CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202408Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	202408
Supporting document/s:	SDN 004 (Original NDA, eCTD0000) SDN 012 (Nonclinical response to information request; eCTD 0007)
Applicant's letter date:	SDN004: 5-31-2013 SDN012: 9-19-2013 SDN021: 3-18-2014 SDN031: 12-24-2015
CDER stamp date:	SDN004: 5-31-2013 SD021: 3-18-2014 SDN031: 12-24-2015
Product:	Acyclovir ophthalmic ointment, 3.0%
Indication:	(b) (4)
Applicant:	Fera Pharmaceuticals, LLC 15 R Birch Hill Rd Ste A Locust, NY 11560
Review Division:	DTOP
Reviewer:	Aaron M Ruhland, PhD
Supervisor/Team Leader:	Lori E Kotch, PhD, DABT
Division Director:	Renata Albrecht, MD
Project Manager:	Lois Almoza

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202408 are owned by Fera Pharmaceuticals or are data for which Fera Pharmaceuticals has obtained a written right of reference.
Any information or data necessary for approval of NDA 202408 that Fera Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202408.

1 Executive Summary

1.1 Introduction

The subject of this NDA is topical acyclovir ophthalmic ointment 3.0% (b) (4) This document represents a revision to the review of the initial NDA submission (SDN004; review submit date 2-24-2014). Revisions were made to the original review to update the proposed labeling to PLLR format and provide basis for data conveyed elsewhere in that labeling. In the original submission, a 505(b)(2) application, the Applicant referenced several listed drugs on Form 356h. The applicant has not conducted any nonclinical studies to support the safety of topical ophthalmic acyclovir but rather relies on published literature to establish ophthalmic safety and the Agency's past findings of general safety for a single topical acyclovir listed drug (as now listed on Form 356h):

- Acyclovir topical ointment 5% (Zovirax®; NDA 18604)

Due to limiting the number of applications referenced on Form 356h, statements made in the labeling regarding nonclinical studies can no longer rely on those drugs which were no longer listed. Published data were found which continue to support the statements and are reviewed in this document.

1.2 Brief Discussion of Nonclinical Findings

All nonclinical data conveyed in the labeling were derived from the referenced listed drug or published literature reports. For review of publications supporting embryofetal toxicity, neonatal toxicity, fertility and genotoxicity, see nonclinical review of the original application (SDN004) dated 2-24-2014 in DARRTS. In this review, statements made regarding results of nonclinical carcinogenicity studies are supported by review of published literature. Additionally, the labeling has now been converted to PLLR format.

1.3 Recommendations

1.3.1 Approvability

The application is approvable from a Pharmacology/Toxicology perspective

1.3.2 Labeling (Applicant's version)

Reviewer's note: No draft labeling was submitted with the Applicant's resubmission. The following labeling is the most recent version proposed by the applicant in SDN021 (3-18-2014). This version reflects the Applicant's acceptance of the Agency's recommended edits sent during the first review cycle (letter date: 3-14-2014). For the

Applicant's original proposed labeling, see SDN004 or the nonclinical review of this submission (review dated 2-24-2014). The following sections of the applicant's proposed labeling are relevant to the Pharmacology/Toxicology discipline only (full proposed label included as Appendix A).

Applicants proposed label:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy:

(b) (4)

A prospective epidemiologic registry of acyclovir use during pregnancy was established in 1984 and completed in April 1999. There were 749 pregnancies followed in women exposed to systemically administered acyclovir during the first trimester of pregnancy resulting in 756 outcomes. The occurrence rate of birth defects approximates that found in the general population. However, the small size of the registry is insufficient to evaluate the risk for less common defects or to permit reliable or definitive conclusions regarding the safety of acyclovir in pregnant women and their developing fetuses. The maternal plasma level of acyclovir following ocular administration is unknown. (b) (4)

8.3 (b) (4) *Acyclovir concentrations have been documented in breast milk* (b) (4)

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility:

Acyclovir was not shown to be carcinogenic in mouse and rat bioassays at oral doses up to 450mg/kg ((b) (4) *times the* (b) (4) *[RHOD], on a mg/m2 basis).*

Acyclovir was tested in 16 in vitro and in vivo genetic toxicity assays. Acyclovir was found to be negative in the Ames test, positive in in vitro mouse lymphoma assay (TK locus), and positive in in vitro and in vivo assays for chromosomal effects.

In reproduction studies acyclovir did not impair fertility or reproduction at oral doses up to 450 mg/kg/day in mice ((b) (4) *times the RHOD), or at subcutaneous doses of 25 mg/kg/day in rats (125 times the RHOD). At* (b) (4) *in rats and rabbits* (b) (4) *implantation* (b) (4) *was decreased.*

1.3.3 FDA's proposed changes to labeling (Redline version of sections relevant to nonclinical Pharmacology/Toxicology). Suggested deletions are notated as a strikethrough font and suggested additions are notated as a double-underlined **red** font.

A final decision regarding the inclusion of human data in the Risk Summary and Human Data sections is deferred to the clinical team.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy:

Risk Summary

(b) (4)

A prospective epidemiologic registry of acyclovir use (b) (4) from 1984 (b) (4) ~~-~~to1999~~.~~ indicated that the occurrence rate of birth defects (b) (4) in women exposed to systemically administered acyclovir during the first trimester of pregnancy (period of organogenesis) (b) (4) ~~-~~approximate~~s~~d that found in the general population. Likewise, oral and subcutaneous administration of acyclovir to pregnant mice, rats and rabbits during organogenesis did not produce teratogenicity at clinically relevant doses [*see Animal Data*]. (b) (4)

Data

Human Data

A prospective epidemiologic registry of acyclovir use during pregnancy was established in 1984 and completed in April 1999. There were 749 pregnancies followed in women exposed to systemically administered acyclovir during the first trimester of pregnancy resulting in 756 outcomes. The occurrence rate of birth defects approximates that found in the general population. However, the small size of the registry is insufficient to evaluate the risk for less common defects, or to permit reliable or definitive conclusions regarding the safety of acyclovir in pregnant women and their developing fetuses. The human maternal plasma level of acyclovir following ocular administration is unknown.

Animal Data

In published animal reproduction studies, acyclovir was not maternally toxic and did not produce teratogenicity in the mouse at oral doses up to 450 mg/kg/day (1100 times the maximum recommended human ophthalmic dose [RHOD] on a mg/m^2 basis, assuming 100% absorption), or in the rat and rabbit at subcutaneous doses up to 50 mg/kg/day (approximately 250 and 500 times the RHOD, respectively) when administered throughout the period of organogenesis.

Administration of acyclovir from postnatal days 3 to 21 did not produce adverse effects in neonatal rats at subcutaneous doses less than or equal to 20 mg/kg/day (100 times the RHOD).

Reviewers note: The pregnancy registry data presented in Section 8.1 of the labeling are present in the current labeling for Zovirax® ointment referenced by the Applicant. No additional references are needed to support these data. The animal data regarding embryofetal teratogenicity are presented in approved labeling for other acyclovir formulations but not labeling for NDA 18604 (Zovirax ointment) referenced by the applicant. However, these data are also published (Moore, H.L., *et al*., 1983, "Preclinical toxicology studies with acyclovir: teratogenic, reproductive and neonatal tests", *Fundam Appl Toxicol*, 3(6): 560 – 568), therefore, these data may remain in the labeling (see review of publication in Nonclinical review dated 2-24-2014).

Data regarding the effect of acyclovir on neonatal development in rats are not present in the labeling for NDA 18604. In other approved labeling (not referenced by the Applicant), data from a neonatal study in rats are conveyed as follows: "In a rat peri- and post-natal study at 50 mg/kg/day, s.c., there was a statistically significant decrease in group mean numbers of corpora lutea, total implantation sites, and live fetuses." This statement is unsubstantiated by published data and the labeling containing this information is not referenced by the Applicant, therefore these data are not to be included in the labeling for this application. Data supporting the proposed statement "Administration of acyclovir from postnatal days 3 to 21 did not produce adverse effects in neonatal rats at subcutaneous doses less than or equal to 20 mg/kg/day (100 times the RHOD)" are published and were reviewed previously (see Nonclinical review dated 2-24-2014).

(b) (4) **8.3 Lactation**:

Risk Summary

(b) (4)

-There is no information regarding the presence of acyclovir in human milk following ocular administration, the effects on the breastfed infant, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for

acyclovir, and any potential adverse effects on the breast-fed child from acyclovir or from the underlying maternal condition.

Reviewer's note: These statements are supported by the data presented in the labeling for NDA 18604. The applicant-proposed [redacted] have been deleted in the current label. No further justification is required.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility:

Acyclovir was not shown to be carcinogenic in mouse and rat bioassays at oral doses up to 450 mg/kg (approximately [redacted] 1100- [redacted] 2200 times the [redacted] ~~[~~maximum RHOD~~]~~, on a mg/m^2 basis, assuming 100% absorption).

Acyclovir was tested in 16 ~~in vitro~~*in vitro* and ~~in vivo~~in vivo genetic toxicity assays. Acyclovir was found to be negative in the Ames test, positive in the ~~in vitro~~*in vitro* mouse lymphoma assay (TK locus), and positive in the ~~in vitro~~*in vitro* and ~~in vivo~~*in vivo* assays for chromosomal effects.

In reproduction studies, acyclovir did not impair fertility or reproduction at oral doses up to 450 mg/kg/day in mice ([redacted] 1100 times the RHOD), or at subcutaneous doses of 25 mg/kg/day in rats (125 times the RHOD). At [redacted] a dose of 50 mg/kg/day in rats and rabbits ([redacted] 250 and 500 times the RHOD, respectively), implantation [redacted] efficiency was decreased.

Reviewer's note: The data are presented in approved labeling for other acyclovir formulations but not labeling for NDA 18604 (Zovirax ointment) referenced by the applicant. In those approved labels, data regarding positive and negative results of 16 genotoxicity tests are described however the details of which assays were positive or negative is not included. These statements are supported by data presented in (Clive, D., *et al*., 1983, *Fundam Appl Toxicol*, 3:587–602). This publication was previously reviewed (see Nonclinical review dated 2-24-2014).

Data in the labeling regarding carcinogenicity are presented in approved labeling for other acyclovir formulations but not labeling for NDA 18604 (Zovirax ointment) referenced by the applicant. These data are, however, published (Tucker, W.E., *et al*., 1983, "Preclinical toxicology studies with acyclovir: Carcinogenicity bioassays and chronic toxicity tests", *Fundam Appl Toxicol*, 3(6): 579 – 586). They are now reviewed below.

Regarding the statement describing no effect on fertility, these published data were previously reviewed (see Nonclinical review dated 2-24-2014 regarding Moore, H.L., *et al*., 1983, "Preclinical toxicology studies with acyclovir:

teratogenic, reproductive and neonatal tests", *Fundam Appl Toxicol*, 3(6): 560 – 568).

2 Drug Information

2.1 Drug

CAS Registry Number: 59277-89-3

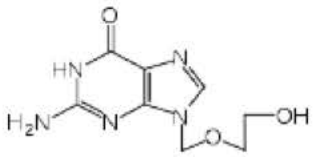
Generic Name: Acyclovir ophthalmic ointment (3.0%)

Code Name: AVACLYR (proposed)

Chemical Name: 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one

Molecular Formula/Molecular Weight: $C_8H_{11}N_5O_3$ / 225.21 g/mol

Structure or Biochemical Description



Pharmacologic Class: Acyclic purine nucleoside analogue

2.2 Relevant INDs, NDAs, BLAs and DMFs

- NDA 018604 (Zovirax® topical ointment 5%, approved 3-29-1982)
- DMF (b) (4) (LoA provided)

2.3 Drug Formulation

Ingredient	Amount w/w (%)	Function
Acyclovir, USP	3	Active
White petrolatum, USP	(b) (4)	Base

2.6 Proposed Clinical Population and Dosing Regimen

Acyclovir topical ophthalmic ointment is indicated for the treatment of acute herpetic keratitis (dendritic ulcers) in patients with herpes simplex (HSV-1 and HSV-2) virus. The recommended dosing regimen is (b) (4)

Reviewer's note: Based on the 1 cm ribbon and the tube's average orifice internal diameter of 0.05 inches, the dose of the drug product is 12.9 mg when a specific gravity of 0.89 g/cm^3 for white petrolatum is used. For 3% drug substance, this calculates as 0.387 mg per dose. The maximum total daily dose (i.e. administered 5 times per day) of drug product is 64.5 mg/day containing **1.935 mg** of drug substance.

3 Studies Reviewed

Tucker, W.E., et al., 1983, "Preclinical toxicology studies with acyclovir: Carcinogenicity bioassays and chronic toxicity tests", *Fundam Appl Toxicol*, 3(6): 579 – 586

Reviewer's note: The data presented in the published study are to support the following labeling statements:

> Acyclovir was not shown to be carcinogenic in mouse and rat bioassays at oral doses up to 450mg/kg (approximately 1100-2200 times the RHOD, on a mg/m^2 basis, assuming 100% absorption).

Rats (n=85/sex/treatment group) and mice (n=100/sex/treatment group) were treated once daily by gastric intubation with suspensions of acyclovir given at 0, 50, 150 and 450 mg/kg/day (dose volume of 10 mL/kg) . All rats and mice were observed twice each day for signs of toxicity or changes in physical condition; detailed physical inspections were carried out weekly. Individual food consumption and body weights were recorded weekly. The animals were dosed each day until mortality decreased a group size to approximately 20% of the number of animals of that sex present in the test group when the study started. At that point all remaining animals of that sex were killed and necropsied.

Results:
Rats: The study was terminated at week 110 for male rats and week 122 for female rats. Mean body weights for control and treated rats were similar for the duration of the study. Life table methods used to assess the effect of treatment on the overall incidences of benign tumor bearing animals (TBAs), malignant TBAs, and total TBAs revealed no significant differences between control and treated rats. None of the rats had unusual neoplasms and there were no more benign or malignant neoplasms in rats treated for their lifetime with 50, 150 and 450 mg/kg/day acyclovir than in control rats. Acyclovir was considered not carcinogenic as tested in rats.

Mice: The 20% survival cut off was at 126 weeks for male mice and at 111 weeks for female mice. Mean body weights for control and treated male mice were similar for the duration of the study. Life table methods did not reveal any significant effects of treatment on the incidence of benign TBAs, malignant TBAs, or total TBAs for either male or female mice. The incidence of lung adenomas was significantly ($p < 0.05$) larger

in treated mice as determined by the Cochran- Armitage test but only in the interval from month 18 to the end of the study. The authors note that since lung adenomas occur spontaneously at a high incidence in old CD-1 mice and since female mice treated with 150 and 450 mg/kg ACV survived significantly longer than control female mice, this result can be explained. The incidence of lung adenomas was not significantly different in either male or female mice treated with ACV when the data were analyzed by the log rank test and Cox's life-table regression model. None of the mice had unusual neoplasms. The results of gross and microscopic examinations also demonstrated that chronic treatment with ACV did not produce non-neoplastic lesions or alter the incidence or severity of common spontaneous disease processes in mice.

10 Integrated Summary and Safety Evaluation

This review represents a revision to the review of the original NDA application. The original application received a complete response letter regarding CMC issues and was not approved during the first cycle. In this second cycle of review, the applicant only references a single listed drug (NDA 018604: Zovirax® topical ointment 5%) for 505(b)(2) approval which no longer allows reference to other approved formulations of acyclovir which describe the lack of carcinogenic potential of acyclovir. A published reference was found, however, and the data which describe carcinogenicity assays can be included in the labeling. The second cycle of review also allows the opportunity to convert the label to PLLR format.

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/s/

AARON M RUHLAND
05/23/2016

LORI E KOTCH
05/23/2016

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant's letter date:	SDN004: 5-31-2013
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CDER stamp date:	SDN004: 5-31-2013
Product:	Acyclovir ophthalmic ointment, 3.0%
Indication:	(b) (4)
Applicant:	Fera Pharmaceuticals, LLC
	15 R Birch Hill Rd Ste A
	Locust, NY 11560
Review Division:	DTOP
Reviewer:	Aaron M Ruhland, PhD
Supervisor/Team Leader:	Lori E Kotch, PhD, DABT
Division Director:	Renata Albrecht, MD
Project Manager:	Lois Almoza

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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

The subject of this NDA is topical acyclovir ophthalmic ointment 3.0% (b) (4) [redacted] Acyclovir is a nucleoside analogue which upon conversion by viral and cellular kinases is incorporated into viral DNA, resulting in premature chain termination. Systemic exposure following topical ocular administration is very low and resultant toxicity is not expected. The applicant has not conducted any nonclinical studies to support the safety of topical ophthalmic acyclovir but rather relies on published literature to establish ophthalmic safety and the Agency's past findings of general safety for systemic and topical acyclovir listed drugs:

- Acyclovir for injection (NDA 01603)
- Acyclovir topical ointment 5% (NDA 18604)
- Acyclovir capsules (NDA 18828)
- Acyclovir suspension (NDA 19909)
- Acyclovir tablets (NDA 20089)
- Acyclovir topical cream 5% (NDA 021478)

1.2 Brief Discussion of Nonclinical Findings

All nonclinical data were derived from the referenced drugs or published literature reports. Literature referenced by the applicant supports previous findings detailed in the labeling for listed drugs and is not reviewed. (b) (4) [redacted]

The applicant did not include citations or data that characterize the genotoxic potential of acyclovir. The labeling for systemic acyclovir contains the statement: "Acyclovir was tested in 16 *in vitro* and *in vivo* genetic toxicity assays. Acyclovir was positive in 5 of the assays". An independent review of a published article (see below) confirmed the genotoxic potential of acyclovir though plasma concentrations following intravenous or topical ophthalmic doses do not reach threshold levels shown to induce genotoxicity *in vitro* or *in vivo*.

The applicant has also submitted published nonclinical studies to support the ocular safety and pharmacokinetics of acyclovir. These studies showed that acyclovir

distributes to the aqueous humor following topical ocular administration. Distribution to other ocular tissues was not explored. In rabbits following topical ocular application, a dose-dependent increase in mild conjunctival irritation was noted following treatment with acyclovir and its white petrolatum base compared to saline control. The irritation was most pronounced following the last dose of the day and was almost completely resolved by the following morning's dose. No other signs of ocular pathology were noted upon biomicroscopy, funduscopy or histological analysis. A NOAEL was established at 6% acyclovir, 5 times per day (6.3 mg/eye/day) which provides a ~3.25-fold safety margin over the maximum recommended human dose.

1.3 Recommendations

1.3.1 Approvability

The application is approvable from a Pharmacology/Toxicology perspective
.

1.3.2 Labeling (Applicant's version)

The following sections of the applicant's proposed labeling are relevant to the Pharmacology/Toxicology discipline (full proposed label included as Appendix A).

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy:

(b) (4)

A prospective epidemiologic registry of acyclovir use during pregnancy was established in 1984 and completed in April 1999. There were 749 pregnancies followed in women exposed to (b) (4) acyclovir during the first trimester of pregnancy resulting in 756 outcomes. The occurrence rate of birth defects approximates that found in the general population. However, the small size of the registry is insufficient to evaluate the risk for less common defects or to permit reliable or definitive conclusions regarding the safety of acyclovir in pregnant women and their developing fetuses. (b) (4)

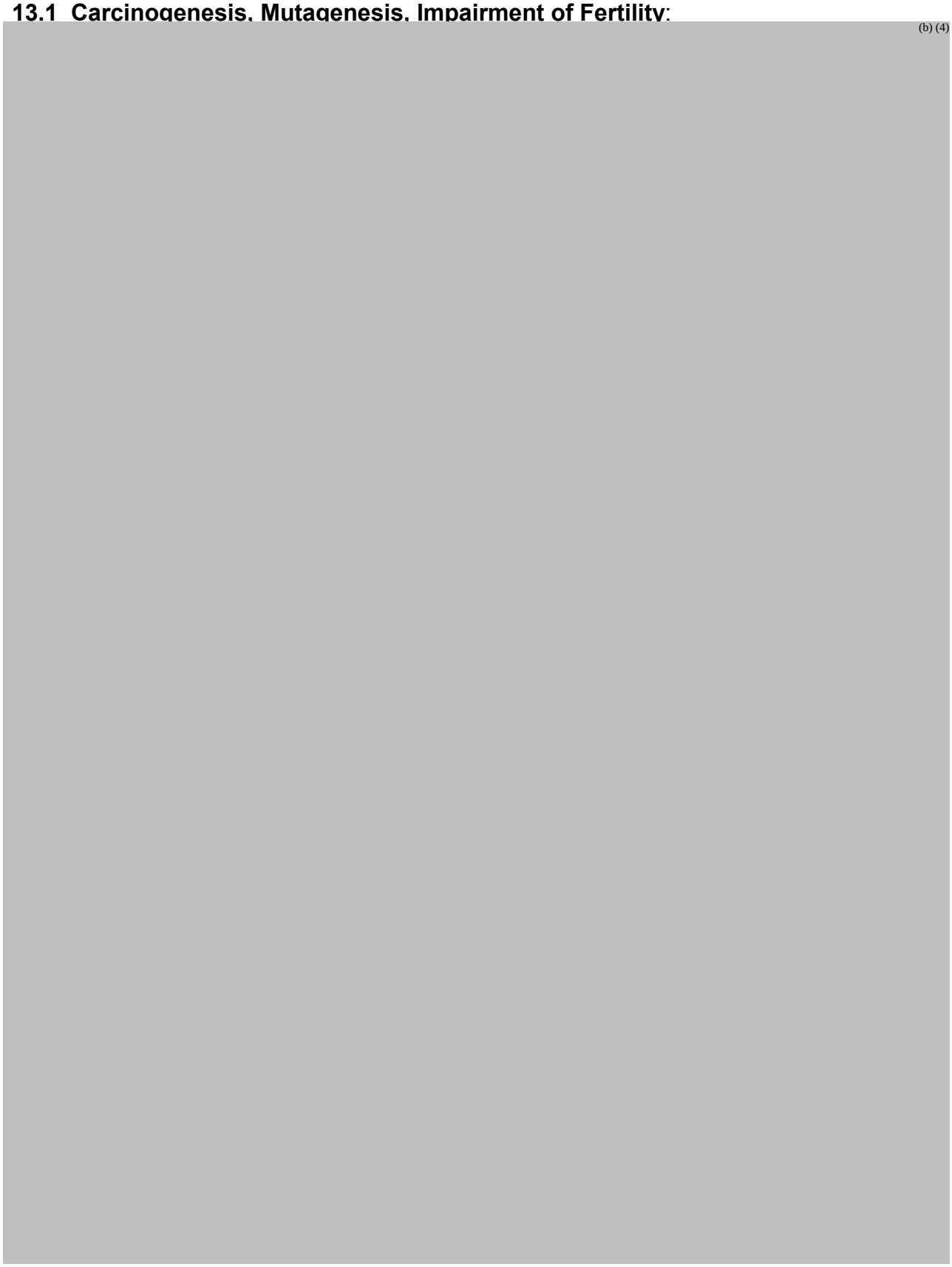
8.3 (b) (4)

acyclovir, concentrations have been documented in breast milk (b) (4)

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility:

(b) (4)



1.3.3 FDA's proposed changes to labeling (Redline version of sections relevant to nonclinical Pharmacology/Toxicology). Details regarding proposed changes are included in this review in the relevant sections. Suggested deletions are notated as a strikethrough font and suggested additions are notated as a thick underlined font.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy:

(b) (4)

There are no adequate and well-controlled studies ~~of systemic~~ with acyclovir in pregnant women. A prospective epidemiologic registry of acyclovir use during pregnancy was established in 1984 and completed in April 1999. There were 749 pregnancies followed in women exposed to (b) (4) systemically administered acyclovir during the first trimester of pregnancy resulting in 756 outcomes. The occurrence rate of birth defects approximates that found in the general population. However, the small size of the registry is insufficient to evaluate the risk for less common defects or to permit reliable or definitive conclusions regarding the safety of acyclovir in pregnant women and their developing fetuses. The maternal plasma level of acyclovir following ocular administration is unknown. (b) (4)

Reviewer's note: (b) (4)

Additional language derived from the labeling for acyclovir for injection has been added and is supported by the published nonclinical study submitted to the NDA by the applicant (Moore, *et al*., 1983, reviewed below).

8.3 (b) (4)

acyclovir, concentrations have been documented in breast milk (b) (4)

Reviewer's note: (b) (4)

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility:

(b) (4)

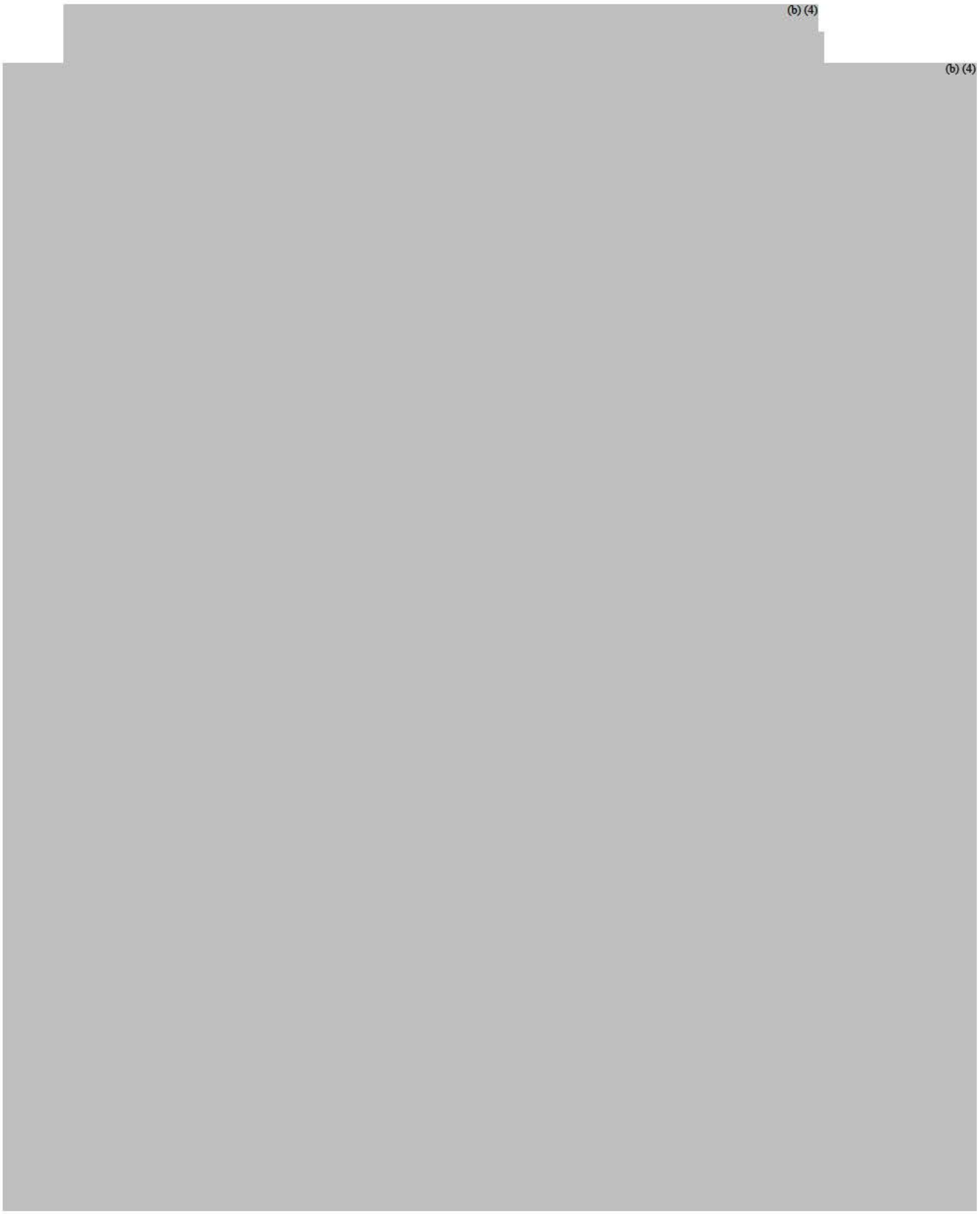
Acyclovir was not shown to be carcinogenic in mouse and rat bioassays at oral doses up to 450mg/kg ((b) (4) times the (b) (4) [RHOD] on a mg/m^2 basis, assuming 100% absorption).

Acyclovir was tested in 16 *in vitro* and *in vivo* genetic toxicity assays. Acyclovir was found to be negative in the Ames test, positive in *in vitro* mouse lymphoma assay (TK locus), and positive in *in vitro* and *in vivo* assays for chromosomal effects.

In reproduction studies acyclovir did not impair fertility or reproduction at oral doses up to 450 mg/kg/day in mice ((b) (4) times the RHOD), or at subcutaneous doses of 25 mg/kg/day in rats (125 times the RHOD). At (b) (4) in rats and rabbits (b) (4) (b) (4) implantation (b) (4) was decreased.

Reviewer's note: This applicant's proposed section of the labeling is (b) (4) (4)

(b) (4)


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

Reviewer's note: (b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 59277-89-3

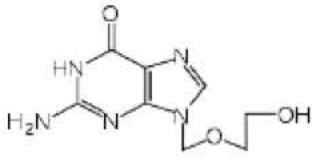
Generic Name: Acyclovir ophthalmic ointment (3.0%)

Code Name: AVACLYR (proposed)

Chemical Name: 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one

Molecular Formula/Molecular Weight: $C_8H_{11}N_5O_3$ / 225.21 g/mol

Structure or Biochemical Description



Pharmacologic Class: Acyclic purine nucleoside analogue

2.2 Relevant INDs, NDAs, BLAs and DMFs

- NDA 018604 (Zovirax® topical ointment 5%, approved 3-29-1982)
- NDA 021478 (Zovirax® topical cream 5%, approved 12-30-2002)
- NDA 018828 (Zovirax® oral capsule 200 mg, approved 1-25-1985)
- NDA 019909 (Zovirax® oral suspension 200mg/5mL, approved 12-22-1989)
- NDA 20089 (Zovirax® oral tablet 400 or 800 mg, approved 4-30-1991)
- NDA 018603 (Zovirax® injection, discontinued, approved on 10-22-1982)
- DMF (b) (4) (LoA provided)

2.3 Drug Formulation

Ingredient	Amount w/w (%)	Function
Acyclovir, USP	3	Active
White petrolatum, USP	(b) (4)	Base

2.4 Comments on Novel Excipients

White petrolatum is qualified for topical ophthalmic use as an ointment formulation at concentrations up to (b) (4)% and for other topical use up to (b) (4)%.

Reviewer's note: The formulation has been in use in humans for many years with no implication of the excipients contributing to toxicity. Limited nonclinical studies submitted by the applicant did not associate any toxicity with the vehicle. The (b) (4) excipient should be considered qualified.

2.5 Comments on Impurities/Degradants of Concern

The Chemistry, Manufacturing and Controls (CMC) reviewer for this application asked for consultation regarding specifications proposed (b) (4)

(b) (4)

(b) (4)

The applicant submitted the updated specifications to the NDA in a response dated 2-14-2014 (SDN016, eCTD sequence number 0012).

2.6 Proposed Clinical Population and Dosing Regimen

Acyclovir topical ophthalmic ointment is indicated for the treatment of acute herpetic keratitis (dendritic ulcers) in patients with herpes simplex (HSV-1 and HSV-2) virus. The recommended dosing regimen is (b) (4)

Reviewer's note: Based on the 1 cm ribbon and the tube's average orifice internal diameter of 0.05 inches, the dose of the drug product is 12.9 mg when a specific gravity of 0.89 g/cm^3 for white petrolatum is used. For 3% drug substance, this calculates as 0.387 mg per dose. The maximum total daily dose of drug product is 64.5 mg/day containing 1.935 mg of drug substance.

3 Studies Submitted

3.1 Studies Reviewed

Toxicology

- Davidson R, *et al*., 1981, " Inhibition of Herpes Simplex Virus Transformed and Nontransformed Cells by Acycloguanosine: Mechanisms of uptake and toxicity", *Virology,* 113:9–13.
- Lass J, *et al.,*1984, "Antiviral medications and corneal wound healing", *Antiviral Res*, 4:143–150.
- Moore J Jr , *et al*., 1983, "Preclinical toxicology studies with acyclovir: Teratologic, reproductive, and neonatal tests", *Fundam Appl Toxicol*, 3:560–568.
- Steele R, *et al*., 1980, "Comparative *in vitro* immunotoxicology of acyclovir and other antiviral agents", *Infect Immun*, 28:957–962.

- Tucker, W, *et al*., 1983, "Preclinical toxicology studies with acyclovir: ophthalmic and cutaneous tests", *Fundam Appl Toxicol*, 3:569-572

3.2 Studies Not Reviewed

In the nonclinical overview and list of nonclinical references, the applicant described or included many published articles which were found to be irrelevant to characterizing the nonclinical safety or nonclinical pharmacokinetics of topical ophthalmic acyclovir. Many articles pertained to Varicella virus (not HSV) or described the mechanism of resistance to acyclovir. These studies are listed below with a short explanation describing reason for irrelevance to nonclinical safety and pharmacology.

Pharmacology of acyclovir/ Pathology of disease

- Biron K, Elion G, 1980, "*In vitro* Susceptibility of Varicella Zoster Virus to Acyclovir", *Antimicrob Agents Chemotherapy*, 18:443–447
  - Microbiology study cited by applicant to support effective tissue levels of acyclovir against Varicella virus (not HSV)
- Boivin G, *et al*., 1994, "Phenotypic and Genotypic Characterization of Acyclovir-Resistant Varicella Zoster Virus Isolates from Persons with AIDS", *J Infect Dis*, 170:68–95.
  - Characterization of resistance mechanisms of Varicella zoster (not HSV)
- Collum L, *et al.*, 1985, "Oral Acyclovir in Herpetic Keratitis", *Trans Ophthalmol Soc UK* 104:629.
  - Human study of oral acyclovir treatment of herpetic keratitis. The article makes mention of aqueous humor levels of acyclovir which exceed the ED_{50} of HSV-1.
- Dorsky D, and C. Crumpacke, 1987, "Drugs Five Years Later: Acyclovir", *Ann Intern Med*, 107:859–874.
  - Review of mechanism and clinical uses
- Englund J, *et al*., 1990, "Herpes Simplex Virus Resistant to Acyclovir", *Ann Intern Med*, 112:416–422.
  - Determination of resistance to acyclovir in clinical isolates of HSV
- Feldman S, *et al*., 1988, "Excessive serum concentrations of acyclovir and neurotoxicity", *J Infect Dis*, 157:385–388.
  - Human case report
- Field H, *et al*., 1982, "Atypical Patterns of Neural Infection Produced in Mice by Drug Resistant Strains of Herpes Simplex Virus", *J Gen Virol*, 569: 91-99.
  - Study of infection (no acyclovir treatment)
- Hirsch MS, and R Schooley, 1989, "Resistance to antiviral drugs: The end of innocence", *N Engl J Med*, 320: 313-314.
  - Editorial on rise in drug resistant virus strains
- Ida M, *et al*., 1999, "Emergence of Resistance in Acyclovir and Penciclovir in Varicella Zoster Virus and Genetic Analysis of Acyclovir-Resistant Variants", *Antiviral Res,* 40:155–66.
  - *In vitro* generation and characterization of acyclovir resistant HSV strains

- Jacobson M, *et al*., 1990, "Acyclovir-Resistant Varicella Zoster Virus Infection After Chronic Oral Acyclovir Therapy in Patients with the Acquired Immunodeficiency Syndrome (AIDS)", *Ann of Inter Med,* 112: 187-191
    - Human case reports
- Keller PM, Fyfe JA, Beauchamp L *et al*., 1981, "Enzymatic phosphorylation of acyclic nucleoside analogues and correlations with antiherpetic activities", *Biochem Pharmacol*, 30:3071-3077.
    - Specificity of viral thymidine kinase for nucleoside analogues
- Markham R, *et al*., 1977, "Double-blind Clinical Trial of Adenine Arabinoside and Idoxuridine in Herpetic Corneal Ulcers", *Trans Ophthalmol Soc UK*, 97: 333 - 340.
    - Human clinical trial
- McKendrick M, *et al*., 1984, "Oral acyclovir in herpes zoster", *J Antimicrob Agents Chemother,* 14:661–665.
    - Human clinical study
- McKendrick M, *et al*.,1986, "Oral acyclovir in acute herpes zoster", *Br Med J,* 293: 529–1532.
    - Human clinical study
- Mertz G, *et al*., 1988, "Prolonged continuous versus intermittent oral acyclovir treatment in normal adults with frequently recurring genital herpes simplex virus infection", *Am J Med,* 85:14–19.
    - Human clinical study
- Morfin F, *et al*., 1999, "Phenotypic and genetic characteristics of thymidine kinase from classical strains of varicella zoster virus resistant to acyclovir", *Antimicrob Agents Chemother*, 43:2412–2416
    - Characterization of target enzyme in Varicella zoster (not HSV)
- Strauss S, Smith H, Brickman C et al., 1982, Acyclovir for Chronic Mucocutaneous Herpes Simplex Virus Infection in Immunosuppressed Patients. Ann of Intern Med, 96:270-271.
    - Human case reports
- Wade J, *et al*, 1982, "Treatment of cytomegalovirus pneumonia with high-dose acyclovir", *Am J Med*, 73:249.
    - Human clinical trial of acyclovir against cytomegalovirus infection

Pharmacokinetics

- de Miranda P, Krasney H, Page D *et al*., 1982, Species Differences in the Disposition of Acyclovir. *Am J Med*., 73:31-35.
    - Species differences in acyclovir pharmacokinetics following systemic administration, no pharmacokinetic assessment following ocular administration
- de Miranda P, *et al.,* 1982, Metabolic fate of Acyclovir in Humans. *Am J Med*, 73:215–220.
    - Human pharmacokinetic data
- de Miranda P, and M. Blum, 1983, Pharmacokinetics of Acyclovir after Intravenous and Oral Administration. *J Antimicrob Chemotherapy* 12:29–37.

- Human pharmacokinetic data
- Hung S, *et al*., 1984, "Pharmacokinetics of Oral Acyclovir (Zovirax) in the eye", *Br J Ophthalmol*, 68:192–195.
  - Human ocular pharmacokinetics following oral administration of acyclovir
- Laskin O, 1983, "Clinical pharmacokinetics of acyclovir", *Clin Pharmacokinet* 8:187–193.
  - Human pharmacokinetic review

4 Pharmacology

4.1 Primary Pharmacology

(b) (4)

Reviewer's note: The applicant proposes (b) (4)

(b) (4)

Reviewer's note: The applicant proposes (b) (4)

5 Pharmacokinetics

5.1 Systemic pharmacokinetics

The applicant cites several articles regarding pharmacokinetics of acyclovir:

1) **Hung S, Patterson A, Rees, P, 1984, Pharmacokinetics of Oral Acyclovir (Zovirax) in the eye. *Br J Ophthalmol*, 68:192–195.**
   a. This study was performed in humans following oral administration of acyclovir and therefore will not be reviewed.

2) **de Miranda P, Krasney H, Page D et al., 1982, Species Differences in the Disposition of Acyclovir. *Am J Med*., 73:31-35.**
   a. This study compared oral bioavailability and distribution of acyclovir in different animal species. Since there is no ocular administration studies, this article is of limited utility because comparative bioavailability of oral versus ocular dosage forms cannot be determined.

3) **Biron K, Elion G, 1980, In vitro Susceptibility of Varicella Zoster Virus to Acyclovir. *Antimicrob Agents Chemotherapy*, 18:443–447.**
   a. This study determined the *in vitro* susceptibility of varicella zoster virus (VZV) to acyclovir. VZV appears less susceptible to acyclovir when compared to HSV, but is not a relevant organism for the proposed indication (corneal ulcers due to Herpes Simplex virus)

4) **de Miranda P, Good S, Krasney H *et al*., 1982, Metabolic fate of Acyclovir in Humans. Am J Med, 73:215–220.**
   a. This study was performed in humans following intravenous administration of acyclovir and therefore will not be reviewed.

5) **Strauss S, Smith H, Brickman C *et al*., 1982, Acyclovir for Chronic Mucocutaneous Herpes Simplex Virus Infection in Immunosuppressed Patients. Ann of Intern Med, 96:270-271.**
   a. This study was performed in humans with immunodeficiency and regarded efficacy in treating chronic infection

5.2 Ocular Pharmacokinetics

Study Title: Preclinical toxicology studies with acyclovir: ophthalmic and cutaneous tests

Reference: Tucker, WE, *et al*., 1983, *Fundam Appl Toxicol*, 3: 569 – 572.

New Zealand White rabbits (n= 3 male/time point) with normal corneas (i.e. no pre-test fluorescein staining) were treated with a 1 cm ribbon of 3% acyclovir (~0.63 mg acyclovir) in white petrolatum vehicle applied on to the corneal surface of the right eye. The eye was then closed and lightly massaged to spread the test article. Rabbits were sacrificed at 0.5, 1, 2, 4, and 6 hours after treatment for determination of acyclovir concentration in the aqueous humor and blood.

Reviewer's note: The author notes that a 1 cm of 3% acyclovir would contain approximately 0.63 mg acyclovir. In order for this to calculate correctly, the 1 cm ribbon would have a mass of 21 mg. Based on a calculation provided by the CMC reviewer, a 1 cm ribbon of the drug product would weigh 12.9 mg and would contain 0.387 mg acyclovir per dose.

The analysis showed that acyclovir crossed the cornea which resulted in detectable levels of acyclovir in the aqueous humor. Acyclovir levels in the aqueous humor peaked between 1 and 2 hours following application. By 4 hours, ~25 – 50% of the peak concentration remained. In plasma, acyclovir was not detectable in 11 of the 15 treated animals. In those animals with detectable systemic acyclovir, concentrations were low

and not detectable by 6 hours. The lower limit of detection of the assay was 0.25 µM. Distribution to other ocular tissues was not assessed.

Time after treatment	Acyclovir in aqueous humor (µM)	Acyclovir in plasma (µM)
0.5 hour	1.05 ± 0.35 (236 ng/mL)	None detected
1 hour	2.15 ± 0.41 (484 ng/mL)	0.27 (61 ng/mL)
2 hours	2.53 ± 0.11 (570 ng/mL)	0.62 (140 ng/mL)
4 hours	0.59 ± 0.25 (133 ng/mL)	0.67 (151 ng/mL)
6 hours	0.39 ± 0.19 (88 ng/mL)	None detected

6 General Toxicology

6.1 Systemic toxicology

(b) (4)

Reviewer's note: No other information on the nonclinical toxicology of acyclovir was presented by the applicant. (b) (4)

6.2 Ocular/Dermal Toxicology

Study Title: Preclinical toxicology studies with acyclovir: ophthalmic and cutaneous tests

Reference: Tucker, WE, *et al.*, 1983, *Fundam Appl Toxicol*, 3: 569 – 572.

Conducting laboratory and location:	Wellcome Research Laboratories 3030 Cornwallis Road Research Triangle Park, NC 27709
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not specified

Dermal toxicity:

Experiment 1: Subchronic dermal irritation and systemic toxicity of acyclovir ointment (polyethylene glycol vehicle)

Polyethylene glycol based ointments containing 5% or 10% acyclovir were tested for dermal irritation and systemic toxicity in Hartley albino guinea pigs (10/sex/group). Dermal toxicity was tested in a model of intact skin and a model of abraded skin. For abraded skin, four linear abrasions 1 cm apart and deep enough to penetrate the stratum corneum were made to the lumbar region every 3 or 4 days. Intact skin was shaved. 200 mg of the ointment was applied over the test area which represented approximately 10% of the total body area. Ointment was applied 4-times daily (800 mg ointment per day) which would represent total doses of 40 mg and 80 mg acyclovir for the 5% and 10% formulations, respectively).

Clinical assessment (daily):

- No changes were attributed to the test article.

Body weights (pretest, and Days 1, 4, 11, 18, and 21):

- No changes were attributed to the test article.

Food consumption (Days 4, 11, and 18):

- No changes were attributed to the test article.

Skin irritation assessment (Draize assessment; pretest and Days 2, 9, 16 and 23):

- Slight erythema observed in all groups and attributed to the shaving procedure.

Hematology (hematocrit, hemoglobin, red blood cell count, leukocyte count; pretest and Days 8, 15, and 23):

- A difference in absolute values for lymphocyte (females only) and erythrocyte counts (males and females) was observed in guinea pigs treated with 10% acyclovir applied to abraded skin.

Treatment	Sex	Pre-dose	Day 8	Day 15	Day 23
		Lymphocytes (cells per cm^2)			
Polyethylene glycol vehicle	Male	3909	3339	4414	4535
	Female	4133	3668	5282	5175
10% acyclovir	Male	3237	2912	4024	3447
	Female	3295	2306* (-37%)	3521** (-33%)	3765** (-27%)
		Erythrocytes (x 10^6 per cm^2)			
Polyethylene glycol vehicle	Male	5.12	5.12	5.19	5.21
	Female	5.06	5.07	5.05	5.20
10% acyclovir	Male	4.8	4.7**	4.8*	4.7*
	Female	5.1	4.6**	4.5*	4.8*

* $p \leq 0.05$ (as compared to control values for same time period)
** $p \leq 0.01$ (as compared to control values for same time period)

Clinical chemistry (BUN, glucose, total protein, albumin, alkaline phosphatase, AST, ALT; pretest and Days 8, 15, and 23):

- No changes were attributed to the test article

Organ weights (brain, liver, kidneys, testes, thyroid glands, adrenal glands, pituitary gland):

- No changes were attributable to the test article

Histology ("wide variety of tissues" from control and high dose):

- No changes were attributable to the test article

Toxicokinetics (1 hour after first daily dose on Days 1 and 18):

- Systemic exposure was apparent but appeared to vary widely. The data indicate that acyclovir is absorbed through intact and abraded skin. While it appears that systemic exposure was greater on Day 18 compared to Day 1, the significance or repeatability of this observation was not explored.

Treatment	Sex	Acyclovir in plasma (μM)
		Dose Day 1
5% acyclovir ointment – abraded skin	Male	0.22
	Female	0.31
10% acyclovir ointment – abraded skin	Male	0.05
	Female	3.07
10% acyclovir ointment – intact skin	Male	0.19
	Female	1.41
		Dose Day 18
5% acyclovir ointment – abraded skin	Male	4.51
	Female	1.78
10% acyclovir ointment – abraded skin	Male	2.41
	Female	5.52
10% acyclovir ointment – intact skin	Male	4.00
	Female	3.60

Experiment 2: Skin sensitization (Draize)

Male Hartley albino guinea pigs (58 days old; 400 – 500 g) were treated intradermally with 0.1% acyclovir in a 0.9% saline vehicle (n=8). Positive controls consisted of sensitizing agents 1-chloro-2,4,-dinitrobenzene (n=4). A total of 0.1 cubic centimeter (100 μL) was administered via intradermal injection into a shaved area on the lumbar region of the test animal. The doses were given three times weekly (Monday-Wednesday-Friday) until a total of 10 doses were given. Each injection was given at a new site.

Erythema (measured at site 24 and 48 hours after injection with vernier calipers):

- Slight erythema was noted in all groups and was attributed to the shaving procedure

Challenge (Additional intradermal 50 μL dose given on 14 days after final sensitizing dose; erythema measured with vernier calipers):

- Sensitization did not occur. Guinea pigs treated with the positive control displayed sensitization to the known sensitizer.

Ocular toxicity

Experiment 1:

New Zealand white rabbits (n=4/sex/group) were treated unilaterally with 1, 3, and 6% acyclovir in white petrolatum vehicle 5-times per day (90-minute intervals) for 21 days. The ointment was applied topically as a 1 cm ribbon to the lower conjunctival sac. The

authors note that this is approximately 21 mg which would correspond to 1.26 mg of acyclovir for the 6% acyclovir ointment. The authors describe the rabbits as “young” and weighing 2.3 – 3.3 kg. Control rabbits received 0.9% saline (placebo control) or a 1 cm ribbon of white petrolatum (vehicle control).

Clinical observations (daily):

- No differences were attributable to the test article

Body weight (Pre-dose and weekly):

- No changes were attributable to the test article

Ophthalmic examinations:

- Signs of irritation in cornea, iris, and conjunctiva with scoring according to Draize scale (pretest, immediately before each dose on first 2 days of treatment, then before the first daily dose (AM) and before the last daily dose each day (PM) thereafter. Recovery was determined on post-dose days 1, 2, 4, 7, 14 and 21)
- Biomicroscopy (pretest, Day 21 and Day 22 post-dose):
- Funduscopy (pretest, Day 21 and Day 22 post-dose recovery):

Ocular Histopathology (n=5 at end of dose period, n=3 on day 24 of recovery): The actual ocular tissues examined were not noted by the authors.

A dose dependent increase in mild conjunctival irritation was noted following treatment with acyclovir and its white petrolatum base compared to saline control. The irritation was most severe following the last dose of the day and was almost completely resolved by the following morning’s dose. Draize scores are solely based on conjunctival findings, as the scores for iridic or corneal irritation were always 0. No other signs of ocular pathology were noted upon biomicroscopy, funduscopy or histological analysis.

This experiment establishes a NOAEL as 6% acyclovir, 5 times per day (6.3 mg/eye/day). The authors estimate that a 1 cm ribbon weighs 21 mg which exceeds the approximation of 12.9 mg made by the CMC reviewer for a 1 cm ribbon of the applicant’s drug product. One potential reason for this difference is a difference in the internal diameter of the orifice of the tube containing the drug product. Based on the estimation of the maximum daily dose of 1.935 mg/eye/day, the NOAEL established in this study yields a ~3.25-fold safety margin over the applicant’s proposed dose.

Treatment	Average scores for conjunctival irritation in rabbits treated with acyclovir ophthalmic ointments			
	Pre-dose	AM	PM	Post-dose
0.9% saline	0.00	0.00	0.00	0.00
White petrolatum vehicle	0.00	0.02	1.75	0.00
1% acyclovir ointment	0.00	0.00	1.65	0.00
3% acyclovir ointment	0.00	0.15	2.59	0.00
6% acyclovir ointment	0.00	0.02	2.95	0.00

Experiment 2:

New Zealand White rabbits (n=9 male) were treated with a single dose of 100 mg of 5% acyclovir (5 mg acyclovir) formulated in polyethylene glycol meant for topical dermal application. In 3 of the rabbits, the test material was washed from the test eye 20 seconds after application.

Ophthalmic examinations:

- Eye irritation (scored according to Draize scale at 24, 48, 72 and 96 hours after treatment and on Day 6):
  - No acute irritation was attributed to the formulation
- Fluorescein stain (Day 6):
  - No signs of corneal damage were attributed to the test article

Reviewer's note: This experiment supports the ocular safety profile of acyclovir but is of limited utility because of formulation differences.

Study Title: Antiviral Medications and Corneal Wound Healing.

Reference: Lass J, *et al.*, 1984, *Antiviral Res*, 4:143–150.

Conducting laboratory and location:	Not specified. Authors are listed as having business addresses of: 1) Division of Ophthalmology Case Western Reserve University Cleveland, OH 2) Department of Ophthalmology The Cleveland Clinic Foundation Cleveland, OH 3) Department of Corneal Research Retina Foundation Boston, MA
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not specified

Methods

Concentration:	3% (exact dose not specified)
Frequency of dosing:	5x/day
Route of administration:	Topical ocular
Dose volume:	Not specified
Formulation/Vehicle:	Petrolatum base
Species/Strain:	New Zealand White rabbit
Number/Sex/Group:	Males/group
Age:	Not specified
Weight:	2 – 3 kg

Experiment 1: Epithelial would healing

A circular 8.5 mm epithelial defect was created on the cornea and the epithelium then removed using a Bard Parker blade. Acyclovir ointment (3%) in petrolatum was applied 5-times per day for 7 days. Petrolatum vehicle and a saline control were included. Wounds were examined daily with slit lamp and scored according to the condition of the epithelium:

- Score 0: No visible abnormality
- Score 0.5: Intraepithelial edema visible with difficulty only by retro illumination
- Score 1.0: Edema readily apparent by retro illumination, but not by direct illumination
- Score 2.0: edema apparent by direct illumination
- Score 3.0: epithelium grossly thickened and cloudy
- Score 4.0: epithelium thickened and cloudy with a rough and irregular surface

Conjunctival injection, stromal edema and iritis were also scored on a 0 – 4+ basis. At the end of treatment, selected eyes were evaluated for histologic examination. Corneas were fixed with formalin following sacrifice, eyes were enucleated and the whole eye fixed in formalin. Paraffin embedded sections were stained with hematoxylin and eosin before examination.

No differences in epithelial healing rate were observed for rabbits treated with acyclovir compared to controls. No toxicity was associated with the vehicle. No toxic effects on the regenerating epithelium were noted. Neither edema in the stroma nor iritis were observed.

Experiment 2: Stromal wound healing

A full thickness 1.5 mm corneal button was excised from the eye. After a fibrin epithelialized plug had formed 3 days later, the acyclovir solution along with atropine 1% drops were instilled twice daily throughout the study. During treatment, daily or alternate day observations were made to confirm that no leaks were formed in the corneal wound and that the anterior chamber was deep and no synechiae had formed. Animals were sacrificed at 3 weeks when the fibrin plugs had converted to scar tissue. The collagen content of the stroma was then assessed with hydroxyproline staining. No significant difference in collagen content was noted and acyclovir did not induce stromal vascularization like other antivirals studied.

7 Genotoxicity

The applicant did not submit any nonclinical data characterizing the genotoxic potential of acyclovir. In the labeling for systemic formulations of acyclovir, statements are made regarding the positive findings in nonclinical genotoxicity assays. A published study was found which accurately reflects the genotoxicity findings reported in the labeling for systemic acyclovir.

Study Title: Preclinical toxicology studies with acyclovir: genetic toxicity tests

Reference: Clive, D., *et al*., 1983, *Fundam Appl Toxicol*, 3:587–602

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

Study title:

GLP compliance:	Not specified
QA statement:	No
Drug, lot #, and % purity:	Not specified

Methods

Strains:	*S. typhimurium*: TA1535, TA1537, TA1538, TA98, TA100
Concentrations in definitive study:	0.1 – 300,000 µg/mL +/- S9 activation
Basis of concentration selection:	Not specified
Negative control:	Not specified
Positive control:	Not specified
Formulation/Vehicle:	Not specified
Incubation & sampling time:	48 hours

Study Validity

No positive control or positive control results were included for the Ames, *E. coli* polA, or yeast cell assays. Positive controls performed as expected for the L5178Y and CHO cell assays.

Results

Acyclovir with or without S9 metabolic activation did not increase the number of revertants over control and was considered negative for genotoxic potential with this assay.

E. coli DNA repair assay

Methods:
E. coli strains W3110/polA$^+$ and p3478/polA$^-$ cultures were incubated with up to 10,000 µg of acyclovir +/- S9 mix and plates incubated for 48 hours. The zone of inhibition was then measured.

Results
Acyclovir did not inhibit growth and was considered negative for genotoxic (or cytotoxic) potential in this assay.

Yeast conversion assay

Methods:
Saccharomyces cerevisiae D4 cells were incubated with acyclovir (0.1 – 500 µg/plate) +/- S9 mix. After 4 hours, cells were removed, diluted and re-plated on selective medium (tryptophan deficient) to determine viability (try$^+$ gene convertants). Plates were incubated for 3 – 5 days before scoring.

Results:
Acyclovir did not induce gene conversion and was considered negative for genotoxic potential in this assay.

Mammalian L5178Y and Chinese Hamster Ovary (CHO) cell assays

Methods:

The $TK^{+/-}$ L5178Y mouse lymphoma cell line or CHO cell TK/APRT heterozygotes were incubated with acyclovir or control compounds for 4 hours with or without metabolic S9 activation. Concentrations of acyclovir ranged 400 – 2400 µg/mL for L5178Y cells and 1000 – 3000 µg/mL for CHO cells and limited based on solubility. For L5178Y cells, the TK mutants were selected using trifluorothymidine after 2 - 3 days expression. The cells were also assayed at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus after 6 days expression and at the Na^+/K^+ ATPase locus (OUA; base pair substitution rendering ouabain resistance) after 2 – 3 days expression. Mutants were cloned onto soft agar and counted after 11 days incubation.

For CHO cells, the cultures were incubated with the test substance for 4 hours, washed, and then cultured to determine survival at Day 7. CHO cells were assessed for mutations at the OUA, HGPRT, adenine phosphoribosyltransferase (APRT) and TK loci.

Results:
L5178Y: Acyclovir did not induce mutations at the HGPRT or OUA loci either in the presence or absence of S9 activation. In the TK assay, acyclovir was considered mutagenic causing a dose dependent increase in the number of TK mutants that was not due to selection of TK mutants decreasing cytotoxicity (since acyclovir at high concentrations can be converted by TK).

Concentration (µg/mL)	Relative growth (% of control)	Relative count (% of control)	Relative total growth (% of control)	Total number of TFT^R colonies	Number of mutants per 10^6 survivors
0	100	100	100	63	24
400	77	84	65	305	136
800	88	94	83	404	162
1200	103	85	87	198	88
1600	85	93	79	455	185
2000	81	82	66	534	246
2400	65	72	47	663	344
Hycanthone (10µg/mL)	42	46	19	919	753

CHO cells: Acyclovir did not induce mutations at the HGPRT or OUA loci either in the presence or absence of S9 activation. Acyclovir did not induce mutations at the APRT locus.

Human lymphocyte chromosome aberration assay

Methods: Fresh donor lymphocytes were stimulated for 24 hours with phytohemagglutinin before addition of acyclovir at concentration range 65 – 500 µg/mL or the positive control, ethyl methanesulfonate (250 µg/mL) and incubated for an

additional 48 hours. Cells were treated with colcemid and fixed prior to microscopic examination.

Results: At 48 hours, an increase in the incidence of aberrant metaphases was observed in cells treated with 250 or 500 µg/mL.

Conc (µg/mL)	Avg. % aberrant cells	Combined average number of aberrations/cell
0	2.0	0.020
65	2.7	0.027
125	3.0	0.036
250	15.3	0.190
500	24.3	0.350
EMS (250)	27.0	0.524

Balb / c-3T3 transformation assay

Methods:
Assay 1: The 3T3 cell line was incubated with acyclovir dose range 0.4 – 50 µg/mL or positive/negative control for 72 hours before washing and additional incubation for 4 weeks. Cells were fixed and stained then scored for types I, II and III foci.

Assay 2: The 3T3 cell line was incubated with 50 µg/mL acyclovir or negative/positive controls. Type III foci from each treatment were isolated and grown to confluency. After 3 passages, cells were either plated on supplemented agar and incubated for an additional 3 weeks or injected subcutaneously into syngeneic, immunosuppressed BALB/c male weanling mice. When tumors reached 1 cm in diameter, they were prepared for histologic examination.

Results:
Assay 1: Following exposure to 50 µg/mL acyclovir, a positive result for type III foci was obtained. At this concentration, acyclovir induced an approximate 2-fold increase in the number of scored type III foci. All other concentrations tested negative in the assay.

Assay 2: The type III foci grew in soft agar and induction of tumors were noted in immunosuppressed weanling mice.

C3H/10T1/2 transformation assay

Methods: Cultures of C3H/10T1/2 cells were incubated with acyclovir dose range 8 – 64 µg/mL for 18 hours before washing and further incubation for 35 days. Plates were then scored for type III foci.

Results: In this assay, acyclovir did not induce the formation of Type III foci.

Mouse *in vivo* dominant lethal study

Methods: Male and virgin female BKA mice were injected intraperitoneally with 0, 12.5 or 25 mg/kg acyclovir BID for 5 days. Prior to dosing, each male was housed with one female for a period of 7 days and then individually housed for the dosing period. After completion of dosing, each male was housed with two untreated, virgin females, each week, for 8 consecutive weeks. Females were sacrificed 18 days after first caging with the male and counts of live and dead implants were made.

Results: Acyclovir did not induce dominant lethality in this assay.

***In vivo* cytogenetic test (Rat)**

Methods: Male and female CD rats (n=12/group) received intravenous acyclovir at 0, 25, 50 or 100 mg/kg or injected intraperitoneally with the positive control, triethylenemelamine (0.4 mg/kg). Animals were sacrificed at 6, 24, or 48 hours and chromosome analysis performed on bone marrow smears.

Results: No increase in chromosomal breakage was induced by acyclovir at any dose. A dose of 80 mg/kg was previously found to result in plasma levels of acyclovir as high as 118 µg/mL which is below the concentration which induced DNA damage *in vitro*.

***In vivo* cytogenetic test (Chinese hamster)**

Methods: Female Chinese hamsters were injected intraperitoneally with acyclovir dose range 0 – 1000 mg/kg (n≥3/dose). Cyclophosphamide (80 or 100 mg/kg) served as the positive control. Animals were sacrificed 24 hours after dosing and bone marrow slides prepared for chromosomal analysis. Three separate experiments were carried out with different combinations of doses.

Results: At doses equal to or higher than 500 mg/kg, acyclovir induced clastogenicity in some hamsters. In those hamsters effected, high frequencies of chromosome damage were observed (e.g. 99 out of 108 cells scored in one animal had a chromosome break). Plasma levels at these doses were as high as 710 µg/mL which is above the in vitro clastogenic concentrations of 250 µg/mL. A high degree of specificity was noted as to the type of breakage induced. OF the 99 breaks scored in the above mentioned hamster, 97 occurred at the centromere of a single one of the six intermediate size metacentric chromosomes resulting in a telocentric chromosome and elsewhere in the spreak an acentric fragment. No cells had more than one chromosome break. The authors argue that a heteromorphic chromosome pair must be involved to generate this

response. Since only female hamsters were used in the study, the authors implicate the heteromophic X-chromosomes.

Dose (mg/kg)	Exp #	No. of animals	Total No. of nuclei	No. of aberrations	
				Total	Centromeric breaks
0	1	4	181	0	0
	3	1	100	0	0
25	2	3	279	2	0
	3	3	223	0	0
50	2	3	181	2	1
	3	5	380	1	1
100	2	5	500	2	0
	3	3	219	2	1
500*	1	1	108	99	97
		1	95	3	0
		1	108	3	0
		1	101	67	62
	2	1	106	6	0
		1	100	2	0
		1	105	54	51
		1	101	1	0
		1	105	0	0
	3	1	110	4	2
		1	104	34	33
		1	107	12	3
		1	62	2	1
Cyclophosphamide					
80	1	3	272	20	0
100	3	1	44	4	1

* All animals in the high dose group (500 mg/kg) are included to demonstrate the all-or-nothing effect described above.

Genotoxicity summary:

Acyclovir was not found to be negative for genotoxic potential in the following assays:

- Ames reverse mutation assay (5 strains)
- *E. coli* DNA repair assay
- Yeast conversion assay
- Mammalian L5178Y cell assay at:
  - HGPRT locus
  - OUA^R locus
- Chinese Hamster Ovary (CHO) cell assay (3 loci)
- C3H/10T1/2 transformation assay

- Mouse *in vivo* dominant lethal study
- Rat *in vivo* cytogenetic test

Acyclovir produced positive genotoxic results in the following assays:

- Mammalian L5178Y cell assay at TK locus only
- Human lymphocyte chromosome aberration assay
- Balb / c-3T3 transformation assay (2 assays)
- Chinese hamster i*n vivo* cytogenetic test

Positive results were only obtained at high concentrations of acyclovir relative to the plasma levels obtained following intravenous administration of the approved dose. While no human systemic exposure data was presented in the NDA for the topical ophthalmic formulation, plasma levels are expected to be much less than those obtained with the intravenous formulation.

8 Reproductive and Developmental Toxicology

Study Title: Preclinical toxicology studies with acyclovir: Teratologic, reproductive, and neonatal tests

Reference: Moore J Jr , *et al*., 1983, *Fundam Appl Toxicol*, 3:560–568.

Studies were performed to assess potential teratogenicity or other reproductive toxicity associated with acyclovir. Studies were performed in mice, rats and rabbits.

Mice

Acyclovir was administered by oral gavage (50, 150 and 450 mg/kg/day; dose volume 10 mL/kg) to F0 generation CD-1 mice. All breeding mice were observed daily for clinical signs and weighed weekly. All treated males on study were administered acyclovir starting 64 days before mating. No changes in male fertility were observed.

To determine teratogenic potential, 15 females were treated with acyclovir daily starting 21 days before mating until gestation day 17. Fetuses were collected at sacrifice and 1/3 were fixed in Bouins solution for visceral exam (Wilson technique) while 2/3 were fixed in alcohol and underwent skeletal exam (Dawson technique). Acyclovir was not associated with any malformations or toxic effect.

For fertility studies, 15 females were treated with acyclovir daily starting from 15 days before mating to gestation day 13. At sacrifice, quantitative results for implantation and corpora lutea were presented. Acyclovir did not affect fertility of the females.

For neonatal behavior/reproduction studies, 15 females were treated with acyclovir starting 15 days before mating and continuing through 21 days of lactation (Lactation day 1 defined as parturition). The F1 generation was assessed for:

- Count, sex, weight at birth
- Weekly weight
- Pinna detachment
- Static righting reflex (Day 7)
- Cliff aversion (Day 12)
- Homing to litter (Day 12)
- Eye opening
- Air drop righting reflex (Day 14)
- Open field testing (Day 30)
- Rotating rod assay (Day 35)
- At 8 weeks, 1 male and 1 female from each of the 15 litters were mated (non-brother-sister mating)
- Following gestation and live birth, F2 pups were then counted, sexed, and weighed. Necropsy of the F2 pups was performed at 21 days of age

The applicant notes that the high dose, 450 mg/kg, was found to result in plasma levels of acyclovir of 11 µg/mL at one hour after oral administration. Results showed that acyclovir did not affect male or female fertility, visceral or skeletal malformations, or postnatal development/fertility.

Rats

Female Sprague-Dawley rats (n=30/treatment group) were treated daily with acyclovir given via subcutaneous injection starting on gestation day 6 and continuing to gestation day 15. Acyclovir doses of 12, 25, and 50 mg/kg/day were administered in two daily doses, administered 6 hours apart. Blood concentrations of acyclovir were measured in random rats sacrificed on gestation day 15. Acyclovir was also measured in pooled amniotic fluid. The number of implantation sites, live and dead embryos was recorded. Whole body homogenates were assayed for acyclovir. All other rats were sacrificed on gestation day 19. Gross anomalies were noted and 1/3 fixed in Bouin's solution for visceral examinations (Wilson technique). Whole body transverse sections of the head, thorax, and abdomen were examined. The remaining 2/3 of fetuses were processed for skeletal abnormalities.

It was concluded that acyclovir was not teratogenic or maternally toxic. A 34% decrease in live fetuses per dam at high dose (50 mg/kg/day) on the Day 15 sacrifice was not substantiated upon examination of a larger sample sacrificed on Day 19. In the high dose group, acyclovir concentrations in the maternal plasma were smaller than in amniotic fluid and fetal whole body homogenates.

Study Day	Parameter (mean per pregnant dam)	Dose of acyclovir (mg/kg/day)			
		0	12	25	50
Day 15 (n= 7 – 8 pregnant dams per treatment group)	Implantation sites	10.9	11.3	11.4	7.6
	Resorption sites	0.6	0.4	0.3	0.8
	Live fetuses	10.3	10.9	11.1	6.8
	Dead fetuses	0	0	0	0
Day 19 (n= 21 – 23 pregnant dams per treatment group)	Corpora lutea	12.9	12.4	12.6	12.7
	Implantation sites	9.8	11.3	10.4	9.9
	Resorption sites	0.6	0.6	0.8	0.9
	Live fetuses	9.2	10.6	9.6	8.9
	Dead fetuses	0	0	0	0.1

The mean concentration of acyclovir in maternal plasma was 0.26, 0.69 and 1.59 µg/mL for the 12, 25, and 50 mg/kg/day treatment groups, respectively. These levels were attained 1 hour after the first daily dose (i.e. half of daily dose) on gestation day 15. The study was repeated using the same doses. On day 15, maternal plasma was collected 15 minutes after the second daily dose. The mean concentration of acyclovir in maternal plasma was 16.42 µg/mL for the 50 mg/kg/day treatment group.

Rabbits

The study design (including method of treatment, doses tested, animal husbandry and tissues collected and examined) was the same as for the rat teratology study. Female New Zealand White rabbits were impregnated via artificial insemination. The females were treated by subcutaneous injection from gestation day 6 to gestation day 18. Random rabbits were sacrificed on day 18 for toxicokinetic determinations (described above for rat) and the remaining does were sacrificed on day 29 for collection of conventional teratology data. All fetuses (unfixed) were dissected and examined with a dissecting microscope to detect soft tissue and visceral anomalies (Staples). The heads were removed from 2/3 of the fetuses, fixed in Bouin's solution and sectioned. All fetuses were fixed in 95% ethanol, processed and examined for skeletal alterations.

Acyclovir was not associated with maternal or fetal toxicity. The mean concentration of acyclovir in maternal plasma was 0.25, 0.25 and 0.39 µg/mL for the 12, 25, and 50 mg/kg/day treatment groups, respectively. These levels were attained 1 hour after the first daily dose (i.e. half of daily dose) on gestation day 15. Additional rabbits were administered the same doses. In the 25 mg/kg/day and 50 mg/kg/day treatment groups, the mean peak plasma concentrations of acyclovir were 11.8 and 17.0 µg/mL, respectively. These concentrations were attained at 15 minutes post-dose. Acyclovir concentrations were higher in amniotic fluid than in maternal plasma or fetal whole body homogenates (0.89, 8.03 and 6.16 µg/mL at 1 hour post-dose for the 12.5, 25, and 50 mg/kg/day treatment groups, respectively).

Neonatal toxicology

Untreated, pregnant CD rats (15) were allowed to deliver and raise their natural litters. Each litter was culled to five male and five female neonates in each of four test groups. The neonates were treated subcutaneously (once daily) for 19 consecutive days beginning at 3 days of age with 0.9% saline control or 5, 20 or 80 mg/kg/day acyclovir. A random group of rats were sacrificed on day 22 (day after weaning) and day 45, for tissue ("wide variety of tissues" including brain and eye sections) light microscopic examination. On postdose day 18 (day 39 of age), rats were bled for hematologic evaluation (PCV, hemoglobin, RBC, WBC, differential counts, platelet counts, reticulocyte counts and clotting time). On post-dose day 21 (42 days of age), blood was collected for serum chemistry (BUN, glucose, total protein, creatinine, cholesterol, total bilirubin, transaminase). On post-dose day 23, mice underwent ophthalmologic examination (slit lamp and indirect ophthalmoscope).

Local effects were noted in the high dose group at the site of administration (alopecia, focal discoloration, scabs). Male and female neonates treated with 20 or 80 mg/kg/day acyclovir gained less body weight than controls. No increase in mortality or other overt signs of toxicity were noted. Values for BUN were significantly increased in rats treated with 80 mg/kg/day ($p \leq 0.01$ for males and $p \leq 0.05$ for females) on day 16 of the dosing phase by 34% and 27%, for males and females respectively. The increase was not fully recovered by post-dose day 22 as BUN remained increased by 17% and 27% in high dose males and females, respectively. Correlates of minimal accumulations of cellular debris in renal collecting ducts and loops of Henle were observed in 1/6 males and 5/6 females in the high dose group sacrificed at end of treatment. Plasma levels of acyclovir ranging from 30 – 99 μg/mL were achieved in high dose animals.

Parameter: Mean body weight (g)	Acyclovir dose (mg/kg/day)							
	0		5		20		80	
	M	F	M	F	M	F	M	F
Mean body weight: Dosing Day 1	10.1	9.5	10.4	9.7	10.3	9.7	10.4	9.6
Mean body weight: Dosing Day 5	19.1	17.7	18.4	17.1	17.2*	16.0*	16.6*	15.2*
Mean body weight: Dosing Day 10	30.3	28.1	30.1	28.3	28.9	27.1	27.4*	25.9*
Mean body weight: Dosing Day 19	59.1	55.8	59.6	55.6	55.2*	52.6*	50.1*	48.0
Mean body weight: Post dose Day 15	157.6	133.8	155.4	132.3	156.0	129.9	142.7*	120.5*
Mean body weight: Post dose Day 23	217.1	164.0	211.1	161.0	209.0	160.0	200.3*	154.6*
Parameter: BUN (mg/100mL)	Acyclovir dose (mg/kg/day)							
	0		5		20		80	
	M	F	M	F	M	F	M	F
BUN: Dose day 16	15.4	19.2	17.2	18.6	15.7	15.9	23.4*	26.6**
BUN: Post dose Day 22	11.6	11.6	11.1	10.4	15.3*	19.2	14.0*	16.0**

9 Special Toxicology

Steele R, *et al*., 1980, "Comparative *in vitro* immunotoxicology of acyclovir and other antiviral agents", *Infect Immun*, 28:957–962.

In this study, the *in vitro* lymphocyte proliferative response to mitogens such as phytohemagglutinin, pokeweed, and concanavalin A were evaluated in the presence of acyclovir added to the culture. Lymphocytes were isolated from healthy volunteers and stimulated with mitogen/acyclovir. Cellular proliferation (blastogenic response) was measured by incorporation of ^{3}[H]-thymidine into stimulated cells. The blastogenic index (BI) was expressed as the average counts per minute of triplicate samples dividing by the uptake after incubation with medium alone. No depression of the BI was observed for acyclovir concentrations ≤ 20 μg/mL (~89 μM). At higher concentrations, acyclovir inhibited the lymphocyte response to mitogen. Based on nonclinical data

described above, concentrations of acyclovir in the aqueous humor or plasma are not expected to exceed 20 μg/mL.

Acyclovir concentration (μg/mL)	% of control BI		
	PHA (5μg/mL)	Con A (10μg/mL)	PWM (1μg/mL)
200	39	27	49
100	66	39	70
50	93	74	89
20	100	100	100
16	100	100	100
12	100	100	100
10	100	100	100
8	100	100	100
4	100	100	100
2	100	100	100
0.4	100	100	100
Control BI	46	31	30

Specific antigen-induced proliferative responses, including responses to herpes group antigens, were determined in the presence and absence of acyclovir. Results showed that acyclovir did not inhibit the donor lymphocyte proliferative response to antigen.

Antigen	Control BI	% of control BI with acyclovir (20μg/mL)
C. albicans	16.3	100
Tetanus toxoid	13.7	100
Herpes group viruses		
HSV-1	11.4	100
HSV-2	17.7	100
Varicella-zoster virus	8.5	98
Cytomegalovirus	6.8	100

Lymphocyte cytotoxicity to herpes group-infected target cells in the presence of acyclovir was determined. A ^{51}Cr release assay using target cell lines previously infected with HSV-1, HSV-2 or varicella-zoster virus was employed. The quantitative release of ^{51}Cr from target is an index of cell mediated cytotoxicity. Specific immune release (SIR) was calculated by subtraction of the percentage of ^{51}Cr released from uninfected control cells from the percentage released from infected cells. The presence of acyclovir did not decrease or enhance lymphocyte mediated cellular cytotoxicity against virus infected cells.

Cells infected with	Control SIR*	% of control SIR for acyclovir treated cells (20 μg/mL)
HSV-1	24.5	100
HSV-2	13.1	100
Varicella-zoster virus	9.9	100
Cytomegalovirus	17.4	100

*SIR was expressed as the percentage of specific release of ^{51}Cr attributable to intracellular virus

The release of leukemia inhibitory factor (LIF) in the presence of acyclovir by leukocytes in response to virus infected cells was examined. A herpes infected cell line was incubated with donor leukocytes in soft agar wells. Upon antigenic stimulation, LIF is released and retards leukocyte migration out of the well and into the agar. The inhibition of migration (MI) of the leukocytes into an agar medium surrounding the culture was measured. The presence of acyclovir did not affect the LIF mediated inhibition of migration of the cells into the medium.

Cells infected with	Control MI (%)	% of control MI for acyclovir treated cells (20 μg/mL)
HSV-1	31	100
HSV-2	19	100
Varicella-zoster virus	26	100
Cytomegalovirus	22	100

10 Integrated Summary and Safety Evaluation

The literature referenced by the applicant supports previous findings detailed in the labeling for the listed acyclovir drug products. Systemic exposure following topical ocular administration is minimal, particularly when compared to the approved intravenous dose. Systemic toxicity following topical ophthalmic administration was not observed. In neonatal rats administered acyclovir subcutaneously, lower body weight gain, injection site reactions and findings related to renal pathology including increased BUN and cellular debris in the collecting ducts and Loop of Henle were observed in animals treated in rats treated with 80 mg/kg/day. Intravenous acyclovir is approved in neonates at a dose of 10 mg/kg infused at a constant rate over 1 hour, every 8 hours for 10 days.

Acyclovir did not affect epithelial healing rate following corneal injury in rabbits. No toxicity was associated with the vehicle. No toxic effects on the regenerating epithelium were noted. Neither edema in the stroma nor iritis was observed.

Following deep injury to the eye, acyclovir did not affect stromal wound healing or induce stromal vascularization.

In a study of immunotoxicity, acyclovir reduced human lymphocyte proliferative response to mitogens *in vitro* at concentrations exceeding 20 μg/mL. At a concentration of 20 μg/mL, acyclovir did not inhibit the donor lymphocyte proliferative response to specific viral antigen. The presence of acyclovir did not decrease or enhance lymphocyte mediated cellular cytotoxicity against virus infected cells or LIF mediated inhibition of migration of cells.

The applicant submitted published nonclinical studies of the ocular safety and pharmacokinetics of acyclovir in rabbits. These studies showed that acyclovir distributes to the aqueous humor following topical ocular administration. Following topical ocular application in rabbits, a dose-dependent increase in mild conjunctival irritation was noted following treatment with acyclovir and its white petrolatum base compared to saline control. The irritation was most pronounced following the last dose of the day and was almost completely resolved by the following morning's dose. No other signs of ocular pathology were noted upon biomicroscopy, funduscopy or histological analysis. A NOAEL as 6% acyclovir, 5 times per day (6.3 mg/eye/day) yields a ~3.25-fold safety margin over the applicant's proposed starting dose.

Appendix A: Sponsor Proposed Label (P/T proposed changes in red)

(b) (4)

2 Pages of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

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/s/

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02/24/2014

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