without any analytical validation report provided, it is very difficult to draw any firm conclusions regarding the study or to assess the reliability of the data provided. Hence, this application does not meet the requirements from the biopharmaceutics standpoint. More complete information would be needed in order to make any judgments regarding this study.

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NDA:	20-961(amendemnt)	SUBMISSION DATE: 07/20/98
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Response to comments in NDA review

In this amendment the sponsor has responded to the deficiencies stated in the review of NDA 20-961. [redacted] The sponsor has submitted a detailed analytical validation report for detecting ISIS 2922 in plasma and vitreous humor. The analytical validation is satisfactory and acceptable. However, the inability to assess the results of study # ISIS 2922-CS5 (An open label pharmacokinetic study of intravitreal ISIS 2922 in AIDS patients with cytomegalovirus retinintis) still remains the same due to limited number of patients enrolled and completed the study. The sponsor has mentioned in reponse 1 that the study report is a preliminary report of the protocol and patient recruitment is continuing.

From the pharmacokinetics standpoint no definitive judgements can be made at this time. No information is available on the vitreal kinetics of the higher dose (330 µg) and few subjects in the lower dose of 165 µg. Plasma concentrations of fomivirsen were undetectable for 165 µg dose at Day 1, 7 and 15 and after 1 hour post dosing for the 330 µg dose. But, no information is available regarding systemic exposure from the 330 µg dose at Day 3, 8 and 15. Further comments can be made upon completion of the study.

/S/ 7/28/98

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