CLINICAL PHARMACOLOGY REVIEW

NDA:	22-308
Submission Date:	30MAY2008
Trade Name	TBD
Generic Name	Besifloxacin hydrochloride
OCP Reviewers	Ryan P. Owen, Ph.D.
OCP Team Leader	Charles Bonapace, Pharm.D.
OCP Division	DCP4
OND division	DAIOP (520)
Sponsor	Bausch & Lomb
Relevant IND(s)	IND 64,335
Submission Type; Code	Original NDA (NME)
Formulation; Strength(s)	Besifloxacin hydrochloride ophthalmic suspension, 0.6% as base
Indication	Bacterial Conjunctivitis
Dosage and Administration	One drop of 0.6% besifloxacin hydrochloride ophthalmic suspension TID for 7 days

Table of Contents

1. Executive Summary

Bausch & Lomb submitted a New Drug Application to market besifloxacin hydrochloride ophthalmic suspension 0.6% for the treatment of bacterial conjunctivitis in adults and pediatric patients one year and older. The proposed dosing regimen of besifloxacin is one drop in the affected eye 3 times a day (TID) for 7 days.

The sponsor performed four Phase 1 studies (Study C-02-403-001, Study 507, Study ROC2-05-070, and Study 424) to assess the safety and tolerability of besifloxacin. Studies C-02-403-001 and 424 characterized besifloxacin pharmacokinetics in plasma and tears respectively. Study 507 examined the corneal endothelial cell density changes in healthy subjects administered 0.6% besifloxacin TID for five days, and did not feature any pharmacokinetic data. Study ROC2-05-070 compared the ocular tolerability of a single dose of 0.6% besifloxacin to moxifloxacin. One Phase 2 (Study 478) and three Phase 3 (Studies 373, 433, and 478) clinical studies were performed to evaluate the safety and efficacy of besifloxacin for the treatment of bacterial conjunctivitis. The treatment emergent adverse events were predominantly ocular in nature, and were considered minor. The incidence of AEs was similar in the besifloxacin treated patients as compared to patients receiving vehicle or the active control.

The dosing regimen of besifloxacin evaluated in the Phase 3 trials was based on the tolerability of 0.6% besifloxacin in the Phase 1 trials, the ocular retention observed in nonclinical studies, and the calculated AUC/MIC and C_{max}/MIC based on the concentrations of besifloxacin in tears, and the MIC_{90} of target pathogens.

Omissions from this application include the impact of intrinsic factors on the pharmacokinetics of besifloxacin, a thorough QT study, and the potential for besifloxacin to inhibit the cytochrome P450 enzymes. However, based on the low systemic availability of besifloxacin, the impact of intrinsic factors on the pharmacokinetics of besifloxacin would be expected to be minimal. Additionally, the *in vitro* hERG study suggested that concentrations greater than 10,000 times the highest observed plasma concentration induced a 13% inhibition in hERG tail current, suggesting that it is unlikely that besifloxacin would impact cardiac repolarization. *In vitro* incubation with human hepatocytes suggest that besifloxacin is not significantly metabolized, and the plasma concentrations observed in clinical trials suggest that it is unlikely that besifloxacin would reach a high enough concentration to interfere with the metabolism of other drugs.

The dosing regimen of one drop of 0.6% besifloxacin TID for seven days is acceptable based on the high rate of microbial eradication and low incidence of clinically significant adverse events.

1.1 Recommendation

The Office of Clinical Pharmacology Division 4 has reviewed NDA 22-308 and has determined that it is acceptable from a Clinical Pharmacology perspective.

1.2 Phase 4 Commitments

No Phase 4 commitments are recommended.

_____.
Ryan P. Owen, Ph.D.
Reviewer
Clinical Pharmacology
DCP4/OCP

Concurrence: _____.
Charles Bonapace
Team Leader
Clinical Pharmacology
DCP4/OCP/OTS

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Besifloxacin is an 8-chloro fluoroquinolone anti-infective for topical ophthalmic use in the treatment of bacterial conjunctivitis. To support product approval, the sponsor conducted two Phase 3 studies examining the safety and efficacy of besifloxacin ophthalmic suspension as compared to vehicle in the treatment of bacterial conjunctivitis. An additional Phase 3 study which compared besifloxacin ophthalmic suspension to the active control moxifloxacin was included for the safety analysis. In addition to the Phase 3 trials, three clinical pharmacology clinical studies were included in the application. Study C-02-403-001 was an initial dose-finding study that evaluated the safety and tolerability of 0.3% and 0.6% besifloxacin ophthalmic suspension dosed QID for one week. Study 424 examined the ocular pharmacokinetics of besifloxacin in the tears of healthy volunteers following a single administration of 0.6% besifloxacin. Study 478 examined the plasma concentrations of besifloxacin in patients with suspected bacterial conjunctivitis who had been administered topical ocular 0.6% besifloxacin TID for five days. The proposed dose of besifloxacin ophthalmic suspension is 0.6% TID for seven days.

Absorption

In humans, besifloxacin is absorbed into the ocular tissues following topical ophthalmic application. Figure 1 shows the mean concentration-time profile of besifloxacin in human plasma.

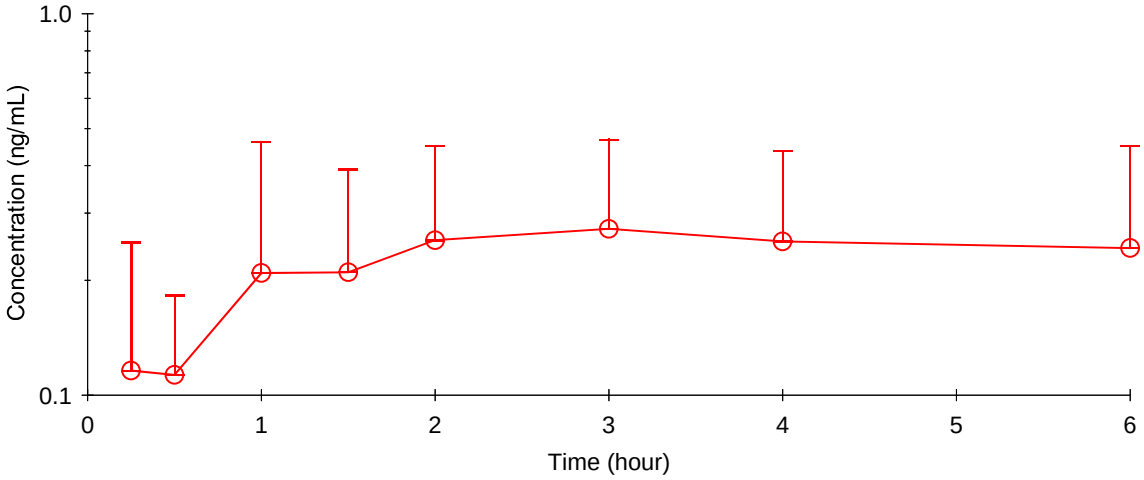


Figure 1: Mean (+SD) plasma concentrations versus time profile (log-linear scale).

Distribution

The protein binding of besifloxacin in humans is estimated to be 39-44% in human plasma. The distribution of besifloxacin into human tissues was not examined.

Metabolism

Besifloxacin is metabolically stable, and undergoes little or no chiral interconversion to its enantiomer. There was no detectable decrease in besifloxacin concentration following a two hour incubation with human hepatocytes.

Excretion

The excretion of besifloxacin in humans has not been evaluated. In animal models, the majority of besifloxacin (73%) was recovered in the feces, and a smaller fraction (23%) was recovered in the urine.

Intrinsic Factors

The impact of intrinsic factors on the pharmacokinetics of besifloxacin were not evaluated.

Drug-drug interactions

No drug-drug interactions are anticipated with besifloxacin

Clinical dose and regimen selection

The 0.6% TID dosing regimen for besifloxacin was selected on the basis of the safety and tolerability of the 0.6% QID dosing regimen in the initial Phase 1 clinical trial. The ocular retention observed in nonclinical trials and the AUC/MIC and C_{max}/MIC parameters calculated based on the concentrations of besifloxacin achieved in human tears and the MIC_{90} of select target pathogens support the dosage regimen.

Exposure-response: efficacy

One dosing strength (0.6% besifloxacin TID for five days) was tested in the Phase 3 trials. Table 1 shows the clinical response at Visit 3 for the combined Phase 3 efficacy results. Besifloxacin demonstrated superior clinical resolution to vehicle at Visit 3.

Table 1: Clinical resolution at Visit 3 (Study 373 and 433 mITT population)

	Besifloxacin Suspension (N = 259)	Vehicle (N = 249)
Subjects with Non-Missing Data	251	223
Clinical Resolution (Data 'as observed')		
Yes	205 (81.7%)	151 (67.7%)
No	46 (18.3%)	72 (32.2%)
p-Value for Comparison of Treatments[1]	0.0009 / 0.0006	
95% CI for Difference[2]	(6.14%, 21.78%)	
Subjects with Missing Data	8	26
Clinical Resolution (Missing or Discontinued Subjects Imputed as 'no')		
Yes	205 (79.2%)	151 (60.6%)
No	54 (20.8%)	98 (39.4%)
p-Value for Comparison of Treatments[1]	<0.0001 / <0.0001	
95% CI for Difference in Percentages[2]	(10.52%, 26.49%)	

[1] p-Values from CMH test stratified by center / exact Pearson chi-squared test, respectively.
[2] Difference calculated as besifloxacin minus vehicle. Positive values favor besifloxacin.

Exposure-response: safety

Non-ocular adverse events were generally low. Table 2 shows the treatment-emergent ocular adverse events of besifloxacin, vehicle, and Vigamox (moxifloxacin 0.5% ophthalmic solution). The frequency of ocular adverse events in the besifloxacin treatment group was in general similar to or lower than the frequency of adverse events in the vehicle and moxifloxacin populations.

Table 2: Treatment-emergent, ocular AEs occurring in ≥ 0.5% of eyes in either treatment group, all treated eyes, safety population.

	Besifloxacin Suspension (N^1 = 1810)	Vehicle (N^1 = 961)	Vigamox (N^1 = 855)	p-Value[2]
Total Number of AEs	327	258	153	
Number of Eyes With at Least One AE	249 (13.8%)	190 (19.8%)	120 (14.0%)	<0.0001
EYE DISORDERS	243 (13.4%)	189 (19.7%)	113 (13.2%)	<0.0001
Conjunctivitis	47 (2.6%)	41 (4.3%)	33 (3.9%)	0.0223
Vision blurred	38 (2.1%)	39 (4.1%)	4 (0.5%)	0.0035
Conjunctivitis bacterial	32 (1.8%)	27 (2.8%)	22 (2.6%)	0.0736
Eye irritation	26 (1.4%)	27 (2.8%)	12 (1.4%)	0.0187
Eye pain	28 (1.5%)	17 (1.8%)	9 (1.1%)	0.6396
Eye pruritus	18 (1.0%)	18 (1.9%)	3 (0.4%)	0.0761
Conjunctival haemorrhage	7 (0.4%)	5 (0.5%)	4 (0.5%)	0.7622
Eye discharge	6 (0.3%)	6 (0.6%)	3 (0.4%)	0.3615
Eyelid oedema	6 (0.3%)	4 (0.4%)	5 (0.6%)	0.7457
Conjunctival hyperaemia	10 (0.6%)	3 (0.3%)	0 (0.0%)	0.5612
Punctate keratitis	5 (0.3%)	3 (0.3%)	5 (0.6%)	>0.9999
Ocular hyperaemia	6 (0.3%)	5 (0.5%)	1 (0.1%)	0.5290
Conjunctivitis viral	10 (0.6%)	0 (0.0%)	1 (0.1%)	0.0185
Dry eye	5 (0.3%)	2 (0.2%)	4 (0.5%)	>0.9999
Limbal hyperaemia	4 (0.2%)	3 (0.3%)	4 (0.5%)	0.6994
Lacrimation increased	1 (0.1%)	6 (0.6%)	2 (0.2%)	0.0085

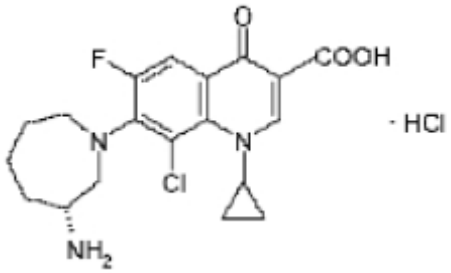
[1] N= all treated eyes for the specified treatment group and includes study and fellow eyes.
[2] p-Value based on Fisher's Exact test, comparing besifloxacin ophthalmic suspension and vehicle.
NOTE: Treatment-emergent refers to: subsequent to the treatment of the study eye. The total number of AEs counts all AEs for eyes. Eyes may have more than 1 AE per body system and preferred term. A subject could be counted twice for a specific AE, if both eyes had the event while being treated. Percentages are based on the number of eyes that received the indicated study treatment.

Question-Based Review

2.1. General attributes of the drug

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to the clinical pharmacology and biopharmaceutics review?

Besifloxacin hydrochloride ophthalmic suspension is a sterile, ophthalmic suspension. It is an 8-chloro fluoroquinolone anti-infective for topical ophthalmic use. The chemical name for besifloxacin is 7-[(3R)-3-Aminohexahydro-1*H*-azepin-1-yl]-8-chloro-1-cyclopropyl-6 fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid monohydrochloride. The empirical formula of besifloxacin hydrochloride is $C_{19}H_{21}ClFN_3O_3 \cdot HCl$. The chemical structure is represented below:



Besifloxacin hydrochloride is a white to pale yellowish-white powder. Each mL of Besifloxacin ophthalmic suspension contains 6.63 mg besifloxacin hydrochloride equivalent to 6 mg besifloxacin base (active ingredient), 0.01% benzalkonium chloride (preservative), and several inactives (polycarbophil (b) (4) mannitol, poloxamer 407, sodium chloride, edentate disodium dehydrate, sodium hydroxide, and purified water).

2.1.2. What are the proposed mechanism(s) of action and therapeutic indications(s)?

The proposed mechanism of action for besifloxacin is the inhibition of both bacterial DNA gyrase and topoisomerase IV. DNA gyrase is an essential enzyme required for replication, transcription, and repair of bacterial DNA. Topoisomerase IV is an essential enzyme required for partitioning of the chromosomal DNA during bacterial cell division.

The proposed indication for besifloxacin is the treatment of bacterial conjunctivitis caused by the following organisms:

<u>Aerobic and facultative Gram-positive microorganisms</u>
CDC coryneform group G
*Corynebacterium pseudodiphitheriticum**
*Corynebacterium striatum**
Staphylococcus aureus
Staphylococcus epidermidis

*Staphylococcus hominis**
*Staphylococcus lugdunensis**
Streptococcus mitis group
Streptococcus oralis
Streptococcus pneumoniae
*Streptococcus salivarius**

Aerobic and facultative Gram-negative microorganisms
Haemophilus influenzae
*Moraxella lacunata**

*Efficacy for this organism was studied in fewer than 10 infections.

2.1.3. What are the proposed dosage(s) and route(s) of administration?

The proposed dose is one drop of 0.6% besifloxacin ophthalmic suspension in the affected eye 3 times a day for 7 days. Besifloxacin is being developed solely as a topical ophthalmic suspension.

2.2. General clinical pharmacology

2.2.1. What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The clinical development program for besifloxacin featured four Phase 1 studies (C-02-403-001, Study 507, ROC2-05-070, and Study 424), one Phase 2 study (Study 478), and three Phase 3 studies (Studies 373, 433, and 434). The initial Phase 1 dose finding trial, C-02-403-001, evaluated the safety, tolerability, and pharmacokinetics of 0.3% and 0.6% topical ocular besifloxacin dosed four times a day for seven days (see Appendix 4.2.3.). Since preclinical data (Study I07U0102) demonstrated that greater ocular exposure (defined as the AUC in tears) to besifloxacin occurred at the higher dose, and both doses were well-tolerated in Study C-02-403-001, the 0.6% dose was used for all further clinical trials.

In Study 507 besifloxacin 0.6% was administered three times daily for five days to healthy volunteers. The purpose of this study was to evaluate corneal endothelial cell density changes (no statistically or clinically significant change in corneal endothelial cell density was observed in this study). ROC2-05-070 was a one day randomized trial of besifloxacin ophthalmic suspension compared to moxifloxacin to evaluate visual performance following single dose administration of study drug in healthy volunteers. The eyes that received besifloxacin ophthalmic suspension had statistically significant worse high contrast/high illumination visual acuity immediately after drop instillation, and a longer recovery time to baseline visual acuity compared to eyes receiving moxifloxacin. The average time to recover to baseline visual acuity was 58 seconds for besifloxacin, which was deemed to be clinically acceptable.

Study 424 was a Phase 1 study that examined the pharmacokinetics of besifloxacin in the tears of healthy volunteers after a single instillation of 0.6% besifloxacin ophthalmic suspension (see Appendix 4.2.4.). Study 478 was a Phase 2 study that examined the systemic pharmacokinetics of besifloxacin after single and multiple TID instillations of 0.6% besifloxacin ophthalmic suspension in patients with suspected bacterial conjunctivitis (see Appendix 4.2.5.).

Three independent Phase 3 studies (Study 373, 433, and 434) were conducted to assess the safety and efficacy of 0.6% besifloxacin ophthalmic suspension, dosed TID for the treatment of bacterial conjunctivitis. Studies 373 and 433 were superiority designs, and compared besifloxacin to vehicle, and Study 434 was a non-inferiority design that compared besifloxacin to an active comparator (0.5% moxifloxacin ophthalmic solution). The Agency indicated that Study 434 may not have the assay sensitivity necessary to serve as an adequate and well-controlled clinical study. Accordingly, the sponsor presented Studies 373 and 433 (both conducted entirely in the United States) as the two Phase 3 trials required to demonstrate safety and efficacy. See Table 3 for a comparison of Study 373 and 433.

Table 3: Clinical efficacy and safety studies conducted in the US for Besifloxacin

Study	Type	Indication	Dosing Regimen	N per arm	Primary Endpoint
373	Multi-center, randomized, double-masked, parallel clinical trial	Bacterial Conjunctivitis	0.6% Besifloxacin Ophthalmic Suspension or Vehicle TID for five days	Besifloxacin treated: 49 Vehicle treated:49	Clinical resolution at Visit 3 and microbial eradication at Visit 3
433	Multi-center, randomized, double-masked, vehicle-controlled, parallel-group clinical trial	Bacterial Conjunctivitis	0.6% Besifloxacin Ophthalmic Suspension or Vehicle TID for five days	Besifloxacin treated: 473 Vehicle treated: 484	Clinical resolution microbial eradication at Day 5/Visit 2 and Day 8/Visit 3

2.2.2. What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

Study 373: All efficacy analyses were performed on an intent-to-treat (ITT) basis, based on all randomized subjects who received at least one drop of the study medication and

had baseline cultures indicating pathogenic bacteria levels (ranging from 1 to 1000 CFU/mL depending on the species). If subjects were missing Visit 3 (Day 8 +1 day) data, the last available on-treatment clinical and bacteriological data were carried forward. Clinical resolution was defined as the absence of conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection. Microbial eradication was defined as the absence of the baseline pathogens.

Study 433: The primary efficacy endpoints included clinical resolution and microbial eradication at Day 5/Visit 2 and Day 8/Visit 3, recorded as a binary response. Clinical resolution was defined as the absence of both ocular discharge and bulbar conjunctival injection at Visit 2 (Day 5 ± 1 day). Microbial eradication was defined as the absence at Visit 2 (Day 5 ± 1 day) of all accepted ocular bacterial species that were present at or above threshold at baseline. For the ITT study population, these analyses were performed in two manners: (i) assuming missing values and discontinued subjects as failures; and (ii) using only observed data. The imputation of failures was not performed for the analyses of the primary efficacy endpoints on the Per Protocol (PP) study population as missing information was infrequent in the PP study population.

2.2.3. Are the active moieties in the plasma (or other biological fluid) appropriately measured to assess pharmacokinetic parameters and exposure response relationships?

Besifloxacin was the active moiety measured in human plasma and in human tears in clinical trials. In Study 414135, besifloxacin was incubated with hepatocytes from several different species (see Appendix 4.2.1.). Besifloxacin was shown to be metabolically stable, and to not undergo chiral interconversion after incubation with human hepatocytes.

2.2.4. Exposure-response

2.2.4.1. What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

One dosing regimen of besifloxacin was evaluated in the Phase 3 trials: 0.6% TID for five days. The safety and tolerability of the 0.6% dose was assessed in Study C-02-403-001 and Study 424. Study 424 evaluated the concentrations of besifloxacin in tears, and calculated C_{max}/MIC and AUC/MIC values for select target pathogens based on tear concentrations and the MIC_{90} of the target pathogens (see **2.2.4.4.**).

The data from the Phase 3 studies 373 and 433 were pooled by the sponsor to support the efficacy of besifloxacin. A third Phase 3 study, Study 434, was only included in the safety analysis. Table 4 shows the clinical signs at baseline in the combined mITT population for besifloxacin suspension as compared to vehicle.

Table 4: Clinical signs at baseline (Visit 1, Day 1), Studies 373 and 433 mITT 'as randomized' population

		Besifloxacin Suspension (N = 259)	Vehicle (N = 249)
	Subjects with Non-Missing Data	259	249
Ocular Discharge	Distribution of Scores n (%)		
	Absent	0 (0.0%)	0 (0.0%)
	Mild	109 (42.1%)	104 (41.8%)
	Moderate	124 (47.9%)	120 (48.2%)
	Severe	26 (10.0%)	25 (10.0%)
Bulbar Conjunctival Injection	Distribution of Scores n (%)		
	Normal	0 (0.0%)	0 (0.0%)
	Mild	71 (27.4%)	72 (28.9%)
	Moderate	148 (57.1%)	139 (55.8%)
	Severe	40 (15.4%)	38 (15.3%)

NOTE: Percentages are based on the number of subjects presenting non-missing data.

In Study 373, clinical resolution was defined as the absence of conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection. In Study 433, clinical resolution was defined as the absence of conjunctival discharge and bulbar conjunctival injection. For both studies, microbial eradication was defined as the absence of all accepted ocular bacterial species that were present at or above threshold at baseline. The primary efficacy endpoints for Study 373 were clinical resolution and microbial eradication of baseline bacterial infection at Visit 3 (Day 8, +1 day). In Study 433, the primary efficacy endpoints were clinical resolution and microbial eradication of baseline bacterial infection at Visit 2 (during therapy, Day 5, ± 1 day). Tables 5 and 6 show the clinical and microbial success rates, respectively at Visit 3.

Table 5: Clinical resolution at Visit 3 (Study 373 and 433 mITT population)

	Besifloxacin Suspension (N = 259)	Vehicle (N = 249)
Subjects with Non-Missing Data	251	223
Clinical Resolution (Data 'as observed')		
Yes	205 (81.7%)	151 (67.7%)
No	46 (18.3%)	72 (32.2%)
p-Value for Comparison of Treatments[1]	0.0009 / 0.0006	
95% CI for Difference[2]	(6.14%, 21.78%)	
Subjects with Missing Data	8	26
Clinical Resolution (Missing or Discontinued Subjects Imputed as 'no')		
Yes	205 (79.2%)	151 (60.6%)
No	54 (20.8%)	98 (39.4%)
p-Value for Comparison of Treatments [1]	<0.0001 / <0.0001	
95% CI for Difference in Percentages [2]	(10.52%, 26.49%)	

[1] p-Values from CMH test stratified by center / exact Pearson chi-squared test, respectively.
[2] Difference calculated as besifloxacin minus vehicle. Positive values favor besifloxacin.

Table 6: Microbial eradication at Visit 3

	Besifloxacin Suspension (N = 259)	Vehicle (N = 249)
Subjects with Non-Missing Data	249	222
Microbial Eradication (Data 'as observed')		
Yes	229 (92.0%)	172 (77.5%)
No	20 (8.0%)	50 (22.5%)
p-Value for Comparison of Treatments [1]	<0.0001 / <0.0001	
95% CI for Difference [2]	(8.04%, 20.94%)	
Subjects with Missing Data	10	27
Microbial Eradication (Missing or Discontinued Imputed as 'no')		
Yes	229 (88.4%)	172 (69.1%)
No	30 (11.6%)	77 (30.9%)
p-Value for Comparison of Treatments [1]	<0.0001 / <0.0001	
95% CI for Difference [2]	(12.23%, 26.45%)	

[1] p-Values from CMH test stratified by center/ exact Pearson chi-squared test, respectively.
[2] Difference calculated as besifloxacin minus vehicle. Positive values favor besifloxacin.

Besifloxacin ophthalmic suspension 0.6% TID for five days showed superior clinical and microbial success rates as compared to vehicle at both Visit 2 and Visit 3.

2.2.4.2. What are the characteristics of the exposure-response relationships (dose-response, concentration response) for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.

The vast majority of subjects who participated in the clinical trials conducted in support of besifloxacin received the proposed clinical dose of 0.6% besifloxacin. The only study in which another dose was given was the initial Phase 1 dose-finding study C-02-403-001, which featured 12 subjects who received the 0.3% dose, 14 subjects who received the 0.6% dose, and 28 subjects who received the vehicle (see Appendix 4.2.3. for further details). Eight of the 54 total subjects (14.8%) treated in the study reported 12 adverse events (AEs) during the course of the study. Six subjects in the vehicle group reported a total of 10 AEs, and 2 subjects in the 0.6% besifloxacin ophthalmic suspension group each reported a single adverse event. There were no adverse events reported in the 0.3% besifloxacin ophthalmic suspension group. The AE conclusion from this trial is that 0.6% besifloxacin ophthalmic suspension was tolerated as well as vehicle.

A total of 6.5% (154/2387) of subjects in the safety population experienced a total of 216 treatment-emergent, non-ocular AEs during the three Phase 3 studies [(6.3% (75/1192), 7.8% (48/616) and 5.4% (31/579) for subjects in the besifloxacin ophthalmic suspension, vehicle, and moxifloxacin ophthalmic solution treatment groups, respectively]. There was no statistically significant difference between besifloxacin ophthalmic suspension and vehicle treatment groups in the occurrence of treatment-emergent, non-ocular AEs. Headache was the most common non-ocular AE in all treatment groups. In the besifloxacin ophthalmic suspension treatment group, 21/1192 subjects (1.8%) reported

headaches. The rates of headaches in the vehicle and moxifloxacin dosing group were nearly identical.

Treatment-emergent ocular AEs were collected for all treated eyes in the safety population, which included both study and fellow eyes. Table 7 shows the ocular treatment emergent AEs among patients from the Phase 3 trials who received besifloxacin ophthalmic suspension, vehicle, and moxifloxacin.

Table 7: Treatment-emergent, ocular AEs occurring in ≥ 0.5% of eyes in either treatment group, all treated eyes, safety population.

	Besifloxacin Suspension (N^1 = 1810)	Vehicle (N^1 = 961)	Vigamox (N^1 = 855)	p-Value[2]
Total Number of AEs	327	258	153	
Number of Eyes With at Least One AE	249 (13.8%)	190 (19.8%)	120 (14.0%)	<0.0001
EYE DISORDERS	243 (13.4%)	189 (19.7%)	113 (13.2%)	<0.0001
Conjunctivitis	47 (2.6%)	41 (4.3%)	33 (3.9%)	0.0223
Vision blurred	38 (2.1%)	39 (4.1%)	4 (0.5%)	0.0035
Conjunctivitis bacterial	32 (1.8%)	27 (2.8%)	22 (2.6%)	0.0736
Eye irritation	26 (1.4%)	27 (2.8%)	12 (1.4%)	0.0187
Eye pain	28 (1.5%)	17 (1.8%)	9 (1.1%)	0.6396
Eye pruritus	18 (1.0%)	18 (1.9%)	3 (0.4%)	0.0761
Conjunctival haemorrhage	7 (0.4%)	5 (0.5%)	4 (0.5%)	0.7622
Eye discharge	6 (0.3%)	6 (0.6%)	3 (0.4%)	0.3615
Eyelid oedema	6 (0.3%)	4 (0.4%)	5 (0.6%)	0.7457
Conjunctival hyperaemia	10 (0.6%)	3 (0.3%)	0 (0.0%)	0.5612
Punctate keratitis	5 (0.3%)	3 (0.3%)	5 (0.6%)	>0.9999
Ocular hyperaemia	6 (0.3%)	5 (0.5%)	1 (0.1%)	0.5290
Conjunctivitis viral	10 (0.6%)	0 (0.0%)	1 (0.1%)	0.0185
Dry eye	5 (0.3%)	2 (0.2%)	4 (0.5%)	>0.9999
Limbal hyperaemia	4 (0.2%)	3 (0.3%)	4 (0.5%)	0.6994
Lacrimation increased	1 (0.1%)	6 (0.6%)	2 (0.2%)	0.0085

[1] N= all treated eyes for the specified treatment group and includes study and fellow eyes.
[2] p-Value based on Fisher's Exact test, comparing besifloxacin ophthalmic suspension and vehicle.
NOTE: Treatment-emergent refers to: subsequent to the treatment of the study eye. The total number of AEs counts all AEs for eyes. Eyes may have more than 1 AE per body system and preferred term. A subject could be counted twice for a specific AE, if both eyes had the event while being treated. Percentages are based on the number of eyes that received the indicated study treatment.

Treatment emergent ocular AEs were more common than non-ocular AEs. A total of 559 of the 3626 (15.4%) eyes in the safety population experienced at least 1 treatment-emergent ocular AE during the studies [(249 (13.8%), 190 (19.8%) and 120 (14.0%) eyes in the besifloxacin ophthalmic suspension, vehicle, and moxifloxacin treatment groups, respectively]. The most prevalent AEs were consistent with the underlying ocular disease being studied. Five ocular AEs were reported at statistically different rates between the besifloxacin ophthalmic suspension and vehicle treatment groups. Conjunctivitis, blurred vision, eye irritation and increased lacrimation were reported at

lower rates in the eyes treated with besifloxacin ophthalmic suspension than vehicle, but viral conjunctivitis was observed at a statistically significantly higher rate in the besifloxacin ophthalmic suspension patients.

2.2.4.3. Does this drug prolong the QT or QTc interval?

A thorough QT study was not conducted for besifloxacin. However, the potential for besifloxacin to inhibit the hERG channel was examined in stably transfected HEK-293 cells. Besifloxacin at 10^{-5} M (4303 ng/mL) induced 13 ± 1% inhibition of hERG tail current. Since the only concentration of besifloxacin examined failed to produce a greater than 70% inhibition of hERG tail current, no IC_{50} value could be determined. Under the same conditions, the positive control (E-4031 at 100 nM) induced a 90 ± 4% inhibition of hERG tail current (see Appendix 4.2.2.). Besifloxacin at 10^{-5} M represents a concentration more than 10,000 times larger than the highest mean plasma concentration seen in the clinical trials.

2.2.4.4. Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The dosing regimen of 0.6% besifloxacin TID was chosen based on tolerability in the Phase 1 trials. In the first human clinical trial (C-02-403-001), it was shown that a dose of 0.6% besifloxacin QID for 7 days was well tolerated by healthy volunteers. In Study 424 (see Appendix 4.2.4), the values of the PK/PD parameters (AUC/MIC and C_{max}/MIC) shown to be predictive of success with other fluoroquinolone drugs were calculated based on the concentrations in tears following a single dose of 0.6% besifloxacin ophthalmic and the MIC_{90} of two target pathogens (see Table 8).

Table 8: Mean PK and PK/PD Parameters calculated from the full analysis set (FAS)

	N	T_{max} (h)	C_{max} (µg/g)	$AUC_{0->24}$ (h*µg/g)	$T_{1/2}$ (h)	C_{max}/MIC_{90} (*S. aureus*)	C_{max}/MIC_{90} (*H. influenzae*)	$AUC_{0>24}/MIC_{90}$ (*S. aureus*)	$AUC_{0>24}/MIC_{90}$ (*H. influenzae*)
FAS	62	0.17	610	3429	3.34	610	10167	3429	57150

Therapeutic levels of besifloxacin were achieved in tears after a single instillation as indicated based on the besifloxacin tear levels compared to the MIC_{90} of 1 µg/mL for *S. aureus* and 0.06 µg/mL for *H. influenzae*. The resulting C_{max}/MIC and AUC/MIC ratios are in excess of the historical minimum values associated with anti-infective efficacy (e.g. C_{max}/MIC ratio >10 and AUC/MIC ratio > 100-125). Average tear levels were sustained in excess of 46 µg/g through 8 hours, and were measurable in most patients through 24 hours after a single administration.

2.2.5. What are the PK characteristics of the drug and its major metabolite?

Three clinical trials evaluated the pharmacokinetics of besifloxacin in humans. Study C-02-403-001 evaluated the single and multiple dose pharmacokinetics of 0.3% and 0.6% besifloxacin in healthy volunteers. Study 424 evaluated the ocular pharmacokinetics of single and multiple doses of 0.6% besifloxacin in the tears of healthy volunteers. Study 478 evaluated the systemic pharmacokinetics of patients with suspected bacterial conjunctivitis who received single and multiple doses of 0.6% besifloxacin.

2.2.5.1. What are the single dose and multiple dose PK parameters?

Single dose PK

Single dose pharmacokinetics of a topical ocular dose of besifloxacin were evaluated in the plasma of patients with suspected bacterial conjunctivitis and in the tears of healthy volunteers.

Study 478

The mean plasma concentration-time profile of besifloxacin in the plasma of patients with suspected bacterial conjunctivitis is shown in Figure 2. Descriptive statistics for the concentration time profile, and the relevant pharmacokinetic parameters following topical ocular administration of besifloxacin 0.6% are described in Tables 9 and 10, respectively

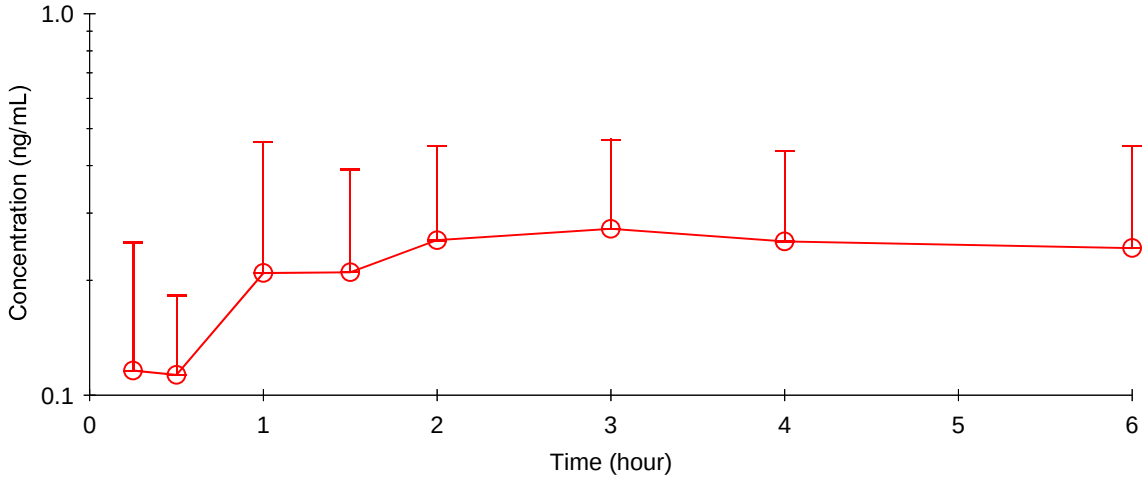


Figure 2: Mean (+SD) plasma concentration versus time profile in patients with suspected bacterial conjunctivitis following a single dose of 0.6% besifloxacin

Table 9: Descriptive statistics of besifloxacin plasma concentrations in patients with suspected bacterial conjunctivitis following a single dose

		Besifloxacin						
	Pre-Dose	.25	.5	1	1.5	2	3	4	6
Total Non-Missing		22	22	22	22	22	22	22	22
Mean (SD)		0.1162 (0.1347)	0.1134 (0.0690)	0.2090 (0.2515)	0.2102 (0.1813)	0.2554 (0.1946)	0.2729 (0.1946)	0.2532 (0.1842)	0.2426 (0.2067)
95% CI		(0.0565, 0.1760)	(0.0828, 0.1440)	(0.0975, 0.3206)	(0.1298, 0.2906)	(0.1691, 0.3417)	(0.1867, 0.3592)	(0.1716, 0.3349)	(0.1509, 0.3342)
Coefficient of Variation		115.9	60.9	120.3	86.3	76.2	71.3	72.7	85.2
Median		0.0978	0.1045	0.1350	0.1585	0.1905	0.2035	0.1780	0.1590
Min, Max		(b) (4)							
Geometric Mean		0.0815	0.0904	0.1311	0.1507	0.1840	0.2057	0.1942	0.1704
95% CI on Geometric Mean		(0.0565, 0.1175)	(0.0648, 0.1260)	(0.0839, 0.2049)	(0.1009, 0.2252)	(0.1226, 0.2762)	(0.1420, 0.2982)	(0.1367, 0.2759)	(0.1137, 0.2553)

Table 10: Besifloxacin single dose pharmacokinetic parameter values in plasma among patients with suspected bacterial conjunctivitis

PK parameter	Mean	SD	CV
AUC_{0-6} (ng·hr/mL)	1.45	0.865	59.8
$AUC_{0-\infty}$ (ng·hr/mL)	3.07	2.60	84.7
T_{max} (hr)	3.17	1.74	54.8
C_{max} (ng/mL)	0.368	0.274	74.6
K_{el} (1/hr)	0.189	0.065	34.2
$t_{1/2}$ (hr)	4.27	2.22	51.9

The pharmacokinetic sampling used to calculate the parameters in Table 10 was only done for six hours. Therefore, the K_{el} may not be accurate; similarly, parameters dependent on K_{el} such as the $AUC_{0-\infty}$ and $t_{1/2}$ may not be accurate.

Study 424

The mean tear concentration-time profiles of besifloxacin is shown in Figure 3. The descriptive statistics for the concentration-time profile, and the relevant pharmacokinetic parameters following a single topical ocular administration of besifloxacin 0.6% are described in Tables 11 and 12, respectively.

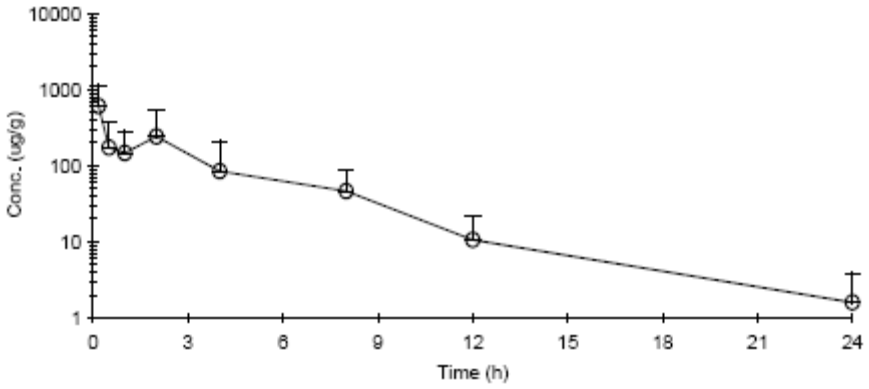


Figure 3: Mean (+SD) tear concentrations versus time profile in the full analysis set (FAS) population (log-linear scale)

Table 11: Descriptive statistics of besifloxacin tear concentrations in the full analysis set population

	Time (h)							
	0.17	0.50	1.00	2.00	4.00	8.00	12.00	24.00
N	8	8	8	8	8	8	8	8
Mean	610	173	147	242	84.7	46.4	10.6	1.60
SD	540	200	135	300	127	39.5	11.0	2.28
Min	(b) (4)							
Median	678	118	106	156	44.0	43.2	9.63	0.361
Max	(b) (4)							
CV%	89	115	92	124	150	85	104	143
95%CI	158; 1062	7; 340	34; 260	-9; 493	-21; 191	13; 79	1; 20	0; 4

Table 12: Besifloxacin pharmacokinetic parameter values in tears using the full analysis set (FAS) and per protocol (PP) population

	N	T_{max} (h)	C_{max} (µg/g)	$AUC_{0\text{-}24h}$ (h*µg/g)	$T_{1/2}$ (h)
FAS	64	0.17	610	1232	3.43
PP	51	0.17	811	1523	3.51

FAS: Subjects who received besifloxacin and from whom sampling data after the first instillation were available
PP: Subjects of the FAS who did not deviate from the protocol in any way likely to seriously affect the primary outcome of the study

The parameters derived from the FAS and PP populations were similar. The C_{max} in both groups occurred at the first time point measured, and the fits of both data showed a $t_{1/2}$ of approximately 3.5 hours. A multiple dosing arm of this study was originally planned, but never conducted, so the results are limited to the single dose PK (see Appendix 4.2.4. for a more detailed study report).

Multiple Dose PK

Multiple dose pharmacokinetics of besifloxacin were evaluated in the plasma of both healthy volunteers and patients.

Study C-02-403-001

Two dosing regimens were evaluated in healthy volunteers in Study C-02-403-001: 0.3% besifloxacin QID for 7 days, and 0.6% besifloxacin QID for 7 days. The mean plasma concentrations at different time points of multiple topical ocular doses of 0.3% or 0.6% besifloxacin are displayed in Figure 4.

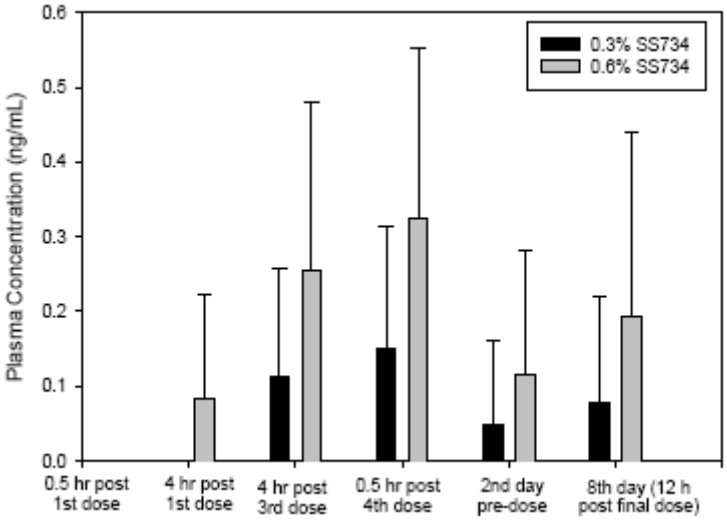
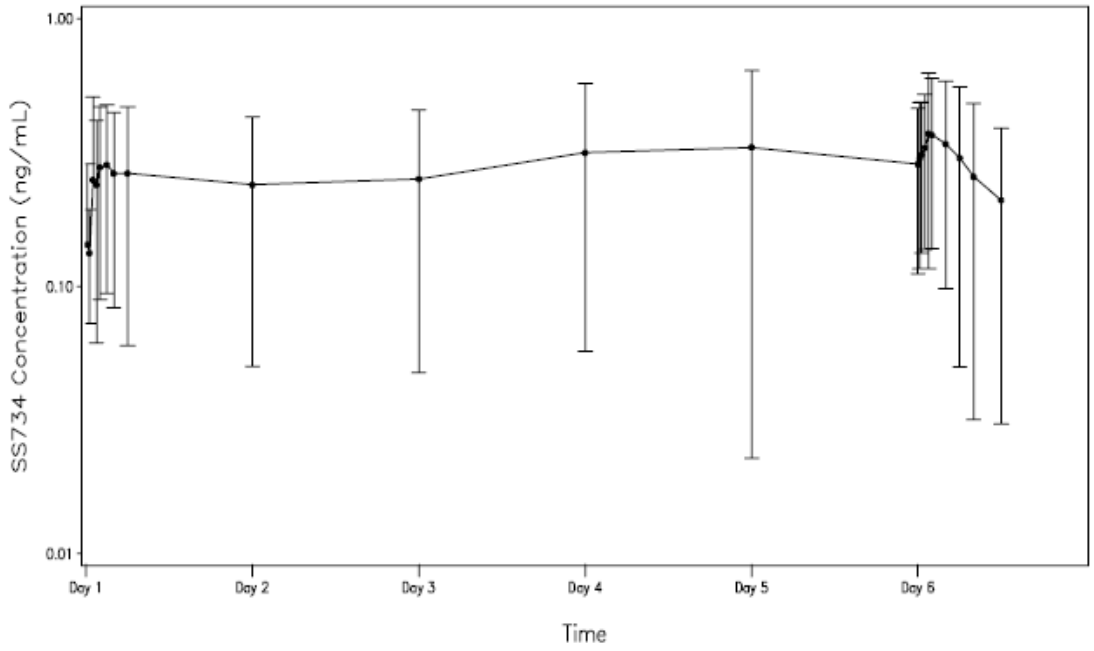


Figure 4: Comparison of plasma concentrations of 0.3% and 0.6% besifloxacin at specified time points

The individual maximum plasma concentrations following topical ocular administration of besifloxacin were all less than 0.4 ng/mL for subjects receiving the 0.3% dosing regimen, and were less than 0.7 ng/mL for subjects receiving the 0.6% besifloxacin dosing regimen. Both dosing strengths were well-tolerated by the healthy volunteers.

Study 478

The mean plasma concentration-time profile of 0.6% besifloxacin TID for five days (with the final dose on the morning of Day 6) to patients with suspected bacterial conjunctivitis is shown in Figure 5. The relevant pharmacokinetic parameters following administration of multiple topical ocular doses of besifloxacin 0.6% is shown in Table 13.



SS734 = besifloxacin

Figure 5: Mean (±SD) plasma concentration-time profile for besifloxacin throughout Study 478 following administration of 0.6% besifloxacin TID for 5 days.

Table 13: Besifloxacin multiple dose pharmacokinetic parameter values in plasma among patients with suspected bacterial conjunctivitis

PK parameter	Mean	SD	CV
AUC_{0-6} (ng·hr/mL)	1.95	1.31	67.2
AUC_{0-12} (ng·hr/mL)	3.21	2.50	77.9
T_{max} (hr)	2.41	2.41	100.4
C_{max} (ng/mL)	0.428	0.299	69.9
K_{el} (1/hr)	0.112	0.032	28.3
$t_{1/2}$ (hr)	6.75	2.14	31.6

The half-life of 6.75 hours following multiple dosing is likely more accurate than the estimated 4.27 hour half-life following single dosing because the pharmacokinetic sampling used to calculate the multiple dose half-life went out to 12 hours as opposed to 6 hours for the single dose half-life estimation. The plasma concentrations that were achieved following multiple administrations of 0.6% besifloxacin were similar between healthy volunteers and patients, which indicates that the disease state of bacterial conjunctivitis does not alter the absorption of besifloxacin into the systemic circulation.

2.2.5.2. How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

The maximum concentrations of besifloxacin that were achieved in the plasma of healthy volunteers (see Figure 5) are similar to the maximum concentrations obtained in the

plasma of patients with suspected bacterial conjunctivitis. This suggests that the disease state does not heavily influence the absorption of drug into the systemic circulation.

2.2.5.3. What are the characteristics of drug absorption?

Repeated topical ocular administration of besifloxacin (0.6% besifloxacin QID for 7 days) resulted in plasma concentrations of besifloxacin less than 0.7 ng/mL.

2.2.5.4. What are the characteristics of drug distribution?

The protein binding of besifloxacin in humans is estimated to be 39-44% in human plasma. Besifloxacin was approximately evenly distributed between plasma and the cellular components of rat and human blood. Maximum besifloxacin concentrations were generally observed between 5 min and 1 hour post dosing in all ocular tissues across species.

2.2.5.5. Does the mass balance study suggest renal or hepatic as the major route of elimination?

A mass balance study was not performed in humans.

2.2.5.6. What are the characteristics of drug metabolism?

Results from *in vitro* and *in vivo* studies suggest that besifloxacin is metabolically stable, with little or no chiral interconversion (see Appendix 4.2.1.). There was no detectable decrease in the concentration of besifloxacin following a two hour incubation with human hepatocytes.

2.2.5.7. What are the characteristics of drug excretion?

The characteristics of drug excretion have not been evaluated in humans. In rats given an oral dose of ^{14}C-besifloxacin, 96% of the radioactive dose was recovered within 120 hours after dosing, with more than 80% of the dose excreted within 24 hours after dosing. About 73% of the dose was recovered in the feces, and 23% of the dose was recovered in the urine.

2.2.5.8. Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Two concentrations of besifloxacin were tested in a single clinical trial. The mean plasma concentrations of 0.3% and 0.6% besifloxacin at the same time points appear to be dose proportional.

2.2.5.9. How do the PK parameters change with time following chronic dosing?

A slight accumulation of besifloxacin occurs with multiple dosing. The mean C_{max} of besifloxacin following single dosing was 0.368 ng/mL whereas the C_{max} on day 6 of TID dosing was 0.428 ng/mL. Additionally, the $t_{1/2}$ is longer following the multiple dosing pharmacokinetics of besifloxacin. However, this likely represents a more accurate estimation of the half-life as the single dose calculation is based on plasma concentrations that were collected out to six hours.

2.2.5.10. What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

The intra-subject variability was not evaluated. The inter-subject variability in the plasma concentrations was considerable (see Tables 9 and 11 for descriptive statistics of besifloxacin concentrations in plasma and tears, respectively) in all of the PK studies conducted. The most likely cause of this variability is that the plasma concentrations of besifloxacin were low and often near the limit of quantification. The C_{max} of besifloxacin in nearly all subjects was below 1 ng/mL, indicating that the systemic absorption of besifloxacin following topical ocular administration was low.

2.3. Intrinsic Factors

2.3.1. What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The impact of intrinsic factors on the pharmacokinetics of besifloxacin was not evaluated.

2.3.2. Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended based upon exposure-response relationships, describe the alternative bases for the recommendation.

2.3.2.1. Elderly

Rates of clinical resolution tended to be higher in the younger age groups (less than 2 years, 2-19 years, 20-59 years) at both Visit 2 and Visit 3 for both besifloxacin ophthalmic suspension and vehicle treatment groups. In general, the treatment differences on clinical resolution rates within each age group were similar to overall, with besifloxacin ophthalmic suspension rates higher than vehicle, with the exception of Visit 2 in the >60 year old age group where clinical resolution rates were low overall. No dosage adjustment is recommended based on age.

2.3.2.2. Pediatric patients

The safety and effectiveness of besifloxacin in infants below 1 year of age has not been established. The efficacy of besifloxacin in treating bacterial conjunctivitis in pediatric patients one year or older has been demonstrated in controlled clinical trials. No overall differences in safety and effectiveness have been observed between pediatric patients one year and older and adult patients. No dosage adjustment is recommended in pediatric patients ≥ 1 year of age.

2.3.2.3. Gender

Clinical resolution rates at Visit 2 tended to be slightly lower for males than females in the besifloxacin ophthalmic suspension treatment group and slightly higher for males than females in the vehicle treatment group. Similar trends were seen in the besifloxacin group, but not the vehicle group, at Visit 3. At Visits 2 and 3, the difference in clinical resolution rates between the besifloxacin ophthalmic suspension and vehicle treatment group was statistically significant for females. For males, the difference in clinical resolution rates between the besifloxacin ophthalmic suspension and vehicle treatment groups was only statistically significant at Visit 3. No dosage adjustment is recommended based on gender.

2.3.2.4. Race

Due to small sample sizes, statistical comparisons within race groups do not have sufficient power to be conclusive. At Visit 2, rates of clinical resolution for "Blacks or African Americans" were consistent with the rates observed for the overall mITT (total mITT: N=255 on besifloxacin, N=235 on vehicle. African American mITT: N=19 on besifloxacin, N=24 on vehicle) as randomized population. At Visit 3, rates of clinical resolution for this subgroup were higher in the vehicle treatment group (78.3% clinical success for African Americans on vehicle at Visit 3 compared to an overall success rate of 67.7% for the vehicle group). The efficacy in the besifloxacin group was also somewhat different (African American mITT Visit 3 besifloxacin clinical success rate: 72.2%; total mITT Visit 3 besifloxacin clinical success rate 81.7%). No dosage adjustment is recommended.

2.3.2.5. Renal impairment

The impact of renal impairment on the pharmacokinetics of besifloxacin was not examined.

2.3.2.6. Hepatic impairment

The impact of hepatic impairment on the pharmacokinetics of besifloxacin was not examined.

2.3.2.7. What pregnancy and lactation information is there in the application?

There are no adequate and well-controlled studies in pregnant or lactating women with besifloxacin.

2.4. Extrinsic Factors

2.4.1. What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or response and what is the impact of any differences in exposure on response?

The impact of extrinsic factors has not been examined. However, given the topical ocular dosing route and the site of action, extrinsic factors are unlikely to play a significant role in impacting exposure-response.

2.4.2. Drug-drug interactions

2.4.2.1. Is there an in vitro basis to suspect in vivo drug-drug interactions?

No. Topical ophthalmic use of besifloxacin is not expected to elicit any potential systemic PK drug interactions. This is based on the local administration of besifloxacin to the eye, which results in a mean C_{max} of ~ 0.4 ng/mL following six days of dosing.

2.4.2.2. Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

Following a two hour incubation with human hepatocytes, no detectable decrease in the besifloxacin concentration was observed. *In vitro* and *in vivo* metabolic data suggest that besifloxacin is metabolically stable, and a majority of the drug is recovered unchanged. Therefore, it does not appear to be a substrate of CYP enzymes.

2.4.2.3. Is the drug an inhibitor and/or inducer of CYP enzymes?

The sponsor did not investigate whether besifloxacin inhibited or induced CYP enzymes. The low systemic concentrations of besifloxacin following multiple dosing of the proposed dosing strength suggest that inhibition or induction of CYP enzymes would be unlikely.

2.4.2.4. Is the drug a substrate and/or inhibitor of P-glycoprotein transport processes?

The sponsor did not investigate whether besifloxacin acted as an inhibitor of P-glycoprotein. Given the ophthalmic route of administration, and the low systemic concentrations achieved following multiple dosing, it is unlikely that inhibition of P-glycoprotein by besifloxacin would be clinically relevant.

2.4.2.5. Are there other metabolic/transporter pathways that may be important?

Besifloxacin is dosed as a topical ophthalmic suspension. Based on the limited absorption and low systemic concentrations, other transporters are unlikely to be important.

2.4.2.6. Does the label specify co-administration of another drug, and if so, has the interaction potential between these drugs been evaluated?

The co-administration of another drug is not specified in the label.

2.4.2.7. What other co-medications are likely to be administered to the target patient population?

Besifloxacin could potentially be administered with other drugs that are used in the treatment of ocular diseases.

2.4.2.8. Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No in vivo drug-drug interactions studies were conducted.

2.4.2.9. Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

There is no known mechanistic basis for pharmacodynamic drug-drug interactions.

2.4.2.10. Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

There are no unresolved questions related to metabolism or protein binding.

2.4.3. What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

There are no unresolved issues related to dose, dosing regimens, or administration.

2.5. General Biopharmaceutics

2.5.8. If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?

The sponsor used *Vigamox* (moxifloxacin 0.5% ophthalmic solution) as the active control in study 434. Thus, no unapproved or unaltered products were used as active controls.

2.6. Analytical section

2.6.1. How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

Besifloxacin in plasma and tears was measured in studies C-02-403-001, 478, and 424 using LC-MS-MS.

2.6.2. Which metabolites have been selected for analysis and why?

Since the *in vitro* metabolism studies demonstrated that besifloxacin is stable in the presence of human hepatocytes, no metabolites were analyzed.

2.6.3. For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?

Total besifloxacin was the only moiety measured. This is appropriate because unbound besifloxacin concentrations are likely to be below the lower limit of quantification in plasma and the protein binding is anticipated to be low in tears.

2.6.4. What bioanalytical methods are used to assess concentrations?

See **2.6.1.**

2.6.4.1. What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

C-02-403-001
The range of the standard curve is from 0.200 – 10.0 ng/mL. The peak area ratios (y) of besifloxacin to the internal standard and the concentrations of the calibration standards (x) were fitted by weighted ($1/x^2$) linear least squares regression analysis to the equation $y = a + bx$, where "a" is the y-intercept and "b" is the slope of the calibration curve. No plasma concentration exceeded 10.0 ng/mL.

Study 478
The range of the standard curve is from 0.05 – 10.0 ng/mL. Linear regression with $1/x^2$ weighting was determined to be the best curve fit for the validation of besifloxacin in human plasma. No plasma concentration exceeded 10.0 ng/mL.

Study 424
The range of the standard curve is from 2.00 ng/mL to 2000 ng/mL. A quadratic regression with $1/\text{concentration}^2$ weighting and quantification by peak area ratio was used. No tear concentration exceeded 2000 ng/mL.

2.6.4.2. What are the lower and upper limits of quantification (LLOQ/ULOQ)?

Table 14 shows the LLOQ and ULOQ for assay used in the quantification of besifloxacin during development.

Table 14: Summary of the validated bioanalytical methods used for the quantitation of besifloxacin in clinical PK studies.

Method Format	Species	Matrix	Concentration Range (LLOQ-ULOQ)	Study Supported
LC/MS/MS	Human	Plasma	0.200 – 10.0 ng/mL	C02-403-001
LC/MS/MS	Human	Plasma	0.0500 – 10.0 ng/mL	Study 478
LC/MS/MS	Human	Tears	2.00 – 2000 ng/mL (ca. 0.200- 200 µg/g)[a]	Study 424

[a]Approximate range based on a tear sample weight of 0.01g.

2.6.4.3. What are the accuracy, precision, and selectivity at these limits?

Table 15 shows the accuracy, precision and selectivity for the assays used in the quantitation of besifloxacin during development.

Table 15: Summary of the accuracy, precision, and selectivity of the different assay methods that were used to measure besifloxacin concentrations.

Study	Matrix	Accuracy	Precision	Selectivity
C02-403-001	Plasma	(b) (4)		Acceptable
Study 478	Plasma	(b) (4)		Acceptable
Study 424	Tears	(b) (4)		Acceptable

2.6.4.4. What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

C02-403-001

Benchtop, freeze/thaw, and extract stability in human plasma were performed. Three replicates from the high (8.00 ng/mL) and low (0.600 ng/mL) QC pools were thawed and placed on the benchtop at room temperature for approximately 24h prior to extraction. A different set of triplicate samples from high and low QC pools was taken through four freeze/thaw cycles and then analyzed. A third set of triplicate samples was extracted and injected; the extracts were then reinjected after approximately 100h at room temperature. All of the stability samples in each pool were within ±15% of their theoretical concentrations.

Study 478
See Table 16

Table 16: Stability conditions tested for the assay used to detect besifloxacin in Study 478.

Stability Condition	Time or Cycles tested
Room Temperature in Plasma	24 hours
Freeze/Thaw (-20 ºC) in Plasma	3 cycles
Freeze/Thaw (-70 ºC) in Plasma	3 cycles
Frozen Storage Stability (-20 ºC)	40 days
Frozen Storage Stability (-70 ºC)	40 days
Whole Blood (Room Temperature)	1 hour
Whole Blood (Ice Bath)	1 hour
Stock Solution (Room Temperature)	18 hours
Stock Solution (4 ºC)	57 days
Working Solution (Room Temperature)	18 hours
Working Solution (4 ºC)	43 days

Study 424
See Table 17

Table 17: Stability conditions tested for the assay used to detect besifloxacin in Study 424.

Stability Condition	Time or Cycles tested
Matrix Stability at Room Temperature	At least 24 hours for buffer and 2 hours for Schirmer strips
Extract Stability at Room Temperature	At least 74 hours in substitute matrix and human tears
Freeze/Thaw Stability	3 cycles in buffer and Schirmer strips at approximately -20 ºC
Long Term Matrix Stability	At least 57 days for buffer and 51 days for Schirmer strips at approximately -20 ºC
Stock Solution Stability at Room Temperature	At least 6 hours for besifloxacin and 2 hours for sparfloxacin
Stock Solution Stability at -20 ºC	At least 14 days for besifloxacin and 25 days for sparfloxacin

2.6.4.5. What is the QC sample plan?

C02-403-001
High, medium, and low QC samples were prepared at 8.00, 4.00, and 0.600 ng/mL. A very high dilution QC pool was prepared at 50.0 ng/mL.

Study 478
Intra- and inter-assay results from QC samples prepared at 0.500, 0.150, 4.00, 8.00, and 50.0 ng/mL for besifloxacin were analyzed in three precision and accuracy runs.

Study 424
At least six replicates of a high, meddle, low, and LLOQ QC samples are included in at least three runs. QC samples were prepared at 2.00, 6.00, 1000, and 1500 ng/mL.

3. Detailed Labeling Recommendations

See Appendix 4.1. Proposed Package Insert

4. Appendices

4.1 Proposed Package Insert

Detailed Labeling Recommendation (1/27/2008) is included with Track Changes

7 pages of draft labeling has been withheld after this page as B4.

4.2 Individual Study Reports

4.2.1 In vitro study of interspecies chiral interconversion and liver metabolism of (+)-SS734 using hepatocytes in suspension

OBJECTIVES:
The aim of this study was to determine the interspecies chiral interconversion and liver metabolism of (+)-SS734 using suspensions of cryopreserved hepatocytes of CD1 mouse, Sprague-Dawley rat, New England White rabbit, beagle dog, and human (from 2 donors).

METHODS:
(+)-SS734 hepatocyte incubations with all species were performed at 2, 30, 60, and 120 minutes in duplicate at a final concentration of 10 μM at 37ºC. Trypan blue dye tests were performed on all incubated samples of the 120 min period in order to assess hepatocyte viability at the end of the incubation. The metabolic viability of the hepatocytes was confirmed by measuring one cytochrome P450-dependent enzymatic activity (phenacetin metabolism) and phase II enzymatic activity (7-hydroxycoumarin metabolism).

A chiral selective LC-PDA method was used to evaluate racemization of (+)-SS734 in the incubation samples. The stability of (+)-SS734 under incubation conditions was determined after the 120 min incubation period to test for chemical degradation.

Each incubation mixture was divided into four vials. At the end of the incubation time period, one vial was cooled down with ice, centrifuged, and analyzed with LC-PDA-MS and chiral LC-PDA methods.

RESULTS:
Results from the 2h hepatocyte incubation indicate that (+)-SS734 is metabolically stable in most species tested. Only incubations with dog hepatocytes resulted in a detectable decrease in (+)-SS734 concentration after two hours (16% decrease). Although some metabolites were observed in other species, they are likely to be very minor since no detectable decrease in the parent compound was observed. Table 1 shows the presence of metabolites in the five species evaluated. The metabolic viability of all hepatocytes was confirmed for each species by measuring one cytochrome P450-dependent enzymatic activity (phenacetin metabolism) and phase II enzymatic activity (7-hydroxycoumarin metabolism).

Table 1: Summary of the (+)-SS734 metabolities detected following a 2 hour incubation with hepatocytes

Metabolite		Presence of metabolites				
m/z value	Approximate retention time (min)	Mouse	Rat	Rabbit	Dog	Human
392	11.6	+/-	+/-	+/-	+	+*
376	11.8	+/-	+*	+*	+	-
410	13.3	-	+*	-	+	-
426	13.4	-	+*	+*	+	+*
376	15.0	-	-	-	+	-
397	24.2	+*	+*	+*	+	+*
359	24.2	+*	+*	+*	+	+*
356	28.2	+*	+*	+*	+	+*

+: present

+*: although clearly detectable the amount of the metabolite formed is relatively low compared to the dog hepatocyte incubations because a decrease in (+)-SS734 concentration was not observed after 2 hours incubation.

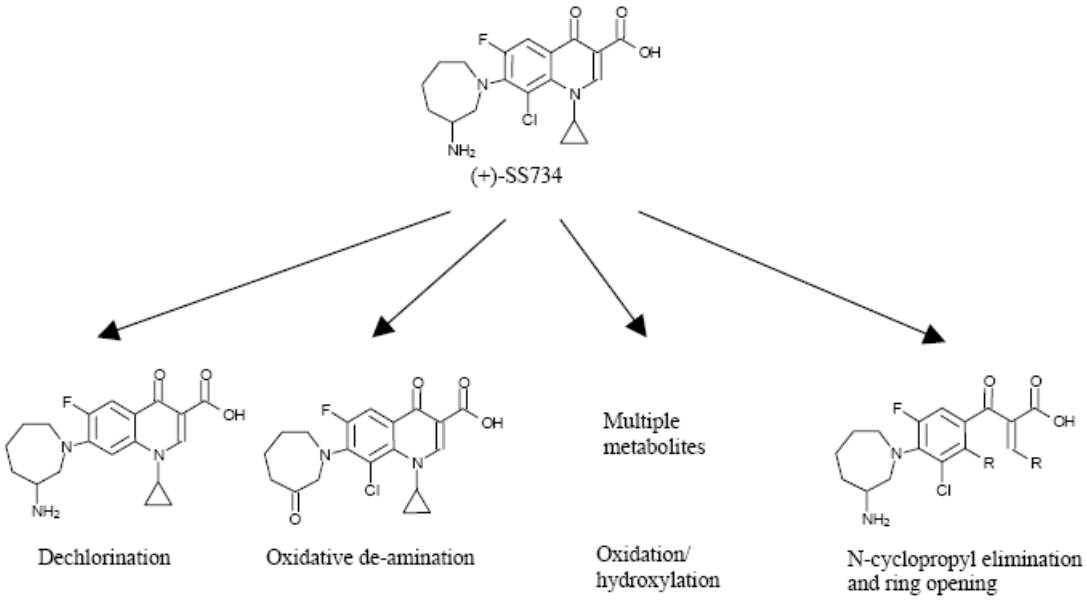
+/-: probably present but in very low amount

-: below detection limit

Source: study report 414135

There were a total of 8 metabolites detected in dog, although there were fewer in other species. Although exact chemical structures could not be determined, the sponsor was able to establish the likely metabolic pathways that would result in the observed mass changes. Figure 1 shows the likely metabolic pathways for (+)-SS734.

Figure 1: Likely metabolic pathways for (+)-SS734



Source: study report 414135

Seven of the 8 metabolites identified were the result of more than 1 metabolic reaction.

The chiral interconversion studies indicated that (+)-SS734 was stable, and was not converted to the (-)-SS734 enantiomer at any of the time periods tested in any of the species. Table 2 is the result of the chiral analysis in the human hepatocytes.

Table 2: Chiral metabolic stability results for (+)-SS734 in incubations with male human hepatocytes

Incubation time (min)	Determination 1			Determination 2		
	Peak area		Percentage	Peak area		Percentage
	(-)-SS734	(+)-SS734	(+)-SS734	(-)-SS734	(+)-SS734	(+)-SS734
1	0	163332	100	0	147567	100
30	0	173586	100	0	149133	100
60	0	169495	100	0	150794	100
120	0	174035	100	0	152840	100

Source: study report 414135

CONCLUSIONS:
(+)-SS734 was determined to be metabolically stable in all species except for dog following 2h incubations with hepatocytes. Therefore, it is unlikely that there will be extensive metabolism of (+)-SS734 in humans. Additionally, the chiral metabolic stability results suggest that (+)-SS734 is not converted to its enantiomer following incubation with hepatocytes. The eight metabolites indentified in dog were characterized with mass spectroscopy, and the likely metabolic pathways that led to the formation of the observed metabolites were identified.

4.2.2 Evaluation of effects on HERG current in stably transfected HEK-293 cells

OBJECTIVES:
To assess the effects of SS734 on HERG tail current in stably transfected HEK-293 cells as compared to the positive control E-4031

METHODS:
(b) (4)

RESULTS:
Prior to the initiation of the experiment, values of the HERG tail current amplitude were determined to be within the range of acceptability in HERG-1 cDNA transfected HEK-293 cells (1478 ± 59 pA, Table1). No significant effects of the vehicle (0.1% sterile water in Tyrode) were observed on HERG tail current. SS734 at 10^{-5}M (4.3 µg/mL) induced 13 ± 1% inhibition of HERG tail current (n=6). An IC_{50} value was not determined since SS734 failed to produce more than 70% inhibition of HERG tail current. Under the same experimental conditions and after a washout period of 5 minutes in Tyrode, the positive control E-4031 at 100 nM induced 90 ± 4% inhibition of HERG tail current.

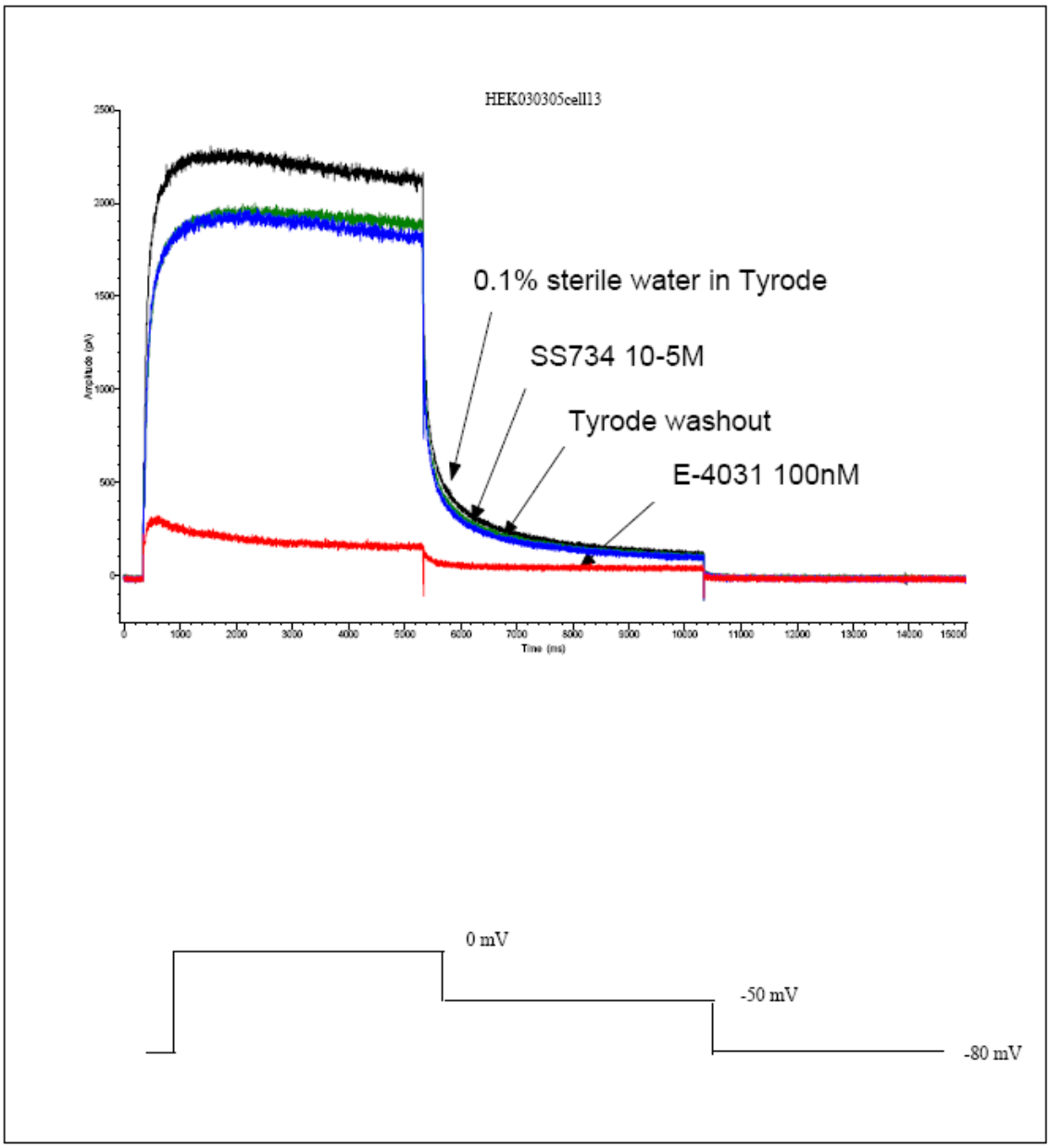
Table 1: Mean results of SS734 on hERG tail current amplitude

Treatment		Amplitude
Predose values (Tyrode)	Mean	1478
	SEM	59
	N	6
Vehicle	Mean	5
	SEM	2
	N	6
SS734 10^{-5} M	Mean	13
	SEM	1
	N	6
	P	⋆
Tyrode washout	Mean	18
	SEM	2
	N	6
	P	⋆⋆
E-4031 100 nM	Mean	90
	SEM	4
	N	6
	P	⋆⋆
	Threshold	7

Predose values are expressed in pA.
Results are expressed in percentage of inhibition of hERG tail current calculated in relation to predose values.
Vehicle: 0.1% sterile water in Tyrode
NS:P>0.05, ⋆:P≤0.05, ⋆⋆:P≤0.01, when compared with vehicle
Analysis of variance with Newman-Keuls test if P ≤0.05
Threshold : smallest difference being statistically significant (P ≤ 0.05) estimated from Newman-Keuls test
Electronic signature: approved by **(b) (6)** on 15-MAR-2005 at 10:39:13.272
Study 20040783PEHP

SS-734 induces a small but significant inhibition of the HERG tail current at a concentration of 10^{-5}M. This inhibition is less than the positive control which was tested at a concentration 100 fold less. Figure 1 shows a graphical representation of a single cell tested under the experimental conditions.

Figure 1: HED030305cell13 hERG tail current



CONCLUSIONS:

SS734 at 10^{-5}M produced a slight but significant inhibition of hERG tail current (13% as compared to the vehicle control) in stably transfected HEK-293 cells. This value did not approach what was seen with the positive control of E-4031 at 100 nM, which produced a 90% inhibition of hERG tail current.

4.2.3 Study C-02-403-001: A Study to Evaluate the Systemic Safety and Ocular Safety/Tolerability of Topical Administration of 0.3% and 0.6% ISV-403 Compared to Vehicle When Dosed QID for 7 Days in Normal Volunteers.

Principal Investigator: Thomas Walters, MD Texan Eye Care, Austin, TX
Dates: 4/12/03-5/24/03

OBJECTIVES:

- To evaluate the systemic safety and ocular safety/tolerability of topical administration of 0.3% and 0.6% ISV-403 compared to vehicle when dosed QID for 7 days in normal volunteers

BACKGROUND:

ISV-403 is the name of the ophthalmic formulation which contains the novel fluoroquinolone antibiotic SS-734 (also known as besifloxacin in later studies), and InSite Vision's patented delivery system DuraSite®, a polycabophil-based preparation known to increase ocular contact time. This formulation is intended to be administered as a topical eye drop for the treatment of bacterial conjunctivitis.

Ocular administration of 0.1% to 0.6% ISV-403 four times daily for 12 days was found to be safe in an animal ocular toxicology study (Study # 5618B). Ophthalmic examination and macroscopic/microscopic ocular findings showed no evidence of ocular toxicity. No systemic toxicities were observed during the 12-day treatment period.

The current study is a Phase 1 trial to assess the safety and tolerability of two dosing regimens of ISV-403 0.3% and 0.6% four times per day for seven days in healthy human subjects.

FORMULATION:

All formulations are ISV-403-P2. These formulations are all topical ophthalmic eye drop solutions.

<u>0.3% ISV-403</u>
Lot Number: B02P
Contains 0.3% SS734, sodium hydroxide, mannitol, poloxamer 407, and DuraSite® (polycarbophil, sodium chloride, EDTA disodium and sterile water for irrigation). The formulation is preserved with benzalkonium chloride 0.01%.
<u>0.6% ISV-403</u>
Lot Number: B03P
Contains: 0.6% SS734, sodium hydroxide, mannitol, poloxamer 407, and DuraSite® (polycarbophil, sodium chloride, EDTA disodium and sterile water for irrigation). The formulation is preserved with benzalkonium chloride 0.01%.
<u>ISV-403 Vehicle</u>
Lot Number: B04P

Contains sodium hydroxide, mannitol, poloxamer 407, and DuraSite® (polycarbophil, sodium chloride, EDTA disodium and sterile water for irrigation). The formulation is preserved with benzalkonium chloride 0.01%.

All test products were manufactured by (b) (4) All investigational products were supplied in 5 mL multi-use containers (bottles) and after shipment to the investigator sites were stored at room temperature.

STUDY DESIGN:
In this single site, randomized, double-masked, parallel study, fifty-four (54) normal volunteers were evaluated in ascending dose groups. The first group of 26 subjects was randomized to receive either 0.3% ISV-403 or vehicle. The second group of 28 subjects was randomized equally to receive either 0.6% ISV-403 or vehicle. One drop of study medication was instilled in both eyes four times a day at approximately 4-hour intervals for one week. The second dosing group was to start only if the first dosing group did not present any significant safety issues. The ISV-403 and Vehicle were administered from identical multi-dose containers.

On the first day of administration, blood samples for pharmacokinetic analysis were taken before, 30 minutes, and 4 hours after the first instillation of the study medications, and again before and 30 minutes after the fourth (final) instillation of the study drug on Day 1. On Day 2, a blood sample was taken prior to the first instillation of the day. On Day 8, the subjects gave one final blood sample for pharmacokinetic analysis 12 hours after the final dose. Ocular comfort and safety were assessed during the scheduled visits throughout the trial (Days 1, 2, and 8 relative to the start of dosing).

ASSAY METHODOLGY:

Criterion	Results	Comments
Linear Range	0.2 – 10.0 ng/mL	Satisfactory
LLOQ	0.2 ng/mL	Satisfactory
Accuracy	(b) (4)	Satisfactory
Precision	(b) (4)	Satisfactory

RESULTS:
Subject Disposition
Fifty-four of the 73 subjects screened were randomly assigned to one of the three treatment groups. Sixteen of the 73 screened subjects failed to meet the eligibility criteria and three subjects withdrew consent. Table 1 shows the subject disposition

Table 1: Disposition of all randomized subjects.

	Treatment									
	Dose Group 1				Dose Group 2					
	Vehicle		0.3% ISV-403		Vehicle		0.6% ISV-403		All	
	N	%	N	%	N	%	N	%	N	%
Completed	14	100	12	100	14	100	14	100	54	100
Total Randomized	14	100	12	100	14	100	14	100	54	100

Source: Study Report C-02-403-001, Table 2

Plasma Levels

In the Sponsor's analysis, samples below the limit of quantitation (BLQ, 0.2 ng/mL) were given a value of zero prior to computing the summary statistics. Table 2 shows the Sponsor calculations treating all BLQ samples as 0, and Figure 1 shows the same data in graphical format. The reviewer's analysis is also provided in Table 3. In the analysis of the reviewer, all BLQ samples are removed from the calculations, and are not assigned a value. The calculations of the reviewer represent means that will be higher than the true means, as all BLQ values were removed from the analysis. Still, even in this worst case scenario, the highest mean plasma level achieved was less than 0.5 ng/mL.

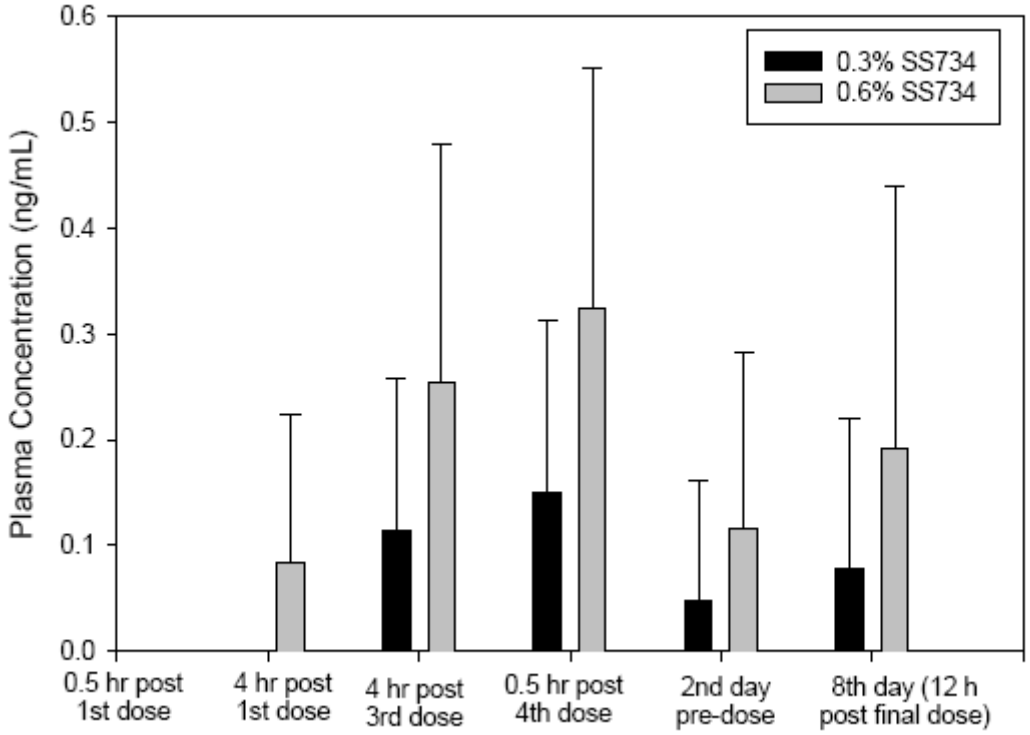
Table 2: Plasma concentrations (ng/mL)- Besifloxacin. Mean (SD), minimum and maximum concentrations by visit and hour. Samples below the detection limit (0.2 ng/mL) assigned a value of zero.

Visit	Dose	Time of Last Dose	Hour		0.3% ISV-403 (N= 12)	0.6% ISV-403 (N= 14)
Visit 2 - Day 1	1	30 minutes	1.0	Mean(SD)	0.000 (0.000)	0.000 (0.000)
				Min - Max	0.000 to 0.000	0.000 to 0.000
				Samples Quantifiable	0	0
		4 Hours	4.5	Mean(SD)	0.000 (0.000)	0.083 (0.140)
				Min - Max	0.000 to 0.000	(b) (4)
				Samples Quantifiable	0	4
	3	4 Hours	12.5	Mean(SD)	0.114 (0.143)	0.255 (0.225)
				Min - Max	(b) (4)	
				Samples Quantifiable	5	9
	4	30 minutes	13.0	Mean(SD)	0.149 (0.164)	0.325 (0.227)
				Min - Max	(b) (4)	
				Samples Quantifiable	6	11
Visit 3 - Day 2	4	Previous day	0.0	Mean(SD)	0.048 (0.114)	0.116 (0.166)
				Min - Max	(b) (4)	
				Samples Quantifiable	2	5
Visit 4 - Day 8	QID 7 days	Previous day	0.0	Mean(SD)	0.078 (0.142)	0.192 (0.247)
				Min - Max	(b) (4)	
				Samples Quantifiable	3	6

Table 3: Reviewer's calculation of besifloxacin plasma concentrations (ng/mL). All BLQ values were excluded from the analysis.

Visit	Dose	Time of Last Dose	Hour		0.3% ISV-403 (N=12)	0.6% ISV-403 (N=14)
Visit 2-Day 1	1	30 min	1.0	Mean	BLQ	BLQ
				Min-Max	N/A	N/A
				# Quantifiable	0	0
		4 hours	4.5	Mean	BLQ	0.289
				Min-Max	N/A	(b) (4)
				# Quantifiable	0	4
	3	4 hours	12.5	Mean	0.273	0.397
				Min-Max	(b) (4)	
				# Quantifiable	5	9
	4	30 min	13.0	Mean	0.297	0.414
				Min-Max	(b) (4)	
				# Quantifiable	6	11
Visit 3 –Day2	4	Previous Day	0.0	Mean	0.291	0.324
				Min-Max	(b) (4)	
				# Quantifiable	2	5
Visit 4 –Day8	QID 7 days	Previous Day	0.0	Mean	0.313	0.444
				Min-Max	(b) (4)	
				# Quantifiable	3	6

Figure 1: Mean plasma concentrations (ng/mL) of SS734



Adverse events

All 54 subjects used the study drugs for 7 days as planned in the protocol. A total of 8 subjects reported 12 adverse experiences. No serious adverse experiences were reported. Six subjects in the Vehicle groups had a total of 10 adverse experiences, and two subjects in the 0.6% ISV-403 group each had a single adverse experience. There were no adverse experiences reported in subjects in the 0.3% ISV-403 group. The majority of adverse experiences occurred during the first two days of study drug use. The investigator indicated that all of the adverse experiences were not related or unlikely related to the study medication. Two subjects in the Vehicle group had superficial punctuate keratitis that did not resolve during the study. The investigator believed that the punctuate keratitis was caused by the many eye exams performed in the study and the use of Fluress (fluorescein sodium 0.25%, benoxinate 0.4%) with the eye exams.

APPLICANT'S CONCLUSIONS:

ISV-403 in concentrations of 0.3% and 0.6% given four times a day for one week is as well tolerated as its vehicle and is as safe as the vehicle with respect to adverse experiences, and effects on visual acuities, intraocular pressures, and biomicroscopic and ophthalmoscopic examination findings, vital signs, blood pressure, heart rate, EKG analyses, blood chemistry, hematology and urinalysis. The findings from this study in normal subjects support the clinical evaluation of ISV-403 up to 0.6%.

REVIEWER'S ASSESSMENT:
The safety, tolerability, and pharmacokinetics of besifloxacin were assessed in this Phase 1 trial. No serious adverse events occurred in the study, and no subjects withdrew from the trial due to an adverse event. The adverse events that did occur were minor in severity, and were comparable between the vehicle and treatment groups. The Sponsor made an attempt to assess the pharmacokinetics of besifloxacin with an assay which had a lower limit of quantitation of 0.2 ng/mL. Most of the concentrations, particularly in the 0.3% ISV-403 group, were BLQ. However, concentrations in the 0.6% group were detectable at some time points, suggesting that the assay is sensitive enough to detect plasma concentrations of besifloxacin. The results of this trial indicate that 0.3% and 0.6% ISV-403 are safe and well-tolerated in human volunteers.

4.2.4. Study 424: Ocular Pharmacokinetics of 0.6% ISV-403 Eye Drops After a Single Instillation and During Repeated Instillations for 5 Days in Healthy Volunteers

Principal Investigator: Pr. Claude Dubray, Centre de Pharmacologie Clinique Clermont Ferrand Cedex, France
Dates: 4/3/06-4/26/06

OBJECTIVES:

- To assess the ocular pharmacokinetics of SS-734 (active ingredient, besifloxacin) after single instillation of 0.6% ISV-403 (name of formulation) eye drops in both eyes
- To establish that the calculated PK/PD parameters AUC/MIC and C_{max}/MIC are in excess of the minimum values associated with anti-infective efficacy

BACKGROUND:
ISV-403 is the ophthalmic formulation which contains the novel fluoroquinolone antibiotic SS-734 (also known as besifloxacin in later studies), and InSite Vision's patented delivery system DuraSite®, a polycabophil-based preparation known to increase ocular contact time. This formulation is intended to be administered as a topical eye drop for the treatment of bacterial conjunctivitis.

A previous Phase 1 study (C-02-403-001) evaluated the systemic safety and ocular safety/tolerability of 0.3% and 0.6% ISV-403 compared to vehicle when dosed QID for seven days. Adverse events resulting from this study were infrequent, and the study conclusion was that it was safe to proceed with the further clinical development of doses of ISV-403 up to 0.6%.

The purpose of this study is to evaluate the ocular pharmacokinetics of SS-734 following a single administration of 0.6% ISV-403 to a population of healthy volunteers. A secondary endpoint is to ensure that the calculated PK/PD parameters that have previously been shown to be relevant for fluoroquinolone efficacy have been exceeded for some of the common pathogens which cause bacterial conjunctivitis.

FORMULATION:
ISV-403 (contains 0.6% of SS-734). Batch number J04Q
Expiry or Retest date: 9/15/06

STUDY DESIGN:
In this single-center, open, prospective study, one 37 μL drop of 0.6% ISV-403 was administered with a micropipette (to ensure consistent volume) by study personnel in each eye of 64 healthy male and female participants. There were eight time points evaluated: 0.17h, 0.5h, 1h, 2h, 4h, 8h, 12h, and 24h. Each time period contained eight subjects. The volunteers were assigned to a time point based on their enrollment number (e.g. for the 0.17h time period, subjects 1, 9, 17, 25, 33, 41, 49, and 57 were used). For the purposes of PK sampling, all samples were collected from the right eye of the

subjects using a Schirmer tear test strip. The tear concentrations were later determined, and the following pharmacokinetic/pharmacodynamic parameters were calculated: C_{max}, T_{max}, $AUC_{0\rightarrow24}$, $t_{1/2}$, C_{max}/MIC_{90}, $AUC_{0\rightarrow24}/MIC_{90}$. There was no rationale provided as to why only one tear concentration was obtained per subject. There was no placebo group tested, and no other formulations of ISV-403 were examined. Concentrations that were below the limit of quantification (2ng/mL) were included in the calculation as one half of the limit of quantification. Parameters were calculated from both the per protocol set (PP) and the full analysis set (FAS). The PP set was a subset of the FAS set which excluded likely outliers in the calculation of mean concentrations at given time points. Likely outliers were defined as a point that was either 10x higher or lower than the mean of all concentrations in the group.

ASSAY METHODOLGY:
Concentrations of SS-734 in tears were determined using LC/MS/MS

SS-734:

Criterion	Tears	Comments
Concentration Range	2 to 2000 ng/mL	Satisfactory
LLOQ	2ng/mL	Satisfactory
Linearity	$R^2 \geq 0.998$	Satisfactory
Accuracy	(b) (4)	Satisfactory
Precision	(b) (4)	Satisfactory

RESULTS:
Study Population and Disposition
A total of 91 subjects were screened, and 64 healthy subjects (8 per time point) were enrolled. All 64 subjects completed the study. There were two protocol deviations; Subject 110's tear sample at 30 min was taken 2 min after the appointed time, and one subject (118) who continued on a previously used medication that was not specified as being allowed under the protocol for treatment of oesophagal reflux. These protocol deviations were deemed to be minor, and are not believed to have had any impact on the study results.

Demographics
A summary of demographic and baseline characteristics for the study population is presented in Table 1.

Table 1. Demographics and Baseline Characteristics – Study 424

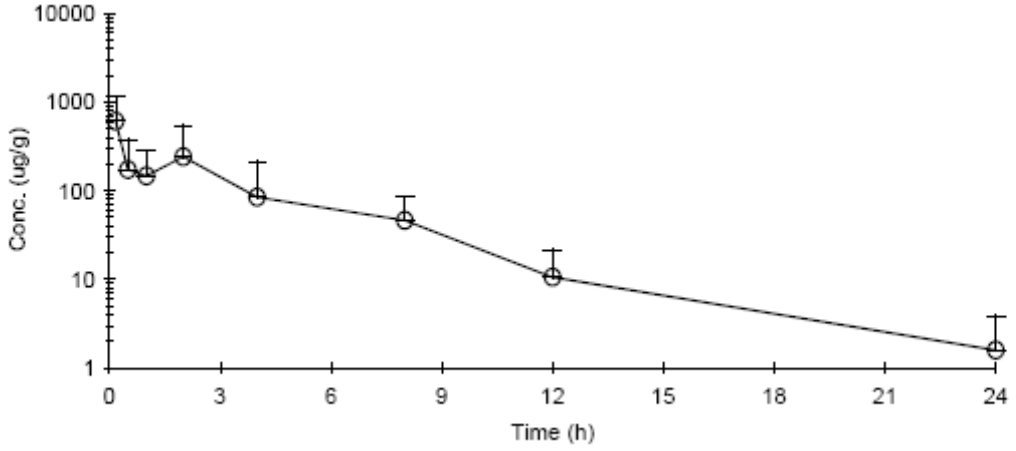
Demographic Characteristic	
Age (yr) ± SD	23.7 ± 4.2
Gender (% male)	50.0%
Blue/Green/Gray Eye Color	57.8%
Hazel Eye Color	6.3%
Brown Eye Color	35.9%
Addiction to Smoking (% of patients who smoked, but <10 Cigarettes per Day)	32.8%

Source: (b) (4) study report 424, Table 14.1.1-1

Pharmacokinetic Results

PK parameters were calculated according to standard methods, using WinNonLin version 5.01. For mean concentration calculations (plotted in Figure 1.1-1, and listed in Table 4.1.1-3), concentrations below the limit of quantification were replaced by a value of one-half of the lower limit of quantification (corrected for the weight of the individual tear sample). This included data from two subjects, 123 and 124. The pharmacokinetic parameters listed in the table 4.1.1-3 were estimated. The $AUC_{0\rightarrow 24h}$ was calculated using the linear trapezoidal rule with linear interpolation. The $T_{1/2}$ was estimated from the terminal rate constant k_e, which was estimated by log-linear regression analysis on data points visually assessed to be on the terminal log-linear phase. For the slope of the terminal elimination phase to be accepted as reliable, the terminal data points were to be distributed about a single straight line on visual inspection, and a minimum of three data points were to be used, including the last measured point, and excluding C_{max}.

Figure 1: Mean tear concentration-time profile of SS-734 in the FAS population



Source: (b) (4) study report 424, Figure 11.4.1

Table 2: Descriptive statistics of SS-734 tear concentrations in the FAS population

	Time (h)							
	0.17	0.50	1.00	2.00	4.00	8.00	12.00	24.00
N	8	8	8	8	8	8	8	8
Mean	610	173	147	242	84.7	46.4	10.6	1.60
SD	540	200	135	300	127	39.5	11.0	2.28
Min	(b) (4)							
Median	678	118	106	156	44.0	43.2	9.63	0.361
Max	(b) (4)							
CV%	89	115	92	124	150	85	104	143
95%CI	158; 1062	7; 340	34; 260	-9; 493	-21; 191	13; 79	1; 20	0; 4

Source: (b) (4) study report 424, Table 11.4-1

The measured concentrations showed a great deal of variability (%CV ranged from 85-150, Table 4.1.1-2). However, tear levels were maintained in excess of 46 µg/g through 8 hours, and only two subjects had tear levels that were below the limit of quantification of the LC/MS/MS assay (one at 12h and one at 24h).

Table 3: Mean PK and PK/PD Parameters calculated from the FAS and PP sets.

	N	T_{max} (h)	C_{max} (µg/g)	AUC_{0-24h} (h*µg/g)	$T_{1/2}$ (h)	C_{max}/MIC_{90}a	C_{max}/MIC_{90}b	AUC_{0-24h}/MIC_{90}a	AUC_{0-24h}/MIC_{90}b
FAS	64	0.17	610	1232	3.43	610	≥10167	1232	≥20533
PP	51	0.17	811	1523	3.51	811	≥13517	1523	≥25383

FAS: Full Analysis Set
PP: Per Protocol
MIC_{90}a *staphylococcus aureus* = 1 µg/mL
MIC_{90}b *haemophilus influenzae* ≤ 0.06 µg/mL

Source: Legacy study report 424 Table 11.4-2

The sponsor calculated the pharmacokinetic parameters for two different sets: FAS and PP. FAS, or full analysis set, contains the PK values of every patient, whereas the PP, or per protocol set excludes values that were more than 10x higher or lower than the mean concentration at any time point. The calculated parameters do not vary much between the FAS set or the PP set (see Table 3).

Table 4: Recalculated parameters for the FAS set

	N	T_{max} (h)	C_{max} (µg/g)	$AUC_{0->24}$ (h*µg/g)	$T_{1/2}$ (h)	C_{max}/MIC_{90} (*S. aureus*)	C_{max}/MIC_{90} (*H. influenzae*)	$AUC_{0->24}/MIC_{90}$ (*S. aureus*)	$AUC_{0->24}/MIC_{90}$ (*H. influenzae*)
FAS	62	0.17	610	3429	3.34	610	10167	3429	57150

MIC_{90} for *S. aureus* = 1 µg/mL
MIC_{90} for *H. influenzae* ≤ 0.06 µg/mL

The reviewer recalculated the parameters for the FAS set excluding the two subjects who were below the limit of quantification. The reviewer used a noncompartmental model with IV-bolus dosing as the model. The AUC_{0-24} was calculated by multiplying the AUC_{0-8} (1143 h*µg/g) by three to reflect the proposed TID dosing, whereas the Sponsor

calculated the AUC_{0-24} based on a single dose (1331 h*µg/g was the reviewer's corresponding value for single dose AUC_{0-24}). The reviewer's recalculated parameters (Table 4) are quite close to the sponsor's FAS calculations (Table 3). The reviewer's calculations show a slightly shorter half-life, and a slightly larger AUC, indicating that excluding the two subjects that could not accurately be assigned a variable does not dramatically affect the calculations.

The calculated C_{max}/MIC_{90} and the $AUC_{0\rightarrow24h}/MIC_{90}$ values for both *S. aureus* and *H. influenzae* are both well in excess of what has previously been shown to be predictive of fluoroquinolone antibacterial efficacy in plasma (C_{max}/MIC ratio >10 and AUC/MIC ratio 100-125).

Safety Results

The purpose of study 424 was to assess the pharmacokinetics of ISV-403 in tears of healthy volunteers. There were so serious AEs, and a total of three AEs (see Table 5).

Table 5: Adverse events that occurred during Study 424

Subject No.	AE No.	- AE System Organ Class - AE Preferred Term - AE Verbatim	- Duration [1] - Day of onset	- Intensity - Relationship	Action taken [2]
102	1	- General Disorders and Administration Site Conditions - Instillation Site Irritation - Burning appeared 5 min after Instillation (During 30 min)	- 30 min - Day 0	- Mild - Probably	No Action
134	1	- Eye Disorders - Vision Blurred - Blurred Vision	- 2 min 30 - Day 0	- Severe - Probably	No Action
150	1	- Eye Disorders - Vision Blurred - Blurred Vision	- 6 min 04 - Day 0	- Severe - Probably	No Action

[1] Expressed as number of days or Day(s)/hh:mm - [2] Include action on investigational drug (reduced or stopped), hospitalisation, or corrective treatment

Source: (b) (4) study report 424 Table 12.2-1

In addition, the sponsor also included a table (see Table 6) about the local tolerance upon instillation of the dose. Symptoms lasted between 2 seconds and 30 min except for one case of dry eye that was reported to last for 4 hours.

Ta**ble 6: Severity of initial ocular reactions to administration of ISV-403**

			N	%
Type of sensation and intensity	Blurred Vision	None	18	28.1
		Light	34	53.1
		Moderate	10	15.6
		Severe	2	3.1
	Stinging	None	45	70.3
		Light	17	26.6
		Moderate	2	3.1
	Foreign Body Sensation	None	46	71.9
		Light	16	25.0
		Moderate	2	3.1
	Other	None	60	93.8
		Light	4	6.3

Source: (b) (4) study report 424 Table 12.4-1

APPLICANT'S CONCLUSIONS:
Following single instillation of 0.6% ISV-403, maximal tear concentrations (610 ± 540 µg/g) were observed following the first time point examined (10 min). Inter-subject variability was relatively large. Average tear levels were sustained in excess of 46 µg/g through 8h, and were measurable in most patients through 24h after a single administration. Therapeutic levels of SS-734 (Besifloxacin) were achieved in tears after a single instillation as indicated by comparing the SS-734 tear levels to the MIC_{90} values of 1 µg/mL for *S. aureus* and 0.06 µg/mL for *H. influenzae*. The resulting C_{max}/MIC ratios and the AUC/MIC ratios are in excess of the minimum values associated with anti-infective efficacy (e.g. C_{max}/MIC ratio > 10 and AUC/MIC ratio > 100-125). Only three adverse events occurred in the study, and all were resolved within 30 minutes, which indicates the overall safety of SS-734.

REVIEWER'S ASSESSMENT:
Study 424 demonstrated the safety and tolerability of a single dose of 0.06% ISV-403, and provided pharmacokinetic data in tears. Although the PK data was variable, the concentrations achieved were well above the target PK/PD parameters likely required for efficacy. The low rate and minor nature of adverse events suggests that the chosen dose is safe. The Sponsor's conclusions regarding the PK and safety data following a single instillation of ISV-403 are acceptable from a Clinical Pharmacology perspective.

4.2.5. Systemic Pharmacokinetics of SS-734 after Single and Multiple TID Instillations of 0.6% ISV-403 Ophthalmic Suspension in Subjects with Suspected Bacterial Conjunctivitis (Study 478)

TITLE:
Systemic Pharmacokinetics of SS-734 after Single and Multiple TID Instillations of 0.6% ISV-403 Ophthalmic Suspension in Subjects with Suspected Bacterial Conjunctivitis

Principal Investigators: Andrew Cottingham, MD San Antonio, TX
Bruce Kanengiser, MD Piscataway, NJ
Michael Tepedino, MD High Point, NC
Analytical site: (b) (4)
Study Dates: October 2006 – October 2007

OBJECTIVES:
- The primary objective is to assess the extent of systemic exposure to besifloxacin following single and multiple 3 times daily topical administration of besifloxacin hydrochloride ophthalmic suspension, 0.6% as base in subjects with suspected bacterial conjunctivitis
- The secondary objective is to measure the patients' visual acuity using the Snellen VA method, and to evaluate the effectiveness of the therapy in resolving the bacterial conjunctivitis.

BACKGROUND:
ISV-403 the ophthalmic formulation which contains the novel fluoroquinolone antibiotic SS-734 (also known as besifloxacin in later studies), and InSite Vision's patented delivery system DuraSite®, a polycabophil-based preparation known to increase ocular contact time. This formulation is intended to be administered as a topical eye drop for the treatment of bacterial conjunctivitis.

A previous Phase 1 study (C-02-403-001) in 54 healthy human volunteers examined the plasma concentration of besifloxacin in subjects who were dosed QID for 7 days with the ISV-403. Plasma C_{max} values obtained during this study on days 1, 2, and 8 did not exceed 1 ng/mL, indicating low systemic exposure. The current study is designed to evaluate the systemic pharmacokinetics of SS-734 in patients with suspected bacterial conjunctivitis in order to investigate whether the pathophysiological changes encountered in the target disease state could alter anterior ocular tissues to result in increased besifloxacin absorption.

FORMULATION:
ISV-403 (contains 0.6% of SS-734). Batch number 037931
Manufactured by Bausch & Lomb (Tampa, FL)

STUDY DESIGN:
This was a multi-center, open-label, single and multiple dose pharmacokinetic study which was conducted at 3 sites in the United States. Eligible subjects had a clinical

diagnosis of bilateral bacterial conjunctivitis. Each subject received 1 drop in each eye, 3 times daily of ISV-403 for 5 days, with a final single dose on the morning of Day 6. Pre- and post-dose blood samples were collected to assess single-dose PK and steady-state PK after multiple TID dosing.

In order to assess single dose PK, 5-10 mL blood samples were drawn from subjects on Day 1 at the following time points: predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6h post dose. Samples to determine steady-state PK were taken on Day 6 at predose, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 12h post dose. Additionally, samples were collected before the first dose of the day on Days 2-5 in order to determine C_{min}. Other pharmacokinetic parameters were calculated from the concentration-time data including AUCs at different time intervals, T_{max}, K_{el}, and $t_{1/2}$. Concentrations were determined by a validated LC-MS/MS method with a LLOQ of 0.05 ng/mL.

In addition to the PK analysis, subjects were given a biomicroscopy examination at the beginning and end of the study to rate the ocular discharge and bulbar conjunctival injection. In order to be included in the study, subject eyes were required to have ocular discharge at the beginning of the study. The visual acuity of each subject was measured at every day during the study using the Pin-holed Snellen visual acuity test.

ASSAY METHODOLOGY:
Concentrations of SS-734 in plasma were determined using a validated LC/MS/MS method.

Table 1: SS-734 analytical method parameters

Criterion	Results	Comments
Concentration Range	0.05-10 ng/mL	Satisfactory
LLOQ	0.05 ng/mL	Satisfactory
Linearity	$R^2 \geq 0.995$	Satisfactory
Accuracy	(b) (4)	Satisfactory
Precision	(b) (4)	Satisfactory

RESULTS:
Study Population and Disposition
A total of 24 subjects were enrolled in this study. All subjects were considered to be part of the safety population, but only 22 subjects were considered part of the pharmacokinetic population. Subject #005 withdrew consent on day 2, and the PK samples from subject #004 were never received by the bioanalytical lab. There were a total of 28 protocol deviations, 19 of which were for samples taken outside of the specified time interval, and all of which were considered minor. None of the protocol deviations are believed to have impacted the study results.

Demographics
A summary of the demographic characteristics of the study population is shown in Table 2.

Table 2: Subject Demographics of the population for study 478

	(N = 24)
Age (years)	
Mean (SD)	38.9 (14.5)
Minimum, Maximum	19, 70
Gender	
Male	9 (37.5%)
Female	15 (62.5%)
Ethnicity	
Not Hispanic and Not Latino	22 (91.7%)
Hispanic or Latino	2 (8.3%)
Race	
White	17 (70.8%)
Black/African American	6 (25.0%)
American Indian/Alaskan Native	0 (0.0%)
Asian	1 (4.2%)
Native Hawaiian/Pacific Islander	0 (0.0%)
Other	0 (0.0%)
Distribution of Age Categories	
18-29	6 (25.0%)
30-39	8 (33.3%)
40-49	4 (16.7%)
50-59	3 (12.5%)
60-69	2 (8.3%)
70-79	1 (4.2%)
80 or older	0 (0.0%)

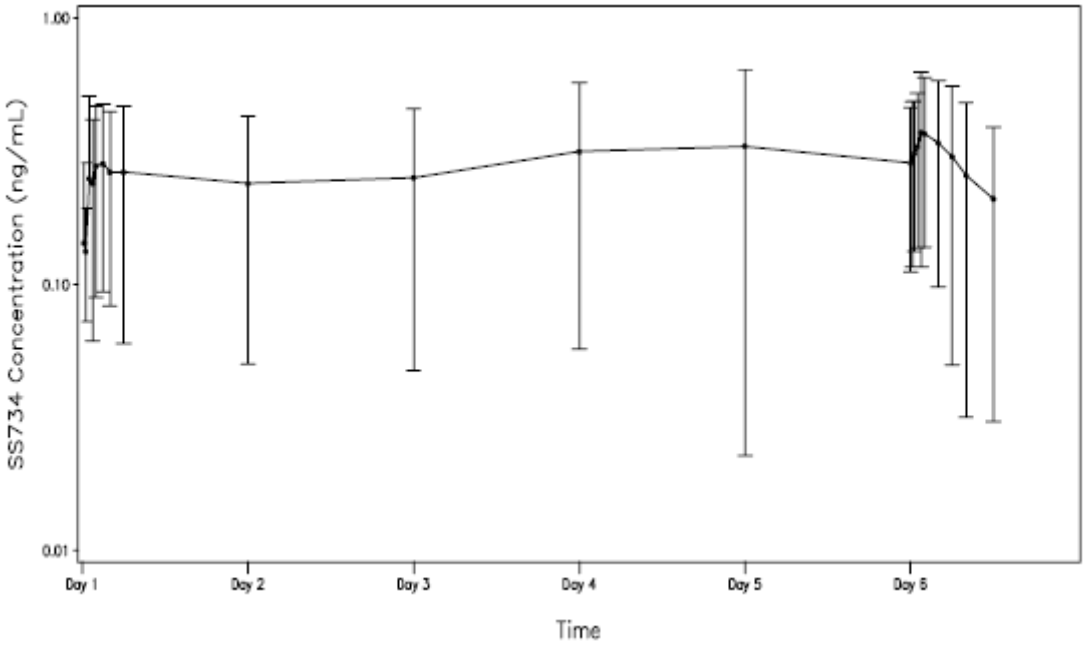
Source: Study 478 study report, Table 6

Pharmacokinetic Results

Pharmacokinetic analysis was performed using model-independent methods with either Microsoft Excel or Pharsight WinNonlin Version 4.1. C_{min}, C_{max}, and T_{max} were taken

directly from the individual plasma data. The elimination rate constant was estimated from the slope of the semilogarithmic plot of the apparently linear terminal phase of the plasma concentration-time curve calculated by linear regression. The elimination half-life was calculated based on the elimination rate constant. The AUC was calculated by using the linear trapezoidal method with the extrapolation of areas to infinity done by adding the value of C_{last}/K_{el} to the calculated AUC_{0-t}. Concentrations that were below the limit of quantification were treated as 0.5 * the LLOQ or 0.025 ng/mL for the purposes of PK calculations. Exceptions to this rule are predose levels, which are given a value of 0, and samples collected after the time of the last measurable concentration, which were excluded from the PK analysis. Two patients had predose plasma concentrations on Day 1 that were measurable; these were set to zero prior to analysis. The reviewer also analyzed the plasma concentration data excluding all BLQ values, but did not get appreciably different results for the PK parameters from what was calculated by the Sponsor. Figure 1 shows the mean concentration-time profile compiled for the entire study, and the calculated PK parameters are shown for Day 1 (single dose, up to six hours) in Table 3 and for Day 6 (multiple dose) in Table 4.

Figure 1 Mean plasma concentration-time profile of besifloxacin (±SD)



Source: Study 478 study report, Figure 1

Table 3: Mean PK parameters following a single dose of ISV-403

PK parameter	Evaluable subjects	Mean ±SD	CV (%)
AUC_{0-6} (ng*h/mL)	20	1.45 ± 0.865	59.8
T_{max} (h)	22	3.17 ± 2.60	54.8
C_{max} (ng/mL)	22	0.368 ± 0.274	74.6
K_{el} (1/h)	8	0.189 ± 0.065	34.2
$t_{1/2}$ (h)	8	4.27 ± 2.22	51.9

Source: Adapted from Table 8 in the study report for Study 478

Table 4: Mean PK parameters following multiple doses of ISV-403 on Day 6.

PK parameter	Evaluable subjects	Mean ±SD	CV (%)
AUC_{0-6} (ng*h/mL)	22	1.95 ± 1.31	67.2
AUC_{0-12} (ng*h/mL)	22	3.21 ± 2.50	77.9
T_{max} (h)	22	2.41 ± 2.41	100.4
C_{max} (ng/mL)	22	0.428 ± 0.299	69.9
K_{el} (1/h)	14	0.112 ± 0.032	28.3
$t_{1/2}$ (h)	14	6.75 ± 2.14	31.6
AUC accumulation ratio	20	1.60 ± 0.742	46.4
Cmax accumulation ratio	22	1.45 ± 0.656	45.2

Source: Adapted from Table 8 in the study report for Study 478

The plasma concentrations of besifloxacin increased following the initial dose, reaching a maximal concentration at approximately 3h before beginning to decline with a calculated $t_{1/2}$ of approximately 4h based on a single dose or approximately 7 hours based on multiple dosing. The terminal $t_{1/2}$ could not be reported for the majority of subjects on Day 1 because the acceptance criteria were not met for the linear regression. The lack of a well-defined terminal phase on Day 1 may be related, in part, to the short sample collection interval relative to the $t_{1/2}$. Besifloxacin was present at low systemic levels; most concentrations were below 1 ng/mL. Variability in plasma concentration between subjects was high, with CV values ranging from 61-120%.

The TID dosing regimen resulted in an accumulation of besifloxacin (see Figure 1). However, the mean C_{max} of the final dose was 0.428 ng/mL (Table 4), indicating that the extent of accumulation was minimal. C_{min} plasma concentrations were measurable in most subjects, and were similar across different days, suggesting that steady-state is reached by Day 2.

Safety Results

There were no ocular Adverse Events (AEs) during the study. There was only non-ocular AE that was considered possibly related to the study drug (mild headache, reported by subject #026).

Pharmacodynamic Results

The Biomicroscopy results at the beginning and the end of the study are shown in Table 5. The N in the table represents eyes, and not individual subjects

Table 5 Biomicroscopy Results

	Besifloxacin Suspension N = 48 n (%)	
	Visit 1 (Day 0)	Visit 7 (Day 6)
Ocular Discharge		
Total Non-Missing	48	46
Absent	0 (0.0)	42 (91.3)
Mild	29 (60.4)	4 (8.7)
Moderate	18 (37.5)	0 (0.0)
Severe	1 (2.1)	0 (0.0)
Missing	0	0
Bulbar Conjunctival Injection		
Total Non-Missing	48	46
Normal	0 (0.0)	38 (82.6)
Mild	21 (43.8)	8 (17.4)
Moderate	17 (35.4)	0 (0.0)
Severe	10 (20.8)	0 (0.0)
Missing	0	0

Source: Study 478 study report, Table 11

Both eyes of a subject were required to have ocular discharge in order to enter the study. At Day 6, the majority of eyes in the study no longer had ocular discharge. Four eyes from two subjects (subject #008 and #017) had ocular discharge remaining at the completion of the study period. Similar success rates were observed with the bulbar conjunctival injection treatment. Eight eyes from four subjects (subjects #001, #008, #017, and #028) had mild bulbar conjunctival injection at the completion of the study. No eye regressed in either category.

Visual acuity was monitored throughout the study. All study eyes had pin-holed Snellen VA of 20/70 or better at each visit. By the completion of the study, the VA was either unchanged or improved as compared with the pre-study levels (Table 6)

Table 6: Pin-holed Snellen visual acuity at each visit

	Besifloxacin Suspension N = 48 n (%)						
	Visit 1 (Day 0)	Visit 2 (Day 1)	Visit 3 (Day 2)	Visit 4 (Day 3)	Visit 5 (Day 4)	Visit 6 (Day 5)	Visit 7 (Day 6)
Total Non-Missing	48	48	46	46	46	46	46
20/20	19 (39.6)	23 (47.9)	24 (52.2)	23 (50.0)	24 (52.2)	24 (52.2)	24 (52.2)
20/25	16 (33.3)	17 (35.4)	16 (34.8)	17 (37.0)	16 (34.8)	16 (34.8)	16 (34.8)
20/30	10 (20.8)	5 (10.4)	3 (6.5)	3 (6.5)	3 (6.5)	3 (6.5)	3 (6.5)
20/40	1 (2.1)	1 (2.1)	1 (2.2)	1 (2.2)	1 (2.2)	1 (2.2)	1 (2.2)
20/70	2 (4.2)	2 (4.2)	2 (4.3)	2 (4.3)	2 (4.3)	2 (4.3)	2 (4.3)
20/80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
20/100	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
20/200	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Missing	0	0	2	0	0	0	0

Source: Study 478 study report, Table 13

APPLICANT'S CONCLUSIONS:
The data from a previous Phase 1 study indicated that the dosing of ISV-403 into the eyes of healthy volunteers did not result in systemic exposures over the level of 1 ng/mL. The current study was conducted in patients to see whether the pathophysiological changes observed with suspected bacterial conjunctivitis would impact the systemic concentrations of besifloxacin. The results of this study are in agreement with the previous Phase 1 study in that the C_{max} values achieved in plasma were found to be low (mean C_{max} below 0.5 ng/mL in this study). There was a slight accumulation of besifloxacin following TID administration, consistent with a terminal half-life of 6.8h.

Adverse events were minimal, with only one non-ocular adverse event, a headache which was rated mild in severity and determined to be possibly related to the study drug. Bilateral slit lamp biomicroscopy results showed that no eyes had more than mild ocular discharge or bulbar conjunctival injection on Day 6, with no eyes regressing. Visual acuity was maintained or improved at the end of the trial.

REVIEWER'S ASSESSMENT:
Study 478 demonstrated that plasma levels of besifloxacin were not significantly different in when dosed in patients as compared to what was previously observed in healthy volunteers. This suggests that the suspected bacterial conjunctivitis disease state does not lead to pathophysiological changes that significantly impact the amount of besifloxacin able to enter the systemic circulation. The maximal concentrations achieved in plasma are low, and suggest that the chances of any systemic effects are minimal. The

sponsor also offers some evidence as to the effectiveness of the treatment regimen, but this is difficult to evaluate since there was no comparator group in this study. The Sponsor's pharmacokinetic conclusions are acceptable for a Clinical Pharmacology perspective.