# CENTER FOR DRUG EVALUATION AND RESEARCH

*APPLICATION NUMBER:*

**22-308**

# PHARMACOLOGY REVIEW(S)

Comments on N22308 besifloxacin 0.6% ophthalmic suspension
From: Abby Jacobs
Date: Feb 27, 2009

1. I agree that there are no pharm/tox issues affecting approval.
2. I concur that the appropriate pregnancy category is C.

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

/s/

Abby Jacobs
2/27/2009 12:25:53 PM
PHARMACOLOGIST

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

# PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

| | |
|---|---|
| NDA NUMBER: | **22-308** |
| SERIAL NUMBER: | **000** |
| DATE RECEIVED BY CENTER: | **5/30/08 (EDR)** |
| PRODUCT: | **Besifloxacin 0.6% Ophthalmic Suspension (Besivance™)** |
| INTENDED CLINICAL POPULATION: | **Adult and Pediatric Patients with bacterial conjunctivitis** |
| SPONSOR: | **Bausch & Lomb, Inc.** |
| DOCUMENTS REVIEWED: | **M-000, N-000** |
| REVIEW DIVISION: | **Division of Anti-Infective and Ophthalmology Products** |
| PHARM/TOX REVIEWER: | **Amy L. Ellis** |
| SUPERVISORY PHARMACOLOGIST: | **Wendelyn Schmidt** |
| ACTING DIVISION DIRECTOR: | **Wiley Chambers** |
| PROJECT MANAGER: | **Alison Rodgers** |

## *TABLE OF CONTENTS*

# *EXECUTIVE SUMMARY*

## I. Recommendations

A. Recommendation on approvability

The pharmacologist has no objection to the approval of this NDA.

B. Recommendation for nonclinical studies

No additional nonclinical studies are recommended.

C. Recommendations on labeling

Where possible, the animal-to-human dose comparisons in the label should be converted from nominal dose to systemic exposure, as pharmacokinetic data are available for animals and humans. The discussion of the rabbit developmental toxicity data in the *Pregnancy* section (8.1) should be removed, as this was not an appropriate species for testing besifloxacin and the data were confounded by severe maternal toxicity related to general antimicrobial administration. The photomutagenicity data in the *Carcinogenesis, mutagenesis, impairment of fertility* section should be removed as these data are not, in general, considered useful for hazard identification. Section 13.2 *Animal Toxicology and/or Pharmacology* should be deleted. It should be reserved for the discussion of toxicities identified in animals that are of concern and that may be clinically relevant. There are no such issues with this product. The specific changes recommended for this product label can be found at the end of this review.

## II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Systemic and ocular toxicology studies were conducted with besifloxacin. Ocular studies in rabbits and dogs demonstrated adequate local tolerance to the 0.6% ophthalmic suspension. No signs of inflammation were observed and no ocular histopathologic changes were observed when these species were dosed 4 times daily for up to one month. Repeat dose oral toxicity studies were performed in rats and dogs. When doses of approximately 400 mg/kg/day were given to rats for 28 days, neither clinical signs of toxicity nor histopathologic changes were observed. Dogs that received 50 mg/kg/day of besifloxacin demonstrated salivation and emesis (typical signs following quinolone administration in this species), but no histopathologic changes were observed. Besifloxacin had a genotoxicity profile typical of a fluoroquinolone. It was mutagenic in some bacterial strains, clastogenic in Chinese hamster ovary cells, and positive in a mouse micronucleus assay *in vivo*. When administered orally, besifloxacin did not reduce fertility in male or female rats at doses up to 500 mg/kg/day. It was not associated with fetal skeletal or visceral malformations when administered to pregnant rats at maternally toxic doses up to 1000 mg/kg/day. Besifloxacin was fetotoxic (postimplantation loss, neonatal mortality, developmental delays) at 1000 mg/kg/day, which provided exposure levels far in excess of what would occur after topical ocular administration. There was a clear NOAEL of 100 mg/kg/day for

reproductive toxicity, and systemic exposure following this dose would still be far in excess of exposure that would occur after ocular dosing.

B. Pharmacologic activity

Besifloxacin has a broad spectrum of bactericidal activity against strains commonly isolated from patients with bacterial conjunctivitis. It acts by inhibiting bacterial DNA gyrase and topoisomerase IV.

C. Nonclinical safety issues relevant to clinical use

None.

APPEARS THIS WAY ON ORIGINAL

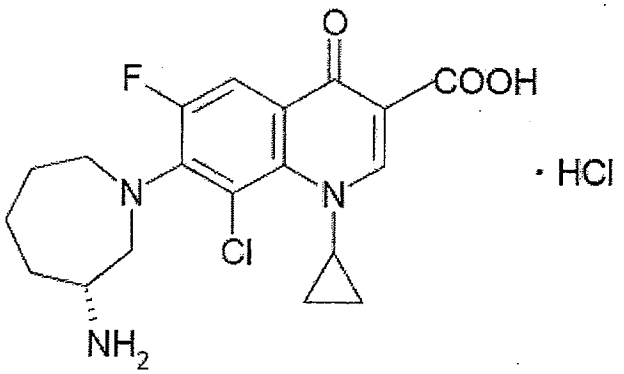
## *2.6 PHARMACOLOGY/TOXICOLOGY REVIEW*

### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number**: 22-308
**Review number**: 1
**Sequence number/date/type of submission**: M-000/21-DEC-2007/presubmission;
N-000/30-MAY-2008/orig NDA submission
**Information to sponsor**: Yes (X) No ( )- labeling recommendations
**Sponsor and/or agent**: Bausch & Lomb (Rochester, NY)
**Manufacturer for drug substance**: [redacted] b(4)
**Reviewer name**: Amy L. Ellis
**Division name**: Anti-Infective and Ophthalmology Products
**Review completion date**: 2/6/09

**Drug**:

Trade name: Besivance™ (proposed)
Generic name: Besifloxacin hydrochloride Ophthalmic Suspension 0.06%
Code names: SS734; BOL-303224A
Chemical name: (+)-7-[(3*R*)-3-aminohexahydro-1*H*-azepin-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride
CAS registry number: 405165-61-9
Molecular formula/molecular weight: $C_{19}H_{21}ClFN_3O_3 \cdot HCl$ / 430.30
Structure: From the NDA:




**Relevant INDs/NDAs/DMFs**: IND 64,335

**Drug class**: Fluoroquinolone antimicrobial

**Intended clinical population**: Adult and pediatric patients (≥ 1 year of age) with bacterial conjunctivitis.

**Clinical formulation**: From the NDA:

**Table 2.3.P.1-1: Qualitative and Quantitative Composition of Besifloxacin HCl Ophthalmic Suspension, 0.6% as Base**

| Component | Reference to Quality Standard | Function | Concentration (mg/mL) | % w/v‡ |
|---|---|---|---|---|
| Besifloxacin HCl salt^ (equivalent free base) | In-house | Active | 6.63 (6.06) | 0.663 (0.606) |
| Benzalkonium Chloride, | NF | Preservative | 0.10 | 0.01 |
| Polycarbophil* | USP | [redacted] | | |
| Mannitol | USP | | | |
| Poloxamer 407 | NF | | | |
| Sodium Chloride* | USP | | | |
| Edetate Disodium* | USP | | | |
| Sodium Hydroxide*† | NF | | | |
| Purified Water* | USP | | | |

b(4)

[redacted] b(4)

The polycarbophil-containing vehicle (DuraSite®) is used in the marketed ophthalmic product AzaSite™, also used to treat bacterial conjunctivitis.

**Route of administration**: Topical ophthalmic

**Disclaimer**: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:**

Secondary Pharmacodynamics:

Effects of besifloxacin on lipopolysaccharide (LPS)-induced cytokine production in human THP-1 monocytes (Study PH07025)

Effects of besifloxacin on lipopolysaccharide (LPS)-induced cytokine production in human corneal epithelial cells (Study PH07026)

Effects of besifloxacin on IL-1ß-induced cytokine production in human corneal epithelial cells (Study PH07038)

Safety Pharmacology:

SS734: Evaluation of effects of besifloxacin on HERG current in stably transfected HEK-293 Cells (Study 20040783PEHP)

SS734: Evaluation of effects on cardiac action potential in isolated canine Purkinje fibers (Study 20040782PECM)

SS734: Evaluation of effects on blood pressure, heart rate, and electrocardiogram after single oral administration to conscious dogs (Study 20040781PCC)

Pharmacokinetics/Metabolism:

Investigation of the Ocular and Systemic Pharmacokinetics of BOL-303224, Moxifloxacin, Gatifloxacin after Topical Administration to Rabbits (Study BL06137)

Investigation of the Anterior Ocular and Systemic Pharmacokinetics of ER-3224 Following a Single Topical Ocular Administration in the Pigmented Rabbit (Study BL05046)

Investigation of the Anterior Ocular and Systemic Pharmacokinetics of BOL-303224-A Following a Single Topical Ocular Administration in the Rabbit (Study BL06032)

Investigation of the Anterior Ocular Pharmacokinetics of BOL-303224 Following Topical Ocular Administration of 0.6% ISV-403 to Rabbits: Comparison of Two Methods for Conjunctiva Sampling (Study BL06115)

Investigation of the Ocular Pharmacokinetics of BOL-303224, after Repeated (TID) Topical Ocular Administration to Pigmented Rabbits (Study BL07050)

Investigation of the Ocular Pharmacokinetics of BOL-303224, after Repeated (BID) Topical Ocular Administration to Pigmented Rabbits (Study BL07051)

Investigation of the Anterior Ocular and Systemic Pharmacokinetics of BOL-303224-X Following a Single Topical Ocular Administration of ISV-403 to Cynomolgus Monkeys (Study BL07045)

Ocular and systemic PK of carbon-14 following topical ocular administration of [$^{14}$C]besifloxacin (Study B16F0205)

Ocular and systemic PK of carbon-14 following repeated topical ocular administration of [$^{14}$C]besifloxacin (Study B06U0106 (BL06016))

Investigation of the Ocular Pharmacokinetics of BOL-303224 following Subconjunctival Administration of ISV-403 to Pigmented Rabbits (Study BL07009)

Investigation of the Ocular Pharmacokinetics of BOL-303224 following Intravitreal Administration of ISV-403 to Pigmented Rabbits (Study BL07015)

Single and Repeat Dose Toxicity:

ISV-403: 28-Day ocular tolerance study in pigmented rabbits using a QID dosing regimen (Study B16F0904)

4-week ocular tolerance study of ISV-403 administered 4 times daily in the beagle dog (Study AA25934)

Evaluation of the ocular irritation of two besifloxacin suspension formulations following multiple dosing in New Zealand White rabbits (Study P1007002)

Reproductive and Developmental Toxicity:

Study for toxic effects of SS734 on pre- and postnatal development, including maternal function in rats (Study 967-010)

**Studies not reviewed within this submission:**

These studies were reviewed under IND 64,335:

Safety Pharmacology:

Evaluation of effects of besifloxacin on respiratory system (Study 20040780PCR)

Evaluation of besifloxacin on urinary system (Study 20040778PGR)

Pharmacokinetics/Metabolism:

Single oral dose PK study (Study SS734-PKKT-0211)

Protein binding of [$^{14}$C]besifloxacin and blood/plasma ratio determination (Study SS734-PKKT2-0210)

Ocular PK after topical ocular administration of 0.1%, 0.3% or 0.6% besifloxacin (Study I07U0102)

Effect of corneal wound and DuraSite vehicle on ocular PK (Study B16F0604)

Chiral interconversion and liver metabolism in hepatocytes (Study 414135)

Single and Repeat Dose Toxicity:

Ocular tolerance of besifloxacin ophthalmic formulation (Study 5618B)

Single-dose/7-day repeated dose intravenous injection/oral gavage toxicity and toxicokinetic study with besifloxacin (Study 967-001)

28-day oral gavage toxicity study of besifloxacin (Study 967-003)

Escalating dose range-finding toxicokinetic study with besifloxacin (Study 967-002)

28-day oral gavage toxicity study of besifloxacin followed by a 2-week recovery (Study 967-004)

Genotoxicity:

Ames test mutation assay with a confirmatory assay with besifloxacin *in vitro* (Study 7281-106)

Ames test mutation assay in the presence of solar-simulated light with besifloxacin *in vitro* (Study 7281-105)

Chromosomal aberration assay with besifloxacin *in vitro* (Study 7281-102)

Micronucleus test with besifloxacin (racemate) (Study AM-M5-3)

Micronucleus test with besifloxacin (Study 7281-103)

*In vivo/in vitro* Unscheduled DNA Synthesis assay (Study 7281-104)

Reproductive and Developmental Toxicity:

Fertility and early embryonic development study with besifloxacin (Study 967-009)

Dose-range finding embryo-fetal development study with besifloxacin (Study 967-005)

Embryo-fetal development study with besifloxacin (Study 967-007)

Dose-range finding embryo-fetal development study with besifloxacin (Study 967-006)

Embryo-fetal development study with besifloxacin (Study 967-008)

Special Toxicity:

Photoirritation study with besifloxacin (Study JBS-02-MOPT-491)

Photoirritation study with besifloxacin ophthalmic formulation (Study 0216)

Photosensitization study with besifloxacin ophthalmic formulation (Study 857410)

This chronic ocular toxicity/tolerance study of the DuraSite™ excipient was previously reviewed under approved NDA 50-810 for AzaSite™ (belonging to InSite Pharmaceuticals, the company that is licensing besifloxacin ophthalmic suspension to Bausch & Lomb):

Ocular tolerance of the ophthalmic formulation excipient (Study 2584-100)- this report was submitted to the current NDA, 22-308

APPEARS THIS WAY ON ORIGINAL

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Besifloxacin inhibits bacterial DNA gyrase and topoisomerase IV and has a broad spectrum of bactericidal activity against strains commonly isolated from patients with bacterial conjunctivitis. In addition, some exploratory *in vitro* data suggest that besifloxacin inhibits cytokine formation in human corneal epithelial cells and monocytes, but the relevance of this finding to therapeutic efficacy is unknown.

### 2.6.2.2 Primary pharmacodynamics

Mechanism of action: Besifloxacin inhibits bacterial DNA gyrase and topoisomerase IV.

Drug activity related to proposed indication: Besifloxacin has a broad spectrum of antimicrobial activity. It is bactericidal against strains that are commonly associated with bacterial conjunctivitis (e.g., *Streptococcus sp., Staphylococcus sp., Haemophilus sp., Corynebacterium sp.,* and *Moraxella sp.*).

### 2.6.2.3 Secondary pharmacodynamics

In addition to its antimicrobial activity, some *in vitro* data from human THP-1 monocytes and human corneal epithelial cells suggests that besifloxacin inhibits cytokine production in these cells. The sponsor considers it possible that this activity may improve the therapeutic efficacy of besifloxacin in ocular infections that have an inflammatory, as well as infectious, component. It is noted that inhibition of cytokine production was also observed with another fluoroquinolone (moxifloxacin) that has been approved for ophthalmic use. These exploratory, nonGLP *in vitro* studies (PH07025, PH07026, and PH07038) did not demonstrate whether this secondary pharmacologic activity of besifloxacin plays any meaningful therapeutic role in its effectiveness at treating bacterial conjunctivitis.

### 2.6.2.4 Safety pharmacology

Safety pharmacology data are not relevant to the topical ophthalmic use of besifloxacin because systemic exposure following clinical ocular dosing is very low. However, several safety pharmacology studies were performed with besifloxacin.

Besifloxacin (SS734) caused delayed diuresis in rats at oral doses of 100-300 mg/kg and had an antidiuretic effect at 1000 mg/kg. Oral doses of up to 1000 mg/kg did not have any effect on the respiratory parameters of rats. A Functional Observational Battery evaluation conducted prior to the initiation of dosing and during week 4 of a repeat dose toxicity study in rats did not reveal any changes that appeared drug-related at doses of besifloxacin up to 500 mg/kg/day. Besifloxacin was not a potent inhibitor of HERG current in HEK-293 cells, causing a 13% inhibition at 100 μM. It caused only a 4% increase in $APD_{90}$ in isolated canine Purkinje fibers. When given to dogs orally at 10 mg/kg, besifloxacin had no effect on cardiac parameters. Doses

of 30 and 100 mg/kg were associated with an increase in the QT interval corrected using Sarma's method, but not uncorrected QT or QT corrected using Bazett's or Fridericia's formulas.

**SS734: Evaluation of effects of besifloxacin on HERG current in stably transfected HEK-293 Cells**

**Study no.**: 20040783PEHP
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: 3/2/05
**GLP compliance**: GLP (French, OECD, US, Japanese)
**QA report**: yes (x) no ( )
**Drug, lot #, and % purity**: SS734, Batch No. 04113J, 99.3% pure

**Summary:** HEK-293 cells stably transfected with HERG-1 cDNA were exposed to SS734 in Tyrode's solution. E-4031 was used as a positive control. Patch-clamp techniques were used to monitor the HERG current in individual cells. The HERG tail current was inhibited by only 13% when cells were exposed to $10^{-5}$ M (100 μM) SS-734. Thus, an IC50 value for HERG current inhibition could not be calculated for SS734 under the conditions of this assay and this compound was not considered to be a potent inhibitor of the HERG current by the investigators. In contrast, the positive control, E-4031, caused approximately 90% inhibition of HERG current at a concentration of 100 nM.

**SS734: Evaluation of effects on cardiac action potential in isolated canine Purkinje fibers**

**Study no.**: 20040782PECM
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: 2/24/05
**GLP compliance**: GLP (French, OECD, US, Japanese)
**QA report**: yes (x) no ( )
**Drug, lot #, and % purity**: SS734, Batch No. 04113J, 99.3% pure

**Summary:** Two male dogs (6 and 8 months old) were heart donors for this study. Three Purkinje fiber preparations were obtained from each dog and bathed in Tyrode's solution. SS734 was studied at concentrations from $10^{-8}$ to $10^{-5}$ M, in ascending order. Finally, after a washout with fresh Tyrode's solution, each preparation was exposed to cisapride at $3 \times 10^{-7}$ M as a positive control. A stimulation rate of 1 Hz was used to induce action potential in the Purkinje fibers (pulses of 2 volts of 1 msec duration at 1 second intervals), followed by a 0.33 Hz rate. Concentrations of SS734 $10^{-6}$ to $10^{-8}$ had no effect on action potential under the conditions of this study. A small, but statistically significant ($p \leq 0.05$) increase in $APD_{90}$ (approximately 4%) was observed at both 1 and 0.33 Hz with the $10^{-5}$ M concentration of SS734. Neither early nor delayed after depolarization was observed, however. The positive control performed as expected.

**SS734: Evaluation of effects on blood pressure, heart rate, and electrocardiogram after single oral administration to conscious dogs**

**Study no.**: 20040781PCC
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: 2/2/05
**GLP compliance**: GLP (French, OECD, US, Japanese)
**QA report**: yes (x) no ( )
**Drug, lot #, and % purity**: SS734, Batch No. 04113J, 99.3% pure

**Summary:** Telemeterized male and female beagle dogs (n=3 per sex) received single oral 10, 30, and 100 mg/kg doses of SS734. A vehicle group received 0.5% carboxymethylcellulose. Cardiac parameters were monitored at 5 minute intervals for 24 hours prior to the initiation of the study and for 48 hours after each dose. QT interval duration was corrected using 3 different formulas- Bazett's, Fridericia's, or Sarma's. There was a 48 hour washout period between treatments (given in ascending order). In a separate follow-up study using the same animals, dogs were given 100 mg/kg of SS734; 6-lead ECG was performed prior to each dose and 1 and 4 hours after administration of drug. Blood samples for PK were drawn prior to each dose, then 0.5, 1, 2, 4, 8, and 24 hours after dosing. None of the doses caused a significant change in blood pressure (MAP, systolic or diastolic) or heart rate. The only change in ECG that appeared related to drug was a statistically significant increase in the QT interval duration when corrected for heart rate using Sarma's method at the 30 ($p \leq 0.05$) and 100 ($p \leq 0.01$) mg/kg doses. The maximum increases at these doses were 12 and 22 ms, respectively, at 3 and 4 hours after administration. Uncorrected QT intervals at these doses were not significantly different from controls and QT intervals using Bazett's or Fridericia's formula did not differ significantly between control and drug-treated dogs. It is difficult to determine the biological significance of the corrected QT data in this study, as drug studies in dogs with other quinolone antimicrobials did not use Sarma's method for correction, but used one of the other 2 methods that did not detect a difference between the mid and high dose SS734 animals and controls. The fact that the observation was dose-related increases the possibility that it is drug-related, however. Regardless, the plasma level of SS734 following topical ocular administration would be well below that following oral administration of 10 mg/kg/day, a clear NOAEL for cardiac findings. There were no clinical signs of toxicity at any of these doses. The plasma Cmax of SS734 in the dogs following oral administration of 100 mg/kg ranged from 6.1-15.7 μg/ml, with Tmax at around 2-4 hours for most dogs. $AUC^{0-24\ hr}$ ranged from 92-167 μg·hr/ml.

### 2.6.2.5 Pharmacodynamic drug interactions

Nothing relevant reported in the nonclinical data.

## 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Primary Pharmacodynamics- These studies are reviewed by the Microbiologist.

Copied from the NDA, below:

Secondary Pharmacodynamics

Test Article: Besifloxacin

| Organ Systems Evaluated | Species/ Strain | Method of Admin. | Doses | Gender and No. per Group | Noteworthy Findings | GLP | Study Number |
|---|---|---|---|---|---|---|---|
| Immune System | Human THP-1 monocyte | In vitro | 0.1, 1, 10, 30 µg/mL | Triplicate groups of $4 \times 10^5$ cells | Besifloxacin inhibited LPS-induced cytokine production in human THP-1 monocytes with a comparable or higher potency compared with moxifloxacin. | No | PH07025 |
| Immune System | Human Corneal Epithelial Cell | In vitro | 0.1, 1, 10, 30 µg/mL | Triplicate groups of $4 \times 10^5$ cells | Besifloxacin inhibited LPS-induced cytokine production in HCEpiC with better potency compared with moxifloxacin. | No | PH07026 |
| Immune System | Human Corneal Epithelial Cell | In vitro | 0.1, 1, 10, 30 µg/mL | Triplicate groups of $4 \times 10^5$ cells | Besifloxacin inhibited IL-1β-induced cytokine production in HCEpiC with comparable or better potency compared with moxifloxacin. | No | PH07038 |

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

Although high levels in tears and ocular tissues are observed, systemic exposure to besifloxacin is low following ocular administration. Plasma levels were measurable after the 0.6% besifloxacin ocular suspension was applied to the eyes of rabbits, dogs, and monkeys but even at Cmax they were not far above the limit of quantitation. Data from rabbits indicated that the DuraSite® vehicle enhanced the penetration of besifloxacin through intact corneas, but did not alter penetration through wounded corneas.

When administered orally to rats, besifloxacin was rapidly absorbed and widely distributed throughout the body with the highest tissue levels outside of the GI tract seen in the liver, kidney, and testes. Besifloxacin was excreted rapidly, mostly as unchanged drug, primarily in the feces (73%) with less in the urine (23%).

### 2.6.4.2 Methods of Analysis

Validated HPLC methods were used to measure besifloxacin in plasma, tears, conjunctiva, and cornea. The initial plasma method ( [redacted] for mouse, rat, rabbit, and dog) used UV detection and had a quantitation range of 100-10000 ng/ml. A second method [redacted] used MS detection for dog plasma (4-256 ng/ml), rabbit plasma (2-128 ng/ml) and rabbit cornea (10-640 ng/g). The final method [redacted] used tandem MS detection for rabbit plasma (2-200 ng/ml), rabbit tears (20-10000 ng/ml), and rabbit conjunctiva (100-50000 ng/g). Additional HPLC-MS-MS methods were used for some nonGLP ocular PK studies. $^{14}$C-besifloxacin was used to study the distribution of this drug following topical ocular administration to pigmented rabbits. b(4)

**2.6.4.3 Absorption**

Besifloxacin is rapidly absorbed following ocular administration with Cmax concentrations in ocular tissues generally observed within an hour of application.

After oral administration to rats, besifloxacin is also absorbed quickly. Tmax is dose-dependant (higher dose/later Cmax). Following administration of a 20-100 mg/kg dose, the Tmax of besifloxacin was about 0.5-1 hour and after a 1000 mg/kg dose, it was 1.7-2.7 hours.

**2.6.4.4 Distribution**

Following ocular administration, the highest concentrations of besifloxacin are found in tears, conjunctiva, and cornea. Lesser concentrations are found in aqueous and vitreous humors. Besifloxacin appears to have an affinity for melanin, which has been observed with other fluoroquinolones. The corneal penetration of besifloxacin appeared to be enhanced by the DuraSite® vehicle when applied to intact rabbit corneas, but not wounded corneas. Plasma protein binding is relatively low in both rats (about 30%) and humans (about 40%). Relative distribution into red blood cells is about 50% in both of these species.

After oral administration to rats, besifloxacin was widely distributed throughout the body with the highest tissue levels outside of the GI tract seen in the liver, kidney, and testes.

**Investigation of the Ocular and Systemic Pharmacokinetics of BOL-303224, Moxifloxacin, Gatifloxacin after Topical Administration to Rabbits** (Study BL06137)

**Summary:** When 50 µl of either 0.6% besifloxacin (BOL-303224), 0.5% moxifloxacin, or 0.3% gatifloxacin were applied to the eyes of Dutch-belted rabbits, the lowest plasma levels were observed with besifloxacin. Its levels in aqueous humor and cornea were also lower than the other 2 drugs, though the besifloxacin concentration in tears and vitreous humor was higher.

**Investigation of the Anterior Ocular and Systemic Pharmacokinetics of ER-3224 Following a Single Topical Ocular Administration in the Pigmented Rabbit** (Study BL05046)

**Summary:** Besifloxacin (ER-3224) 0.6% suspensions with 2 different particle sizes were applied to the eyes of New Zealand Composite rabbits, 100 µl per eye. Particle size diameters of the 2 suspensions were 0.76 µm (Lot J04Q) and 3.90 µm (Lot 965701). Levels of besifloxacin in tears, aqueous humor, conjunctiva, and plasma tended to be higher following administration of the Lot with the smaller particle size. However, the mean plasma levels were quite low with both formulations (<20 ng/ml) and the mean levels of besifloxacin in conjunctiva samples for both formulations far exceeded the MICs for key microorganisms of interest.

**Investigation of the Anterior Ocular and Systemic Pharmacokinetics of BOL-303224-A Following a Single Topical Ocular Administration in the Rabbit** (Study BL06032)

**Summary:** New Zealand White rabbits received 100 µl of 0.6% besifloxacin suspension or 0.3% besifloxacin solution into each eye. Plasma levels were similar (low) for both formulations. Tear levels were 6-9 times higher for the suspension and levels in conjunctiva

were about 3 times higher for the suspension. In contrast, corneal levels were about 2 times higher for the solution.

**Investigation of the Anterior Ocular Pharmacokinetics of BOL-303224 Following Topical Ocular Administration of 0.6% ISV-403 to Rabbits: Comparison of Two Methods for Conjunctiva Sampling** (Study BL06115)

**Summary:** This study was an exploratory evaluation of an alternate sampling method for conjunctival tissue in Dutch-Belted rabbits. The conjunctival scraping samples contained besifloxacin levels closer to those in tears than to excised conjunctivae (a 100-fold difference).

**Investigation of the Ocular Pharmacokinetics of BOL-303224, after Repeated (TID) Topical Ocular Administration to Pigmented Rabbits** (Study BL07050)

**Summary:** New Zealand Composite pigmented rabbits (n=3 per time point in each group) were dosed 3 times daily with 50 µl of 0.6% besifloxacin. One group was sacrificed at the end of one day of treatment, others received 4 days of treatment.

From the study report:

| Dosing Regimen | Tissue/Matrix | Cmax (ng/g) | Tmax (hr) | AUC(0-4hr) (µg*hr/g) | AUC(0-24 hr) (µg*hr/g) |
|---|---|---|---|---|---|
| Group 1 (TID dosing for 1 day) | Tears | 2160000 ± 707000 | 0.25 | 2710 | n/a |
| | Conjunctiva | 21000 ± 10300 | 0.25 | 14.3 | n/a |
| | Cornea | 3480 ± 2710 | 0.25 | 3.26 | n/a |
| | Aqueous Humor | 266 ± 169 | 2.0 | 0.398 | n/a |
| | Vitreous Humor | 19.3 ± 12.5 | 0.25 | [a]NC | n/a |
| | Retina | 477 ± 505 | 0.25 | 0.214 | n/a |
| Group 2 (TID dosing for 4 days) | Tears | 3190000 ± 773000 | 0.25 | 2370 | 2750 |
| | Conjunctiva | 27200 ± 15300 | 0.25 | 33.6 | 64.6 |
| | Cornea | 4260 ± 2030 | 0.25 | 3.59 | 6.99 |
| | Aqueous Humor | 93.0 ± 101 | 0.25 | 0.220 | 0.440 |
| | Vitreous Humor | 608 ± 659 | 24 | 0.0759 | 2.56 |
| | Retina | 532 ± 486 | 0.25 | 0.415 | 1.31 |

Note: For aqueous humor the relevant units for Cmax and AUC are ng/mL and µg*hr/mL respectively.
NC = Not calculated. [a]The number of quantifiable samples are too few (7/36) to calculate a meaningful estimation of AUC.

**Investigation of the Ocular Pharmacokinetics of BOL-303224, after Repeated (BID) Topical Ocular Administration to Pigmented Rabbits** (Study BL07051)

**Summary:** New Zealand Composite pigmented rabbits (n=3 per time point in each group) were dosed 2 times daily with 50 µl of 0.6% besifloxacin. One group was sacrificed at the end of one day of treatment, others received 4 days of treatment.

From the study report:

| Dosing Regimen | Tissue/Matrix | Cmax (ng/g) | Tmax (hr) | AUC(0-4hr) (µg*hr/g) | AUC(0-24 hr) (µg*hr/g) |
|---|---|---|---|---|---|
| Group 1 (BID dosing for 1 day) | Tears | 2150000 ± 719000 | 0.25 | 1540 | n/a |
| | Conjunctiva | 54900 ± 32100 | 0.25 | 37.1 | n/a |
| | Cornea | 1970 ± 661 | 0.25 | 2.37 | n/a |
| | Aqueous Humor | 124 ± 104 | 1.5 | 0.240 | n/a |
| | Vitreous Humor | 152 ± 179 | 0.25 | 0.0528 | n/a |
| | Retina | 759 ± 313 | 0.25 | 0.287 | n/a |
| Group 2 (BID dosing for 4 days) | Tears | 1900000 ± 896000 | 0.25 | 1703 | 1940 |
| | Conjunctiva | 42400 ± 26700 | 0.25 | 37.9 | 95.6 |
| | Cornea | 1520 ± 701 | 0.25 | 2.73 | 8.45 |
| | Aqueous Humor | 123 ± 31.3 | 1.5 | 0.213 | 0.398 |
| | Vitreous Humor | 36.4 ± 26.8 | 24 | 0.0196 | 0.236 |
| | Retina | 314 ± 378 | 0.5 | 0.291 | 1.44 |

**Investigation of the Anterior Ocular and Systemic Pharmacokinetics of BOL-303224-X Following a Single Topical Ocular Administration of ISV-403 to Cynomolgus Monkeys** (Study BL07045)

**Summary:** Cynomolgus monkeys (n=3 per time point) received 50 µl of 0.6% besifloxacin suspension into each eye. As has been the case in both rabbits and dogs, plasma levels of besifloxacin in the monkeys were very low after topical ocular administration compared to the high levels found in ocular tissues. There was considerable variation in besifloxacin tissue levels between animals.

From the study report:

| Dose Regimen | Matrix | $C_{max}$ (ng/g) | Tmax (hr) | AUC(0-t) (hr*µg/g) |
|---|---|---|---|---|
| 6 mg/mL Suspension (0.6%) Monkey BOL-303224-A | Tears | 2310000 ± 353000 | 0.083 | 6440 |
| | Cornea | 2100 ± 2560 | 0.25 | 12.4 |
| | Bulbar Conjunctiva | 6430 ± 1700 | 0.50 | 29.4 |
| | Palpebral Conjunctiva | 40000 ± 44000 | 0.25 | 120 |
| | Aqueous Humor | 796 ± 808 | 0.50 | 2.14 |
| | Iris/Ciliary Body | 1120 ± 767 | 0.50 | 2.88 |
| | Vitreous Humor | 163 ± 216 | 0.083 | 0.534 |
| | Retina | 103 ± 143 | 0.083 | 0.454 |
| | Choroid | 71.8 ± 92.3 | 1.00 | 0.289 |
| | [a]Plasma | 9.19 ± 10.2 | 2.17 ± 1.44 | 0.0468 ± 0.0465 |

Note: for plasma and aqueous humor, Cmax is in ng/mL and AUC is (hr*µg/mL)
[a] Plasma PK data were obtained from serial blood samples of 3 animals.

**Ocular and systemic PK of carbon-14 following topical ocular administration of [$^{14}$C]besifloxacin** (Study B16F0205)

**Summary:** Following one ocular administration of $^{14}$C-besifloxacin to Fauve de Bourgogne rabbits, radiolabel was present at its highest levels in the ocular tissues, but small amounts of

radiolabel were detected throughout the bodies, including the contralateral eye. Outside of the ocular tissues of the dosed eye, the greatest quantities of radiolabel were detected in skin, urinary bladder, ileum, jejunum, and duodenum. The first time point for this study was 0.5 hr and the final was 16 hours.

**Ocular and systemic PK of carbon-14 following repeated topical ocular administration of [$^{14}$C]besifloxacin** (Study B06U0106 (BL06016))

**Summary:** Following 4 daily ocular administrations of $^{14}$C-besifloxacin to Fauve de Bourgogne rabbits, the pattern of radiolabel distribution was similar to that observed after a single dose. Again, the highest levels of radiolabel (outside of tissues associated with the dosed eye) were detected in excretory organs (small and large intestine, kidney, urinary bladder). The first time point for this study was 0.5 hr after the last dose and the final time point was 16 hours later.

**Investigation of the Ocular Pharmacokinetics of BOL-303224 following Subconjunctival Administration of ISV-403 to Pigmented Rabbits** (Study BL07009)

**Summary:** Dutch-belted rabbits (n=4 per time point) received 100 µl subconjunctival injections of 0.6% besifloxacin suspension into the right eyes. Ocular tolerability observations in these animals included corneal haze 1 hr after administration; anterior chamber flare, corneal infiltration, and ocular discharge were seen at 8 hours.

From the study report:

| Formulation | Matrix | Cmax (ng/g) | Tmax (hr) | AUC(0-24 hr) (µg*hr/g) | AUC(0-336 hr) (µg*hr/g) |
|---|---|---|---|---|---|
| BOL-303224-A 600 µg/eye | Iris/Ciliary body | 267 ± 297 | 4.0 | 2.05 | 21.9 |
| | Conjunctiva | 60900 ± 74400 | 0.25 | 367 | 416 |
| | Cornea | 2850 ± 2780 | 0.25 | 8.19 | 14.6 |
| | Aqueous Humor | 461 ± 558 | 4.0 | 1.35 | 1.79 |
| | Vitreous Humor | 389 ± 741 | 4.0 | 1.38 | 20.6 |
| | Retina | 13200 ± 20200 | 8.0 | 83.3 | 94.3 |
| | Choroid | 1610 ± 1420 | 8.0 | 189 | 781 |
| | Plasma | 8.79 ± 1.39 | 2.0 | 0.118 | 0.384 |
| | Tear | 3540 ± 4600 | 0.25 | 5.96 | 37.2 |

For aqueous humor and plasma the relevant units are ng/mL and ng*hr/mL for Cmax and AUC, respectively.

**Investigation of the Ocular Pharmacokinetics of BOL-303224 following Intravitreal Administration of ISV-403 to Pigmented Rabbits** (Study BL07015)

**Summary:** Dutch-belted rabbits (n=4 per time point) received 50 µl intravitreal injections of 0.6% besifloxacin suspension into the right eyes. The investigators considered this route of administration to have been poorly tolerated. Ocular observations included anterior chamber flare, Tyndall effect, conjunctival discharge, and corneal opacity

From the study report:

| Formulation | Matrix | Cmax (ng/g) | Tmax (hr) | AUC(0-24 hr) (µg•hr/g) | AUC(0-672 hr) (µg•hr/g) |
|---|---|---|---|---|---|
| 0.6 % ISV-403 Suspension (BOL-303224) | Cornea | 2450 ± 530 | 1.0 | 12.4 | 32.9 |
| | Aqueous Humor | 204 ± 36.7 | 6.0 | 3.72 | 26.8 |
| | Vitreous Humor | 144000 ± 68900 | 0.25 | 1580 | 1980 |
| | Retina | 24900 ± 19600 | 3.0 | 309 | 625 |
| | Choroid | 3950 ± 1730 | 8.0 | 53.2 | 176 |
| | Plasma | 4.24 ± 0.919 | 6.0 | 0.068 | 0.122 |

For aqueous humor and plasma the relevant units are ng/mL and ng*hr/mL for Cmax and AUC, respectively.

### 2.6.4.5 Metabolism

In an *in vitro* metabolism study, besifloxacin was not metabolized by hepatocyte suspensions from humans, mice, rats, or rabbits during a 2 hour incubation. Some metabolism occurred when besifloxacin was incubated with dog hepatocytes, although 84% of the drug remained unchanged at the end of the 2 hour incubation. Eight minor dog metabolites were seen, but not specifically identified. They appeared to result from dechlorination, oxidative deamination, oxidation/hydroxylation, N-cyclopropyl elimination, ring opening, and combinations thereof. Chiral interconversion from the (+) isomer to the (-) isomer of besifloxacin did not occur in any of these species. *In vivo*, over 90% of besifloxacin was excreted unchanged by rats following oral administration. A small number of metabolites were detected using thin layer chromatography in plasma (three), urine (one) and feces (one), but none of these was specifically identified.

### 2.6.4.6 Excretion

Besifloxacin is primarily excreted in the feces following oral administration. In rats, approximately 73% of radiolabeled besifloxacin was recovered in the feces and approximately 23% in the urine. Most of the drug (>80%) was excreted within 24 hours of administration.

### 2.6.4.7 Pharmacokinetic drug interactions

Nothing in the NDA.

### 2.6.4.8 Other Pharmacokinetic Studies

Nothing in the NDA.

### 2.6.4.9 Discussion and Conclusions

Ocular distribution of besifloxacin occurs quickly after topical application to the eye. The highest levels are found in the tears and conjunctiva. In rats, besifloxacin is excreted (mostly as unchanged drug) primarily in the feces, with lesser urinary excretion. Very low systemic exposure following ocular administration renders systemic ADME data of limited significance for this ocular suspension.

### 2.6.4.10 Tables and figures to include comparative TK summary

Not provided.

## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The sponsor did not provide an appropriate summary table in the application.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

Systemic and ocular toxicology studies have been conducted with besifloxacin. Ocular studies in rabbits and dogs demonstrated adequate local tolerance to the 0.6% ophthalmic suspension. No signs of inflammation were observed and no ocular histopathologic changes were observed when these species were dosed 4 times daily for up to one month. Repeat dose oral toxicity studies were performed in rats and dogs. When doses of approximately 400 mg/kg/day were given to rats for 28 days, neither clinical signs of toxicity nor histopathologic changes were observed. Dogs that received 50 mg/kg/day of besifloxacin demonstrated salivation and emesis (typical signs following quinolone administration in this species), but no histopathologic changes were observed.

Besifloxacin was mutagenic in some bacterial strains and clastogenic in Chinese hamster ovary cells. It was also positive in a mouse micronucleus assay *in vivo*. Besifloxacin did not induce unscheduled DNA synthesis in rat hepatocytes, but this assay is notoriously insensitive. This drug had a genotoxicity profile typical for a fluoroquinolone.

When administered orally, besifloxacin did not reduce fertility in male or female rats at doses up to 500 mg/kg/day. It was not associated with fetal skeletal or visceral malformations when administered to pregnant rats at doses up to 1000 mg/kg/day, although it did cause maternal toxicity (including mortality) at this high dose. Both embryo-fetal development and peri- postnatal development studies demonstrated that besifloxacin is fetotoxic. It was associated with increased postimplantation loss, reduced fetal body weights, and developmental delays in pups born to dams that received 1000 mg/kg/day. Pregnancy Category C is recommended, but these findings are unlikely to be relevant following ocular administration of besifloxacin due to low systemic exposure following this route of administration. There was a clear NOAEL of 100 mg/kg/day for reproductive toxicity and systemic exposure following this dose would be far in excess of exposure that would occur after ocular dosing.

**2.6.6.2 Single-dose toxicity**

A single oral 2000 mg/kg dose of besifloxacin was not associated with mortality. Solubility limited the amount of drug that could be administered intravenously, so data from a single dose IV study were complicated by mortality associated with the administration of a large volume of dosing solution (25 ml/kg).

**2.6.6.3 Repeat-dose toxicity**

No signs of ocular irritation or inflammation was observed in Dutch-belted rabbits that received 0.6% besifloxacin in its clinical vehicle 4 times daily for 14 days. No treatment-related changes in ocular tissues were observed and maximum plasma concentrations were barely above the limit of quantitation (2 ng/ml). When 0.6% besifloxacin in its clinical vehicle was applied to the eyes of Fauve de Bourgogne rabbits or beagle dogs 4 times daily for 28 days, it did not cause inflammation. Neither ERG changes nor changes in corneal sensitivity were observed. Minor signs of ocular irritation (e.g., redness, mild chemosis) were occasionally observed in rabbits, but not dogs. Ocular pressure was measured in the dogs and it was not altered following besifloxacin exposure. No treatment-related histopathologic changes to ocular tissues were observed in either species. Systemic exposure to besifloxacin was very low in both species (mean Cmax values of 12 ng/ml or less) and unlikely to be of toxicological significance. Two different Lots of 0.6% besifloxacin ophthalmic suspension with different impurity profiles did not demonstrate different irritation/toxicity profiles when applied to the eyes of New Zealand White rabbits 4 times per day for 2 weeks.

An oral repeat dose toxicity study in rats found no clinical or microscopic evidence of toxicity at besifloxacin doses of up to approximately 400 mg/kg given daily for 28 days. The Cmax and AUC at this dose were approximately 10 μg/ml and 70 μg·hr/ml, respectively. In a 7-day rat study, mild bone marrow depletion was observed in some animals that received daily oral doses of 2000 mg/kg/day.

Salivation and emesis were observed in dogs given oral 50 mg/kg of doses of besifloxacin for 28-days, but microscopic evaluation did not reveal histopathologic changes. The Cmax at this dose was approximately 12 μg/ml. The systemic exposures achieved in the animals following oral doses of besifloxacin far exceeded those measured in animals or humans after topical ocular application of the drug.

**ISV-403: 28-Day ocular tolerance study in pigmented rabbits using a QID dosing regimen**

**Key study findings**: When ISV-403 containing up to 0.6% SS734 in its clinical vehicle was applied to the eyes of Fauve de Bourgogne rabbits 4 times daily for 28 days, it did not cause inflammation. No treatment-related histopathologic changes to ocular tissues were observed. Systemic exposure to SS734 was very low and unlikely to be of toxicological significance.

**Study no.**: B16F0904
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: 5/19/05
**GLP compliance**: French GLP
**QA reports**: yes (X) no ( )

**Drug, lot #, and % purity**: ISV-403 ophthalmic suspension 0.6% SS734; Lot No. E04Q; purity of drug substance not provided

**Methods**

Doses: Untreated, 0 (vehicle control) and 0.6% SS734
Species/strain: Fauve de Bourgogne rabbits (pigmented)
Number/sex/group (main study): 5/sex/group
Route, formulation, volume: Topical ocular, formulated in clinical vehicle, 50 µl per dose
Satellite groups used for toxicokinetics/recovery: One animal per group was kept for an additional week to assess recovery; blood samples for TK were drawn from all animals.
Age: approximately 11 weeks old
Weight: 2.1-2.7 kg (males), 1.7-2.4 kg (females)
Sampling times for TK: Blood samples for TK were drawn on Days 1 and 28 of dosing approximately 30 minutes after the third daily dose.
Unique study design or methodology: Rabbits were dosed 4 times daily into the right eye, approximately 3 hours apart, for 28 days. The left eye served as an untreated controls. Animals were sacrificed on Day 30 (main study) or Day 35 (recovery).

**Results:**

Mortality/Clinical Signs: Rabbits were checked for viability and general cage-side observations were recorded once daily. Ocular observations (using an ophthalmoscope) were recorded each day before the first and last daily doses were given. The Draize scoring system was used to evaluate ocular irritation.

There were no unscheduled deaths in the study. No clinical signs of toxicity due to SS734 were observed. Redness and occasional chemosis (minimal) and corneal opacity (slight) were observed in both the vehicle control and drug-treated eyes. These signs were not observed after the recovery period.

Body weights: Rabbits were weighed prior to the initiation of dosing then at weekly intervals during the dosing period. There were no treatment-related effects on body weight.

Food and water consumption: Data were recorded every other day and compiled weekly. There were no treatment-related effects on food consumption.

Ocular Examinations: Indirect ophthalmoscopy and slit lamp examination were performed prior to the initiation of dosing and weekly thereafter. Corneal observations were reported using a McDonald-Shadduck scale. Evaluation of corneal sensitivity (using a esthesiometer) was performed on Days 1 and 28 prior to the first daily dose and 10, 20, 30, and 60 minutes after. Ocular examination did not reveal any changes that appeared drug-related. Corneal staining was observed in the drug and vehicle treated animals at a higher incidence than control, as was corneal opacity. These lessened or were no longer evident after recovery. Corneal sensitivity was not affected by ISV-403 or its vehicle.

Electroretinography: ERG responses were recorded prior to the initiation of dosing and on Day 22 on rabbits that were dark-adapted overnight. There did not appear to be any drug-related changes in ERG.

Hematology/Clinical Chemistry/Urinalysis: Not done.

Gross necropsy/Organ weights: Gross necropsy did not reveal any findings that appeared related to treatment with SS734 or vehicle. A standard list of organs was weighed. No drug-related differences in organ weights were observed.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

A standard list of tissues was preserved, but only ocular tissues (lens, cornea, conjunctiva, globe, eyelid, iris, retina, optic nerve, Hardarian and lachrymal glands, nictitating membrane), extraocular muscle, and gross lesions were examined microscopically.

There were no histopathologic changes in these tissues that appeared to be related to drug. There was no sign of inflammation in the ocular tissues of rabbits treated with vehicle or ISV-403.

Toxicokinetics: Plasma levels of SS734 were measured using a validated HPLC-MS assay. On Day 1, plasma levels of SS734 in the drug-treated group were below the limit of quantitation (2 ng/ml) in 6/10 rabbits (3 males, 3 females). On Day 28, 4/10 rabbits (2 males, 2 females) had plasma levels below the limit of quantitation. These animals were considered "0" for calculating mean plasma levels. One Day 1, the mean plasma level was $1.22 \pm 1.67$ ng/ml and on Day 28, the mean plasma level was $3.87 \pm 5.02$ ng/ml. This demonstrates very low systemic exposure to SS734.

**4-week ocular tolerance study of ISV-403 administered 4 times daily in the beagle dog**

**Key study findings**: When ISV-403 containing up to 0.6% SS734 in its clinical vehicle was applied to the eyes of beagle dogs 4 times daily for 28 days, it did not cause irritation or inflammation. No treatment-related histopathologic changes to ocular tissues were observed. Systemic exposure to SS734 was very low and unlikely to be of toxicological significance.

**Study no.**: AA25934
**Conducting laboratory and location**: [redacted] b(4)
**Date of study initiation**: 3/17/05
**GLP compliance**: U.S., Japanese, OECD, French GLPs
**QA reports**: yes (X) no ( )
**Drug, lot #, and % purity**: ISV-403 ophthalmic suspension 0.6% SS734; Lot No. D05Q; purity of drug substance not provided

**Methods**

Doses: 0 (vehicle control) and 0.6% SS734
Species/strain: Beagle dogs

Number/sex/group (main study): 3/sex/group
Route, formulation, volume: Topical ocular, formulated in clinical vehicle, 50 µl per dose
Satellite groups used for toxicokinetics/recovery: Blood samples for TK were drawn from all animals treated with ISV-403; there were no animals used for recovery.
Age: approximately 11 weeks old
Weight: 8.0-9.9 kg (males), 7.4-8.2 kg (females)
Sampling times for TK: Blood samples for TK were drawn on Days 1 and 24 of dosing before each dose was administered, then 30 and 60 minutes afterward, and 24 hours after the first daily dose. Frozen plasma was sent to [redacted] for quantitation of SS734. b(4)
Unique study design or methodology: Dogs were dosed 4 times daily into the left eye, approximately 4 hours apart, for 28 days. The right eye served as an untreated controls. Animals were sacrificed on the day after the last dose was given.

**Results:**

Mortality/Clinical Signs: Dogs were checked for viability and general cage-side observations were recorded twice daily. Animals underwent physical and behavioral examinations prior to the initiation of dosing and weekly during treatment. Visual ocular observations were recorded each day before and after the third daily dose were given.

There were no unscheduled deaths in the study. No clinical signs of toxicity due to SS734 were observed. Visual ocular examinations revealed no signs of irritation or abnormality.

Body weights: Dogs were weighed at weekly intervals during the dosing period. There were no treatment-related effects on body weight.

Food and water consumption: Data were recorded daily and reported as a weekly mean. There were no treatment-related effects on food consumption.

Ocular Examinations: Indirect ophthalmoscopy, tonometry, and slit lamp examination were performed prior to the initiation of dosing and weekly thereafter. Evaluation of corneal sensitivity (using a esthesiometer) was also performed weekly. Ocular examination did not reveal any changes that appeared drug-related. Neither corneal sensitivity nor ocular pressure was affected by ISV-403 or its vehicle.

Electroretinography: ERG responses were recorded prior to the initiation of dosing and during Week 4. There did not appear to be any drug-related changes in ERG.

Hematology/Clinical Chemistry/Urinalysis: Not done.

Gross necropsy/Organ weights: Gross necropsy did not reveal any findings that appeared related to treatment with SS734 or vehicle. A standard list of organs was weighed. No drug-related differences in organ weights were observed.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes (x), no ( )

A standard list of tissues was preserved, but only ocular tissues (eyes, eyelids, optic nerves, lachrymal glands, nictitating membranes) and gross lesions were examined microscopically.

There were no histopathologic changes in these tissues that appeared to be related to drug. There was no sign of inflammation in the ocular tissues of dogs treated with vehicle or ISV-403.

Toxicokinetics: Plasma levels of SS734 were measured using a validated HPLC-MS assay. On Day 1, plasma levels of SS734 in the drug-treated group were below the limit of quantitation in 2 of the 3 males. The other male had plasma levels of 4.7 and 4.3 ng/ml one hour after the first and second daily doses of ISV-403. The females had a mean Cmax of 6.7 ng/ml and $AUC_{0\text{-}12.5\ hr}$ of 49.5 ng·hr/ml. On Day 24, mean Cmax for males and females were 12.2 and 10.1 ng/ml and $AUC_{0\text{-}12.5\ hr}$ values were 65.7 and 57.7 ng·hr/ml, respectively. This demonstrates very low systemic exposure to SS734.

**Evaluation of the ocular irritation of two besifloxacin suspension formulations following multiple dosing in New Zealand White rabbits**

**Key study findings**: Despite their different impurity profiles, neither of the two Lots of 0.6% besifloxacin produced significant drug-related ocular adverse effects. When the test articles were applied to the eyes of New Zealand White rabbits 4 times daily for 14 days, they did not cause inflammation. No treatment-related histopathologic changes to ocular tissues were observed. Minor irritation was observed in some vehicle-treated eyes and one animal that received besifloxacin Lot B.

**Study no.**: P1007002 b(4)
**Conducting laboratory and location**: [redacted]
**Date of study initiation**: protocol signed 12/5/07; dosing inspected during 1/08
**GLP compliance**: U.S. GLP
**QA reports**: yes (X) no ( )
**Drug, lot #, and % purity**: Besifloxacin ophthalmic suspension 0.6%; Lot Nos. 2535-167-A (95.9% pure) and 2535-176-B (97.0% pure). These formulations were prepared with Lots of besifloxacin drug substance that had different impurity profiles for comparative purposes.

**Methods**

Doses: Untreated, 0 (vehicle control) and Lots A and B of 0.6% besifloxacin
Species/strain: New Zealand White rabbits
Number/sex/group (main study): 4/sex/group
Route, formulation, volume: Topical ocular, formulated in clinical vehicle, 50 µl per dose
Satellite groups used for toxicokinetics/recovery: Not done.
Age: approximately 18-22 weeks old
Weight: 2.3-2.9 kg
Unique study design or methodology: Rabbits were dosed 4 times daily into the right eye, approximately 3 hours apart, for 14 days. Controls were treated for 16 days.

The left eye served as an untreated controls. Animals were sacrificed on Day 15 (drug-treated) or 16 (vehicle).

**Results:**

Mortality/Clinical Signs: Rabbits were checked for viability and general cage-side observations were recorded once daily. Ocular observations (using an ophthalmoscope) were recorded on Days 1, 5, 9, and 14 before the first and after the last daily doses were given. The Draize scoring system was used to evaluate ocular irritation.

There were no unscheduled deaths in the study. No clinical signs of toxicity due to besifloxacin were observed. Conjunctival irritation (congestion and swelling) and minor findings in the cornea (some loss of transparency, cloudiness) and/or iris (minimal hyperemia), slight fluorescein staining were observed in 6 vehicle control and 1 Lot B drug-treated eyes. Hyperemia was observed in 2 untreated eyes.

Body weights: Rabbits were weighed prior to the initiation of dosing then at weekly intervals during the dosing period. There were no treatment-related effects on body weight.

Food and water consumption: Not done.

Ocular Examinations: Indirect ophthalmoscopy, slit lamp examination, evaluation of corneal sensitivity, and IOP measurement were performed prior to the initiation of dosing and weekly thereafter. Corneal observations were reported using a McDonald-Shadduck scale. Ocular examination did not reveal any changes that appeared related to treatment, with the exception of minor ocular irritation and corneal opacities discussed above. There did not appear to be any drug-related effects on IOP. Corneal sensitivity was not affected by besifloxacin or its vehicle.

Electroretinography: ERG responses were recorded prior to the initiation of dosing and on Day 14 on rabbits that were dark-adapted for an hour. There did not appear to be any drug-related changes in ERG.

Hematology/Clinical Chemistry/Urinalysis: Not done.

Gross necropsy/Organ weights: Not done.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Right and left eyes (globe and calotte), heart, lung, liver, spleen, kidneys, adrenal glands, and popliteal lymph nodes were preserved and examined microscopically. This was an adequate battery for the purposes of this study.

There were no histopathologic changes in any of these tissues that appeared to be related to drug. There was no sign of inflammation in the ocular tissues of rabbits treated with vehicle or 0.6% besifloxacin.

### 2.6.6.4 Genetic toxicology

Besifloxacin did not induce reverse mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or *E. coli* strain WP2*uvr*A at concentrations up to 3.33 µg/plate in the Ames assay regardless of metabolic activation. However, it was mutagenic in *S. typhimurium* strain TA102 and *E. coli* strain WP2(pKM101) which has been observed in the past for other fluoroquinolones. The presence of UV light did not increase the mutagenic effect of besifloxacin, but this assay is no longer considered to be a reliable predictor of phototoxicity. Besifloxacin induced chromosome aberrations in Chinese hamster ovary cells regardless of metabolic activation. *In vivo*, besifloxacin induced micronuclei formation in polychromatic erythrocytes in mouse bone marrow at oral doses $\geq$ 1500 mg/kg. It did not induce unscheduled DNA synthesis in hepatocytes from rats given oral doses of up to 2000 mg/kg. However, the UDS test is notoriously insensitive and a negative result has little meaning.

### 2.6.6.5 Carcinogenicity

Carcinogenicity studies have not been performed with besifloxacin. They are not necessary for a product being developed for short term use in patients with bacterial conjunctivitis.

### 2.6.6.6 Reproductive and developmental toxicology

A complete battery of reproductive and developmental toxicity studies was conducted with besifloxacin. Pregnancy Category C is recommended for this product. Besifloxacin did not impair the fertility of male or female rats given doses of up to 500 mg/kg/day (the highest dose tested in this study). Doses of up to 1000 mg/kg/day were not associated with visceral or skeletal malformations in rat pups in a study of embryo-fetal development, although this high dose was associated with maternal toxicity (reduced body weight gain and food consumption) and maternal mortality. At this dose, the mean Cmax and AUC in the rat dams were 20 µg/ml and 178 µg·hr/ml, respectively. Increased postimplantation loss was observed at 1000 mg/kg/day in the rat embryo-fetal development study. Fetal body weights were less than controls and decreased ossification was also observed at this high dose. The NOAEL for the rat embryo-fetal development study was the mid dose of 100 mg/kg/day (Cmax, 5 µg/ml; AUC, 23 µg·hr/ml). These findings were consistent with a rat study of pre- and postnatal development conducted using the same doses of besifloxacin (10, 100, and 1000 mg/kg/day). $F_1$ pups at the high dose weighed significantly less than controls and had a reduced neonatal survival rate. Attainment of developmental landmarks and sexual maturation were delayed at 1000 mg/kg/day, although surviving pups from this dose group that were reared to maturity did not demonstrate deficits in behavior, including activity, learning and memory, and their reproductive capacity appeared normal. The NOAEL for this pre- and postnatal developmental toxicity study in rats was 100 mg/kg/day. The sponsor also submitted data from besifloxacin embryo-fetal development studies conducted in rabbits. This species did not tolerate repeated dosing with besifloxacin (not unusual for certain classes of antimicrobials) and the dose range finding study should have demonstrated to the investigators that the rabbit was not an appropriate species to use for this type of study. Severe maternal toxicity (generally secondary to upset of gastrointestinal flora in the rabbit) led to the does consuming little food and aborting their litters at 20 mg/kg/day.

Due to the much greater systemic exposure of the rats given oral doses of besifloxacin in reproduction toxicity studies compared to systemic exposure following topical ocular application in humans, the reproductive toxicity of besifloxacin observed in animal studies is unlikely to be clinically relevant for this product.

**Study for toxic effects of SS734 on pre- and postnatal development, including maternal function in rats**

**Key study findings**: The NOAEL for reproductive toxicity in this Segment 3 study was the mid dose of 100 mg/kg/day. There was a small reduction in body weight gain and food consumption during the initial week of dosing in the $F_0$ dams receiving the mid dose that did appear drug-related. Maternal toxicity was evident at the high dose of 1000 mg/kg/day. Clinical signs of toxicity (salivation, unkempt appearance), reduced body weight gain during gestation, and reduced food consumption were observed. Additionally, the death of one $F_0$ dam at the high dose may have been drug-related. Fetotoxicity was also observed at this dose. $F_1$ pups whose dams received 1000 mg/kg/day weighed significantly less than controls and had reduced neonatal survival. Attainment of developmental landmarks and sexual maturation were delayed, although surviving pups from this dose group did not demonstrate deficits in behavior, including activity, learning and memory, and their reproductive capacity appeared normal.

**Study no.**: 967-010 b(4)
**Conducting laboratory and location**: [redacted]
**Date of study initiation**: 10/8/04
**GLP compliance**: U.S., Japanese, OECD GLP
**QA reports**: yes (x) no ( )
**Drug, lot #, and % purity**: SS734, Lot No. 03063J, 100.2% pure

**Methods**

Doses: 0 (vehicle), 10, 100, 1000 mg/kg/day
Species/strain: Time-mated presumed pregnant Sprague-Dawley rats
Number/sex/group: 25
Route, formulation, dose volume: Oral, given at a dose volume of 10 ml/kg in a vehicle of 0.5% carboxymethylcellulose in 0.9% NaCl.
Satellite groups used for toxicokinetics: none; TK not done
Study design: $F_0$ dams were dosed from Gestation Day 6 through Lactation Day 20. $F_0$ dams were allowed to deliver naturally and pups were culled to 4/sex/litter on LD 4. Intact pups that died during the lactation period were necropsied. $F_0$ dams were sacrificed on LD 21 and underwent gross necropsy. The number of uterine implantation sites was determined. Dams that did not deliver by GD 25 were sacrificed, necropsied, and the uteri stained with 10% ammonium sulfide to determine pregnancy status of the animals. Pups that remained after culling were kept until at least Post Natal Day 28. At least one male and one female from each litter were randomly selected for further evaluation of behavior, sexual maturation, and reproductive competence/fertility; the other pups were sacrificed. When dose groups did not have 25 surviving litters, additional pups from the surviving litters were randomly selected to bring the number of pups evaluated for these parameters up to 25. Motor activity was evaluated on approximately PND 35 $\pm$ 2 using an activity chamber with photocell sensors. Passive avoidance

testing occurred between PND 73-86. Dark and light compartments were separated by a mechanical door, animals learned that if they moved to the dark compartment, they would receive a shock. After $F_1$ rats were at least 80 days old and the passive avoidance testing was complete, animals were tested for reproductive competence/fertility. Male and females were paired (sibling pairs avoided) until evidence of mating was observed, for a maximum of 20 days. $F_1$ females were sacrificed on GD 13 (or 13 days after the last day of pairing) and their uterine contents examined. The mated $F_1$ males were sacrificed after cesarean sections were complete. The $F_1$ males and females were necropsied and any congenital abnormalities were noted.

Parameters and endpoints evaluated: Dams ($F_0$): clinical signs, body weight, food consumption, maternal behavior. Offspring ($F_1$): litter size, viability, survival, body weights, sex ratio, external abnormalities, physical development, static righting reflex (LD 2), pinna detachment (LD 2), cliff aversion (LD 11), eye opening (LD 13), air drop righting reflex (LD 16), Irwin screen (LD 21), auditory response (Galton whistle, PND 22). One male and one female per litter that were selected for additional evaluation: sexual maturation (vaginal opening, PND 28; preputial separation, PND 35), behavior (motor activity, learning/memory via step through passive avoidance), reproductive competence/fertility (pregnancy rate, number of implantations/corpora lutea, viability of embryos, litter size, and external embryo abnormalities).

**Results**

$F_0$ in-life: All $F_0$ dams in the control, 10, and 100 mg/kg/day groups survived until scheduled sacrifice. Two $F_0$ dams in the 1000 mg/kg/day group died early. One of these, on GD 13, was the result of a dosing error. The other death occurred nine days after delivery. This dam had no remaining pups by the time of its death- 7 pups were stillborn and 6 pups died within a week of birth (all but one prior to LD 4). Necropsy did not reveal a specific cause of death for this high dose dam and a relationship to drug treatment cannot be excluded.

Clinical signs of toxicity were not observed in the low and mid dose $F_0$ dams that received SS734. Salivation and sparse patches of body hair was observed in the high dose $F_0$ dams. Four rats from this group had hunched posture during the lactation period and 2 of these 4 also appeared unkempt.

SS734 did not affect body weight, body weight gain, or food consumption of the 10 mg/kg/day $F_0$ dams. At the mid dose of 100 mg/kg/day, there was a small reduction in body weight gain during the first week of dosing, statistically significant ($p<0.05$) only during the GD 10-14 period. Food consumption was also reduced during this period ($p<0.01$). By the end of the gestation period, overall body weight gain in this group did not differ significantly from controls for GD 6-20 ($120.8 \pm 23.4$ g vs. $110.4 \pm 15.4$ g) and the body weights in these groups on GD 20 also did not significantly differ ($362.8 \pm 33.5$ vs. $346.8 \pm 22.3$). During the lactation period, this mid dose group gained more weight and consumed more food per day overall than controls. At the end of the lactation period, the mean body weight in the dams from the control and mid dose groups were within 1 g.

The high dose $F_0$ dams gained significantly less weight than controls from GD 6-20 at all intervals ($p<0.01$). On GD 20, overall mean body weight gain in this groups was $79.9 \pm 29.7$ g and mean body weight was $319.1 \pm 32.4$ g. Food consumption in the high dose dams was consistently less than controls during dosing in the gestation period ($p<0.01$ overall), average daily food consumption of $24.9 \pm 2.6$ g vs. $20.5 \pm 3.2$ g. During the lactation period, approximately half of the high dose females were sacrificed because all the pups in their litters

had died. The remaining females had body weights similar to controls during the lactation period. Food consumption in the high dose dams remained suppressed compared to control during the first 10 days of the lactation period, but was similar to controls thereafter.

No evidence of dystocia or protracted labor was observed.

$F_0$ necropsy: No macroscopic treatment-related findings were apparent in the $F_0$ dams. The number of uterine implant scars per dam was comparable among the treatment groups. Pregnancy rates in the drug-treated dams did not differ from controls.

$F_0$ Litter Data: One control $F_0$ dam was not pregnant and a second completely resorbed its litter. One 10 mg/kg $F_0$ dam was not pregnant. All in the 100 and 1000 mg/kg groups were pregnant, though as mentioned above, one high dose $F_0$ dam died on GD 13 due to a dosing error. SS734 did not have an effect on gestation length, litter size, the numbers of dead vs. live pups per litter, and the number of stillborn pups per litter in the low and mid dose groups. SS734 did not affect the sex ratio at any dose. At the high dose, gestation length of the $F_0$ dams was significantly longer than controls. There were fewer live pups per litter at 1000 mg/kg/day and there were more litters with stillborn pups. Neonatal survival was considerably lower than controls at the high dose of SS734. Pups that lived until LD 4 had a 91.4% rate of survival for the remainder of the lactation period, but it was still significantly less than the 100% survival rate of controls.

From the Study Report: b(4)

[redacted] Study Number 967-010

[redacted] for Toxic Effects of SS734 on Pre- and Postnatal Development, including Maternal Function in Rats

Table 10 Summary of P Natural Delivery and Litter Data

| Endpoint | | 0 mg/kg/day (Vehicle Control) | 10 mg/kg/day | 100 mg/kg/day | 1000 mg/kg/day |
|---|---|---|---|---|---|
| No. Live Pups/Litter Day 4 (Preculling) | Mean | 12.1 | 10.9 | 10.9 | 8.5[b] |
| | SD | 2.33 | 1.93 | 2.20 | 3.45 |
| | N | 23 | 24 | 25 | 13 |
| Day 4 (Postculling) | Mean | 8.0 | 7.9 | 7.8 | 6.8[b] |
| | SD | 0.21 | 0.41 | 0.62 | 2.41 |
| | N | 23 | 24 | 25 | 13 |
| Day 7 | Mean | 8.0 | 7.9 | 7.8 | 7.3 |
| | SD | 0.21 | 0.41 | 0.62 | 1.72 |
| | N | 23 | 24 | 25 | 12 |
| Day 14 | Mean | 8.0 | 7.9 | 7.8 | 7.3 |
| | SD | 0.21 | 0.41 | 0.62 | 1.72 |
| | N | 23 | 24 | 25 | 12 |
| Day 21 | Mean | 8.0 | 7.9 | 7.8 | 7.3[b] |
| | SD | 0.21 | 0.41 | 0.62 | 1.71 |
| | N | 23 | 24 | 25 | 12 |
| | SD | 2.41 | 2.03 | 1.75 | 4.66 |
| | N | 23 | 24 | 25 | 24 |
| Liveborn/Litter | Mean | 12.1 | 11.1 | 11.3 | 9.2[b] |
| | SD | 2.34 | 1.95 | 1.80 | 3.27 |
| | N | 23 | 24 | 25 | 24 |

Table 10 Summary of P Natural Delivery and Litter Data

| Endpoint | | 0 mg/kg/day (Vehicle Control) | 10 mg/kg/day | 100 mg/kg/day | 1000 mg/kg/day |
|---|---|---|---|---|---|
| Sex Ratio (% Males per Animal) Pups Day 0 | Mean %/Litter | 51.92 | 48.06 | 49.26 | 53.81 |
| | SD | 12.834 | 16.937 | 17.243 | 20.984 |
| | N | 23 | 24 | 25 | 24 |
| Pups Day 4 (Preculling) | Mean %/Litter | 51.73 | 47.79 | 49.28 | 52.48 |
| | SD | 12.959 | 16.485 | 17.988 | 24.935 |
| | N | 23 | 24 | 25 | 13 |
| Pups Day 4 (Postculling) | Mean %/Litter | 50.93 | 50.00 | 49.01 | 52.88 |
| | SD | 8.763 | 12.769 | 14.191 | 23.094 |
| | N | 23 | 24 | 25 | 13 |
| Pups Day 21 | Mean %/Litter | 50.93 | 50.00 | 49.01 | 48.66 |
| | SD | 8.763 | 12.769 | 14.191 | 18.638 |
| | N | 23 | 24 | 25 | 12 |
| Pup Survival Indices Viability Index | Mean %/Litter | 99.67 | 98.94 | 97.10 | 47.34[b] |
| | SD | 1.604 | 2.871 | 7.917 | 44.509 |
| | N | 23 | 24 | 25 | 23 |
| Lactation Index | Mean %/Litter | 100.00 | 100.00 | 100.0 | 91.35[a] |
| | SD | 0.000 | 0.000 | 0.000 | 27.663 |
| | N | 23 | 24 | 25 | 13 |

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

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

Clinical findings in some pups from the high dose females included decreased activity, cold to touch, and pale color. These observations were consistent with the increased mortality of the pups from this dose group.

Pup body weights were reduced in the 1000 mg/kg/day group compared to controls both at birth and throughout the lactation period. The difference was statistically significant ($p<0.01$) only through LD 4, but it appeared biologically relevant throughout the study and correlated with delay in the attainment of developmental landmarks as discussed below. At birth, the mean weight of the control pups was $6.80 \pm 0.55$ g compared to $5.05 \pm 0.83$ g for the high dose group. On LD 4 (prior to the cull), the mean pup weights were $10.67 \pm 1.10$ vs. $7.42 \pm 1.75$ g. At this point, only 13/24 high dose litters remained viable. On LD 21, the mean pup weights were 53.19 $\pm$ 3.98 vs. $42.68 \pm 7.07$ g. On Day 28 after birth (before pups not selected for behavioral and reproductive testing were sacrificed), mean pup weights were 93.17 + 5.57 and 74.58 + 10.54 g in the control and high dose groups, respectively. On this day, 12/24 high dose litters remained.

$F_1$ physical development: Delays in the attainment of several developmental landmarks were observed in the 1000 mg/kg/day dose group. Pinna detachment and eye opening in the high dose pups occurred approximately a day later than the other groups. Sexual maturation was also delayed in the high dose pups. The physical development of the pups from the 10 and 100 mg/kg/day dose groups was comparable to controls.

From the Study Report:

b(4)

[redacted] Study Number 967-010
Study for Toxic Effects of SS734 on Pre- and Postnatal Development, including Maternal Function in Rats

Table 16 Summary of $F_1$ Physical Development

| Endpoint | | 0 mg/kg/day (Vehicle Control) | 10 mg/kg/day | 100 mg/kg/day | 1000 mg/kg/day |
|---|---|---|---|---|---|
| No. of Litters Evaluated | | 23 | 24 | 25 | 13 |
| Static Righting Reflex (Days) | Mean | 2.4 | 2.4 | 2.6 | 2.67 |
| | SD | 0.26 | 0.39 | 0.34 | 0.44 |
| | N | 23 | 24 | 25 | 13 |
| Pinna Detachment (Days) | Mean | 2.1 | 2.0 | 2.2 | 3.0[b] |
| | SD | 0.23 | 0.05 | 0.34 | 0.47 |
| | N | 23 | 24 | 25 | 13 |
| Cliff Aversion (Days) | Mean | 11.0 | 11.0 | 11.0 | 11.0 |
| | SD | 0.05 | 0.06 | 0.03 | 0.11 |
| | N | 23 | 24 | 25 | 12 |
| Eye Opening (Days) | Mean | 14.0 | 14.0 | 13.9 | 14.7[b] |
| | SD | 0.42 | 0.46 | 0.58 | 0.64 |
| | N | 23 | 24 | 25 | 11 |
| Air Drop Righting Reflex (Days) | Mean | 16.0 | 16.0 | 16.0 | 16.0 |
| | SD | 0.00 | 0.00 | 0.00 | 0.05 |
| | N | 23 | 24 | 25 | 12 |
| Auditory Response Percent Pups Passing/Dam | Mean | 100.0 | 100.0 | 100.0 | 100.0 |
| | SD | 0.00 | 0.00 | 0.00 | 0.00 |
| | N | 23 | 24 | 25 | 12 |

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

No. - Number
SD - Standard Deviation
N - Number of measures used to calculate mean.

Table 17 Summary of $F_1$ Sexual Maturation

| Endpoint | | 0 mg/kg/day (Vehicle Control) | 10 mg/kg/day | 100 mg/kg/day | 1000 mg/kg/day |
|---|---|---|---|---|---|
| Vaginal Opening (Days) | Mean | 32.1 | 32.1 | 32.1 | 33.5[b] |
| | SD | 1.22 | 1.41 | 1.08 | 2.33 |
| No. of Pups Passing | | 25 | 25 | 25 | 25 |
| Body Weight on Day Passed Vaginal Opening, g | Mean | 114.0 | 120.0 | 119.5 | 102.0[b] |
| | SD | 14.67 | 10.49 | 12.56 | 10.93 |
| No. of Pups | | 25 | 25 | 25 | 25 |
| Preputial Separation (Days) | Mean | 42.9 | 43.1 | 43.1 | 45.5[b] |
| | SD | 1.29 | 1.69 | 1.47 | 3.02 |
| No. of Pups Passing | | 25 | 25 | 25 | 25 |
| Body Weight on Day Passed Preputial Separation, g | Mean | 232.8 | 236.02 | 235.0 | 208.1[b] |
| | SD | 18.71 | 20.47 | 12.41 | 24.26 |
| No. of Pups | | 25 | 25 | 25 | 25 |

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

No. - Number
SD - Standard Deviation

$F_1$ behavioral evaluation: No drug-related findings were observed. Although there were some statistically significant differences between controls and treated animals for some of the parameters evaluated, they did not appear to be related to SS734 because they were not of great

magnitude and occurred only in the low or mid dose group or only in a single gender. Thus, SS734 did not appear to have an effect on the behavior or motor activity pups placed in activity chambers. Passive avoidance testing did not reveal any meaningful differences between drug-treated and control pups.

$F_1$ reproduction: No drug-related findings were observed. The mating, fertility, and fecundity indices in the SS734 groups was comparable to control, as was the copulatory interval. Body weight gain during GD 0-13 was similar among all of the $F_1$ females regardless of dose group. There was a small, but statistically significant difference ($p<0.05$) in the number of implantation sites between control and the low/high dose groups (16.5 vs. 14.7/14.3) and a similar difference between the numbers of viable embryos in these groups (15.8 vs. 13.8). However, these differences are considered to be the result of biological variation because both pre- and postimplantation losses did not differ significantly between drug-treated groups and controls.

$F_2$ findings: No drug-related findings were observed.

Toxicokinetics: Not done

**2.6.6.7 Local tolerance**

Ocular toxicity studies in rabbits and dogs (up to 1 month in duration) demonstrated that besifloxacin in DuraSite™ vehicle was generally well tolerated. Minor signs of ocular irritation (e.g., redness, mild chemosis) were occasionally observed in rabbits, but not dogs. There were no drug-related histopathological findings in the eyes of either species.

**2.6.6.8 Special toxicology studies**

Besifloxacin was phototoxic to the ears of mice following oral doses $\geq$ 200 mg/kg/day in conjunction with exposure to UVA light. However, besifloxacin was not phototoxic when solutions containing up to 1% were applied topically to the skin of guinea pigs and followed by exposure to UVA/B light. A photoallergenicity study in guinea pigs was also conducted using topically applied besifloxacin. This study was negative, but this model is not a sensitive predictor of human photoallergy.

**2.6.6.9 Discussion and Conclusions**

Besifloxacin 0.6% in the DuraSite® polycarbophil vehicle appears reasonably safe for the proposed indication of bacterial conjunctivitis in pediatric and adult patients. The drug would be administered to humans 3 times daily for 7 days. This regimen is supported by studies in rabbits and dogs that received 4 daily doses of this product for up to 28 days without evidence of ocular inflammation or adverse microscopic changes in ocular tissues. Minor signs of ocular irritation (e.g., redness, mild chemosis) were occasionally observed in rabbits, but not dogs. ERG measurements and corneal sensitivity were not significantly changed in either species following drug treatment and ocular pressure did not increase in the dogs (it was not measured in the rabbit study).

Oral doses of up to 400 mg/kg in rats and 50 mg/kg in dogs given daily for 28 days were not associated with microscopic changes, although the dogs experienced treatment-associated salivation and vomiting. Systemic exposure following these oral doses was far above what occurs after ocular administration of besifloxacin.

Besifloxacin has a genotoxicity profile typical for fluoroquinlones. It is mutagenic to some strains of bacteria, clastogenic in Chinese hamster ovary cells, and induces micronuclei in mouse bone marrow PCEs. These results are likely related to topoisomerase inhibition.

Although besifloxacin was fetotoxic in reproduction toxicity studies (postimplantation loss, neonatal mortality, developmental delays), these findings occurred at exposure levels far in excess of what would occur after topical ocular administration. Pregnancy Category C is recommended to be consistent with other products of this pharmaceutical class.

There are a number of other marketed fluoroquinolone antimicrobials formulated for ocular use. This product is similar to those products and does not appear to have a significantly different risk profile.

### 2.6.6.10 Tables and Figures

All tables and figures relevant to this NDA have been included in other sections of this review.

## 2.6.7 TOXICOLOGY TABULATED SUMMARY

The sponsor did not provide an appropriate summary table in the application.

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: Besifloxacin 0.6% in the DuraSite® polycarbophil vehicle appears reasonably safe for the proposed indication of bacterial conjunctivitis in pediatric and adult patients. It did not cause ocular inflammation or histopathologic changes in rabbits or dogs. This product is similar to other marketed fluoroquinolones and it does not appear to have a significantly different risk profile. The vehicle has been used in another approved ocular product, AzaSite™, also used to treat bacterial conjunctivitis. The pharmacology/toxicology reviewer has no objection to the approval of this NDA.

Unresolved toxicology issues: None.

Recommendations:. There are no recommendations for additional nonclinical studies.

Suggested labeling:

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Pregnancy Category C. Oral doses of besifloxacin up to 1000 mg/kg/day were not associated with visceral or skeletal malformations in rat pups in a study of embryo-fetal development,

although this high dose was associated with maternal toxicity (reduced body weight gain and food consumption) and maternal mortality. Increased postimplantation loss, decreased fetal body weights, and decreased fetal ossification were also observed. At this high dose, the mean Cmax in the rat dams was approximately 20 µg/ml, over 20,000 times the highest plasma concentrations measured in humans. The No Observed Adverse Effect Level (NOAEL) for this study was 100 mg/kg/day (Cmax, 5 µg/ml). In a prenatal and postnatal development study in rats, the NOAELs for both fetal and maternal toxicity were also 100 mg/kg/day. At 1000 mg/kg/day, pups weighed significantly less than controls and had a reduced neonatal survival rate. Attainment of developmental landmarks and sexual maturation were delayed, although surviving pups from this dose group that were reared to maturity did not demonstrate deficits in behavior, including activity, learning and memory, and their reproductive capacity appeared normal. Since there are no adequate and well-controlled studies in pregnant women, Besivance™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

**8.4 Pediatric Use**

The safety and effectiveness of Besivance™ in infants below 1 year of age have not been established. The efficacy of Besivance™ in treating bacterial conjunctivitis in pediatric patients one year or older has been demonstrated in controlled clinical trials (see 14 CLINICAL STUDIES) [redacted] b(4)

It is unlikely that the ophthalmic administration of Besivance™ has any effect on weight bearing joints, even though systemic administration of quinolones has been shown to cause arthropathy in immature animals.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment Of Fertility

Long-term studies in animals to determine the carcinogenic potential of besifloxacin have not been performed.

No *in vitro* mutagenic activity of besifloxacin was observed in an Ames test (up to 3.33 µg/plate) on bacterial tester strains *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2uvrA. However, it was mutagenic in *S. typhimurium* strain TA102 and *E. coli* strain WP2(pKM101). Positive responses in these strains have been observed with other quinolones and are likely related to topoisomerase inhibition. Besifloxacin induced chromosomal aberrations in CHO cells *in vitro* and it was positive in an *in vivo* mouse micronucleus assay at oral doses ≥ 1500 mg/kg. Besifloxacin did not induce unscheduled DNA synthesis in hepatocytes cultured from rats given the test compound up to 2,000 mg/kg by the oral route.

In a fertility and early embryonic development study in rats, besifloxacin did not impair the fertility of male or female rats at oral doses of up to 500 mg/kg/day. This is over10,000 times higher than the highest recommended total daily human ophthalmic dose.

Reviewer: Amy L. Ellis NDA No.

*Note: I recommend deleting Section 13.2, Animal Toxicology and/or Pharmacology, because it should be reserved for discussions of toxicities identified in animals that are of concern and that may be clinically relevant, especially in cases where clinical data are not available or clear.*

Signatures (optional):

Reviewer Signature _____

Supervisory Pharmacologist Signature_____ Concurrence Yes ___ No ___

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/s/
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Amy Ellis
2/9/2009 05:42:36 PM
PHARMACOLOGIST
The pharmacologist has no objection to the approval of this NDA.
Wendy- You signed the paper copy of this review on 2/9/09.

Wendelyn Schmidt
2/10/2009 10:19:01 AM
PHARMACOLOGIST