

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**22-211**

**PHARMACOLOGY REVIEW(S)**



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## ***EXECUTIVE SUMMARY***

### **I. Recommendations**

- A. Recommendation on approvability  
The approval of NDA 22-211 is recommended.
- B. Recommendation for nonclinical studies  
None
- C. Recommendations on labeling  
See suggested label in page 23.

### **II. Summary of nonclinical findings**

- A. Brief overview of nonclinical findings  
Ganciclovir is a virustatic agent currently approved for human use in the treatment for cytomegalovirus (CMV) infection. Ganciclovir IV, ganciclovir capsules and ganciclovir intravitreal implant are currently marketed in the US. Ganciclovir Ophthalmic Gel 0.15% (Virgan®) is approved and marketed in over 30 foreign countries for the treatment of acute herpetic keratitis. Most nonclinical information in support of this NDA has been submitted for Cytovene® (ganciclovir sodium for injection – Roche Laboratories, NDA 19-661).  
Additional GLP safety studies have been conducted by Laboratoires Théa to evaluate the safety and tolerability of the ophthalmic formulation of ganciclovir (approved in other countries under the brand name Virgan®), for the treatment of herpetic keratitis. In 2007 and 2008, 2 nonclinical studies were conducted that evaluated the local topical tolerance and toxicity of ST-605 compared to trifluridine 1% ophthalmic solution (a marketed product) or placebo gel, after repeated instillation in rabbits. None of the ocular studies showed any systemic adverse reactions resulting from ocular topical instillation of ST-605, regardless of formulation tested. The only noted ocular finding was slight irritation and redness postinstillation. However, each morning the signs of the previous day had disappeared, showing reversibility of the irritation.
- B. Pharmacologic activity  
Ganciclovir is active against the herpes simplex viruses (HSV-1 and HSV-2) most often responsible for human herpetic keratitis. Ganciclovir inhibits replication of human herpes viruses both in vivo and in vitro. It is converted intracellularly by thymidine kinase in infected cells to ganciclovir monophosphate. This monophosphate is then converted to diphosphate and then the active triphosphate. Ganciclovir triphosphate inhibits viral DNA polymerase and also incorporates itself into viral DNA, inhibiting viral replication. Ganciclovir is minimally phosphorylated in uninfected cells, and the concentration of ganciclovir triphosphate is reportedly 10-fold higher in CMV-infected cells than in uninfected cells, lending some degree of specificity for cytotoxicity to infected cells only.

C. Nonclinical safety issues relevant to clinical use

Most of the nonclinical pharmacology and toxicology data summarized in this application were obtained from the literature and by studies conducted by Roche Laboratories in support of the systemic administration of ganciclovir. These nonclinical data were acquired over 10 years ago, and since that time, the US clinical safety database for ganciclovir has been established and is reflected in the current product labeling for Cytovene® (ganciclovir intravenous, NDA 19-661).

In nonclinical studies, ganciclovir, similar to other acyclic purine analogs, has shown mutagenic and reproductive toxicities. The risk/benefit ratio of ganciclovir IV has been described in the label for Cytovene®. The systemic absorption of ocularly administered ganciclovir is relatively low. Since the AUC for patient is not available from the clinical study, the mean plasma level or the administered dose of ganciclovir in patient and animal will be compared in the label to express the margin of safety.

**APPEARS THIS WAY  
ON ORIGINAL**

## 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 22-211

**Review number:** No.1

**Sequence number/date/type of submission:** SN0000/June 25, 2008/Original Submission

**Information to sponsor:** Yes (x) No ( )

**Sponsor and/or agent:** Sirion Therapeutics, Inc.

**Manufacturer for drug substance:**

**Drug Substance:** \_\_\_\_\_

**Drug Product:** Alliance Medical Products, Inc., Irvine, CA 92618

**Reviewer name:** Conrad H. Chen, Ph.D.

**Division name:** Division of Anti-Infective and Ophthalmology Products

**Review completion date:** June 12, 2009

**Drug:**

Trade name: Zirgan or \_\_\_\_\_

Generic name: Ganciclovir

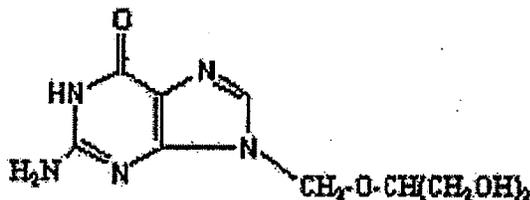
Code name: ST-605, GV 550, DHPG

Chemical name: 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine

CAS registry number: 82410-32-0

Molecular formula/molecular weight: C<sub>9</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>/255.2

Structure:



**Relevant INDs/NDAs/DMFs:**

IND 25,082 and NDA 19-661 (Cytovene® Intravenous Injection RS 21,592)

NDA 20-460 (Cytovene® Capsule)

NDA 20-569 (Vitrasert® Intravitreal Implant)

IND 75,762 (Ganciclovir Ophthalmic Gel, 0.15%)

**Drug class:** Antiviral agent

**Indication:** Treatment of acute herpetic keratitis (dendritic ; \_\_\_\_\_ ulcers)

**Clinical formulation:**

During the clinical development, the preservative used in the ST-605 formulation was changed from sodium mercuriothiolate (0.006%) to benzalkonium chloride (0.0075%). Sodium mercuriothiolate has been used in the FDA approved ophthalmic products at up to 1%. Benzalkonium chloride has been used in the FDA approved ophthalmic products at up to 8.8%.

**Table 1. ST-605 Quantitative and Qualitative Composition**

Component	Quantity (%w/w)	Function	Quality Standard
Ganciclovir	0.15%	Active ingredient	USP
Carbomer			NF
Mannitol			USP
Benzalkonium chloride		Antimicrobial preservative	USP / NF
Sodium hydroxide	As needed	pH adjustment	NF
Water for injection	qs	Aqueous vehicle	USP

qs, quantum sufficient, a sufficient quantity; USP, United States Pharmacopeia; NF, National Formulary

b(4)

**Route of administration:** Topical administration to the eye

**Proposed use:** One drop in the affected eye 5 times per day (approximately every 3 hours while awake) until the corneal ulcer heals, and then 1 drop 3 times per day for 7 days

**2.6.2 PHARMACOLOGY**

For non-clinical studies in this NDA, the sponsor is using the same data previously used in the marketing application in the Europe for Virgan® (0.15% ganciclovir eye gel). These non-clinical data were acquired over 10 years ago. In a pre-NDA meeting comment, the pharmacology reviewer has responded to sponsor’s question that the existing non-clinical data appeared adequate to support an NDA.

**2.6.2.1 Brief summary**

Most of the nonclinical pharmacology and toxicology data summarized in this application were obtained from the literature and by studies conducted by Roche Laboratories in support of the systemic administration of ganciclovir. These nonclinical data were acquired over 10 years ago, and since that time, the US clinical safety database for ganciclovir has been established and is reflected in the current product labeling for Cytovene® (NDA 19-661).

Herpes simplex keratitis is a common cause of corneal ulceration and the most common cause of corneal blindness in the US. Primary ocular herpes simplex is infrequent, but recurrent herpes keratitis outbreaks are more common because of the herpesvirus’ ability to lie dormant along basal ganglion after primary infection. Ganciclovir is active against the herpes simplex viruses (HSV-1 and HSV-2) most often responsible for human herpetic keratitis. Ganciclovir inhibits replication of human herpes viruses both in vivo and in vitro. It is converted intracellularly by thymidine kinase in infected cells to ganciclovir monophosphate. This monophosphate is then converted to diphosphate and then the active triphosphate. Ganciclovir triphosphate inhibits viral DNA polymerase and also incorporates itself into viral DNA, inhibiting viral replication. Ganciclovir is minimally phosphorylated in uninfected cells, and the concentration of ganciclovir

triphosphate is reportedly 10-fold higher in CMV-infected cells than in uninfected cells, lending some degree of specificity for cytotoxicity to infected cells only.

### 2.6.2.2 Primary pharmacodynamics

#### *In Vitro* Pharmacodynamics

Ganciclovir has demonstrated virustatic activity against DNA-replicating viruses, including CMV, adenovirus, and HSV. Using 3 reference isolates of HSV-1 and 3 reference isolates of HSV-2, the 50% inhibitory concentration (IC<sub>50</sub>) of ganciclovir *in vitro* ranged from 0.05 to 0.13 µg/mL (or 0.2–0.5 µM) for HSV-1 and 0.08 to 0.46 µg/mL (or 0.4–1.8 µM) for HSV-2 (Smee, 1985). The cytotoxic dose for the Vero cells used to cultivate these viruses was 230 µg/mL. In another report, the IC<sub>50</sub> of ganciclovir against isolated clinical ocular strains of HSV-1 ranged from 0.06 to 0.13 µg/mL (Smee, 1983). Additional *in vitro* studies were conducted to test the susceptibility of ganciclovir to 9 strains of HSV-1 and 1 strain of HSV-2 that were recently isolated from patients in the US. These results showed an IC<sub>50</sub> for ganciclovir ranging from 307–736 ng/mL, with a mean of 593 ng/mL (0.59 µg/mL) (4.2.1.1.2 *In Vitro* Susceptibility of Low Passage Clinical Isolates of HSV to Ganciclovir). Another *in vitro* plaque reduction assay performed with the McKrae strain of HSV-1 showed an IC<sub>50</sub> for ganciclovir of 61.65 ng/mL (0.061 µg/mL) (4.2.1.4.1 *In Vitro* Synergistic Interaction of Ganciclovir and Trifluridine Against Herpes Simplex Virus Type-1). On the basis of these data, the IC<sub>50</sub> of ganciclovir for the least sensitive herpes virus isolates of HSV-1 or HSV-2 was approximately 0.50 µg/mL. Adenovirus infections also induce expression of kinase activity and are sensitive to ganciclovir, but they are less sensitive than herpesvirus, with an IC<sub>50</sub> of 1.8 µg/mL to 4.0 µg/mL for Ad8 and Ad19, the 2 most commonly isolated ocular clinical strains of adenovirus.

#### *In Vivo* Pharmacodynamics

Topical ocular administration of ganciclovir has been shown to be effective against herpetic keratitis in laboratory animals. Trousdale et al conducted a study in rabbits with experimental acute herpetic keratitis in which ganciclovir 1%, ganciclovir 0.1%, acyclovir 3%, idoxuridine 0.5%, and placebo were administered 3 to 5 times a day for 14 days (Trousdale, 1984). There was no discernable difference between the 1% and 0.1% concentrations of topical ganciclovir. The eyes treated with either concentration of ganciclovir or acyclovir 3% had significantly less punctate keratitis ( $P < 0.05$ ) than the eyes treated with idoxuridine. In turn, the eyes treated with idoxuridine had significantly less punctate keratitis than the eyes treated with placebo ( $P < 0.05$ ).

A study by Shiota et al demonstrated that ganciclovir at concentrations of 0.03% to 1% administered at 2-hour intervals, 5 times a day for 2 days prevented herpetic ulcer formation (Shiota, 1987). In addition to the prevention study, the effect of ganciclovir on dendritic herpetic ulcers was investigated. The therapeutic effect of ganciclovir 0.3% in Vaseline given 5 times per day for 4 days was dramatic, with the infected eyes nearly completely healed. Ganciclovir was also more effective than acyclovir and idoxuridine in treating dendritic ulcers.

Inoue et al (Inoue, 1989) showed that a low concentration of ganciclovir (0.03%) was as effective as acyclovir 3%. A rabbit study conducted by Denis et al (4.2.1.1.1 Evaluation

of the Efficacy of GV550 in the Treatment of Herpetic Keratitis in the Rabbit) found equivalent clinical efficacy for ganciclovir 0.05% and 0.2% when administered as an ophthalmic gel and acyclovir 3% when administered as an ointment.

In a 5-day repeated-dose study conducted in 2008 in 29 rabbits, the efficacy of ST-605 was compared to trifluridine and placebo (4.2.1.1.3 Efficacy of Ganciclovir Ophthalmic Gel in the Treatment of Established Herpetic Keratitis in New Zealand White Rabbits and Comparison to Treatment with Trifluridine Ophthalmic Solution). Two days after instillation of HSV-1 onto their corneas, the animals were randomized into 1 of the 3 treatment groups and received the study drug 5 times per day for 5 days. Efficacy was measured on a 0–4 scale, which assessed the proportion of the cornea covered by the ulcer. Both trifluridine and ST-605 significantly reduced the severity of herpetic keratitis compared to treatment with the placebo gel ( $P < 0.0001$ ).

Therefore, ganciclovir has demonstrated efficacy both *in vitro* and *in vivo* against HSV-1 and HSV-2 ocular infections when administered as a greasy ointment or an aqueous gel or solution, at concentrations ranging from 0.05% to 1%.

#### 2.6.2.3 Secondary pharmacodynamics

Not submitted.

#### 2.6.2.4 Safety pharmacology

Not submitted.

#### 2.6.2.5 Pharmacodynamic drug interactions

Not submitted.

### 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

The pharmacokinetics of systemically administered ganciclovir was published in the literature and has also been submitted to the Agency in support of the marketing approval for Cytovene (NDA 19-661). The pharmacokinetic studies were conducted for ST-605 to characterize the absorption and distribution of ganciclovir to ocular tissues and systemically, following topical ophthalmic administration of the formulated drug product. The rabbits were used in pharmacokinetic studies following the single and multiple instillations of the ST-605 in both intact and abraded (de-epithelialized) corneas, as well as in rabbits with herpetic corneal ulcers, to establish whether there was a difference in absorption in healthy versus ulcerated corneas.

#### 2.6.4.1 Brief summary

In a series of GLP pharmacokinetic studies, the absorption, distribution and metabolism of ST-605 were studied. The following studies were conducted:

1. Ocular Pharmacokinetics of Ganciclovir Ophthalmic Gel after Instillation in Intact and De-epithelialized Eyes of Pigmented Rabbits,
2. H-GV 550 Ocular Autoradiography, Distribution and Metabolism in Blood, After Single Ocular Instillation in Pigmented Rabbits with Intact and De-epithelialized Corneas,
3. Determination of Ganciclovir Pharmacokinetic Parameters in Blood, and

#### 4. Study of the Ocular Penetration of Ganciclovir and Acyclovir in the Rabbit, after Chronic Local Administration.

The study results were summarized in the following sections.

Results of multiple GLP nonclinical studies of ST-605 ophthalmic gel indicate that ganciclovir, when administered topically to the eye, has good corneal penetration and achieves virustatic ganciclovir levels in the anterior segment of the eye that are sustained for several hours following a single instillation. Repeat administration studies of ST-605 also showed very low levels of ganciclovir in the plasma, demonstrating a lack of systemic bioavailability after topical administration.

##### 2.6.4.2 Methods of Analysis

The radiochemical procedures and HPLC procedures were used.

##### 2.6.4.3 Absorption

In a series of GLP-compliant studies, topical ganciclovir was administered to rabbits with normal intact eyes and to rabbits where the cornea had been de-epithelialized to evaluate the absorption and distribution of a single dose of radiolabeled <sup>3</sup>H-ganciclovir. The deepithelialized eye was used as a model for an eye with keratitis over the corneal surface.

Table 1 presents a summary of the radioactivity ocular tissue concentration data obtained for each tissue over time. Maximum radioactivity was detected from 0.5 hour to 1 hour after administration in all ocular structures in both groups of animals (intact and de-epithelialized corneas). Radioactivity then decreased rapidly in all ocular structures except whole blood and plasma, where radioactivity levels decreased and then plateaued between 4 hours and 24 hours postdosing. The highest concentrations of radioactivity were found in the external ocular structures, tears, conjunctiva, and cornea. In the interior eye, the radioactivity was higher in the anterior segment than in the posterior. With the exception of the retina, conjunctiva, and choroids, radioactivity levels in the ocular structures were from 2 to 10 times greater in de-epithelialized eyes than in intact eyes. The calculated peak systemic levels of ganciclovir were very low and were the same in whole blood and plasma: about 5 ng-Eq GV 550/mL in rabbits with intact corneas and about 20 ng-Eq GV 550/mL in rabbits with de-epithelialized corneas, at 1 hour and 0.5 hour, respectively. Detectable levels of radiolabeled drug persisted in the cornea for up to 8 hours after administration, whereas all other tissues showed negligible levels of radioactivity past 2 hours.

**Table 1. Concentrations of Radioactive Material in the Tissues of Rabbits After a Single Instillation of [<sup>3</sup>H]-ganciclovir [Study 046-89]**

Tissue	Mean Concentration ( $\mu\text{g Eq}^*\text{GV 550/g}$ )					
	0.5 h Intact	0.5 h Abraded	1 h Intact	1 h Abraded	2 h Intact	2 h Abraded
Tears	143	244	52	347	6.7	1.3
Conjunctiva	160	44	31	6.4	7.8	2.4
Cornea	17	190	11	55	4.3	20.7
Aqueous humor	0.42	32.1	0.96	19	0.52	10.7
Iris ciliary body	3.8	29.5	1.0	11	0.45	8.9
Lens	0.15	0.42	ND	.20	ND	0.28
Vitreous	0.17	0.23	ND	ND	ND	ND
Retina	1.1	1.8	.23	.47	0.13	0.15
Choroid	6.3	6.1	1.9	1.8	0.69	1.3
	Mean Concentration ( $\text{ng Eq}^*\text{GV 550/mL}$ )					
Plasma	3.5	23.7	4.7	22.5	4.2	22.2
Whole blood	4.4	21.4	4.4	16.9	4.1	13.8

Limit of detection 50-80 disintegrations per minute; ND = levels below 0.1 ng, eq GV 550/g

Source: 4.2.2.2.1 Study 046-89, Table 2

In a second radiolabeled single-dose study in rabbits, the pharmacokinetics and excretion of ganciclovir were determined following a single instillation of <sup>3</sup>H-ganciclovir into the conjunctival sac of pigmented rabbits with de-epithelialized corneas. The topical ophthalmic instillation results were compared with the pharmacokinetic results obtained after intravenous administration of an equivalent amount of <sup>3</sup>H-ganciclovir as an injectable solution (150  $\mu\text{g/mL}$ ). The time to maximum plasma concentration ( $T_{\text{max}}$ ) obtained after ocular instillation of the gel in plasma was 60 minutes, and in whole blood it was 30 minutes. The maximum plasma concentration ( $C_{\text{max}}$ ) represents 1.14% and 1.96% of the ocular dose of ganciclovir administered. Urinary elimination over 24 hours after ocular administration showed that 20% of the dose was excreted via urine (16% after 0 to 6 hours and 4% after 6 to 24 hours). Fecal administration over 24 hours after ocular instillation was approximately 4% of the dose administered. In contrast, ganciclovir was rapidly eliminated from systemic circulation after intravenous administration. Although there were some inconsistencies in the results obtained after ocular instillation and intravenous administration in this study, comparison of the area under the curve ( $\text{AUC}_{(0-6)}$ ) suggest an approximate 60% absorption of ganciclovir via the ocular route. However, based on the 24-hour urinary excretion after intravenous administration, the total excretion was estimated at approximately 20%. Approximately 80% of the radioactivity administered was recovered in the urine over the 24 hours following intravenous administration, with 2% in the feces. This indicates either that the urine collection period should have been longer or that radioactivity was being lost via another route. For safety purposes, therefore, the more conservative 60% absorption rate should be used.

#### 2.6.4.4 Distribution

The tissue distribution of <sup>3</sup>H-ganciclovir following single topical or intravenous doses is described above. The results of these studies are consistent, in that radioactivity was maximal approximately 0.5 hours after instillation in both intact and de-epithelialized eyes before gradually declining and was not detectable 24 hours after instillation. Radioactivity was mainly distributed in the anterior segment (cornea, aqueous humor, iris-ciliary body) and was low in the posterior segment (vitreous, retina, and choroids). No radioactivity was detected in the lens. Radioactivity levels were higher in the de-epithelialized eyes than intact eyes. The levels of radioactivity were higher on the eyelids of the intact eyes than the de-epithelialized eyes. HPLC analysis of the plasma samples from the animals produced a single peak of radioactivity, indicating that ganciclovir had not been extensively metabolized; radioactivity was no longer detectable in the plasma 24 hours after administration.

Plasma and ocular tissue levels of ganciclovir were measured in albino rabbits following repeated ocular treatments with an ophthalmic gel containing ganciclovir 0.0125%, 0.05%, or 0.2% of, administered 4 times a day in both eyes for 12 days. An additional group of animals received acyclovir 3%, using the same dosage regimen. All rabbits had been infected with herpetic virus at Day 0 and presented from Day 3, the first day of treatment with severe corneal lesions. Ocular tissue samples were taken on Day 14 (12th day of treatment), 4 hours after the last dose was administered. The ganciclovir levels obtained were highest in the cornea (2.3 µg/g) and iris (1.2 µg/g). These are relatively low levels based on the results obtained in radiolabeled studies following single <sup>3</sup>instillation of H-ganciclovir in pigmented rabbits. However, different methods of analysis (radioactivity vs. HPLC), the use of albino versus pigmented animals, and different testing facilities make valid comparisons between studies difficult. The results from this study do indicate that there was not a significant accumulation of ganciclovir in the eye structures or the blood and plasma (52 ng/mL) after repeated administration, and that therapeutic levels of ganciclovir can be found in the target tissue—the cornea—after repeat topical administration.

#### 2.6.4.5 Metabolism

Because of the low levels of ganciclovir in the plasma, demonstrating a low systemic availability of ganciclovir after local administration, exposure studies evaluating the metabolites of ganciclovir were not conducted.

#### 2.6.4.6 Excretion

One study characterized the absorption and excretion following a single instillation of <sup>3</sup>H-ganciclovir into the conjunctival sac of 1 eye of pigmented rabbits with de-epithelialized corneas. In addition to distribution of radioactivity in the blood, urine and fecal samples were collected over 24 hours postinstillation. Table 2 summarizes that percentage dose recovered from these tissues over time. Urinary elimination over 24 hours after ocular administration showed that 20% of the dose was excreted via urine (16% after 6 hours). Fecal administration over 24 hours after ocular instillation was approximately 4% of the dose administered.

**Table 2. Excretion of Radioactive Material After Single Instillation of [<sup>3</sup>H]-ganciclovir to Rabbits [018-91]**

Dose (total µg)	Route	Mean Percentage of Administered Dose			
		Urine: 6 hr	Urine: 24 hr	Feces: 24 hr	Total
75	Topical (ophthalmic)	15.9	4.3	3.7	24
75	Intravenously	65.0	14.4	1.2	80

Source: 4.2.2.2.1 Study 018-91. Annex A—Tables 4, 5, 8, and 9

#### 2.6.4.7 Pharmacokinetic drug interactions

Not conducted.

#### 2.6.4.8 Other Pharmacokinetic Studies

Not conducted.

#### 2.6.4.9 Discussion and Conclusions

Results of multiple GLP nonclinical studies of ST-605 ophthalmic gel indicate that ganciclovir, when administered topically to the eye, has good corneal penetration and achieves virustatic ganciclovir levels in the anterior segment of the eye that are sustained for several hours following a single instillation. Repeat administration studies of ST-605 also showed very low levels of ganciclovir in the plasma, demonstrating a lack of systemic bioavailability after topical administration.

### 2.6.6 TOXICOLOGY

#### 2.6.6.1 Overall toxicology summary

Most nonclinical safety information in support of this NDA has previously been submitted for Cytovene® (ganciclovir sodium for injection – Roche Laboratories, NDA 19-661). The systemic toxicity of ganciclovir in animals was investigated in Good Laboratory Practice (GLP)-compliant toxicity studies conducted by Syntex and Roche Pharmaceutical Companies, in support of ganciclovir for injection. Additional GLP safety studies have been conducted by Laboratoires Théa to evaluate the safety and tolerability of the ST-605 ophthalmic formulation of ganciclovir (approved in other countries under the brand name Virgan®), for the treatment of herpetic keratitis.

Two recent nonclinical studies were conducted in 2007 and 2008 at \_\_\_\_\_ b(4) \_\_\_\_\_, on behalf of the Sponsor to evaluate the toxicity of the ST-605 ophthalmic formulation of ganciclovir in rabbits with intact corneas and with total corneal epithelial defects. The GLP status of these studies was not clear. The details of all those studies will be summarized in the following sections.

#### General toxicology:

Four-week IV repeat dose toxicity studies in mouse and dog and 3-month oral toxicity studies in mouse, rat and dog were conducted. These studies have been previously reviewed under NDA 19-661.

In the 4-week IV mouse study, treatment-related clinical signs and symptoms such as hypothermia, inactivity, pallor, rough coat, and wasting were noted and were more pronounced in incidence and severity in the high-dose (135 mg/kg/day) group. During the recovery period, the clinical signs and symptoms improved rapidly, and most animals fully recovered by the end of the first week of recovery. The gross and microscopic organ changes seen were reproductive cell atrophy in both males and females, atrophy of adnexal skin tissue, and some nephropathy; all were less severe or absent at the end of the recovery period. The tests from low-dose (15 mg/kg/day) males exhibited early evidence of recovery; however, little recovery was seen in mid- (45 mg/kg/day) to high- (135 mg/kg/day) dose males.

In the 1-month intravenous study in beagle dogs, at the end of the 1-month recovery period post treatment, testicular changes were present for all treated males, and the severity of hypospermatogenesis increased with increasing dose. For high-dose males (1.8 mg/kg/bid), the testicular changes were more severe at the end of recovery than at the end of the treatment period, reflecting the typical spermatogenesis cycle chronology. Because spermatogonia and primary spermatocytes were present at the end of recovery in all males, these results indicate that full recovery of spermatogenesis would be expected if the animals were allowed a longer recovery period. In the preceding intravenous 1-month dog range-finding study, all high-dose males and females (90 mg/kg/day) and 1 mid-dose female (30 mg/kg/day) died before termination. The cause of death for the high-dose group was diagnosed as enteropathy or gastroenteropathy. The cause of death for the mid-dose female was gastroenteropathy and nephropathy. Changes in hematology and clinical chemistry were seen mainly for mid- and high-dose groups, including reduced levels of leukocytes, platelets, or reticulocytes. In addition, there was dose-related reduction in bone marrow cellularity in all dose groups.

On the basis of the reproductive toxicity results seen in the 1-month studies, 5 GLP 3-month toxicity studies were conducted to evaluate the systemic and reproductive toxicity of ganciclovir in male and female mice, Sprague Dawley rats, and beagle dogs. In all of these studies, ganciclovir at varying dose levels was administered orally for 90 days, with recovery periods ranging from 0 days for the rats to 4 months in mice and 65 days (males and females) and 126 days (males only) in dogs. The effects of ganciclovir on the reproductive system will be described further under the section of reproductive toxicology.

In a three-month oral toxicity study in male mice, effects of DHPG on the male reproductive system were seen at all dose levels at 10, 100, or 1000 mg/kg/day. Recovery from these effects on the male reproductive system occurred only at the lowest dose given: 10 mg/kg/day. The median plasma levels of ganciclovir in male mice at 10 mg/kg/day administered orally were about 0.24 to 0.57 µg/mL. Additionally, males dosed with 1000 mg/kg/day DHPG exhibited decreased food intakes during the treatment period with correspondingly slightly-lower mean body weights than vehicle-control mice. A second three-month oral toxicity study in male mice was conducted to establish a no-observed effect level (NOEL) of DHPG on the testes of mice. The NOEL was established at 1 mg/kg/day (median plasma level = 22.9 ng/mL). In a previous oral three-month toxicity study, testicular effects of DHPG had been seen at the lowest dose of 10 mg/kg/day.

In a three-month oral toxicity study in female mice, no toxic effects of DHPG were seen at doses up to 1000 mg/kg/day.

In a three-month oral toxicity study in rats, males exhibited aspermic testes at all doses of DHPG from 0.1 to 5.0 g/kg/day. However, the effects on the testes were less severe at doses of 0.1 g/kg/day than at doses of 0.5 to 5.0 g/kg/day. Females exhibited ovarian suppression at the highest dose given: 5.0 g/kg/day.

In a three-month oral toxicity study in dogs, effects of treatment with DHPG were seen on the integumentary (skin lesion), hematopoietic (anemia), and male reproductive systems (testicular atrophy). The no-observed effect level of DHPG on the hematopoietic system was 2 mg/kg/day. Effects on the integumentary and male reproductive systems, though slight, were seen at the lowest dose given: 0.2 mg/kg/day. All effects of DHPG were reversible. The median plasma levels of ganciclovir at 0.2 mg/kg/day in dogs was 0.16 µg/mL.

#### Genetic toxicology:

The mutagenicity of ganciclovir was investigated in a series of GLP-compliant studies with adequate controls. Ganciclovir gave negative results in plate and suspension bacterial mutation assays, a yeast mutation assay, and a cell transformation assay. Positive results were obtained in the sister chromatid exchange assay, the mouse lymphoma assay, and the mouse micronucleus test. Similar mutagenicity results have been obtained with other acyclic purine analogs. Toxicology studies of ganciclovir have shown increased mouse lymphoma cell mutations and DNA damage in human lymphocytes in vitro at between 50 µg/mL to 500 µg/mL and 250 µg/mL to 2000 µg/mL, respectively. In the mouse micronucleus assay, ganciclovir was clastogenic at doses of 150 to 500 mg/kg (intravenously), which was from 2.8 to 10 times the human exposure of systemically administered ganciclovir for CMV retinitis.

#### Carcinogenicity:

A GLP carcinogenicity study was conducted in Swiss-Webster mice by Syntex Research (Roche Laboratories; Oral Carcinogenicity Study in Mice with Ganciclovir). In this study, ganciclovir was given orally by gavage at doses of 1, 20, or 1000 mg/kg/day to groups of 60 male and 60 female mice for up to 18 months. Control groups of 120 mice per sex received vehicle control only. The high dose selected was determined to be the maximum oral dose of ganciclovir that could be administered on a daily basis because of formulation viscosity. The low dose selected was based on the highest level of ganciclovir that was shown not to cause testicular atrophy in male mice in a 3-month oral toxicity study. Treatment-related tumors were present at the mid and high doses. At the high dose, the principal tumor sites included epithelial tumors of the preputial gland of males; forestomach of males and females; and clitoral gland, vagina, and mammary gland of females, as well as vascular tumors of the ovaries, uterus, mesenteric lymph node, and liver of females and hematopoietic tumors (histiocytic sarcoma) of the liver in males and females. Other sites with a slightly increased incidence of tumors in high-dose animals included epithelial tumors of the digestive tract, pancreas, and skin in males or females. At the mid-dose, a slightly increased incidence of tumors was present in the preputial and Harderian glands of males, the forestomach of males and females, and the liver (histiocytic sarcoma) of females. The increased incidence of Harderian

gland tumors (epithelial in origin) in mid-dose males but not high-dose males was attributed to the longer survival and treatment period of mid-dose mice, as most Harderian gland tumors occurred after the high-dose groups were terminated. It should be noted that the preputial and clitoral glands, forestomach, and Harderian glands of mice do not have human counterparts. No increased incidence of tumors was present in mice treated with 1 mg/kg/day of ganciclovir.

Following 15 months dosing at 1, 20, or 1000 mg/kg/day, the median peak plasma levels (males and females averaged) were 0.05, 0.64, and 6.22 µg/mL, which were within expected ranges based on previous data from the 3-month toxicity study in mice.

#### Reproductive toxicology:

A series of 4 GLP reproductive toxicity studies were conducted by Syntex/Roche, which evaluated fertility, reproductive toxicity, and effects on embryofetal development after intravenous administration of ganciclovir in mice and rabbits. These studies were conducted during 1983-1984 and were previously reviewed under NDA 19-661.

#### Fertility and Embryonic Development

Two GLP studies of the effect of ganciclovir on fertility and general reproductive performance in mice were conducted by Syntex. In the fertility and reproductive study in male mice (Male Fertility and Reproduction Study of DHPG in Mice), 4 groups of 20 male Swiss-Webster mice in each group received daily intravenous doses of placebo (vehicle-control) or ganciclovir at 0.4, 2.0, or 10.0 mg/kg/day. In this study, there were no notable findings except the following: At the end of the treatment period, the fertility of mid- and high-dose male mice was greatly decreased. By the first recovery period mating cycle (2 months of recovery), mid-dose males exhibited complete recovery of fertility, but high-dose males remained infertile. By the second recovery period mating cycle (6 months of recovery), only 2 of 9 surviving high-dose males exhibited regained fertility. These effects were corroborated by histological examination of the male reproductive organs, with testicular atrophy with aspermatogenesis or hypospermatogenesis accompanied by atrophy of the epididymides and decreased numbers of spermatozoa in epididymal tubules. These changes were reversed as males regained fertility during the recovery periods.

In the fertility and reproductive toxicity study of ganciclovir in female mice (Fertility and Reproduction Study of DHPG in Female Mice), 4 groups of 40 adult female Swiss-Webster mice (designated P1) were given daily intravenous doses of either placebo (vehicle control) or 5, 20, or 90 mg/kg/day of ganciclovir, administered daily from 14 days before mating until their pups (F1) were weaned at 21 days of age. After an additional 2-month recovery period, vehicle control and high-dose P1 females were again bred to untreated males.

The notable findings in this study were the following: the overall pregnancy rate for high-dose females (24%) was almost half of the pregnancy rate for the vehicle, low-, and mid-dose females (41%–48%). For females examined midgestation, no treatment-related changes were seen for low- and mid-dose females; high-dose females exhibited increased embryoletality, as evidenced by an increased number of resorptions. For F1 pups necropsied after weaning, no treatment-related changes were seen for the offspring of low- and mid-dose females; however, male offspring of high-dose females exhibited

hypoplastic testes and seminal vesicles, and both male and female offspring of high-dose females exhibited an increased incidence of epithelial hyperplasia and hyperkeratosis of the nonglandular stomach.

For the second-generation mating cycle, the P2 animals from low- and mid-dose P1 dams did not exhibit any treatment-related changes for any parameter evaluated including mating behavior and fertility and viability of the offspring. The offspring of high-dose P1 dams were not mated. In this study, the NOEL of intravenous ganciclovir was 20 mg/kg/day. At doses of 90 mg/kg/day, the receptivity to mating and the pregnancy rate were decreased and the resorption rate was increased. After an approximate 2-month recovery period, the females exhibited complete recovery. After weaning, the offspring of high-dose females exhibited hypoplastic testes and seminal vesicles (males) and changes in the epithelium of the nonglandular stomach (males and females). No effects were seen for the second generation, although the offspring of high-dose females were not mated because of insufficient numbers available for mating.

#### Embryo-Fetal Development

Two GLP toxicity studies were conducted to evaluate the teratogenicity of ganciclovir to mice and rabbits. In the study in mice (Intravenous Teratology Study with DHPG in Mice), 4 groups of 25 females with evidence of mating per group were given daily intravenous doses of either placebo (control vehicle) or 12, 36, or 108 mg/kg/day of ganciclovir from Days 7 through 16 of gestation. On gestation day 18 (day evidence of mating was found considered Day 1), dams were necropsied and fetuses evaluated for teratology. There were no treatment-related changes in body weights for the low- and mid-dose dams; however, the high-dose dams gained weight over the course of the study. No treatment-related changes in maternal or fetal parameters were seen for low- and mid-dose dams and fetuses. For the high-dose dams, the number of early and total resorptions and the Resorption Index were increased, and live litter size was decreased. For high-dose fetuses, fetal weights and sizes were decreased, and there was generalized reduced ossification of all major bones. No treatment-related teratologic changes were noted on external skeletal and visceral examinations of the fetuses. Two fetuses each from a separate mid-dose dam had cleft palates. This was stated as a spontaneous change in mice and was considered unrelated to treatment. The fewer number of live fetuses and litters available for teratologic examination in the high-dose group was a result of the higher number of resorptions in this group, including 5 dams with all fetuses undergoing resorption. The NOEL in this study was 36 mg/kg/day, with maternal/fetal toxicity and embryoletality seen at 108 mg/kg/day.

The second intravenous teratology study was conducted in rabbits by Syntex Laboratories (Intravenous Teratology Study in Rabbits with DHPG). Four groups of 21 artificially inseminated female Dutch-belted rabbits per group were administered daily intravenous dose of either vehicle control or ganciclovir at 6, 20, or 60 mg/kg/day from Days 7 through 19 of gestation. On gestation Day 29 (day of insemination was Day 1), dams were necropsied and the fetuses subjected to teratologic examination. Clinical signs of toxicity occurred mainly for high-dose animals and consisted of cyanosis, inactivity, soft feces, and wasting, which occurred only in the high-dose animals. The mean body weights of control, low-, and mid-dose pregnant females increased during the study, whereas the mean body weights of high-dose pregnant females decreased during the

treatment period; these weights recovered after cessation of dosing. No embryolethal effects were seen for the low-dose group, but in the high-dose group, 12 of 14 litters were undergoing resorption. The 2 high-dose litters with live fetuses showed a decreased live litter size, with a corresponding increase in number of resorptions (all early). Increased values for the number of resorptions and for the proportion of pregnant dams with resorptions were also seen for mid-dose dams. No effects on fetal growth were seen in the low-dose group. In the high-dose group, mean fetal weights decreased 27% from control values, and the weights of the mid-dose groups decreased 8% from control values. In the high-dose group, there appeared to be a correlation between reduced ossification (cranial, hyoid, sternbrae, and pelvic bones) and fetal growth retardation.

No teratogenic effects were seen for the low-dose group. In the high-dose group, only 2 dams contained live fetuses. For these 2 litters, 5 of 5 fetuses exhibited major anomalies that may have been compound related. In 4 fetuses from 1 high-dose litter, 3 fetuses had anophthalmia/microphthalmia, and 2 fetuses also had cleft palate; the fourth fetus had a grossly aplastic kidney and pancreas. In 1 fetus from another high-dose litter, the following were noted: ecchymosis, cleft palate, hydrocephaly, and microphthalmia. In the mid-dose group, 1 fetus had brachygnathia, and 1 fetus had cleft palate. Of the anomalies seen, only cleft palate appeared to follow a dose-response pattern. Cleft palate had not been seen in the historical control data for this species from earlier Syntex teratology studies.

The smaller number of live fetuses and litters available for teratologic examination in the high-dose group was a result of the higher number of resorptions in this group, including 12 dams with all fetuses undergoing resorption. The NOEL in this study was 6 mg/kg/day, with doses of 20 and 60 mg/kg/day causing fetal growth retardation, embryolethality, and teratogenicity.

#### Prenatal and Postnatal Development and Maternal Function

Prenatal and postnatal development was assessed in the ganciclovir intravenous teratology studies described above in Embryo-Fetal Development.

#### Local tolerance:

##### Summary:

Seven local ocular tolerance studies have been conducted with ganciclovir, when administered in the ST-605 ophthalmic gel formulation. Three of the local tolerance studies compared the effects of ST-605 eye gel containing either BAC 0.0075% as the preservative or sodium mercuriothiolate 0.006% (thiomersal) as the preservative. The BAC preservative was selected prior to the Phase 3 clinical study and was also present in the initially marketed Virgan formulation. In these studies, there appeared to be no differences between gel formulations with respect to irritancy to the conjunctiva, iris or cornea, or corneal sensitivity after single instillation, or wound-healing time after repeat instillation. Two studies evaluated the local tolerance of an additional formulation change that was made after initial marketing approval, whereby changes to the carbomer gel and the ganciclovir API were evaluated. Two additional studies have been conducted evaluating the local tolerance and corneal toxicity of ST-605 and trifluridine 1% in rabbits with intact corneas or with total corneal epithelial defects. The ocular toxicity information from the above studies has been previously used in the marketing approval of

ST-605 ophthalmic formulation of ganciclovir (Virgan®) in foreign countries. A summary of the individual studies is presented below.

Individual study:

In a GLP Study 042-92 conducted in 1992 (Title: Acute ocular tolerance/effects of GV 550 0.15% with thiomersal and GV 550 0.15% with benzalkonium chloride), 6 albino male New Zealand rabbits with 3 per group received a single 100- $\mu$ L instillation of either ST-605 containing thiomersal 0.006% or ST-605 containing BAC 0.0075% in 1 eye, with the opposite eye as the untreated control. Eyes were examined at 1 hour and then at 1, 2, 3, 4, and 7 days after instillation with supplementary observation of the cornea after fluorescein staining. In this study, neither formulation induced any eye irritation.

In a GLP Study 040-92 conducted in 1992, the same protocol was followed with a single 50- $\mu$ L instillation of the same test article(s) into 1 eye, with the opposite eye serving as the untreated control. Corneal sensitivity was measured in both eyes before instillation and then at 5, 10, 20, 30, 40, 50, and 60 minutes after instillation (measured by the Cochet-Bonnet esthesiometer). In this study, there were no changes in corneal sensitivity associated with either gel.

Study 001-92 (a GLP study conducted in 1992) evaluated the influence of the same two ST-605 formulations (each containing a different preservative as described above) on corneal epithelial wound healing in albino rabbits. In this study, 30 albino New Zealand rabbits were divided into 3 groups of 10, and the right cornea was débrided under general and local anesthetic using a scalpel. The test article (ST-605 containing either BAC 0.0077% or thiomersal 0.01% as the preservative, or a balanced salt solution as the control) was then instilled as 1 drop (50 mg gel) in the abraded eye 5 times per day until complete re-epithelialization occurred. Daily measurements were made of the corneal ulcer surface using fluorescein dye, with photographs and computer analysis of the images. The average healing period was  $10 \pm 2.2$  days for ST-605 containing BAC,  $8 \pm 3.2$  days for ST-605 containing thiomersal, and  $8.8 \pm 4$  days for the control group. The difference between the groups was not statistically significant, although 3 rabbits (2 from the ST-605 with BAC group and 1 from the control group) were sacrificed prematurely due to severe ocular inflammation. Four rabbits were not completely healed at the end of the study (Day 16): 1 in the ST-605 with BAC group, 2 in the ST-605 with thiomersal group, and 1 in the control group. In this study, the two ST-605 formulations had no influence on corneal healing when compared with balanced salt solution.

Another study (Study T01F1500, a GLP study conducted in 2000) evaluated the ocular tolerance of multiple instillations of ST-605 over 28 days in New Zealand white rabbits. Two ST-605 ophthalmic gel formulations were compared; both contained BAC as the preservative, but one formulation was the Phase 3 and early marketed formulation, and the other was the new proposed commercial formulation containing a new carbomer and ganciclovir API from a new synthetic route. The animals were divided into 2 groups containing 6 males and 6 females per group. The ST-605 test article was instilled into 1 eye as 1 drop (50  $\mu$ L) 5 times per day for 28 consecutive days, then the rabbit necropsied. Ocular histologic examination, hematology, twice-daily ophthalmoscopic examination,

and weekly slit-lamp examination were performed. The opposite eye was untreated and served as a control. Either treatment showed no changes in the behavior or external appearance of the animals. There were no changes in body weights, organ weights. The only ophthalmic finding was a slight thinning of the corneal epithelium. The histological examination of other organs (including the testes) was not conducted. Both formulations showed good ocular tolerance and there were no differences between treatments.

Another ocular tolerance study was performed by Laboratoire Bio-Tox (Study BT-2360, conducted in 1990), which evaluated the marketed formulation of ST-605 in New Zealand rabbits after 42 days of repeat instillation. This study was undertaken to evaluate long-term ocular tolerance after treatment with the commercial ST-605 formulation using the clinical acute dosing regimen of 5 instillations per day, though the normal duration of treatment for herpetic keratitis is 21 days. The rabbits were divided into 2 groups of 6 rabbits (3 males, 3 females) per group. Each rabbit received either the ST-605 test article or vehicle control, as 1 drop (51 mg) in 1 eye 5 times per day for 42 consecutive days. The animals were observed daily for clinical signs or symptoms, and eye irritation by the Draize irritation index. Full clinical and ophthalmologic examinations were performed predosing and on Days 1, 8, 15, 22, 29, 36, and 43. Examination of the retina was performed on Day 43. Blood samples for hematology were collected before treatment and on Day 43. On Day 43, the animals were sacrificed and eyes, ocular appendages, and gonads were taken for histopathologic examination. There were no deaths or changes in body weights in either group. There were no changes in behavior or reflexes, no changes in the cornea, iris, or retina, and no changes found in microscopic examination of the eyes, testes or ovaries. No hematologic changes and macroscopic organ changes were noted. The only findings were a slight reddening of the conjunctiva from Day 5 in 2 of 3 animals treated, then in all animals treated on Day 8. At Day 9, this was accompanied by the presence of tears, primarily noted after the fourth daily instillation. However, each morning the signs of the previous day had disappeared, showing reversibility of the irritation. The GLP status of this study is not stated.

In 2007 and 2008, 2 nonclinical studies were conducted that evaluated the local topical tolerance and toxicity of ST-605 compared to trifluridine 1% ophthalmic solution (a marketed product) or placebo gel, after repeated instillation in rabbits (Toxicity of Ganciclovir Ophthalmic Gel and Comparison to Trifluridine Drops in New Zealand White Rabbits With Intact Corneas).

In the first study, 1 drop of test article was administered 5 times per day for 14 consecutive days, in both eyes of rabbits (5/group). The animals were observed daily for clinical signs or symptoms, and both eyes were examined using a slit lamp, with and without fluorescein. No corneal changes were seen at any time during the study. Only very slight lower limbal congestion of blood vessels was noted in some eyes and that lasted 1 to 2 days. Two animals treated with ST-605 and 2 treated with trifluridine showed no congestion during the entire study. No statistical difference was seen among the 3 treatment groups.

In the second study, 30 rabbits had their right eye corneal epithelium surgically removed (Toxicity of Ganciclovir Ophthalmic Gel and Comparison to Trifluridine Drops in New Zealand White Rabbits with Total Corneal Epithelial Defects). Treatment was

administered 5 times per day for 20 days in the right eye only. Animals were observed daily for clinical signs or symptoms, and both eyes were examined using a slit lamp, with and without fluorescein. There was no significant difference in the healing of the corneal defects among the 3 groups. Treatment with trifluridine produced more corneal edema than treatment with either ST-605 ( $P < 0.0001$ ) or placebo ( $P < 0.0001$ ). Treatment with trifluridine also produced more redness and swelling than placebo or ST-605 ( $P < 0.0001$ ). The studies were conducted at Louisiana State University on behalf of the Sponsor. The GLP status of these studies was not clear.

#### 2.6.6.9 Discussion and Conclusions

Most nonclinical safety information in support of this NDA has been previously submitted for Cytovene® (ganciclovir sodium for injection – Roche Laboratories, NDA 19-661). The systemic toxicity of ganciclovir in animals was investigated in Good Laboratory Practice (GLP)-compliant toxicity studies conducted by Syntex and Roche Pharmaceutical Companies, in support of ganciclovir for injection. Additional GLP safety studies have been conducted by Laboratoires Théa to evaluate the safety and tolerability of the ST-605 ophthalmic formulation of ganciclovir (approved in other countries under the brand name Virgan®), for the treatment of herpetic keratitis.

Two more recent nonclinical studies were conducted in 2007 and 2008 at \_\_\_\_\_ on behalf of the Sponsor to evaluate the toxicity of the ST-605 ophthalmic formulation of ganciclovir in rabbits with intact corneas and with total corneal epithelial defects.

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The repeat-dose intravenous and oral toxicity studies in animals showed that ganciclovir caused anemia and testicular toxicity. Ganciclovir increased mutations in mouse lymphoma cells and DNA damage in human lymphocytes *in vitro* at concentrations of 50 to 500 and 250 to 2000  $\mu\text{g/mL}$ , respectively. In the mouse micronucleus assay, ganciclovir was clastogenic at doses of 150 and 500 mg/kg but not 50 mg/kg. Ganciclovir was not mutagenic in the Ames Salmonella assay at concentrations of 500 to 5000  $\mu\text{g/mL}$ . Ganciclovir was carcinogenic in the mouse at oral doses of 20 and 1000 mg/kg/day. There was a significant increase in the incidence of tumors of the preputial gland in males, forestomach (nonglandular mucosa) in males and females, and reproductive tissues (ovaries, uterus, mammary gland, clitoral gland and vagina) and liver in females. No carcinogenic effect was observed in mice administered at 1 mg/kg/day.

Ganciclovir has been shown to be embryotoxic in rabbits and mice following intravenous administration and teratogenic in rabbits. Fetal resorptions were present in at least 85% of rabbits and mice administered 60 mg/kg/day and 108 mg/kg/day, respectively. Effects observed in rabbits included: fetal growth retardation, embryoletality, teratogenicity and/or maternal toxicity. Teratogenic changes included cleft palate, anophthalmia/microphthalmia, aplastic organs (kidney and pancreas), hydrocephaly and brachygnathia. In mice, effects observed were maternal/fetal toxicity and embryoletality.

Those studies described above have been previously reviewed under NDA 19-661 and have been stated in the label for Cytovene®.

Seven local ocular tolerance studies have been conducted with ganciclovir, when administered in the ST-605 ophthalmic gel formulation. Three of the local tolerance studies compared the effects of ST-605 eye gel containing either BAC 0.0075% as the preservative or sodium mercurothiolate 0.006% (thiomersal) as the preservative. The BAC preservative was selected prior to the Phase 3 clinical study and was also present in the initially marketed Virgan formulation. In these studies, there appeared to be no differences between gel formulations with respect to irritancy to the conjunctiva, iris or cornea, or corneal sensitivity after single instillation, or wound-healing time after repeat instillation. The ocular toxicity information from the above studies has been previously used in the marketing approval of ST-605 ophthalmic formulation of ganciclovir (Virgan®) in foreign countries. Two additional studies have been conducted evaluating the local tolerance and corneal toxicity of ST-605 and trifluridine 1% in rabbits with intact corneas or with total corneal epithelial defects.

None of the ocular studies showed any systemic adverse reactions resulting from ocular topical instillation of ST-605, regardless of formulation tested. The only noted ocular finding was slight irritation and redness postinstillation. However, each morning the signs of the previous day had disappeared, showing reversibility of the irritation.

The clinical dosing regimen of ST-605 (0.15%) is 1 drop per eye, 5 times a day, for up to 21 days. The animal ocular tolerance studies with ST-605 (0.15%) included regimen of 1 drop per eye, 5 times a day, for up to 42 days. Therefore, it appears that there is a sufficient margin of safety.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

### Conclusions:

Ganciclovir IV, ganciclovir capsules and ganciclovir intravitreal implant are currently marketed in the US. Ganciclovir Ophthalmic Gel 0.15% (Virgan®) is approved and marketed in over 30 foreign countries.

Most nonclinical safety information in support of this NDA has been previously submitted for Cytovene® (ganciclovir sodium for injection – Roche Laboratories, NDA 19-661). The systemic toxicity of ganciclovir in animals was investigated in Good Laboratory Practice (GLP)-compliant toxicity studies conducted by Syntex and Roche Pharmaceutical Companies, in support of ganciclovir for injection. Additional GLP safety studies have been conducted by Laboratoires Théa to evaluate the safety and tolerability of the ST-605 ophthalmic formulation of ganciclovir (approved in other countries under the brand name Virgan®), for the treatment of herpetic keratitis.

Two more recent nonclinical studies were conducted in 2007 and 2008 at \_\_\_\_\_ on behalf of the Sponsor to evaluate the toxicity of the ST-605 ophthalmic formulation of ganciclovir in rabbits with intact corneas and with total corneal epithelial defects. None of the ocular studies showed any systemic adverse reactions resulting from ocular topical instillation of ST-605, regardless of formulation tested. The only noted ocular finding was slight irritation and redness postinstillation. However, each morning the signs of the previous day had disappeared, showing reversibility of the irritation.

The label for currently marketed Cytovene® (ganciclovir sodium for injection – Roche Laboratories, NDA 19-661) has incorporated the important findings from animal toxicity studies for ganciclovir. Nonetheless, for the label for this NDA (the ganciclovir ophthalmic gel 0.15%) the current label in use for Cytovene® should be modified. The

ratio of animal dose to the recommended human ocular dose should be expressed if the data is available.

The current label for Cytovene® and the proposed label for ZIRGAN by the sponsor are shown below for comparison:

***The current label in use for Cytovene®-IV and -Capsule***

***Carcinogenesis, Mutagenesis\****: Ganciclovir was carcinogenic in the mouse at oral doses of 20 and 1000 mg/kg/day (approximately 0.1x and 1.4x, respectively, the mean drug exposure in humans following the recommended intravenous dose of 5 mg/kg, based on area under the plasma concentration curve [AUC] comparisons). At the dose of 1000 mg/kg/day there was a significant increase in the incidence of tumors of the preputial gland in males, forestomach (nonglandular mucosa) in males and females, and reproductive tissues (ovaries, uterus, mammary gland, clitoral gland and vagina) and liver in females. At the dose of 20 mg/kg/day, a slightly increased incidence of tumors was noted in the preputial and harderian glands in males, forestomach in males and females, and liver in females. No carcinogenic effect was observed in mice administered ganciclovir at 1 mg/kg/day (estimated as 0.01x the human dose based on AUC comparison). Except for histiocytic sarcoma of the liver, ganciclovir-induced tumors were generally of epithelial or vascular origin. Although the preputial and clitoral glands, forestomach and harderian glands of mice do not have human counterparts, ganciclovir should be considered a potential carcinogen in humans.

Ganciclovir increased mutations in mouse lymphoma cells and DNA damage in human lymphocytes in vitro at concentrations between 50 to 500 and 250 to 2000 µg/mL, respectively. In the mouse micronucleus assay, ganciclovir was clastogenic at doses of 150 and 500 mg/kg (IV) (2.8 to 10x human exposure based on AUC) but not 50 mg/kg (exposure approximately comparable to the human based on AUC). Ganciclovir was not mutagenic in the Ames Salmonella assay at concentrations of 500 to 5000 µg/mL.

***Impairment of Fertility\****: Ganciclovir caused decreased mating behavior, decreased fertility, and an increased incidence of embryoletality in female mice following intravenous doses of 90 mg/kg/day (approximately 1.7x the mean drug exposure in humans following the dose of 5 mg/kg, based on AUC comparisons). Ganciclovir caused decreased fertility in male mice and hypospermatogenesis in mice and dogs following daily oral or intravenous administration of doses ranging from 0.2 to 10 mg/kg. Systemic drug exposure (AUC) at the lowest dose showing toxicity in each species ranged from 0.03 to 0.1x the AUC of the recommended human intravenous dose.

***Pregnancy: Category C\****: Ganciclovir has been shown to be embryotoxic in rabbits and mice following intravenous administration and teratogenic in rabbits. Fetal resorptions were present in at least 85% of rabbits and mice administered 60 mg/kg/day and 108 mg/kg/day (2x the human exposure based on AUC comparisons), respectively. Effects observed in rabbits included: fetal growth retardation, embryoletality, teratogenicity and/or maternal toxicity. Teratogenic changes included cleft palate, anophthalmia/microphthalmia, aplastic organs (kidney and pancreas), hydrocephaly and brachygnathia. In mice, effects observed were maternal/fetal toxicity and embryoletality.

Daily intravenous doses of 90 mg/kg administered to female mice prior to mating, during gestation, and during lactation caused hypoplasia of the testes and seminal vesicles in the

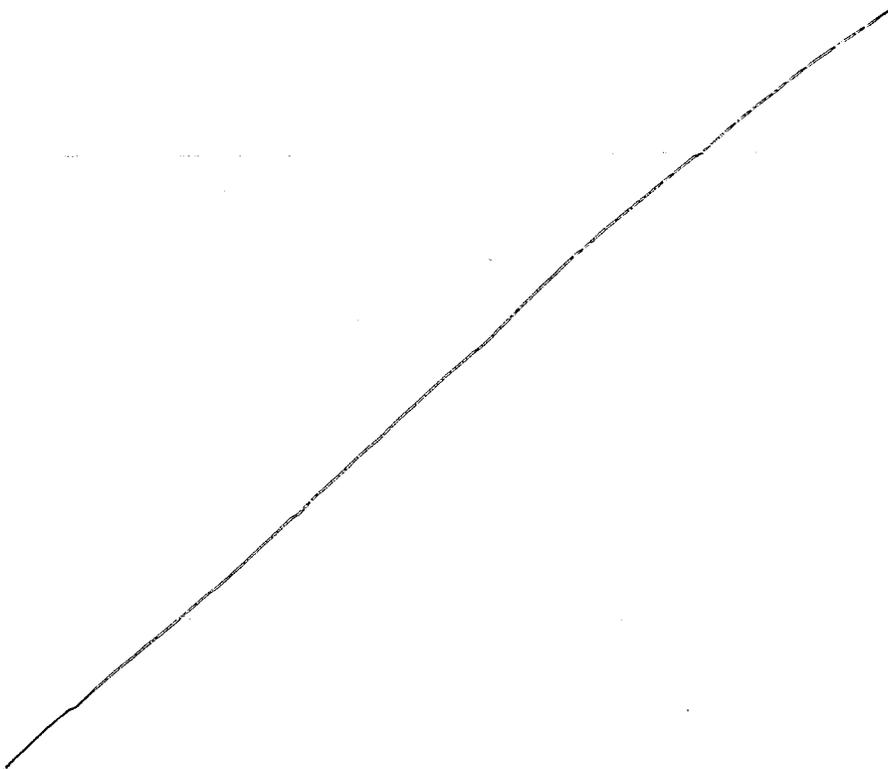
month-old male offspring, as well as pathologic changes in the nonglandular region of the stomach (see Carcinogenesis, Mutagenesis). The drug exposure in mice as estimated by the AUC was approximately 1.7x the human AUC.

Ganciclovir may be teratogenic or embryotoxic at dose levels recommended for human use. There are no adequate and well-controlled studies in pregnant women. CYTOVENE-IV or CYTOVENE should be used during pregnancy only if the potential benefits justify the potential risk to the fetus.

*\*Footnote: All dose comparisons presented in the Carcinogenesis, Mutagenesis, Impairment of Fertility, and Pregnancy subsections are based on the human AUC following administration of a single 5 mg/kg intravenous infusion of CYTOVENE-IV as used during the maintenance phase of treatment. Compared with the single 5 mg/kg intravenous infusion, human exposure is doubled during the intravenous induction phase (5 mg/kg bid) and approximately halved during maintenance treatment with CYTOVENE capsules (1000 mg tid). The cross-species dose comparisons should be divided by 2 for intravenous induction treatment with CYTOVENE-IV and multiplied by 2 for CYTOVENE capsules.*

There are several different versions (June 2008, August 2008, February 2009, and March 2009) of label proposed by the sponsor. The March 2009 version will be adopted for the discussion:

**The proposed label for ZIRGAN by the sponsor: (March 2009 version)**



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1   Page(s) Withheld

       Trade Secret / Confidential (b4)

  ✓   Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

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**Suggested labeling:**

The proposed label by the sponsor does not contain the information for carcinogenicity. This reviewer would recommend adopting the label for Cytovene®-IV with some changes. In the label for ZIRGAN, the human clinical ocular dose (6.25 µg/kg/day, assuming complete absorption) and the animal systemic dose (µg/kg/day) in carcinogenicity and reproductive toxicity studies will be compared.

**13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility:** *Ganciclovir was carcinogenic in the mouse at oral doses of 20 and 1,000 mg/kg/day (approximately 3,200x and 160,000x the human ocular dose of 6.25 µg/kg/day, assuming complete absorption). At the dose of 1,000 mg/kg/day there was a significant increase in the incidence of tumors of the preputial gland in males, forestomach (nonglandular mucosa) in males and females, and reproductive tissues (ovaries, uterus, mammary gland, clitoral gland and vagina) and liver in females. At the dose of 20 mg/kg/day, a slightly increased incidence of tumors was noted in the preputial and harderian glands in males, forestomach in males and females, and liver in females. No carcinogenic effect was observed in mice administered ganciclovir at 1 mg/kg/day (160x the human ocular dose). Except for histiocytic sarcoma of the liver, ganciclovir-induced tumors were generally of epithelial or vascular origin. Although the preputial and clitoral glands, forestomach and harderian glands of mice do not have human counterparts, ganciclovir should be considered a potential carcinogen in humans.*

*Ganciclovir increased mutations in mouse lymphoma cells and DNA damage in human lymphocytes in vitro at concentrations between 50 to 500 and 250 to 2,000 µg/mL, respectively. In the mouse micronucleus assay, ganciclovir was clastogenic at doses of 150 and 500 mg/kg (IV) (24,000x to 80,000x human ocular dose) but not 50 mg/kg (8,000x human ocular dose). Ganciclovir was not mutagenic in the Ames Salmonella assay at concentrations of 500 to 5,000 µg/mL.*

*Ganciclovir caused decreased mating behavior, decreased fertility, and an increased incidence of embryoletality in female mice following intravenous doses of 90 mg/kg/day (approximately 14,400x the human ocular dose of 6.25 µg/kg/day). Ganciclovir caused decreased fertility in male mice and hypospermatogenesis in mice and dogs following daily oral or intravenous administration of doses ranging from 0.2 to 10 mg/kg (32x to 1,600x the human ocular dose).*

**8.1 Pregnancy: Category C**

*Ganciclovir has been shown to be embryotoxic in rabbits and mice following intravenous administration and teratogenic in rabbits. Fetal resorptions were present in at least 85% of rabbits and mice administered 60 mg/kg/day and 108 mg/kg/day (9,600x and 17,280x the human ocular dose of 6.25 µg/kg/day, assuming complete absorption), respectively. Effects observed in rabbits included: fetal growth retardation, embryoletality, teratogenicity and/or maternal toxicity. Teratogenic changes included cleft palate,*

*anophthalmia/microphthalmia, aplastic organs (kidney and pancreas), hydrocephaly and brachygnathia. In mice, effects observed were maternal/fetal toxicity and embryolethality.*

*Daily intravenous doses of 90 mg/kg (14,400x the human ocular dose) administered to female mice prior to mating, during gestation, and during lactation caused hypoplasia of the testes and seminal vesicles in the month-old male offspring, as well as pathologic changes in the nonglandular region of the stomach (see Carcinogenesis, Mutagenesis and Impairment of Fertility).*

*Ganciclovir may be teratogenic or embryotoxic at dose levels recommended for human use. There are no adequate and well-controlled studies in pregnant women. ZIRGAN should be used during pregnancy only if the potential benefits justify the potential risk to the fetus.*

*Footnote: All dose comparisons presented in the Carcinogenesis, Mutagenesis, Impairment of Fertility, and Pregnancy subsections are based on the human ocular dose of 6.25 µg/kg/day to single eye. If both human eyes are treated, the multiple of animal systemic dose to human clinical dose should be divided by 2.*

Eliminate **13.2 Animal Toxicology and/or Pharmacology** subsection proposed by the current sponsor.

Unresolved toxicology issues (if any): None

Recommendations: The approval of NDA 22-211 is recommended.

Signatures (optional):

Reviewer Signature Conrad H. Chen, Ph.D.

Supervisor Signature Wendelyn Schmidt, Ph.D.

Concurrence Yes  No

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/s/  
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Conrad Chen  
7/8/2009 03:22:57 PM  
PHARMACOLOGIST  
The approval of NDA 22-211 is recommended.

Wendelyn Schmidt  
7/8/2009 03:29:19 PM  
PHARMACOLOGIST