

Dermatologic and Ophthalmic Drugs Advisory Committee Meeting Briefing Document

Title: Besifloxacin ophthalmic suspension, 0.6%, for the treatment of bacterial conjunctivitis

NDA Number: 22-308

Product Name: Besifloxacin ophthalmic suspension, 0.6%

Active Ingredient: Besifloxacin hydrochloride

Indication: Treatment of bacterial conjunctivitis

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EXECUTIVE SUMMARY

The data presented in this briefing document demonstrate that besifloxacin ophthalmic suspension, 0.6% has a favorable benefit/risk profile to support the indication for the treatment of bacterial conjunctivitis in patients 1 year of age and above. The proposed dosing regimen is besifloxacin ophthalmic suspension, 0.6%, administered at a dosage of 1 drop in the affected eye(s) three times daily (TID) for 7 days.

Background

Bacterial conjunctivitis is a common external ocular infection that is frequently observed among infants, schoolchildren, and the elderly. The condition is characterized by marked hyperemia or redness of the eye, and mild to moderate purulent conjunctival discharge. Conjunctivitis is contagious and can readily spread within a family, childcare center, or eldercare facility. Children with conjunctivitis may be required to stay home from school or daycare to prevent contagious spread or until they receive treatment for the disease, thus placing a socioeconomic burden on families. Generally, the disease is self-limiting and does not cause permanent loss of vision or structural damage; however, treatment with topical ocular anti-infective agents is standard of care for providing rapid symptomatic relief, reducing the rate of re-infection, possibly preventing the spread of the infection to others, and most importantly, improving the rate of early clinical remission and overall microbial eradication.

The spectrum of causative pathogens continues to evolve, and the incidence of resistance of these organisms to anti-infectives has been increasing. Therefore, there is a continued need for development of novel anti-infectives with improved potency and activity against drug-resistant pathogens. *In vitro* studies with besifloxacin have demonstrated its broad-spectrum antimicrobial activity with potency similar to, if not greater than, antibacterial agents used in other marketed ophthalmic formulations. Furthermore, besifloxacin has been developed exclusively as a topical ophthalmic treatment, thus reducing the potential for encountering resistant organisms resulting from prior use.

Some currently available topical anti-infectives for the treatment of bacterial conjunctivitis, such as ofloxacin, ciprofloxacin, levofloxacin, and gatifloxacin, are dosed as frequently as 8 times per day initially and then tapered to 4 times daily (QID) for the remainder of the treatment period. Besifloxacin ophthalmic suspension, 0.6%, has been developed as a long-acting topical eye drop that can be dosed TID. This less frequent dosing regimen should provide efficacy while enhancing patient convenience in the treatment of bacterial conjunctivitis; this may be particularly advantageous to parents who must administer treatment to young children. In addition, the formulation contains the DuraSite[®] (InSite Vision, Alameda, California, US) delivery system that is designed to increase the retention/dwell time of drug on the eye and reduce the rate of loss of medication caused by blinking and tearing.

Efficacy Results

To support the marketing application of besifloxacin ophthalmic suspension, 0.6%, 3 large and well-controlled safety and efficacy trials (Studies 373, 433, and 434) were conducted in patients aged 1 to 100 years. These studies assessed the clinical and microbial efficacy of besifloxacin ophthalmic suspension compared with vehicle (Studies 373 and 433) or Vigamox[®] (Study 434) for the treatment of bacterial conjunctivitis. Results from these studies demonstrated that besifloxacin ophthalmic suspension administered TID for 5 days was superior to vehicle and non-inferior to Vigamox. The primary efficacy endpoints were met for each of these studies.

Primary endpoint definitions differed between Study 373 and Studies 433 and 434. The primary efficacy endpoints were clinical resolution and microbial eradication at Visit 3 (Day 8 or 9) for Study 373 and clinical resolution and microbial eradication at Visit 2 (Day 5 \pm 1) for Studies 433 and 434. The time point for Visit 2 was defined as Day 4 \pm 1 for Study 373 and Day 5 \pm 1 for Studies 433 and 434; however, the time point for Visit 3 was defined as Day 8 or 9 in all 3 studies. In addition to the difference in timepoint definitions, the definitions for clinical diagnosis and clinical resolution of bacterial conjunctivitis also differed between Study 373 and Studies 433 and 434. In Study 373, patients were required to present with a minimum of grade 1 for conjunctival discharge and a minimum of grade 1 for either bulbar or palpebral conjunctival injection for a clinical diagnosis of bacterial conjunctivitis. For Studies 433 and 434, a minimum of grade 1 for conjunctival discharge and bulbar conjunctival injection was required for diagnosis of bacterial conjunctivitis. Clinical resolution was defined as the absence of 3 clinical signs (conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection) in Study 373 and 2 clinical signs (conjunctival discharge and bulbar conjunctival injection) in Studies 433 and 434. However, microbial eradication was defined similarly in all 3 studies as the absence of all accepted ocular bacterial species that were present at or above threshold levels at baseline. All patients who were randomly assigned to treatment and had culture-confirmed conjunctivitis were evaluated for the primary endpoints in the intent-to-treat (ITT) analysis in Study 373 or the modified intent-to-treat (mITT) analysis in Studies 433 and 434.

Study 373—269 patients were randomized to receive besifloxacin ophthalmic suspension (n = 137) or vehicle (n = 132). A total of 118 (60 besifloxacin and 58 vehicle) patients with culture-confirmed bacterial conjunctivitis at baseline were eligible for the ITT population; efficacy results as follows are data from this ITT, culture-confirmed population. Of these, 2 patients in the vehicle treatment group withdrew from the study prior to Visit 2 (Day 4 \pm 1). The primary efficacy endpoints of clinical resolution and microbial eradication at Visit 3 (Day 8 or 9) were achieved in a significantly greater percentage of patients who received besifloxacin ophthalmic suspension compared with vehicle. At Visit 3 (Day 8 or 9), the clinical resolution rates for the besifloxacin ophthalmic suspension versus vehicle treatment groups were 61.7% versus 35.7%, respectively (Cochran-Mantel-Haenszel (CMH) adjusted for center effects $p = 0.0013$), based on the absence of 3 clinical signs (conjunctival discharge, bulbar and palpebral conjunctival injection). Microbial eradication rates for the besifloxacin ophthalmic suspension versus vehicle treatment groups were 90.0% vs 69.1%, respectively (CMH adjusted $p = 0.0041$). At Visit 2 (Day 4 \pm 1), no statistically significant between-group difference was observed for clinical resolution based on the absence of 3

clinical signs. However, the microbial eradication rates at Visit 2 were significantly greater in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group (90.0% vs 51.8%, respectively; CMH adjusted $p < 0.0001$).

Study 433—957 patients were randomized to receive besifloxacin ophthalmic suspension ($n = 473$) or vehicle ($n = 484$). A total of 390 (199 besifloxacin and 191 vehicle) patients with culture-confirmed bacterial conjunctivitis were eligible for the mITT population; efficacy results as follows are data from this mITT, as-randomized, culture-confirmed population. The primary efficacy endpoints of clinical resolution and microbial eradication at Visit 2 (Day 5 \pm 1) were achieved in a significantly greater percentage of patients who received besifloxacin ophthalmic suspension versus vehicle. At Visit 2 (Day 5 \pm 1), the clinical resolution rates for the besifloxacin ophthalmic suspension versus vehicle treatment groups were 45.2% versus 33.0%, respectively (exact Pearson chi-squared test $p = 0.0169$; CMH adjusted $p = 0.0084$). Similarly, microbial eradication rates were significantly higher in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group (91.5% vs 59.7%; exact Pearson chi-squared test and CMH adjusted $p < 0.0001$). At Visit 3 (Day 8 or 9), the clinical resolution rates in both treatment groups were higher than that observed at Visit 2 (Day 5 \pm 1), and the difference between the 2 groups was statistically significant, favoring the besifloxacin ophthalmic suspension treatment group (84.4% vs 69.1%; exact Pearson chi-squared test $p = 0.0005$, CMH adjusted $p = 0.0011$). Moreover, the benefit of besifloxacin ophthalmic suspension over vehicle in eradicating baseline bacterial infections was maintained at Visit 3 (88.4% vs 71.7%; exact Pearson chi-squared test or CMH adjusted $p < 0.0001$).

Study 434—1161 patients were randomized to receive besifloxacin ophthalmic suspension ($n = 582$) or Vigamox ($n = 579$). A total of 533 (252 besifloxacin and 281 Vigamox) patients with culture-confirmed bacterial conjunctivitis were eligible for the mITT, as-treated population; efficacy results as follows are data from this mITT, as-treated, culture-confirmed population. The primary efficacy endpoint analysis of clinical resolution and microbial eradication at Visit 2 (Day 5 \pm 1) demonstrated that besifloxacin ophthalmic suspension was non-inferior to Vigamox. At Visit 2 (Day 5 \pm 1), besifloxacin ophthalmic suspension was non-inferior to Vigamox for clinical resolution based on the 95% confidence interval (CI) of the difference (58.3% vs 59.4%, respectively; 95% CI, -9.48%, 7.29%), and there was no statistically significant between-group difference. Besifloxacin ophthalmic suspension also was non-inferior to Vigamox for microbial eradication based on the 95% CI of the difference (93.3% vs 91.1%, respectively; 95% CI, -2.44%, 6.74%), and there was no statistically significant between-group difference. At Visit 3 (Day 8 or 9), besifloxacin ophthalmic suspension was non-inferior to Vigamox (based on the 95% CI of the difference) for clinical resolution (84.5% vs 84.0%, respectively; 95% CI, -5.67%, 6.75%) and eradication of baseline bacterial infections (87.3% vs 84.7%, respectively; 95% CI, -3.32%, 8.53%). No statistically significant between-group differences were observed for either of these assessments at Visit 3.

Based on the integrated microbiological data from Studies 373, 433, and 434, the distribution of baseline pathogens was similar across the besifloxacin ophthalmic suspension, vehicle, and Vigamox treatment groups. The relative frequency of the most common organisms isolated at threshold levels or higher from these studies (*H. influenzae*,

S. pneumoniae, *S. aureus*, and *S. epidermidis*) was consistent with common clinical experience in this indication.

Susceptibility testing of clinical trial isolates was performed for besifloxacin and comparator test agents. Overall, isolates cultured in the 3 clinical trials yielded besifloxacin susceptibility patterns similar to those observed in nonclinical studies. A total of 1324 isolates were recovered from culture-confirmed patients in Studies 373, 433, and 434. Overall, MIC₅₀/MIC₉₀ values for the 1324 isolates of all species were 0.06/0.25 µg/mL for besifloxacin. Of the 1324 bacterial isolates, 886 (66.9%) were Gram-positive, while the remaining 438 (33.1%) were Gram-negative. The besifloxacin MIC₅₀/MIC₉₀ values were 0.06/0.25 µg/mL for Gram-positive bacteria and 0.03/0.5 µg/mL for Gram-negative bacteria.

Besifloxacin ophthalmic suspension was active against a wide range of organisms, including antimicrobial-resistant strains. Overall, the sensitivities of the pathogens to besifloxacin (including various drug-resistant isolates) obtained from patients across all treatment groups were similar. In Studies 373, 433, and 434, susceptibility testing of baseline pathogens confirmed that besifloxacin has potent antimicrobial activity against a wide range of current conjunctivitis pathogens.

Safety Results

Overall, a total of 1192 patients in Studies 373, 433, and 434 was exposed to besifloxacin ophthalmic suspension. During the course of these 3 studies, few patients withdrew from treatment due to adverse events (AEs). Adverse events reported were mostly ocular. Ocular AEs were typical of the underlying disease in this study population (ie, patients with bacterial conjunctivitis) and were generally mild in severity and transient in nature. At least 1 ocular AE occurred in 13.8% of eyes in the besifloxacin ophthalmic suspension treatment group, 19.8% of eyes in the vehicle treatment group, and 14.0% of eyes in the Vigamox treatment group. No deaths occurred in studies 373, 433, and 434, and the incidence of serious adverse events (SAEs) was very low in all treatment groups (a total of 4 non-ocular SAEs among 1192 patients, or 0.2% in each of the groups). None of these SAEs was considered treatment related.

In addition, ocular and systemic pharmacokinetic studies have demonstrated that besifloxacin ophthalmic suspension has high ocular retention (≥ 1.6 µg/g in tears for at least 24 hours after a single dose), low systemic exposure (< 0.5 ng/mL), and no effect on corneal endothelial cell density.

Conclusions

There is an ongoing need for development of new treatments for bacterial conjunctivitis that have increased potency and reduced potential for encountering resistance, due to an evolving landscape of causative pathogens and increasing incidence of resistant strains. Besifloxacin is a new chemical entity that is a broad-spectrum, long-acting topical anti-infective with potent antibacterial activity against prevalent and resistant pathogens. The dosing frequency, TID, is convenient for patients and caregivers. In controlled clinical trials in patients aged 1 to 98

years, besifloxacin ophthalmic suspension exhibited statistically and clinically significant improvement in clinical resolution and microbial eradication compared with vehicle alone and clinical and microbial outcomes similar to those observed with Vigamox. Besifloxacin has been developed exclusively as a topical ophthalmic treatment, thus reducing the potential for encountering resistant organisms resulting from prior use. Furthermore, besifloxacin ophthalmic suspension has a favorable safety profile with a very low incidence of AEs and no treatment-related SAEs. Besifloxacin ophthalmic suspension is a step forward in the treatment of bacterial conjunctivitis because it has the characteristics necessary to effectively treat bacterial conjunctivitis and reduce its contagion, duration, and socioeconomic burden.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	Adverse event
AUC	Area under curve
ATCC	American Type Culture Collection
C _{max}	Maximum concentration
CDC	Centers for Disease Control
CFU	Colony forming unit
CI	Confidence interval
CLSI	Clinical Laboratory and Standards Institute
CMH	Cochran-Mantel-Haenszel
ERG	Electroretinography
FAS	Full analysis set
FDA	Food and Drug Administration
ITT	Intent-to-treat (population)
LC/MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LLOQ	Lower limit of quantitation
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
mITT	Modified intent-to-treat (population)
MPC	Mutant prevention concentration
MRSA	Methicillin resistant <i>S. aureus</i>
MRSE	Methicillin resistant <i>S. epidermidis</i>
MSSA	Methicillin susceptible <i>S. aureus</i>
MSSE	Methicillin susceptible <i>S. epidermidis</i>
NDA	New Drug Application
NOAEL	No observable adverse effect level
PD	Pharmacodynamic
PFGE	Pulsed field gel electrophoresis
PK	Pharmacokinetic
PP	Per protocol
PRSP	Penicillin resistant <i>S. pneumoniae</i>
PSSP	Penicillin susceptible <i>S. pneumoniae</i>
QC	Quality control
QID	Four times daily
QT	Electrocardiographic interval of time between the start of Q wave and end of the T wave
SAE	Serious adverse event
SD	Standard deviation
t _{1/2}	Half-life
t _{max}	Time to maximum concentration
TID	Three times daily
VA	Visual acuity

1 PURPOSE OF THE DOCUMENT

This document provides an overview of the efficacy and safety data from the clinical development program with besifloxacin ophthalmic suspension, 0.6%, in patients with bacterial conjunctivitis. In addition, nonclinical data pertaining to the pharmacokinetics/pharmacodynamics, toxicology, mode of action, resistance development, bactericidal activity, and antibacterial spectrum of besifloxacin are provided.

2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Overview of Bacterial Conjunctivitis

The globe of the eye is covered by a thin, transparent, mucous membrane called the conjunctiva. The conjunctiva serves to protect the eye and facilitates eye movement by providing lubrication. Conjunctivitis is an inflammation of this lining of the eye.

Bacterial conjunctivitis is a common external ocular infection that is frequently observed among infants, schoolchildren, and the elderly. The condition is characterized by marked hyperemia or redness of the eye, and mild to moderate purulent conjunctival discharge. Conjunctivitis is contagious and can readily spread within a family, childcare center, or eldercare facility. To prevent contagious spread, children with conjunctivitis may be required to stay home from school or daycare until they receive treatment for the disease or the disease resolves, thus placing a socioeconomic burden on families. Generally, the disease is self-limiting and does not cause permanent loss of vision or structural damage (Jensen & Felix, 1998); however, treatment with topical ocular anti-infective agents is standard of care for providing rapid symptomatic relief, improving the rate of early clinical remission and overall microbial eradication, reducing the rate of re-infection, and most importantly, reducing the potential of spreading the infection to others.

Some of the more common causative organisms of bacterial conjunctivitis can be components of the normal lid flora (eg, *Staphylococcus aureus*) or nasopharyngeal flora (eg, *Haemophilus influenzae*) (Brook et al., 1979; Gigliotti et al., 1981; Hammond & Edmondson, 1997; Leeming, 1999). Other common pathogens include *Streptococcus pneumoniae* and *Moraxella* species, but *Neisseria* species, *Corynebacterium* species, and other *Streptococcus* species also may cause bacterial conjunctivitis.

2.2 Current Practice for the Treatment of Bacterial Conjunctivitis

Intervention with the use of a topical broad-spectrum ocular anti-infective is the standard of care in the management of bacterial conjunctivitis. Treatment often shortens the duration of the disease, reduces contagious spread, and enhances eradication of causative Gram-positive and Gram-negative organisms (Diamant, 1999; Sheikh & Hurwitz, 2006).

Typically, treatment of bacterial conjunctivitis is based on the likely causative pathogens. The choice of empiric therapy should ensure good activity against both Gram-positive and Gram-negative organisms.

2.3 Rationale for Development of Besifloxacin

Some currently available topical anti-infective agents for the treatment of bacterial conjunctivitis, such as ofloxacin, gatifloxacin, levofloxacin, and ciprofloxacin, are dosed as frequently as eight times per day initially and then tapered to four times daily (QID) for the remainder of the treatment period. Bausch & Lomb Incorporated has developed besifloxacin ophthalmic suspension, 0.6%, as a long-acting topical eye drop that can be dosed three times daily (TID). This less frequent dosing regimen should provide efficacy and enhance patient convenience in the treatment of bacterial conjunctivitis. In addition, the formulation contains the DuraSite[®] delivery system (InSite Vision, Alameda, California, United States) that increases the retention/dwell time of drug on the eye and reduces the rate of loss of medication caused by blinking and tearing. *In vitro* studies with besifloxacin demonstrated it to have a broad-spectrum antimicrobial effectiveness with potency similar to, if not greater than, antibacterial agents used in other marketed ophthalmic formulations.

3 BESIFLOXACIN OVERVIEW

3.1 Chemical Name and Structure

The active ingredient, besifloxacin hydrochloride, is a fluoroquinolone anti-infective and is a new chemical entity. The chemical name is (R)-(+)-(3-Amino-2,3,4,5,6,7-hexahydro-1H-azepin-1-yl)-8-chloro-1-cyclopropyl-6-fluoro-1,4,-dihydro-4-oxo-3-quinolinecarboxylic acid hydrochloride. The molecular formula is $C_{19}H_{21}ClFN_3O_3 \cdot HCl$ with a molecular weight of 430.30. The structure is illustrated below in Figure 1.

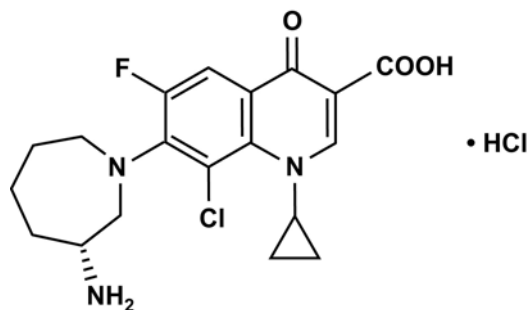


Figure 1. Chemical Structure of Besifloxacin Hydrochloride

3.2 Formulation

The formulation of besifloxacin ophthalmic suspension, 0.6% contains the following:

- **Active ingredient:** besifloxacin 0.6% (6 mg/mL)
- **Inactive ingredients:** mannitol, poloxamer 407, and DuraSite (polycarbophil, sodium chloride, ethylenediaminetetraacetic acid disodium, sodium hydroxide, and water). Besifloxacin ophthalmic suspension may have additional sodium hydroxide to adjust pH to approximately 6.5
- **Preservative:** benzalkonium chloride 0.01%

3.3 Proposed Indication

Besifloxacin ophthalmic suspension, 0.6%, is indicated for the treatment of bacterial conjunctivitis.

3.4 Dosage and Administration

In the clinical safety and efficacy trials conducted in support of this application, patients were treated with besifloxacin ophthalmic suspension at a dose of 1 drop per affected eye(s) TID for 5 days. In the draft product labeling, the FDA-recommended dose of besifloxacin ophthalmic suspension for the treatment of bacterial conjunctivitis is 1 drop per affected eye(s) TID for 7 days.

4 NONCLINICAL EVALUATION OF BESIFLOXACIN

4.1 Microbiology

4.1.1 Mechanism of Action

Besifloxacin is an 8-chloro fluoroquinolone with an N-1 cyclopropyl group. The substituents of the side chain at the 7 position and the chlorine at the 8 position, along with the standard fluoroquinolone core, provide besifloxacin its unique structure and unique activity profile. The compound has broad-spectrum activity against aerobic, facultative, and anaerobic Gram-positive and Gram-negative bacteria due to the inhibition of two essential bacterial enzymes, DNA gyrase and topoisomerase IV. DNA gyrase introduces negative supercoils into DNA during replication and translation, while topoisomerase IV is required for partitioning of the chromosomal DNA during bacterial cell division. Fluoroquinolones, such as besifloxacin, result in the formation of double-stranded DNA breaks that cannot be repaired, leading ultimately to bacterial cell death. Besifloxacin is bactericidal with minimum bactericidal concentrations (MBCs) generally within one dilution of the minimum inhibitory concentrations (MICs). The mechanism of action of fluoroquinolones, including besifloxacin, is different from that of aminoglycoside, macrolide, tetracycline, β -lactam, sulfonamide, and cyclic peptide antibacterial drugs. Therefore, besifloxacin may be active against pathogens that are resistant to these antibacterials and these antibacterial drugs may be active against pathogens that are resistant to besifloxacin.

The mechanism of action of besifloxacin was evaluated in an *in vitro* study that compared besifloxacin to ciprofloxacin and moxifloxacin for catalytic inhibition as well as cleavable complex stimulation with DNA gyrase and topoisomerase IV purified from representative Gram-positive and Gram-negative bacterial pathogens. Catalytic inhibition and cleavable complex stimulation by besifloxacin was 4- to 16-fold more potent than ciprofloxacin and moxifloxacin against *S. pneumoniae* DNA gyrase and 2.5- to 5.0-fold more potent than ciprofloxacin and moxifloxacin against *S. pneumoniae* topoisomerase IV (Table 1). In assays with purified *E. coli* DNA gyrase and topoisomerase IV, both catalytic inhibition and cleavable complex stimulation by besifloxacin were equivalent to that of the ciprofloxacin and moxifloxacin comparators.

Furthermore, this study assessed the mechanism of action of besifloxacin in *S. pneumoniae*, *S. aureus*, and *E. coli* via step selections for isolates with decreased susceptibility to besifloxacin, as well as by testing for altered susceptibilities of ciprofloxacin-resistant variants of all three species containing genetically defined mutations in the quinolone resistance determining regions (QRDRs) of structural genes encoding DNA gyrase and topoisomerase IV (Table 2). Results from this experiment were consistent with well-established mechanisms of action and target-based resistance to other fluoroquinolone inhibitors of type II DNA topoisomerases, including ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and gemifloxacin. DNA sequencing of mutants with altered besifloxacin susceptibilities as well as the MIC testing against ciprofloxacin-resistant isolates indicated that besifloxacin targets DNA gyrase and topoisomerase IV in representative Gram-positive and Gram-negative isolates, with evidence for balanced activity against both essential type II DNA topoisomerase targets in streptococci and staphylococci.

Table 1. Inhibitory Activity (IC₅₀) and Potency of Besifloxacin and Comparators in Stabilizing the Cleavable Complex (CC₂₅) of *S. pneumoniae* and *E. coli* DNA Gyrase and Topoisomerase IV

Quinolones	<i>S. pneumoniae</i> enzymes				<i>E. coli</i> enzymes			
	IC ₅₀		CC ₂₅		IC ₅₀		CC ₂₅	
	Gyrase	Topo IV	Gyrase	Topo IV	Gyrase	Topo IV	Gyrase	Topo IV
Ciprofloxacin								
μM	40	5	40 - 80	2.5-5	1	27	0.15	1.5
μg/mL	15	2	15 - 25	1-2	0.3	9	0.05	0.5
Moxifloxacin								
μM	10	2.5	10 - 20	2.5	1.6	20	0.2	2.3
μg/mL	4	1	4 - 8	1.5	0.7	9	0.07	1
Besifloxacin								
μM	2.5	1	2.5	1	2.3	23	0.1	1.4
μg/mL	1	0.4	1	0.4	1	10	0.04	0.6

Topo IV = Topoisomerase IV.

CC₂₅ is the drug concentration that produces 25% linearization of the DNA under the reaction conditions used.

Table 2. MICs (µg/mL) of Besifloxacin Against Defined Topoisomerase Mutants

Topoisomerase mutants	Besifloxacin	Ciprofloxacin	Moxifloxacin
<i>S. pneumoniae</i>			
Wild type	0.12	1	0.25
<i>parC</i> S79Y	0.25	8	0.25
<i>gyrA</i> S81F	0.5	1	0.5
<i>parC</i> S79Y + <i>gyrA</i> S81F	1	64	4
<i>S. aureus</i>			
Wild type	0.03	1	0.06
<i>parC</i> S80F (or E84K)	0.06	8	0.5
<i>parC</i> S80F + <i>gyrA</i> S84L	0.5	64	2
<i>E. coli</i>			
Wild type	0.12	0.008	0.06
<i>gyrA</i> D87Y (or S83L)	0.5	0.12	0.5
<i>gyrB</i> D426N	0.5	0.03	0.12
<i>gyrA</i> S83L + <i>parE</i> H445L	1	0.12	0.5
<i>gyrA</i> S83L + <i>parC</i> S80R	16	4	4

4.1.2 Development of Resistance to Besifloxacin

Besifloxacin is a potent antibacterial agent by virtue of its efficient biochemical inhibition of type II bacterial topoisomerases at low micromolar levels. Besifloxacin was associated with a low mutant prevention concentration (MPC), especially in the two Gram-positive pathogens, *S. aureus* and *S. pneumoniae*, in which the MPCs were only four times higher than the MICs for those organisms. Correspondingly, very few drug-resistant mutants were obtained in *in vitro* experiments for those two species (< 1 mutant per 10¹⁰ cells).

Consistent with these results is a dual-targeting mechanism of action for besifloxacin, especially in *S. aureus* and *S. pneumoniae*. Dual targeting indicates that DNA gyrase (encoded by *gyrA* and *gyrB*) and topoisomerase IV (encoded by *parC* and *parE*) are both inhibited by the antibacterial drug. As a consequence, strains with high-level resistance would only emerge if both targets were mutated simultaneously, an event that is not very likely in strains that lack predisposing mutations. A dual-targeting mechanism of action for besifloxacin is supported by the following experimental evidence:

- In *S. aureus* and *S. pneumoniae*, single (1st step) mutants were extremely rare (< 1/10¹⁰ cells) and only few or no double (2nd step) mutants were obtained (Table 3).
- Single mutations in the *gyrA* or in the *parC* gene were obtained in *S. aureus* as well as in *S. pneumoniae*. In both species, MIC values for the *gyrA* and the *parC* mutants differed by no more than one 2-fold serial dilution, indicating that besifloxacin has no preference for one enzyme over the other. By comparison, all single mutations in quinolone-resistant strains of *E. coli* were mapped to either the *gyrA* or the *gyrB* gene consistent with the general finding that quinolones primarily target the DNA gyrase in Gram-negative bacteria.

- Biochemical experiments with the purified DNA gyrase and topoisomerase IV enzymes from *S. pneumoniae* and *E. coli* were performed. Inhibitory concentrations of besifloxacin for the *E. coli* gyrase were 10-fold lower than for the topoisomerase IV, suggesting a modest preference for DNA gyrase from Gram-negative bacteria. In contrast, the difference between the inhibitory concentrations for DNA gyrase and topoisomerase IV from *S. pneumoniae* was only 2.5-fold, suggesting that besifloxacin targets both enzymes in Gram-positive bacteria.

Table 3. In Vitro Multistep Selection for Besifloxacin-Resistant Mutants

Species	Selection step	MIC (µg/mL)	MSW (µg/mL)	MPC (µg/mL)	Mutation rate at 4× MIC
<i>S. pneumoniae</i>	1st step	0.12	0.12 - 0.25	0.5	$< 7 \times 10^{-10}$
	2nd step	0.5	0.5 - 2	2 - 4	2.4×10^{-8}
<i>S. aureus</i>	1st step	0.03	0.03 - 0.06	0.12	3.3×10^{-10}
	2nd step	0.25	NMO	0.25	NMO
<i>E. coli</i>	1st step	0.12	0.12 - 2	4	3.8×10^{-8}
	2nd step	2	2 - 8	16	6×10^{-9}

MIC = Minimum inhibitory concentration; MSW = Mutant Selection Window; MPC = Mutant prevention concentration; NMO = No mutants obtained.

4.1.3 Bactericidal Activity of Besifloxacin

The success of *in vivo* antimicrobial action depends to a large extent on the host's defense mechanisms, which ultimately sequester and kill the microorganisms that have been reduced by the bacteriostatic/bactericidal action of the antibacterial agent. Thus, it is also of interest to profile the bactericidal activity of antimicrobial agents. The assessment of *in vitro* bactericidal activity can be accomplished in multiple ways, for example, the time-kill method or the determination of the minimum bactericidal concentration (MBC). The MBC is the drug concentration that leads to a $\geq 99.9\%$ reduction in the viable count (CFU/mL) of the test organism after 24 hours. Bactericidal agents are characterized by low MBC:MIC ratios. (Lorian, 2005)

Figure 2 and Table 4 illustrate the besifloxacin bactericidal activity against recent ocular isolates. Besifloxacin MBCs within 1 to 2 dilutions of the MIC (MBC:MIC ratios ≤ 2) were observed for the majority of ocular isolates tested (*S. pneumoniae*, *S. epidermidis*, *H. influenzae*, and *S. aureus*). Besifloxacin MBCs were within 4-fold of the MIC for more than 80% of the isolates tested. Among staphylococci, equivalent besifloxacin MBC:MIC ratios were observed for both ciprofloxacin-susceptible and -resistant isolates, as well as for methicillin-susceptible and methicillin-resistant isolates. The MBC:MIC ratios observed with besifloxacin were similar to that for comparator fluoroquinolones. Against the majority of ocular isolates tested, the MBC did not exceed 2-fold the initial MIC,

indicating a bactericidal mode of action for besifloxacin. In contrast to all other comparator agents tested (moxifloxacin, azithromycin, tobramycin, gatifloxacin, and ciprofloxacin; data for the latter three not shown), only besifloxacin yielded measurable MIC and MBC values within the test range for all isolates. Time-kill studies confirmed the bactericidal activity of besifloxacin against *S. aureus*, *S. pneumoniae*, and *H. influenzae* (data not shown).

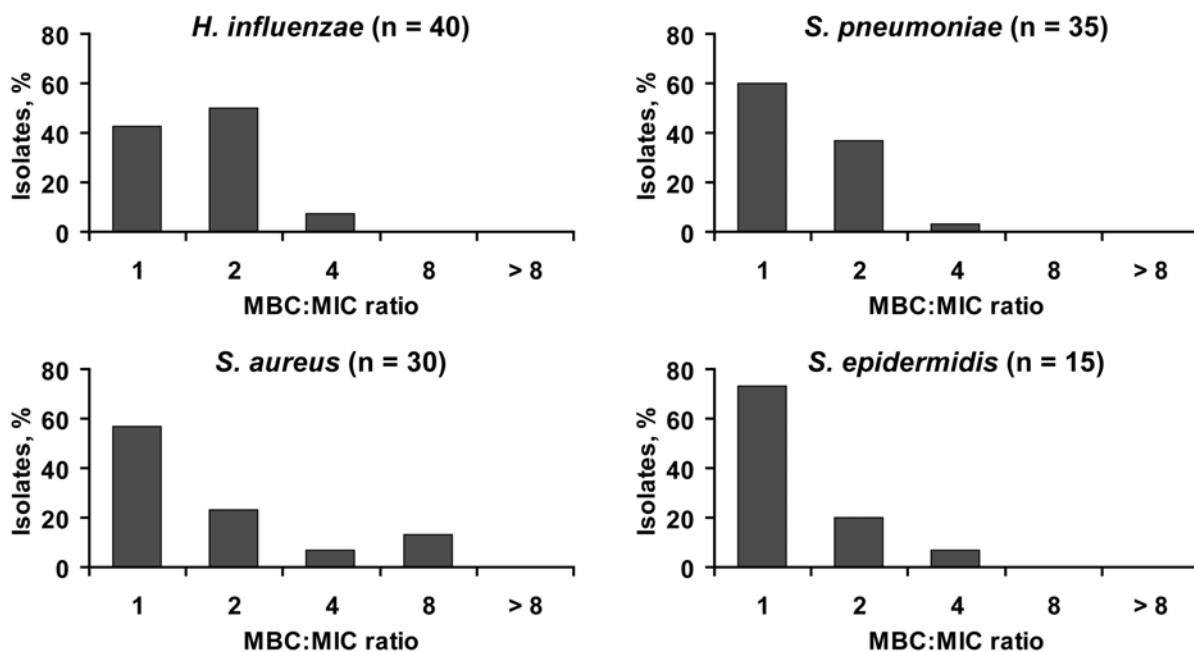


Figure 2. Besifloxacin Bactericidal Activity Against Recent Ocular Isolates

Note: These data are also presented in Table 4 below.

Table 4. In Vitro activity and MBC:MIC Ratio of Besifloxacin and Comparator Agents Against Recent Ocular Isolates

Species (no. of isolates)										
Test drug	MIC (µg/mL)			%S ^a	% of isolates with MBC:MIC ratio					n ^b
	Range	MIC ₅₀	MIC ₉₀		1	2	4	8	≥16	
<i>H. influenzae</i> (N = 40)										
Besifloxacin	≤0.004 - 0.03	0.015	0.015	na	42.5	50.0	7.5	0.0	0.0	40
Moxifloxacin	0.008 - 0.03	0.015	0.03	100.0	60.0	37.5	2.5	0.0	0.0	40
Azithromycin	≤0.004 - 2	0.5	1	100.0	15.8	57.9	15.8	7.9	2.6	38
<i>S. aureus</i> (N = 30)										
Besifloxacin	0.015 - 4	0.12	4	na	56.7	23.3	6.7	13.3	0.0	30
Moxifloxacin	0.015 - >8	0.06	>8	56.7	64.0	16.0	4.0	16.0	0.0	25
Azithromycin	0.5 - >8	1	>8	53.3	0.0	11.1	11.1	22.2	55.6	9
<i>S. epidermidis</i> (N = 15)										
Besifloxacin	0.015 - 4	0.03	4	na	73.3	20.0	6.7	0.0	0.0	15
Moxifloxacin	0.03 - >8	0.06	>8	60.0	66.7	25.0	8.3	0.0	0.0	12
Azithromycin	0.12 - >8	>8	>8	26.7	0.0	33.3	0.0	0.0	66.7	3
<i>S. pneumoniae</i> (N = 35)										
Besifloxacin	0.015 - 0.5	0.06	0.06	na	60.0	37.1	2.9	0.0	0.0	35
Moxifloxacin	0.03 - 2	0.06	0.12	97.1	51.4	40.0	8.6	0.0	0.0	35
Azithromycin	0.06 - >8	0.06	>8	62.9	64.0	4.0	24.0	8.0	0.0	25

Note: This study included, where applicable, isolates that were beta-lactamase positive or resistant to oxacillin, penicillin, and/or ciprofloxacin.

^a Percent of susceptible isolates based on CLSI guidelines.

^b n: number of isolates for which measurable MBC and MIC values were obtained, and thus, an MBC:MIC ratio could be calculated. The n value was used as the baseline (100%) for the calculation of the percentage of isolates with MBC:MIC ratio.

na: not applicable since no systemic susceptibility breakpoints have been established for besifloxacin.

In conclusion, besifloxacin showed bactericidal activity against target pathogens associated with bacterial conjunctivitis, demonstrating activity greater than or equivalent to that of other currently marketed fluoroquinolones against these organisms.

4.1.4 Antibacterial Spectrum of Activity of Besifloxacin

The antibacterial spectrum of activity of besifloxacin was evaluated against a variety of clinical isolates in *in vitro* studies using standard Clinical Laboratory and Standards Institute (CLSI) reference methods. MIC provides an estimate of the inhibitory activity of antimicrobial agents. The MIC, when determined using standard reference methods, is a reproducible parameter for a given antimicrobial agent against most rapidly growing pathogens. Except where noted, MIC values were determined by broth microdilution methods.

Tables 5, 6, and 7 summarize antibacterial activities of besifloxacin and comparator antibacterials against representative Gram-positive, Gram-negative, and anaerobic

pathogens associated with human ocular infections. Table 8 further summarizes besifloxacin antibacterial activity data pooled across multiple nonclinical studies. Overall, results from these studies show that besifloxacin has potent antibacterial activity against a very broad spectrum of bacteria, including all species commonly isolated from patients with bacterial conjunctivitis, such as *Streptococcus* spp., *Staphylococcus* spp., *Haemophilus* spp., *Corynebacterium* spp., and *Moraxella* spp. In addition, besifloxacin is active against a variety of Gram-positive, Gram-negative, and anaerobic pathogens associated with ocular infections. The data demonstrate that the antibacterial potency of besifloxacin is similar to or exceeds the potency of the fluoroquinolone and non-fluoroquinolone comparator antibacterials.

Table 5. Activity of Besifloxacin and Comparators Against Gram-positive Bacteria

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
Staphylococcus aureus (all phenotypes, N = 30)				
Besifloxacin	0.015 - 4	0.12	4	na
Moxifloxacin	0.015 - >8	0.06	>8	56.7
Gatifloxacin	0.03 - >8	1	>8	46.7
Ciprofloxacin	0.12 - >8	2	>8	46.7
Azithromycin	0.5 - >8	1	>8	53.3
Tobramycin	0.12 - >32	0.5	>32	80.0
Levofloxacin	0.06 - >8	1	>8	50.0
Oxacillin	0.12 - >8	0.25	>8	63.3
Staphylococcus aureus (MSSA, N = 19)				
Besifloxacin	0.015 - 4	0.015	0.25	na
Moxifloxacin	0.015 - >8	0.06	1	68.4
Gatifloxacin	0.03 - >8	0.06	2	63.2
Ciprofloxacin	0.12 - >8	0.5	8	57.9
Azithromycin	0.5 - >8	1	>8	78.9
Tobramycin	0.12 - 8	0.25	1	94.7
Levofloxacin	0.06 - >8	0.25	4	68.4
Oxacillin	0.12 - 0.5	0.25	0.5	100.0
Staphylococcus aureus (MRSA, N = 11)				
Besifloxacin	0.015 - 4	0.5	4	na
Moxifloxacin	0.03 - >8	1	>8	36.4
Gatifloxacin	0.06 - >8	2	>8	18.2
Ciprofloxacin	0.12 - >8	>8	>8	27.3
Azithromycin	0.5 - >8	>8	>8	9.1
Tobramycin	0.5 - >32	1	>32	54.5
Levofloxacin	0.12 - >8	4	>8	18.2
Oxacillin	8 - >8	>8	>8	0.0
Staphylococcus aureus (CS, N = 14)				
Besifloxacin	0.015 - 0.25	0.015	0.12	na
Moxifloxacin	0.015 - 0.06	0.03	0.06	100.0
Gatifloxacin	0.03 - 1	0.06	0.25	92.9
Ciprofloxacin	0.12 - 0.5	0.25	0.5	100.0
Azithromycin	0.5 - >8	1	>8	64.3
Tobramycin	0.12 - 8	0.25	1	92.9
Levofloxacin	0.06 - 2	0.12	0.25	92.9
Oxacillin	0.12 - >8	0.25	>8	78.6

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
Staphylococcus aureus (CNS, N = 16)				
Besifloxacin	0.03 - 4	0.25	4	na
Moxifloxacin	0.06 - >8	1	>8	18.8
Gatifloxacin	0.12 - >8	2	>8	6.3
Ciprofloxacin	2 - >8	8	>8	0.0
Azithromycin	0.5 - >8	>8	>8	43.8
Tobramycin	0.25 - >32	0.5	>32	68.8
Levofloxacin	0.25 - >8	4	>8	12.5
Oxacillin	0.12 - >8	0.5	>8	50.0
Staphylococcus epidermidis (all phenotypes, N = 15)				
Besifloxacin	0.015 - 4	0.03	4	na
Moxifloxacin	0.03 - >8	0.06	>8	60.0
Gatifloxacin	0.06 - >8	0.06	>8	60.0
Ciprofloxacin	0.12 - >8	0.12	>8	60.0
Azithromycin	0.12 - >8	>8	>8	26.7
Tobramycin	≤0.008 - 16	0.06	8	86.7
Levofloxacin	0.12 - >8	0.12	>8	60.0
Oxacillin	≤0.06 - 4	1	2	40.0
Staphylococcus epidermidis (MSSE, N = 6)				
Besifloxacin	0.015 - 0.25	0.03	na	na
Moxifloxacin	0.03 - 2	0.06	na	83.3
Gatifloxacin	0.06 - 1	0.06	na	83.3
Ciprofloxacin	0.12 - >8	0.12	na	83.3
Azithromycin	0.12 - >8	0.5	na	66.7
Tobramycin	≤0.008 - 0.06	0.03	na	100.0
Levofloxacin	0.12 - 8	0.12	na	83.3
Oxacillin	≤0.06 - 0.12	≤0.06	na	100.0
Staphylococcus epidermidis (MRSE, N = 9)				
Besifloxacin	0.015 - 4	0.25	na	na
Moxifloxacin	0.03 - >8	1	na	44.4
Gatifloxacin	0.06 - >8	1	na	44.4
Ciprofloxacin	0.12 - >8	2	na	44.4
Azithromycin	>8 - >8	>8	na	0.0
Tobramycin	0.03 - 16	4	na	77.8
Levofloxacin	0.12 - >8	2	na	44.4
Oxacillin	1 - 4	1	na	0.0

Table 5. Activity of Besifloxacin and Comparators Against Gram-positive Bacteria (continued)

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Staphylococcus epidermidis</i> (CS, N = 9)				
Besifloxacin	0.015 - 0.03	0.03	na	na
Moxifloxacin	0.03 - 0.06	0.06	na	100.0
Gatifloxacin	0.06 - 0.06	0.06	na	100.0
Ciprofloxacin	0.12 - 0.12	0.12	na	100.0
Azithromycin	0.25 - >8	>8	na	33.3
Tobramycin	≤0.008 - 8	0.03	na	88.9
Levofloxacin	0.12 - 0.12	0.12	na	100.0
Oxacillin	≤0.06 - 2	0.12	na	55.6
<i>Staphylococcus epidermidis</i> (CNS, N = 6)				
Besifloxacin	0.25 - 4	0.25	na	na
Moxifloxacin	1 - >8	2	na	50.0
Gatifloxacin	1 - >8	1	na	50.0
Ciprofloxacin	2 - >8	>8	na	0.0
Azithromycin	0.12 - >8	>8	na	16.7
Tobramycin	0.06 - 16	2	na	83.3
Levofloxacin	2 - >8	8	na	0.0
Oxacillin	0.12 - 4	1	na	50.0
<i>Staphylococcus haemolyticus</i> (N = 101)				
Besifloxacin	0.015 - 4	0.5	1	na
Moxifloxacin	0.015 - >8	1	8	39.6
Gatifloxacin	0.03 - >8	2	8	40.6
Ciprofloxacin	0.06 - >8	>8	>8	37.6
Azithromycin	0.25 - >8	>8	>8	26.7
Tobramycin	0.015 - >32	2	32	64.4
Levofloxacin	0.06 - >8	4	>8	39.6
Oxacillin	≤0.06 - >8	>8	>8	31.7
<i>Staphylococcus hominis</i> (N = 50)				
Besifloxacin	0.015 - 2	0.25	1	na
Moxifloxacin	0.03 - >8	1	4	34.0
Gatifloxacin	0.03 - >8	1	4	32.0
Ciprofloxacin	0.06 - >8	8	>8	30.0
Azithromycin	0.12 - >8	>8	>8	16.0
Tobramycin	0.015 - >32	16	32	32.0
Levofloxacin	0.06 - >8	8	>8	30.0
Oxacillin	≤0.06 - >8	>8	>8	16.0

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Staphylococcus lugdunensis</i> (N=15)				
Besifloxacin	0.015 - 2	0.06	0.5	na
Moxifloxacin	0.03 - >8	0.12	2	73.3
Gatifloxacin	0.03 - 8	0.12	2	73.3
Ciprofloxacin	0.06 - >8	0.12	>8	66.7
Azithromycin	0.25 - >8	>8	>8	46.7
Tobramycin	0.03 - >32	0.12	32	60.0
Levofloxacin	0.06 - >8	0.25	>8	66.7
Oxacillin	≤0.06 - >8	0.5	>8	60.0
<i>Staphylococcus saprophyticus</i> (N = 101)				
Besifloxacin	0.015 - 0.25	0.06	0.12	na
Moxifloxacin	0.03 - 0.25	0.12	0.12	100.0
Gatifloxacin	0.03 - 0.25	0.12	0.25	100.0
Ciprofloxacin	0.06 - 0.5	0.25	0.5	100.0
Azithromycin	0.12 - >8	1	>8	54.5
Tobramycin	≤0.008 - 32	0.015	0.06	99.0
Levofloxacin	0.06 - 0.5	0.5	0.5	100.0
Oxacillin	≤0.06 - >8	0.5	1	9.9
<i>Staphylococcus warneri</i> (N = 50)				
Besifloxacin	0.015 - 2	0.06	1	na
Moxifloxacin	0.015 - >8	0.06	4	76.0
Gatifloxacin	0.03 - >8	0.12	4	76.0
Ciprofloxacin	0.06 - >8	0.25	>8	74.0
Azithromycin	0.12 - >8	>8	>8	34.0
Tobramycin	0.015 - >32	0.06	8	86.0
Levofloxacin	0.06 - >8	0.12	>8	76.0
Oxacillin	≤0.06 - >8	0.5	>8	46.0
<i>Streptococcus agalactiae</i> (N = 100)				
Besifloxacin	0.03 - 0.12	0.06	0.06	na
Moxifloxacin	0.06 - 1	0.12	0.25	na
Gatifloxacin	0.12 - 1	0.25	0.25	100.0
Ciprofloxacin	0.5 - 8	0.5	1	na
Azithromycin	0.015 - >8	0.06	>8	73.0
Tobramycin	8 - >128	32	64	na
Levofloxacin	0.25 - 4	0.5	1	98.0
Penicillin	≤0.015 - 0.06	0.03	0.06	100.0

Table 5. Activity of Besifloxacin and Comparators Against Gram-positive Bacteria (continued)

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Streptococcus pneumoniae</i> (all phenotypes, N=35)				
Besifloxacin	0.015 - 0.5	0.06	0.06	na
Moxifloxacin	0.03 - 2	0.06	0.12	97.1
Gatifloxacin	0.015 - 4	0.12	0.25	97.1
Ciprofloxacin	0.03 - >8	0.5	1	91.4
Azithromycin	0.06 - >8	0.06	>8	62.9
Tobramycin	8 - 32	16	32	na
Levofloxacin	0.5 - 8	0.5	1	97.1
Penicillin	≤0.015 - >4	≤0.015	4	88.6
<i>Streptococcus pneumoniae</i> (PSSP, N=31)				
Besifloxacin	0.015 - 0.5	0.06	0.06	na
Moxifloxacin	0.03 - 2	0.06	0.12	96.8
Gatifloxacin	0.015 - 4	0.12	0.25	96.8
Ciprofloxacin	0.03 - >8	0.5	1	90.3
Azithromycin	0.06 - >8	0.06	>8	67.7
Tobramycin	8 - 32	16	32	na
Levofloxacin	0.5 - 8	0.5	1	96.8
Penicillin	≤0.015 - 0.5	≤0.015	0.25	100.0
<i>Streptococcus pneumoniae</i> (PISP, N=2)				
Besifloxacin	0.06 - 0.12	na	na	na
Moxifloxacin	0.12 - 0.12	na	na	100.0
Gatifloxacin	0.25 - 0.25	na	na	100.0
Ciprofloxacin	0.5 - 1	na	na	100.0
Azithromycin	>8 - >8	na	na	0.0
Tobramycin	16 - 32	na	na	na
Levofloxacin	0.5 - 1	na	na	100.0
Penicillin	4 - 4	na	na	0.0
<i>Streptococcus pneumoniae</i> (PRSP, N=2)				
Besifloxacin	0.03 - 0.06	na	na	na
Moxifloxacin	0.06 - 0.25	na	na	100.0
Gatifloxacin	0.12 - 0.25	na	na	100.0
Ciprofloxacin	0.5 - 1	na	na	0.0
Azithromycin	0.06 - >8	na	na	50.0
Tobramycin	16 - 32	na	na	na
Levofloxacin	0.5 - 1	na	na	100.0
Penicillin	>4 - >4	na	na	0.0

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Streptococcus pyogenes</i> (N=101)				
Besifloxacin	0.03 - 0.06	0.03	0.06	na
Moxifloxacin	0.06 - 0.5	0.12	0.25	na
Gatifloxacin	0.06 - 0.5	0.12	0.25	100.0
Ciprofloxacin	0.12 - 2	0.5	0.5	na
Azithromycin	0.03 - >8	0.06	8	85.1
Tobramycin	4 - 64	16	16	na
Levofloxacin	0.25 - 2	0.5	0.5	100.0
Penicillin	≤0.015 - 0.06	≤0.015	≤0.015	100.0
Lancefield group C,F,G streptococci (N=50)				
Besifloxacin	0.015 - 0.25	0.03	0.06	na
Moxifloxacin	0.03 - 1	0.12	0.12	na
Gatifloxacin	0.06 - 2	0.12	0.25	98.0
Ciprofloxacin	0.12 - >8	0.5	0.5	na
Azithromycin	0.008 - >8	0.06	>8	74.0
Tobramycin	2 - 32	8	16	na
Levofloxacin	0.12 - 8	0.5	0.5	98.0
Penicillin	≤0.015 - 0.06	≤0.015	0.06	100.0
Viridans streptococci ^c (N=156)				
Besifloxacin	0.015 - 2	0.06	0.12	na
Moxifloxacin	0.03 - 4	0.12	0.25	na
Gatifloxacin	0.03 - 8	0.25	0.5	na
Ciprofloxacin	0.12 - >8	1	4	na
Azithromycin	0.008 - >8	0.06	>8	53.2
Tobramycin	0.5 - 128	16	32	na
Levofloxacin	0.12 - >8	1	1	95.5
Penicillin	≤0.015 - >4	0.06	1	76.3

^a MSSA: methicillin susceptible *S. aureus*, MRSA: methicillin resistant *S. aureus*, MSSE: methicillin susceptible *S. epidermidis*, MRSE: methicillin resistant *S. epidermidis*, CS: ciprofloxacin susceptible, CNS: ciprofloxacin non-susceptible, PSSP: penicillin susceptible *S. pneumoniae*, PISP: penicillin intermediate *S. pneumoniae*, PRSP: penicillin resistant *S. pneumoniae*, VSE: vancomycin susceptible enterococci, VRE: vancomycin resistant enterococci

^b Percent of susceptible isolates. Clinical and Laboratory Standards Institute's breakpoints were not available for some antibacterials for the interpretation as susceptible, intermediate, or resistant.

^c Viridans group streptococci consisted of 2 *S. anginosus*, 13 *S. bovis*, 7 *S. constellatus*, 28 *S. intermedius*, 51 *S. mitis*, 22 *S. oralis*, 2 *S. salivarius*, 17 *S. sanguis*, and 14 other viridans group species.

na = Not applicable.

Table 6. Activity of Besifloxacin and Comparators Against Gram-negative Bacteria

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Citrobacter koseri</i> (N = 100)				
Besifloxacin	0.03 - >8	0.06	0.25	na
Levofloxacin	0.015 - >8	0.03	0.12	99.0
Moxifloxacin	0.015 - >8	0.03	0.25	na
Gatifloxacin	0.008 - >8	0.015	0.12	99.0
Ciprofloxacin	0.004 - >8	0.008	0.06	99.0
Tobramycin	0.25 - 16	0.5	1	99.0
Azithromycin	2 - >8	8	>8	na
Ceftazidime	0.06 - 4	0.12	0.5	100.0
<i>Haemophilus influenzae</i> (all phenotypes, N= 40)				
Besifloxacin	≤0.004 - 0.03	0.015	0.015	na
Moxifloxacin	0.008 - 0.03	0.015	0.03	100.0
Gatifloxacin	≤0.004 - 0.015	0.008	0.008	100.0
Ciprofloxacin	0.008 - 0.015	0.008	0.008	100.0
Azithromycin	≤0.004 - 2	0.5	1	100.0
Tobramycin	0.06 - 4	2	4	na
Levofloxacin	0.008 - 0.015	0.015	0.015	100.0
<i>Haemophilus influenzae</i> (bla negative, N = 24)				
Besifloxacin	≤0.004 - 0.03	0.015	0.015	na
Moxifloxacin	0.008 - 0.03	0.015	0.03	100.0
Gatifloxacin	≤0.004 - 0.015	0.008	0.008	100.0
Ciprofloxacin	0.008 - 0.015	0.008	0.015	100.0
Azithromycin	≤0.004 - 2	0.5	2	100.0
Tobramycin	0.06 - 4	2	4	na
Levofloxacin	0.008 - 0.015	0.015	0.015	100.0
<i>Haemophilus influenzae</i> (bla positive, N = 16)				
Besifloxacin	0.008 - 0.03	0.015	0.03	na
Moxifloxacin	0.008 - 0.03	0.03	0.03	100.0
Gatifloxacin	≤0.004 - 0.015	0.008	0.008	100.0
Ciprofloxacin	0.008 - 0.015	0.008	0.008	100.0
Azithromycin	0.06 - 1	0.5	1	100.0
Tobramycin	0.5 - 4	2	2	na
Levofloxacin	0.015 - 0.015	0.015	0.015	100.0

Test drug ^a	Species (phenotype, no. of isolates)			%S ^b
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Klebsiella oxytoca</i> (N = 50)				
Besifloxacin	0.06 - 8	0.12	1	na
Levofloxacin	0.015 - 8	0.03	0.5	90.0
Moxifloxacin	0.03 - 8	0.06	2	na
Gatifloxacin	0.015 - 8	0.03	0.5	92.0
Ciprofloxacin	0.008 - >8	0.015	0.5	90.0
Tobramycin	0.25 - 8	0.5	1	96.0
Azithromycin	8 - >8	>8	>8	na
Ceftazidime	0.03 - 1	0.12	0.5	100.0
<i>Legionella pneumophila</i> (N = 50)				
Besifloxacin	0.015 - 0.06	0.03	0.03	na
Levofloxacin	0.015 - 0.06	0.03	0.03	na
Moxifloxacin	0.015 - 0.06	0.03	0.06	na
Gatifloxacin	0.015 - 0.06	0.03	0.06	na
Ciprofloxacin	0.015 - 0.06	0.03	0.03	na
Tobramycin	0.25 - 4	1	2	na
Azithromycin	0.03 - 1	0.12	1	na
<i>Moraxella catarrhalis</i> (N = 101)				
Besifloxacin	0.015 - 0.12	0.03	0.03	na
Levofloxacin	0.015 - 0.5	0.015	0.03	100.0
Moxifloxacin	0.015 - 0.12	0.03	0.03	na
Gatifloxacin	0.008 - 0.25	0.015	0.015	na
Ciprofloxacin	0.008 - 0.25	0.015	0.015	100.0
Tobramycin	0.03 - 0.5	0.25	0.25	na
Azithromycin	0.015 - 0.06	0.03	0.03	100.0
Oxacillin	0.25 - >8	4	8	na
<i>Morganella morganii</i> (N = 51)				
Besifloxacin	0.03 - >8	0.12	4	na
Levofloxacin	0.015 - >8	0.06	8	76.5
Moxifloxacin	0.03 - >8	0.25	>8	na
Gatifloxacin	0.015 - >8	0.12	>8	74.5
Ciprofloxacin	0.004 - >8	0.015	>8	76.5
Tobramycin	0.25 - 32	1	4	90.2
Azithromycin	8 - >8	>8	>8	na
Ceftazidime	0.03 - >32	0.12	16	82.4

^a bla: beta-lactamase

^b Percent of susceptible isolates. Clinical and Laboratory Standards Institute's breakpoints were not available for some antibacterials for the interpretation as susceptible, intermediate, or resistant.

na = Not applicable.

Table 7. Activity of Besifloxacin and Comparators Against Anaerobic Bacteria

Test drug	Species (phenotype, no. of isolates)			%S ^a
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Bacteroides fragilis</i> (N=20)				
Besifloxacin	0.25 - 2	0.5	1	na
Moxifloxacin	0.25 - 8	0.5	2	95.0
Gatifloxacin	1 - 16	2	4	na
Clindamycin	0.5 - >8	2	>8	65.0
Metronidazole	2 - 2	2	2	100.0
<i>Clostridium perfringens</i> (N=21)				
Besifloxacin	0.12 - 0.25	0.25	0.25	na
Moxifloxacin	0.25 - 0.5	0.5	0.5	100.0
Gatifloxacin	0.5 - 1	1	1	na
Clindamycin	0.06 - 4	2	4	85.7
Metronidazole	1 - 4	2	4	100.0
<i>Fusobacterium</i> spp.(N=21)				
Besifloxacin	0.12 - 8	0.25	1	na
Moxifloxacin	0.25 - >16	1	2	95.2
Gatifloxacin	0.5 - >16	1	4	na
Clindamycin	0.06 - 8	0.06	2	95.2
Metronidazole	<0.12 - 2	0.25	1	100.0

Test drug	Species (phenotype, no. of isolates)			%S ^a
	MIC (µg/mL)			
	Range	MIC ₅₀	MIC ₉₀	
<i>Prevotella</i> spp. (N=20)				
Besifloxacin	0.06 - 16	1	4	na
Moxifloxacin	0.12 - >16	4	8	45.0
Gatifloxacin	0.25 - >16	8	16	na
Clindamycin	≤0.03 - >8	≤0.03	>8	85.0
Metronidazole	0.25 - 8	4	4	100.0
<i>Propionibacterium acnes</i> (N=21)				
Besifloxacin	0.12 - 0.25	0.25	0.25	na
Moxifloxacin	0.25 - 0.25	0.25	0.25	100.0
Gatifloxacin	0.25 - 0.5	0.25	0.5	na
Clindamycin	≤0.03 - 2	0.06	0.12	100.0
Metronidazole	>16 - >16	>16	>16	0.0

^a Percent of susceptible isolates. Clinical and Laboratory Standards Institute's breakpoints were not available for some antibacterials for the interpretation as susceptible, intermediate, or resistant.
na = Not applicable.

Table 8. Integrated Summary of Besifloxacin MIC Data for Pathogens Associated With Bacterial Conjunctivitis From Preclinical Studies

Organism	No. of Studies	Total N	Besifloxacin		
			MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Range
Combined					
Key Organisms ^a	5	1205	0.06	1	≤ 0.004 - >8
Key Organisms ^a Quinolone-S ^b	5	894	0.06	0.12	≤ 0.004 - 1
Gram-positive					
<i>Corynebacterium</i> species ^c	1	30	0.25	2	≤ 0.06 - 2
<i>Staphylococcus aureus</i> ^d					
MRSA-QR	3	73	1	4	0.25 - 8
MRSA-QS	3	36	0.03	0.06	0.015 - 0.25
MSSA-QR	2	12	0.25	4	0.03 - 4
MSSA-QS	3	80	0.03	0.06	0.015 - 0.12
<i>Staphylococcus epidermidis</i> ^d					
MRSE-QR	2	32	2	4	0.25 - 8
MRSE-QS	2	23	0.03	0.06	0.015 - 0.12
MSSE-QR	2	5	0.5	---	0.25 - 1
MSSE-QS	2	39	0.03	0.06	0.015 - 0.06
<i>Staphylococcus hominis</i> ^b					
Quinolone-S	1	15	0.03	0.06	0.015 - 0.06
Quinolone-R	1	35	0.25	1	0.125 - 2
<i>Staphylococcus lugdunensis</i> ^b					
Quinolone-S	1	10	0.03	0.06	0.015 - 0.06
Quinolone-R	1	5	0.5	2	0.125 - 2
<i>Streptococcus mitis</i> group ^c	1	90	0.06	0.12	0.015 - 2
<i>Streptococcus oralis</i>	1	22	0.06	0.12	0.03 - 2
<i>Streptococcus pneumoniae</i> ^b					
Quinolone-R	1	23	1	4	0.5 - >8
Penicillin-S	3	123	0.12	0.12	0.015 - 1
Penicillin-I	2	28	0.12	0.12	0.03 - 0.25
Penicillin-R	3	61	0.12	0.12	0.03 - 0.25
<i>Streptococcus pyogenes</i>	2	201	0.06	0.12	0.03-0.12
<i>Streptococcus salivarius</i>	1	2	---	---	0.06

Table 8. Integrated Summary of Besifloxacin MIC Data for Pathogens Associated With Bacterial Conjunctivitis From Preclinical Studies (*continued*)

Organism	No. of Studies	Total N	Besifloxacin		
			MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Range
Gram-negative					
<i>Acinetobacter lwoffii</i>	1	13	0.5	0.5	0.12 - 2
<i>Acinetobacter baumannii</i> ^b					
Quinolone-S	1	43	0.5	2	0.25 - 4
Quinolone-R	1	5	>8	---	2 - >8
<i>Acinetobacter baumannii-calcoaceticus</i> ^b					
Quinolone-S	1	23	0.5	1	0.12 - 4
Quinolone-R	1	10	>8	>8	8 - >8
<i>Citrobacter koser</i> ^b					
Quinolone-S	1	99	0.06	0.25	0.03 - 4
Quinolone-R	1	1	---	---	>8
<i>Enterobacter cloacae</i> ^b					
Quinolone-S	1	58	0.25	0.5	0.12 - 2
Quinolone-R	1	1	---	---	>8
<i>Enterobacter aerogene</i> ^b					
Quinolone-S	1	37	0.25	2	0.12 - 4
Quinolone-R	1	2	---	---	4 - >8
<i>Haemophilus influenzae</i> (all phenotypes)	3	243	0.03	0.06	≤ 0.004 - 0.25
β-lactamase +	3	118	0.03	0.06	0.008 - 0.12
β-lactamase –	3	100	0.03	0.03	≤ 0.004 - 0.12
β-lactamase – Ampicillin-R	1	25	0.12	0.25	0.015 - 0.25
<i>Klebsiella oxytoca</i> ^b					
Quinolone-S	1	45	0.12	0.5	0.06 - 1
Quinolone-R	1	5	8	---	1 - 8
<i>Legionella pneumophila</i>	1	50	0.03	0.03	0.015 - 0.06
<i>Moraxella catarrhalis</i>	2	201	0.06	0.12	0.015 - 0.12
<i>Moraxella</i> species ^c	1	30	≤ 0.06	0.13	≤ 0.06 - 0.13
<i>Morganella morganii</i> ^b					
Quinolone-S	1	39	0.12	1	0.03 - 2
Quinolone-R	1	12	4	8	2 - >8
<i>Neisseria gonorrhoeae</i>	1	103	0.015	0.015	0.004 - 2
<i>Pseudomonas aeruginosa</i> ^b					
Quinolone-S	1	49	1	2	0.5 - 4
Quinolone-R	1	51	>8	>8	2 - >8

Table 8. Integrated Summary of Besifloxacin MIC Data for Pathogens Associated With Bacterial Conjunctivitis From Preclinical Studies (*continued*)

Organism	No. of Studies	Total N	Besifloxacin		
			MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Range
Gram-negative (continued)					
<i>Proteus mirabilis</i> ^b					
Quinolone-S	1	98	0.5	1	0.25 - 4
Quinolone-R	1	2	---	---	2 - >8
<i>Serratia marcescens</i> ^b					
Quinolone-S	1	98	1	2	0.25 - 2
Quinolone-R	1	2	---	---	4 - >8
Anaerobes ^c					
<i>Clostridium perfringens</i>	1	21	0.25	0.25	0.12 - 0.25
<i>Propionibacterium acnes</i>	1	21	0.25	0.25	0.12 - 0.25
<i>Bacteroides fragilis</i>	1	20	0.5	1	0.25 - 2
<i>Fusobacterium</i> species	1	21	0.25	1	0.12 - 8
<i>Prevotella</i> species	1	20	1	4	0.06 - 16

MIC₅₀ = Minimum inhibitory concentration required to inhibit the growth of 50% of organisms; MIC₉₀ = Minimum inhibitory concentration required to inhibit the growth of 90% of organisms.

^aPresented are MIC values from nonclinical studies for the following key organisms: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Streptococcus pneumoniae*, *Streptococcus oralis*, *Streptococcus mitis* group, *Streptococcus salivarius*, *Haemophilus influenzae*, and *Moraxella catarrhalis*.

^bIn this table, CLSI breakpoints for additional fluoroquinolones were utilized to define quinolone resistant (QR) or quinolone susceptible (QS) subsets. QR subsets in this table thus include all isolates categorized as intermediate or resistant either to ciprofloxacin, gatifloxacin, levofloxacin, or ofloxacin as appropriate.

^cMIC values were obtained by the agar dilution method.

^dMRSA = Methicillin-resistant *S. aureus*; MSSA = Methicillin-susceptible *S. aureus*; MRSE = Methicillin-resistant *S. epidermidis*; MSSE = Methicillin-susceptible *S. epidermidis*; S = Susceptible; R = Resistant.

^eIn this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

4.1.5 Comparison of Besifloxacin MIC Data From Clinical and Non-clinical Studies

In addition to the nonclinical studies, all baseline pathogens isolated from three besifloxacin safety and efficacy clinical trials (studies 373, 433, and 434) were tested for susceptibility to various ophthalmic antibacterial agents, including besifloxacin.

Overall, isolates cultured in the three besifloxacin clinical efficacy trials yielded besifloxacin activity profiles similar to those seen in the nonclinical data. A total of 1324 isolates were recovered from patients at baseline (Visit 1) in the culture-confirmed population species-specific study eye across all treatment groups (besifloxacin ophthalmic suspension, vehicle, and Vigamox). The MIC₅₀/MIC₉₀ values for the 1324 isolates of all species were 0.06/0.25 µg/mL for besifloxacin. Of the 1324 bacterial isolates, 886 (66.9%) were Gram-positive, while the remaining 438 (33.1%) were Gram-

negative. The besifloxacin MIC₅₀/MIC₉₀ values were 0.06/0.25 µg/mL for Gram-positive bacteria and 0.03/0.5 µg/mL for Gram-negative bacteria.

When the 1324 clinical isolates were compared with 1205 nonclinical isolates of key organisms, a similar besifloxacin MIC distribution was observed. The besifloxacin MIC₅₀ was 0.06 µg/mL for both clinical and nonclinical isolates. Because higher proportions of quinolone-resistant strains were present in the nonclinical studies than were recovered during besifloxacin clinical trials, the overall nonclinical isolate MIC₉₀ value was 4-fold higher than the clinical MIC₉₀ value (1 and 0.25 µg/mL, respectively). However, besifloxacin MIC distributions were similar when clinical isolates were compared to only the 894 quinolone-susceptible nonclinical isolates, with equivalent MIC₅₀/MIC₉₀ values between all clinical isolates and quinolone-susceptible nonclinical isolates (0.06/0.25 and 0.06/0.12 µg/mL, respectively)

4.2 Toxicology

As a class, fluoroquinolones demonstrate a characteristic safety profile that can include QT prolongation, positive genotoxicity, electroretinography (ERG) changes, phototoxicity, and adverse effects on joint tissues. The nonclinical development program for besifloxacin was designed to characterize these potential class effects, as well as other potential toxic effects specific to besifloxacin.

The nonclinical safety testing program for investigating the toxicity profile of besifloxacin, either as the final product formulated in the DuraSite vehicle or as a new chemical substance, was conducted in compliance with Good Laboratory Practice regulations.

The ocular tolerance of besifloxacin was shown to be acceptable with no adverse effects observed in rabbits and dogs after QID dosing for 28 days. ERG measurements were included in the studies due to the known retinal toxicity associated with some fluoroquinolones. A 1-year ocular instillation study with the DuraSite vehicle (which contained up to 1.3% polycarbophil) demonstrated no signs of ocular or systemic toxicity in rabbits.

The systemic toxicity profile of besifloxacin was evaluated in 28-day repeat dose studies in rats and dogs. In the 4-week oral study in rats, the no effect level was established at the highest dose tested. In a 4-week oral study in dogs, the no effect level was based on mild and reversible effects seen at a higher dose. Any systemic effects of besifloxacin in these studies were observed at systemic exposure levels of besifloxacin that were at least 150-fold higher than the systemic exposure observed after topical ophthalmic use in humans. In safety pharmacology studies, besifloxacin was shown, like other fluoroquinolones, to have slight effects on the cardiovascular system. The *in vivo* cardiovascular effects, specifically, an increase in QT duration, following besifloxacin systemic dosing were only observed after doses that were at least 300 times the intended ocular daily dose. There was no change in heart rate, blood pressure, or cardiac conduction.

Comparisons between the identified no observable adverse effect level (NOAEL) in animals and the intended dosing level in patients allowed the calculation of satisfactory safety

margins, indicating an absence of any risks to humans. Although some fluoroquinolones have been reported to possibly affect bones and joints in certain juvenile animal species (Johnson et al., 2000; Nagai et al., 2002), no such changes were detected with besifloxacin.

Besifloxacin is not considered directly DNA reactive, although there were positive responses in some genotoxicity assays. The besifloxacin genotoxicity profile is not regarded as unexpected, but rather characteristic of the fluoroquinolone class of compounds. Fluoroquinolones, as inhibitors of topoisomerases, are well known to indirectly interfere with DNA replication and therefore may confound the interpretation of the *in vitro* or *in vivo* genotoxicity studies (Chetelat, 1996; Gocke, 1991; Mukherjee et al., 1993). Overall, the genotoxicity/carcinogenic risk due to besifloxacin is considered negligible due to the nature of the effects coupled with the low systemic exposure after topical ocular instillation and the short intended duration of treatment.

Results from the reproductive toxicity testing program did not show any potential risk for patients treated with besifloxacin considering the low systemic uptake and the satisfactory safety margin between the NOAEL doses in the toxicity studies and the intended dosing level in patients. In general, any effects observed in these studies occurred at the same or higher concentrations than the parental toxicity with a systemic safety margin of at least 150-fold, based on exposure.

Photosensitivity is a common side effect in patients after oral administration of fluoroquinolones containing a chloride atom at position 8 (Stahlmann & Lode, 1999); therefore, the photosafety of besifloxacin was investigated *in vivo* using systemic and cutaneous routes of administration. Although effects (ear erythema and edema) were seen after very high oral doses of the drug substance in mice, no phototoxicity findings were observed following topical administration of either a preliminary product formulation (up to 1% besifloxacin in aqueous solution) or the final product (0.6% besifloxacin in the DuraSite vehicle) in guinea pigs. Considering the intended local administration route and the short usage pattern, no related effects are expected in patients treated with besifloxacin.

In conclusion, the overall nonclinical profile obtained with besifloxacin is not unexpected, and demonstrates similarities with other fluoroquinolones. Systemic effects of besifloxacin were only observed at plasma concentrations that would not likely be achieved following ocular administration of besifloxacin ophthalmic suspension. Consequently, these effects, while in some cases consistent with the class effects observed with other fluoroquinolones, present no reasonable risk to humans following ocular use of the product.

4.3 Pharmacokinetics

Results from the nonclinical ocular pharmacokinetic (PK) studies in rabbits and monkeys indicate that topical ocular administration of besifloxacin ophthalmic suspension, 0.6%, is associated with rapid absorption and distribution of besifloxacin into ocular tissues. Following the initial rapid absorption into ocular tissues, besifloxacin is eliminated from these tissues with an apparent half-life of more than 5 hours. Repeated (twice daily [BID], TID, and QID) topical ocular administration of besifloxacin was associated with low systemic exposure ($C_{\max} < 0.025 \mu\text{g/g}$ in non-excretory organs), while increased exposure to

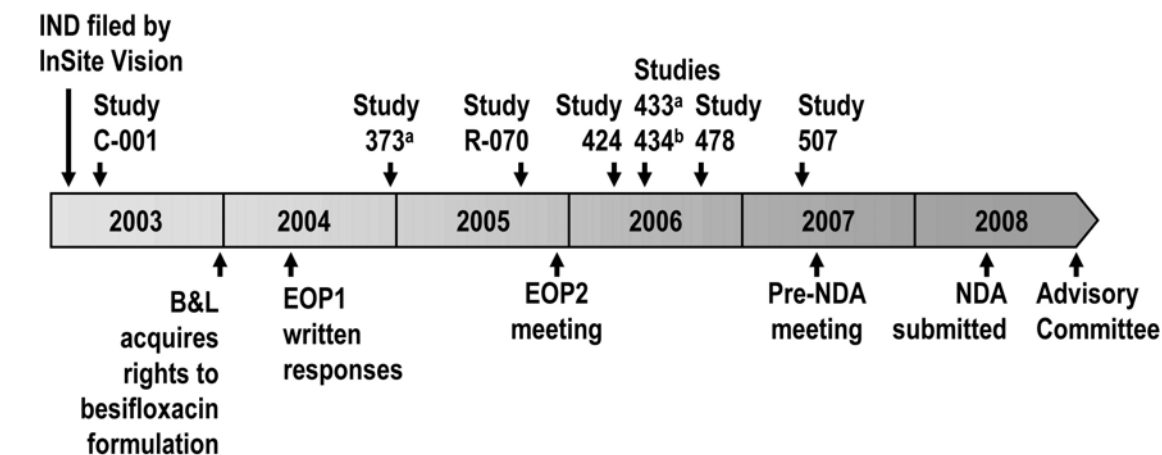
pigmented ocular tissues was observed following QID dosing. The prolonged retention of besifloxacin in pigmented tissues suggests that this compound binds to melanin, which is a characteristic shared by other fluoroquinolones (Ono & Tanaka, 2003; Perez et al., 2002; Siefert et al., 1999; Tanaka et al., 2004). However, based on the extensive ocular safety data available for besifloxacin in animals and humans, any binding of besifloxacin to melanin, if present, appears to occur without adverse consequences. The binding of besifloxacin to plasma proteins was less than 50% in rats and humans, and besifloxacin was not associated with extensive distribution into blood cells.

Systemic exposure to besifloxacin following topical ocular administration in rabbits and monkeys was low, with peak plasma besifloxacin concentrations of 7.6 and 9.2 ng/mL, respectively, on average. Results from ocular and systemic PK studies indicate that besifloxacin distributes widely out of the systemic circulation, with low but measurable levels of radioactivity observed in all tissues studied after topical ocular administration of [¹⁴C]besifloxacin. Besifloxacin is eliminated primarily unchanged via fecal and urinary pathways, with the fecal route predominating and more than 80% of the administered dose eliminated within 24 hours. These *in vivo* findings are consistent with the overall metabolic stability that was observed following *in vitro* incubation of besifloxacin with hepatocytes from mice, rats, rabbits, dogs, and humans.

5 CLINICAL DEVELOPMENT PROGRAM AND REGULATORY HISTORY OF BESIFLOXACIN

5.1 Overview

Besifloxacin ophthalmic suspension is a new molecular entity, developed exclusively for an ophthalmic indication. In 2004, Bausch & Lomb Incorporated (Rochester, New York) acquired the rights to besifloxacin formulation from InSite Vision (Alameda, California). Between 2003 and 2007, a total of 8 clinical studies has been conducted to evaluate the efficacy, safety, and tolerability of besifloxacin ophthalmic suspension (Figure 3).



^aComparator for studies 373 and 433 was vehicle.

^bComparator for study 434 was Vigamox.

Figure 3. Besifloxacin Product Development

5.2 Development of Clinical Studies

Clinical investigation of besifloxacin ophthalmic suspension was initiated by InSite Vision in the spring of 2003. A Phase 1 trial, Study C-02-403-001, was conducted to evaluate the systemic safety, PK, and ocular safety/tolerability of 0.3% and 0.6% ophthalmic suspension formulations of besifloxacin versus vehicle. The results from this trial suggested that both concentrations of besifloxacin ophthalmic suspension were as well tolerated and safe as its vehicle. Based on these results as well as nonclinical data that demonstrated superior ocular exposure to besifloxacin at the higher concentration, besifloxacin ophthalmic suspension, 0.6%, was selected for further clinical development.

In 2004, Bausch & Lomb Incorporated acquired the rights to besifloxacin formulation from InSite Vision, and in December of that year, a US-based safety and efficacy trial (Study 373) was initiated to compare besifloxacin ophthalmic suspension to its vehicle for the treatment of bacterial conjunctivitis. In this study, besifloxacin ophthalmic suspension demonstrated superior clinical resolution of bacterial conjunctivitis and microbial eradication of the infecting organisms when compared to vehicle at Visit 3 (Day 8 or 9).

In September 2005, a 1-day randomized trial of besifloxacin ophthalmic suspension compared to Vigamox was conducted to evaluate the effect of a single dose administration of study drug in healthy volunteers on visual acuity (VA) (Study R0C2-05-070). The immediate post-drop VA (20/37) and recovery time (57.7 sec) associated with besifloxacin treatment was considered clinically acceptable. No statistically significant differences were noted between the test and control eyes with respect to corneal and conjunctival staining. In April 2006, Study 424 was initiated to evaluate the ocular PK of besifloxacin ophthalmic suspension in healthy volunteers. Results from this study demonstrated that besifloxacin ophthalmic suspension resulted in therapeutic levels of besifloxacin in tears that were sustained, on average, for at least 24 hours after a single dose.

In June 2006, 2 safety and efficacy clinical trials were initiated to compare besifloxacin ophthalmic suspension to vehicle (Study 433) and to Vigamox (Study 434) for the treatment of bacterial conjunctivitis. Study 433 was conducted entirely in the US and Study 434 was conducted in the US and Asia. In Study 433, besifloxacin ophthalmic suspension demonstrated superior clinical resolution of bacterial conjunctivitis and microbial eradication of the infecting organisms when compared to vehicle at Visit 2 (Day 5 \pm 1). In Study 434, besifloxacin ophthalmic suspension was found to be non-inferior to Vigamox for clinical resolution and microbial eradication of baseline bacterial infection at Visit 2 (Day 5 \pm 1).

In October 2006, Study 478 was initiated to evaluate the systemic PK of besifloxacin ophthalmic suspension in patients with a clinical diagnosis of acute bilateral bacterial conjunctivitis. Single/multiple-dosing by topical administration (TID for 5 days with a final single dose the morning of Day 6) did not result in meaningful systemic exposure in these patients. In May 2007, Study 507 was conducted in healthy volunteers to evaluate the effect of TID dosing of besifloxacin ophthalmic suspension for 5 days on corneal endothelial cell density. Besifloxacin ophthalmic suspension produced no statistically or clinically significant change in endothelial cell density in this study.

5.3 Summary of Clinical Development

An overview of the 8 clinical studies conducted with besifloxacin ophthalmic suspension is provided in Table 9.

Table 9. Clinical Studies Conducted With Besifloxacin Ophthalmic Suspension

Study Number and Type	Study Design; Control	Study Objectives	Test Product, Dosage Regimen, Route and Duration of Administration	Number of Volunteers or Patients
Study C-02-403-001 Safety/PK	Single-center, randomized, double-masked, parallel-group; Vehicle	Evaluate systemic safety and ocular safety/tolerability of 0.3% and 0.6% formulations of test article vs vehicle	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% • Besifloxacin ophthalmic suspension, 0.3% • Vehicle of besifloxacin ophthalmic suspension —QID for 7 days; topical ocular	54 healthy volunteers
Study 507 Safety	Multicenter, randomized, contralateral eye; No treatment in fellow eye	Evaluate corneal endothelial cell density (cells/mm ²) changes	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% —TID for 5 days; topical ocular	120 healthy volunteers
Study ROC2-05-070 Safety	Single-center, randomized, masked, contralateral eye; Active	Evaluate visual performance after single dose of test article vs active control	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% • Vigamox®—moxifloxacin HCl ophthalmic solution, 0.5% —Single-dose; topical ocular, 1 day	19 healthy volunteers
Study 424 Ocular PK	Single-center, open-label	Assess ocular PK of besifloxacin after single administration of test article	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% —Single dose; topical ocular, 1 day	64 healthy volunteers
Study 478 Systemic PK	Multicenter, open label, single dose/multiple dose	Assess extent of systemic exposure to besifloxacin following single and multiple administrations of test article	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% —TID for 5 days, single dose day 6; topical ocular	24 patients with clinical diagnosis of acute, bilateral bacterial conjunctivitis
Study 373 Safety Efficacy	Multicenter, randomized, double-masked, parallel-group; Vehicle	Evaluate clinical and microbial efficacy of test article vs vehicle in treatment of bacterial conjunctivitis	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% • Vehicle of besifloxacin ophthalmic suspension —TID for 5 days; topical ocular	269 patients with clinical diagnosis of bacterial conjunctivitis
Study 433 Safety Efficacy	Multicenter, randomized, double-masked, parallel-group; Vehicle	Evaluate clinical and microbial efficacy of test article vs vehicle in treatment of bacterial conjunctivitis	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% • Vehicle of besifloxacin ophthalmic suspension —TID for 5 days; topical ocular	957 patients with clinical diagnosis of bacterial conjunctivitis
Study 434 Safety Efficacy	Multicenter, randomized, double-masked, parallel-group; Active	Evaluate clinical and microbial efficacy of test article vs active control in treatment of bacterial conjunctivitis	<ul style="list-style-type: none"> • Besifloxacin ophthalmic suspension, 0.6% • Vigamox®—moxifloxacin HCl ophthalmic solution, 0.5% —TID for 5 days; topical ocular	1161 patients with clinical diagnosis of bacterial conjunctivitis

5.4 Communication with the FDA

The clinical development plan, agreed upon with the FDA at the end of the Phase 2 meeting on December 6, 2005, included 2 controlled safety and efficacy studies, one being vehicle-controlled and the other a non-inferiority trial with the approved agent Vigamox to support the marketing application for besifloxacin ophthalmic suspension. As recommended by the FDA at this meeting, the non-inferiority limits for clinical resolution and microbial eradication in the active-controlled study were set at -0.15 . However, at a pre-New Drug Application (NDA) meeting with the FDA on June 6, 2007, the Agency indicated that its view on active-controlled studies involving anti-bacterial agents had evolved. Specifically, in the case of Study 434, the non-inferiority limit of -0.15 (for each of clinical resolution and microbial eradication) for which this trial was powered, did not maintain at least half the difference between Vigamox and its vehicle, as shown in Vigamox™ – FDA Review Package; NDA-21-598, where the difference for clinical resolution was 0.15 ($0.66 - 0.51$) and the difference for microbial eradication was 0.15 ($0.82 - 0.67$). Therefore, claiming non-inferiority to Vigamox using the originally recommended non-inferiority limits would not yield evidence to conclude that the rates of clinical resolution and microbial eradication for besifloxacin ophthalmic suspension were superior to the rates seen in historic data for the Vigamox vehicle. As a result, Study 434 did not meet the FDA's revised criteria for an acceptable non-inferiority margin and could not serve as an adequate and well-controlled clinical study to support efficacy for the NDA filing. Subsequently, in a letter dated July 19, 2007, the FDA determined that Study 373 could represent an adequate and well-controlled clinical study together with Study 433 to support the NDA filing.

In support of the marketing application for besifloxacin ophthalmic suspension, efficacy data were integrated from the 2 vehicle-controlled studies (Study 373 and Study 433), which independently demonstrated besifloxacin ophthalmic suspension to be superior to vehicle for both clinical resolution and eradication of baseline bacterial infection. For the analyses of bacterial eradication of individual baseline pathogens by species-specific study eyes, microbiological outcome data from the besifloxacin treatment groups in studies 373, 433, and 434 were integrated. Safety data from Studies 373, 433 and 434 have also been integrated.

6 CLINICAL PHARMACOLOGY

6.1 Pharmacokinetics

Three clinical PK studies were conducted to characterize the systemic and ocular exposure to besifloxacin following topical ocular administration. These studies evaluated the systemic exposure to besifloxacin following ascending dose groups in healthy volunteers (Study C-02-403-001), following single and repeated TID ocular administration in patients with clinically diagnosed bacterial conjunctivitis (Study 478), and the ocular exposure to besifloxacin based on levels in tear fluid of healthy volunteers (Study 424). Considering the practical and ethical limitations of performing clinical ocular PK studies in humans, no additional ocular PK studies in humans have been conducted. However, multiple nonclinical studies were conducted to fully characterize the PK and metabolism of besifloxacin (see Section 4.3).

A summary of the three clinical PK studies is presented below.

STUDY C-02-403-001—SYSTEMIC EXPOSURE TO BESIFLOXACIN FOLLOWING TOPICAL OCULAR ADMINISTRATION IN HEALTHY VOLUNTEERS

The objective of this randomized, double-masked, parallel-group study was to evaluate the ocular safety/tolerability of topical administration of 0.3% and 0.6% besifloxacin ophthalmic suspension compared with vehicle when dosed QID for 7 days in healthy volunteers. In addition, the systemic exposure to besifloxacin was assessed.

A total of 54 male and female volunteers with a mean age of 39.1 years (range, 18 to 68 years) were enrolled. Healthy volunteers were evaluated in ascending dose groups, receiving 1 drop of besifloxacin ophthalmic suspension at a concentration of either 0.3% (n = 12) or 0.6% (n = 14), QID in each eye at approximately 4-hour intervals. Fourteen patients in each stage of the study received vehicle with the same dosing schedule. Blood samples were collected before, 30 minutes and 4 hours after the first instillation of study drug, and again before and 30 minutes after the fourth instillation of study drug at Day 1 (Visit 2) to determine besifloxacin levels in plasma. In addition, a blood sample was collected at Day 2 (Visit 3) prior to study drug administration to determine plasma concentration of besifloxacin. Samples were analyzed using a validated LC/MS/MS method, with a LLOQ of 0.2 ng/mL.

All 54 healthy volunteers completed the study. Figure 4 shows the mean + SD besifloxacin concentrations (ng/mL) in plasma at each of the collection times. The plasma besifloxacin concentrations observed were less than 0.35 ng/mL, on average, for both dose groups. In general, plasma besifloxacin concentrations were highly variable and appeared to be dose-proportional. The plasma besifloxacin concentration observed in the 0.6% dose group (0.325 ± 0.227 ng/mL) was approximately twice that observed in the 0.3% dose group (0.149 ± 0.164 ng/mL) 0.5 hours after the fourth instillation at Day 1 (Visit 2).

In conclusion, this study demonstrated that topical ocular administration of besifloxacin ophthalmic suspension (0.3% and 0.6%) QID for 1 week resulted in minimal systemic exposure to besifloxacin in healthy volunteers. On average, the plasma levels of besifloxacin observed in both dose groups were less than 0.35 ng/mL.

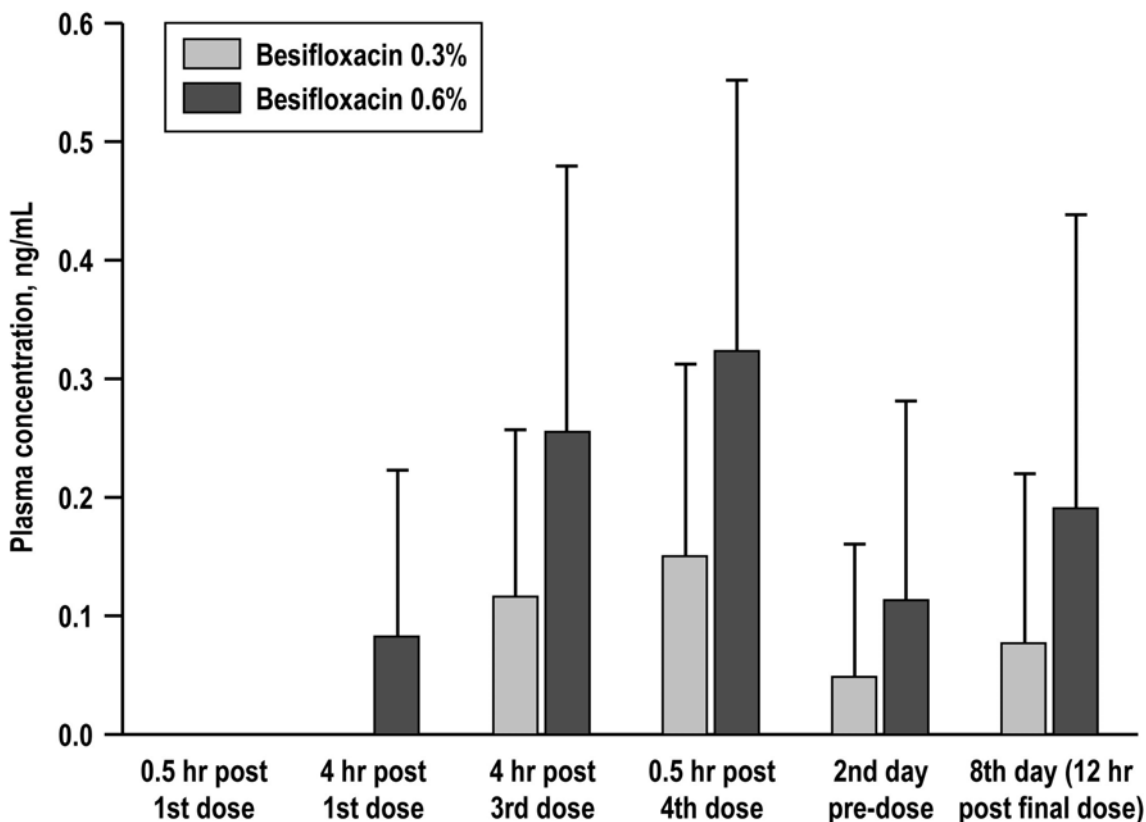


Figure 4. Mean (+ SD) Plasma Concentrations of Besifloxacin Following Single and Repeated Topical Ocular Administration of Besifloxacin Ophthalmic Suspension (0.3% or 0.6%) in Both Eyes of Healthy Volunteers

STUDY 478—SYSTEMIC EXPOSURE TO BESIFLOXACIN FOLLOWING TOPICAL OCULAR ADMINISTRATION IN PATIENTS WITH CLINICALLY DIAGNOSED BACTERIAL CONJUNCTIVITIS

The objective of this study was to assess the extent of systemic exposure to besifloxacin following single and multiple TID topical ocular instillations of besifloxacin ophthalmic suspension, 0.6%, in patients with clinically diagnosed bilateral bacterial conjunctivitis.

A total of 24 male and female patients with clinically diagnosed bacterial conjunctivitis and a mean age of 38.9 years (range, 19 to 70 years) were enrolled. However, only 22 of 24 patients were included in the PK population. Samples from the remaining 2 patients, although collected, were never received by the bioanalytical laboratory for analysis. Patients received 1 drop of besifloxacin ophthalmic suspension, 0.6%, in each eye TID for 5 days at approximately 6-hour intervals, with a final dose the morning of Day 6. Blood samples were collected on Days 1 and 6 at 0 hours (pre-dose), and 0.25, 0.5, 1, 1.5, 2, 3, 4, and 6 hours after dosing, with additional collections at 8 and 12 hours after the last dose (Day 6). In addition, blood samples were collected before administration of the morning dose (0 hours) on Days 2, 3, 4, 5, and 6 for determination of the trough besifloxacin concentration (C_{min}).

Samples were analyzed using a validated LC/MS/MS method, with an LLOQ of 0.05 ng/mL.

A slight apparent accumulation of besifloxacin was observed following TID dosing (Table 10 and Figure 5). Nonetheless, systemic exposure was very low even after the last dose, with an average C_{\max} of 0.428 ng/mL on Day 6. Accumulation ratios based on the plasma C_{\max} and AUC_{0-6} (Day 6/Day 1) values were 1.45 and 1.60, respectively. Pre-dose plasma besifloxacin concentrations were similar in most patients on Days 2, 3, 4, 5, and 6, indicating the achievement of steady-state in all patients by Day 2 of dosing. The mean apparent terminal $t_{1/2}$ was approximately 4.3 hours on Day 1 and approximately 6.8 hours on Day 6; however, the terminal $t_{1/2}$ could not be reported for the majority of patients on Day 1 because the acceptance criteria for linear regression were not met.

In conclusion, these results demonstrate that systemic exposure to besifloxacin is very low (< 0.5 ng/mL) following topical ocular administration in patients with clinically diagnosed bilateral bacterial conjunctivitis.

Table 10. PK Parameter Values for Besifloxacin After the First Dose (Day 1) and at Steady-State (Day 6) Following Topical Ocular Administration of Besifloxacin Ophthalmic Suspension (Study 478)

Parameter	Units	First Dose (Day 1)			Steady-State (Day 6)		
		N	Mean (SD)	% CV	N	Mean (SD)	%CV
C_{\max}	ng/mL	22	0.368 (0.274)	75	22	0.428 (0.299)	70
t_{\max}	hr	22	3.17 (1.74)	55	22	2.41 (2.41)	100
AUC_{0-6}	ng•hr/mL	20	1.45 (0.865)	60	22	1.95 (1.31)	67
$t_{1/2}$	hr	8	4.27 (2.22)	52	14	6.75 (2.14)	32
C_{\max} Accumulation Ratio		--	--	--	22	1.45 (0.656)	45
AUC Accumulation Ratio		--	--	--	20	1.60 (0.742)	46

C_{\max} Accumulation Ratio was calculated as the ratio of C_{\max} for Day 6/Day1.

AUC Accumulation Ratio was calculated as the ratio of AUC_{0-6} for Day 6/Day1.

-- = Not calculated.

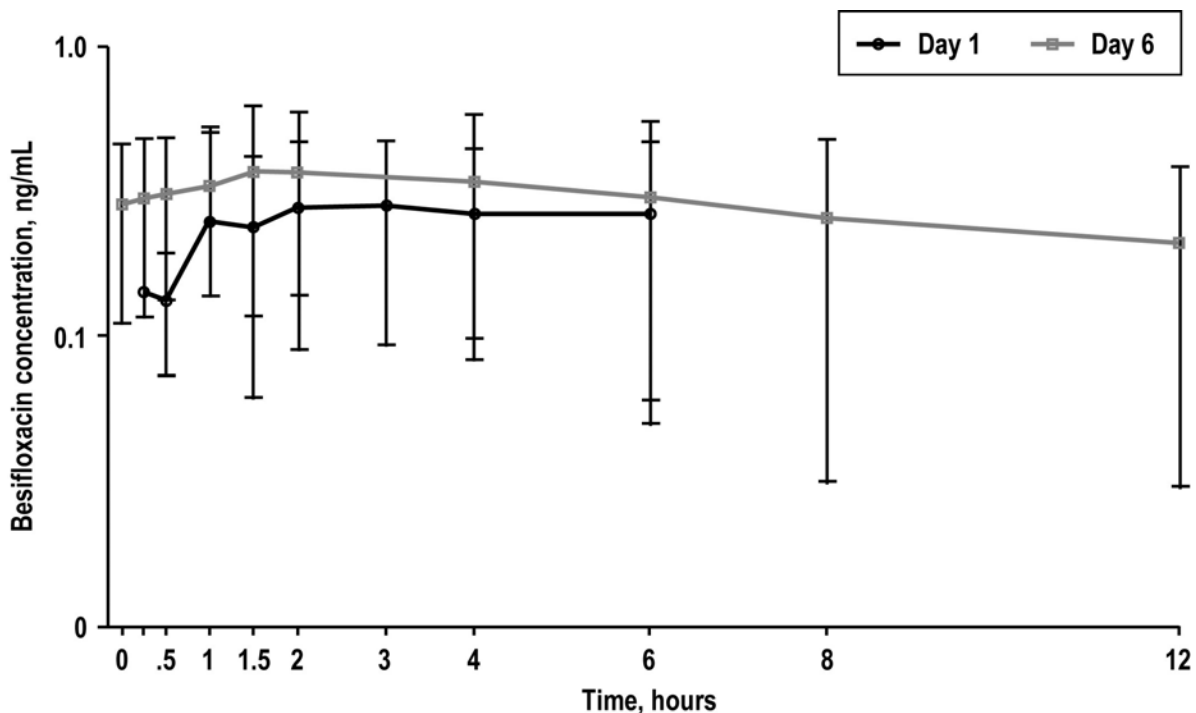


Figure 5. Plasma Concentration-Time Profile for Besifloxacin after Single (Day 1) and Repeated (Day 6) TID Administration of Besifloxacin (Study 478)

STUDY 424—OCULAR (TEAR FLUID) EXPOSURE TO BESIFLOXACIN FOLLOWING TOPICAL OCULAR ADMINISTRATION IN HEALTHY VOLUNTEERS

This study was conducted to evaluate the ocular PK of besifloxacin in tear fluid after a single instillation of besifloxacin ophthalmic suspension, 0.6%, in both eyes of healthy volunteers and to compare the actual besifloxacin exposure to MICs of the most prevalent pathogens associated with bacterial conjunctivitis.

This single-center, open-label, prospective study enrolled 64 healthy male and female volunteers with a mean age of 23.7 years (range, 18 to 39 years). Healthy volunteers received a single instillation (37 μ L by pipette) of besifloxacin ophthalmic suspension, 0.6%, in the conjunctival sac of each eye. A single tear sample was collected on a Schirmer tear strip from each healthy volunteer. Separate subgroups of healthy volunteers (8 volunteers per collection time) were sampled at each of the 8 predetermined collection times over the period of 0.17 hours to 24 hours after dosing: 10 minutes after instillation; 30 minutes after instillation; and 1, 2, 4, 8, 12, and 24 hours after instillation. Samples were analyzed using a validated LC/MS/MS method, and the LLOQ was 2 ng/mL (equivalent to approximately 0.2 μ g/g for a 10-mg tear sample).

Mean tear concentration data were obtained from the per protocol (PP) set and the full analysis (FAS) set. For the purpose of this study, the PP set included all healthy volunteers with the exclusion of apparent outlier values. The FAS set included all healthy volunteers who received besifloxacin, and from whom all sampling data were available (no exclusion of outlier values). The MIC₉₀ values used were those corresponding to the extreme values in

sensitivity from the most frequently encountered bacteria in bacterial conjunctivitis: *S. aureus* (frequent bacteria in adults and elderly people), MIC₉₀ = 1 µg/mL and *H. influenzae* (frequent bacteria in children), MIC₉₀ ≤ 0.06 µg/mL.

Mean maximum besifloxacin concentrations in tears were observed within 10 minutes after instillation (C_{max} 610 ± 540 µg/g) (Table 11 and Figure 6). Concentrations of 1.6 µg/g or higher were sustained for at least 24 hours after dosing. Based on AUC₀₋₂₄, the total exposure to besifloxacin was 1232 µg•h/g. Elimination of besifloxacin from tears occurred at an estimated half-life of 3.4 hours. Therapeutic levels of besifloxacin were achieved in tears after a single instillation, as indicated by comparing the besifloxacin tear levels to the MIC₉₀ values of 1 µg/mL for *S. aureus* and ≤ 0.06 µg/mL for *H. influenzae*. The resulting C_{max}/MIC₉₀ ratios (610 and ≥ 10167) and the AUC₂₄/MIC₉₀ ratios (1232 and ≥ 20533) based on the FAS data for *S. aureus* and *H. influenzae*, respectively, are higher than the published target values associated with bacterial eradication in plasma for fluoroquinolones.

In conclusion, topical ocular application of besifloxacin ophthalmic suspension, 0.6%, resulted in high therapeutic levels of besifloxacin in human tear samples that were sustained at a level of 1.6 µg/g or higher for at least 24 hours after a single dose. The maximum concentration of besifloxacin in tears was approximately 610-fold and 10,000-fold higher than the MIC₉₀ values for *S. aureus* and *H. influenzae*, which are 2 of the most prevalent causative ophthalmic pathogens in patients with bacterial conjunctivitis.

Table 11. PK/PD Parameter Values for Besifloxacin in Tears After Single Topical Ocular Instillation of Besifloxacin Ophthalmic Suspension in Healthy Volunteers (Study 424)

Data Set	N	t _{max} (h)	C _{max} (µg/g)	AUC ₂₄ (µg•h/g)	t _{1/2} (h)	C _{max} /MIC ₉₀ ^a	C _{max} /MIC ₉₀ ^b	AUC ₂₄ /MIC ₉₀ ^a	AUC ₂₄ /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 set.

^aMIC₉₀: *S. aureus* = 1 µg/mL.

^bMIC₉₀: *H. influenzae* ≤ 0.06 µg/mL.

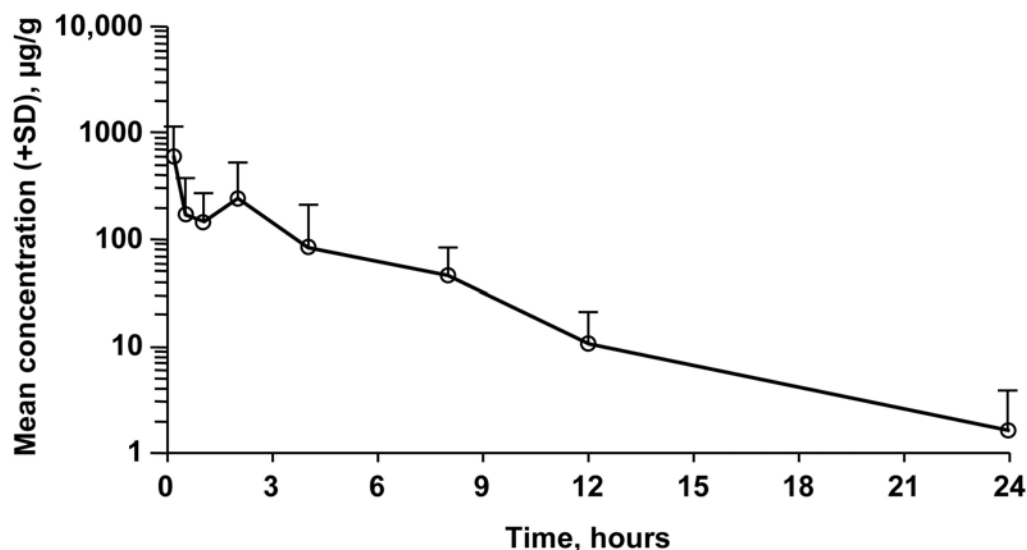


Figure 6. Besifloxacin Concentration-Time Profile in Tears after Single Administration of Besifloxacin to Healthy Volunteers (Study 424)

6.2 Pharmacokinetic/Pharmacodynamic Analyses

To evaluate the PK/pharmacodynamic (PD) relationship of besifloxacin, results from the ocular PK study in humans, Study 424 (described above), were used along with the *in vitro* MIC₉₀ values for prevalent bacterial pathogens isolated from bacterial conjunctivitis patients in besifloxacin clinical safety and efficacy studies 373, 433, and 434.

The relationship between the concentration of besifloxacin in human tear fluid and the concentration required for antimicrobial activity was quantified by calculating the ratios of C_{\max}/MIC_{90} and $\text{AUC}_{24}/\text{MIC}_{90}$. For the purpose of calculating these PK/PD ratios, a PK model was used to simulate besifloxacin concentrations with a TID dosing regimen. An additional consideration in this analysis is the potential role of protein binding, which could effectively lower the concentration of unbound (free) besifloxacin. The inhibitory effect of protein binding on antibacterial efficacy has been reported for β -lactams; however, there is no general consensus about the role of protein binding on the antibacterial activity of fluoroquinolones (Bergogne-Berezin, 2002; Craig & Ebert, 1989; Drusano, 1988; Merrikin et al., 1983; Turnidge, 1999; Zeitlinger et al., 2008). Based on the fact that besifloxacin is approximately 40% bound to proteins in human plasma (similar to other fluoroquinolones), and assuming a similar extent of binding to proteins in ocular tissue, the corresponding C_{\max} and AUC_{24} values for free (unbound) besifloxacin would be approximately 60% of the values determined for total (bound and free) besifloxacin. In order to evaluate the potential theoretical maximum impact of protein binding on besifloxacin activity, C_{\max}/MIC_{90} and $\text{AUC}_{24}/\text{MIC}_{90}$ ratios were calculated based on the PK estimates for total (bound and free) and free (unbound) besifloxacin (Table 12).

Table 12. Predicted PK/PD Ratios for Besifloxacin in Tears After Repeated (TID) Topical Administration of Besifloxacin Ophthalmic Suspension in Healthy Volunteers

Organism	MIC ₉₀ (µg/mL)	C _{max} /MIC ₉₀ ^a		AUC ₂₄ /MIC ₉₀ ^b	
		Total ^c	Free ^d	Total ^c	Free ^d
Gram-positive					
<i>Staphylococcus aureus</i> (MRSA-C ^R)	4	153	92	950	570
<i>Staphylococcus aureus</i> (MSSA-C ^R)	2	305	183	1901	1140
<i>Staphylococcus aureus</i> (all phenotypes)	0.5	1220	732	7602	4561
<i>Streptococcus pneumoniae</i>	0.125	4880	2928	30,408	18,245
<i>Staphylococcus epidermidis</i>	0.5	1220	732	7602	4561
Gram-negative					
<i>Haemophilus influenzae</i>	0.06	10,167	6100	63,350	38,010

^a Calculations based on besifloxacin C_{max} (observed) of 610 µg/g.

^b Calculations based on besifloxacin AUC₂₄ (predicted, TID) of 3801 µg•hr/g.

^c PK/PD ratios calculated based on total (bound and free) besifloxacin.

^d PK/PD ratios calculated based on free besifloxacin levels, which were calculated using the measured value of besifloxacin binding to human plasma proteins (40% bound).

Topical ocular application of besifloxacin ophthalmic suspension, 0.6%, resulted in high therapeutic levels of besifloxacin in human tear samples, with concentrations at 24 hours (1.60 ± 2.28 µg/g), which were above the MIC₉₀ values for prevalent ocular pathogens. Favorable ratios for effective and resistance-limiting levels of anti-infective agents have been proposed to be C_{max}/MIC₉₀ > 10 and AUC₂₄/MIC₉₀ > 30 to 50 for Gram-positive bacteria or > 100 to 125 for Gram-negative bacteria (Allen et al., 2004; Hermesen et al., 2005; Metzler et al., 2004; Smith et al., 2004; Wright et al., 2000). These ratios are useful for evaluating plasma concentrations (in the instance of systemic infections), and also have been proposed for evaluating tissue concentrations in the case of local infections (Nightingale, 2005). The predicted PK/PD ratios for besifloxacin with TID dosing against prevalent pathogens associated with bacterial conjunctivitis demonstrate that the C_{max}/MIC₉₀ and AUC₂₄/MIC₉₀ are substantially above the target values published for fluoroquinolones regardless of whether total besifloxacin concentrations or only free besifloxacin concentrations are considered. Overall, these results provide a PK/PD-based rationale that is consistent with the efficacy observed with besifloxacin in the treatment of bacterial conjunctivitis.

6.3 Summary of Clinical Pharmacology

Topical ophthalmic use of besifloxacin is not expected to elicit any systemic effects. This is based on the fact that besifloxacin ophthalmic suspension is administered locally to the eye, and that the resulting systemic exposure to besifloxacin is minimal (C_{max} ~0.4 ng/mL, on average) following topical administration to humans.

7 CLINICAL AND MICROBIAL EFFICACY OF BESIFLOXACIN

7.1 Background and Overview

Three independent, randomized, doubled-masked, multicenter, parallel-group, controlled studies (Studies 373, 433, and 434) were conducted to assess the safety and efficacy of besifloxacin ophthalmic suspension versus vehicle (Studies 373 and 433) or Vigamox (Study 434), administered TID (at approximately 6-hour intervals) for 5 days, in patients with bacterial conjunctivitis. The overall designs and plans of the 3 clinical studies are described below.

7.1.1 Study Design and Methods

7.1.1.1 Study Population

Adults and children, 1 year of age or older, were eligible for entry into the studies if they had a clinical diagnosis (via biomicroscopy) of bacterial conjunctivitis in at least 1 eye. In all 3 studies, a minimum grade 1 for ocular discharge (crusty or sticky eyelids) was required. In Study 373, a minimum grade 1 for either bulbar or palpebral conjunctival injection was required, whereas for Studies 433 and 434, a minimum of grade 1 for bulbar conjunctival injection was required. In all studies, prospective patients were required to have a pinhole visual acuity (VA) $\geq 20/200$ in both eyes, determined by age-appropriate methods. Females of childbearing potential had to use a reliable means of contraception and have a negative pregnancy test at the baseline visit. Prospective patients were excluded if they had a known hypersensitivity to fluoroquinolones or besifloxacin or any of the ingredients in the study medications, had used topical ophthalmic anti-inflammatory agents within 48 hours before and during the study, used any antibiotic within 72 hours of study entry, had suspected viral or allergic conjunctivitis or suspected iritis, or a history of recurrent corneal erosion syndrome or any active ulcerative keratitis.

7.1.1.2 Study Endpoints

The efficacy endpoints of the 3 clinical studies are summarized in Table 13. The primary efficacy endpoints were clinical resolution and microbial eradication of baseline bacterial infection at Visit 3 (Day 8 or 9) in Study 373 or Visit 2 (Day 5 \pm 1) in Studies 433 and 434. Secondary efficacy endpoints were clinical resolution and microbial eradication at Visit 2 (Day 4 \pm 1) in Study 373 or Visit 3 (Day 8 or 9) in Studies 433 and 434.

Table 13. Efficacy Endpoints of Studies 373, 433, and 434

Endpoints	Endpoints, study visit (day)		
	Study 373	Study 433	Study 434
Primary			
Clinical resolution and microbial eradication	3 (8 or 9)	2 (5 \pm 1)	2 (5 \pm 1)
Secondary			
Clinical resolution and microbial eradication	2 (4 \pm 1)	3 (8 or 9)	3 (8 or 9)

STUDY ENDPOINT DEFINITIONS

Clinical resolution was defined as absence of 3 clinical signs (conjunctival discharge, bulbar and palpebral conjunctival injection) in Study 373 and 2 clinical signs (conjunctival discharge and bulbar conjunctival injection) in Studies 433 and 434. Grading scales for these clinical signs are shown in Table 14.

Table 14. Grading Scales for Ocular Discharge, Bulbar Conjunctival Injection and Palpebral Conjunctival Injection

Grade	Name	Criteria
Ocular Discharge		
0	Absent	No signs of discharge in conjunctiva.
1	Mild	Small amount of mucopurulent or purulent discharge noted in the lower cul-de-sac. No true matting of the eyelids in the mornings upon awakening.
2	Moderate	Moderate amount of mucopurulent or purulent discharge is noted in the lower cul-de-sac. Frank matting together of the eyelids in the morning upon awakening.
3	Severe	Profuse amount of mucopurulent or purulent discharge is noted in the lower cul-de-sac and in the marginal tear strip. Eyelids tightly matted together in the morning upon awakening, requiring warm soaks to pry the lids apart.
Bulbar Conjunctival Injection ^a		
0	Normal	Normal vascular pattern.
1	Trace	Awareness eye is slightly pink in any one quadrant.
2	Moderate	Diffuse pink color in at least 3 quadrants.
3	Severe	Vasodilation in at least 3 quadrants, reddish hue.
Palpebral Conjunctival Injection ^b		
0	Normal	Normal vascular pattern.
1	Trace	Trace hyperemia.
2	Moderate	Moderate hyperemia or definable papillary reaction.
3	Severe	Diffuse vasodilation.

^a Bulbar conjunctival injection was assessed by evaluating 4 quadrants (inferior, superior, temporal, and nasal) per grading scale provided to each clinical investigator.

^b Palpebral conjunctival injection was only assessed as a primary endpoint in Study 373.

In all 3 studies, microbial eradication was defined as the absence of all accepted ocular bacterial species that were present at or above threshold levels at baseline.

To be considered culture-confirmed bacterial conjunctivitis, a patient had to have bacterial species identified in ocular cultures obtained at baseline from a list of accepted ocular bacterial species and corresponding colony forming unit (CFU)/mL threshold levels as defined by Leibowitz in 1991 and referred to as bacterial threshold criteria (“Cagle list”) (Leibowitz, 1991). According to these criteria, an ocular specimen is considered “culture

confirmed” or “culture positive” if the CFU count equals or exceeds the threshold values given for any of the following groups of organisms in Table 15. Using current bacterial nomenclature standards, study personnel at the central laboratory (Covance Central Laboratory Services in Indianapolis, Indiana, United States) assigned bacterial species identified in culture obtained from patients in the 3 studies to the appropriate Cagle group for evaluation of pathogenic threshold levels. Based on current international standards for bacterial nomenclature, sponsor personnel confirmed that each pathogenic species and associated CFU/mL threshold level was assigned to the microbiologically appropriate corresponding pathogen group defined in the Cagle list.

Table 15. Bacterial Threshold Criteria (“Cagle List”)

Group	Threshold (CFU/mL)	Bacterial Species
I	1	<i>Acinetobacter</i> sp., <i>Achromobacter</i> sp., <i>Citrobacter</i> sp., <i>Enterobacter</i> sp., other Enterobacteriaceae, <i>Escherichia</i> sp., <i>Haemophilus</i> sp., <i>Klebsiella</i> sp., <i>Moraxella</i> sp. (other than <i>M. catarrhalis</i>), <i>Neisseria</i> sp., <i>Proteus/Morganella</i> sp., <i>Pseudomonas</i> sp., <i>Serratia marcescens</i> , <i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i>
II	10	<i>Moraxella catarrhalis</i> , <i>Staphylococcus aureus</i> , Group B, C, D, G, and viridians streptococci
III	100	<i>Bacillus</i> sp., <i>Micrococcus</i> sp., <i>Staphylococcus epidermidis</i> , other coagulase-negative <i>Staphylococcus</i> sp.
IV	1000	<i>Corynebacterium</i> sp.

CFU = Colony forming unit.

7.1.1.3 Microbial Culture Methods

Covance Test Method for Study 373

Microbial cultures were taken from the conjunctival cul-de-sac on each Visit prior to the administration of the morning dose. All specimens were shipped to Covance for analysis. Culture tests for bacteria, yeast, and virus were performed by Covance using test methods detailed in Covance Standard Operating Procedures. Quantitative plate counts were performed on bacteria and yeast test specimens. Viral test specimens were evaluated for the presence of adenovirus, herpes simplex virus, varicella zoster virus, and enterovirus.

Representative bacterial and yeast colony types were chosen from the quantitative ocular specimen plates based on similar colony morphology and were identified. Covance study personnel assigned bacterial species identified in this study to the appropriate Cagle group listed in Table 15 above for evaluation of pathogenic threshold levels. If the isolate met the bacterial threshold criteria, MIC testing was performed for besifloxacin and comparator test agents following CLSI document M7-A6 (2003) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.” MIC test drug concentration ranges included 0.004 to 8 µg/mL for besifloxacin, azithromycin, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, and ofloxacin; 0.06 to 2 µg/mL for penicillin, and 0.12 to 4 µg/mL for oxacillin. CLSI document M100-S14 (2004) “Performance Standards

for Antimicrobial Susceptibility Testing” was used to determine the MIC ($\mu\text{g/mL}$) Interpretive Standards for the comparator drugs. Covance Central Laboratory MIC test QC measures were performed according to CLSI documents (M7-A6, January 2003) relating to 30-day QC validations, weekly QC, and daily QC. The CLSI defined QC ranges for antimicrobials that were available for the selected American Type Culture Collection (ATCC) bacterial strains (M100-S14, 2004) were used to monitor the proper performance of the antimicrobial susceptibility test for comparator test agents. Pulsed Field Gel Electrophoresis (PFGE) was used for strain typing of bacteria with the same species recovered at or above threshold from the first and subsequent patient visits. PFGE testing was performed per Covance standard procedures. Bacteria recovered as pathogens were stored in duplicate per Sponsor direction.

Covance test method for Studies 433 and 434 were identical to the ones described above with two exceptions:

1. MIC test drug concentration ranges were 0.015 to 8 $\mu\text{g/mL}$ for penicillin, and 0.03 to 8 $\mu\text{g/mL}$ for oxacillin.
2. The CLSI-defined QC ranges for antimicrobials that were available for the selected ATCC bacterial strains (M100-S16, 2006) were used to monitor the proper performance of the antimicrobial susceptibility test for comparator test agents. MIC values obtained during study 373 were used to calculate tentative besifloxacin QC ranges used for studies 433 and 434.

7.1.1.4 Analysis Populations

For presentation of the clinical efficacy analyses, patients were analyzed as randomized in the vehicle-controlled Studies 373 and 433 and as treated in the active-controlled Study 434. For the species-specific microbial eradication, baseline pathogens with levels at or above threshold were analyzed as treated.

In the 3 clinical studies, the primary efficacy analyses were performed on the intent-to-treat (ITT; Study 373) or modified intent-to-treat (mITT; Studies 433 and 434) culture-confirmed populations, defined as eyes of patients with a clinical diagnosis of bacterial conjunctivitis who received at least 1 drop of study medication and had baseline culture results indicating bacterial levels at or above threshold for any accepted ocular species defined in the protocol.

Definitions of analysis populations used in Studies 373, 433, and 434 are summarized in Table 16.

Table 16. Definitions of Analysis Populations

Study Number	Analysis Populations			
	ITT	mITT	PP	Safety
Study 373	Culture confirmed ^a	—	Culture confirmed without major protocol deviation ^b	Received ≥ 1 dose of study drug
Study 433	Clinically diagnosed	Culture confirmed	Culture confirmed without major protocol deviation ^b	Received ≥ 1 dose of study drug
Study 434	Clinically diagnosed	Culture confirmed	Culture confirmed without major protocol deviation ^b	Received ≥ 1 dose of study drug

ITT = Intent-to-treat; mITT = Modified intent-to-treat; PP = Per protocol.

^a Used for mITT integrated analysis.

^b Discontinuations also excluded.

7.1.1.5 Designation of Study Eyes and Species-Specific Study Eyes

Each randomized patient had a single eye represented in the study eye analyses of all non-species-specific endpoints. For analyses by individual microbial species, a species-specific study eye was defined that could be different from the baseline-designated study eye defined above. The key criteria used to designate study eyes and species-specific study eyes are summarized below:

- At baseline (Visit 1), patients included in the mITT and PP populations had at least one eye that (i) met clinical criteria for acute conjunctivitis, (ii) was treated with besifloxacin or control, and (iii) yielded bacterial cultures at or above defined threshold levels for that pathogen.
- If only one eye met criteria (i)-(iii), then this eye was designated as the study eye. The terms baseline-designated study eye and study eye are used interchangeably.
- If both eyes met criteria (i)-(iii), then the eye with the highest clinical score was designated as the study eye. If both eyes met criteria (i)-(iii) with the same clinical score, then the right eye was designated as the study eye. The eye that was not the study eye was designated as the fellow eye.
- In all cases, any baseline (Visit 1) bacterial species isolated at or above threshold from an individual study eye was used in any species-specific study eye tabulations for that species.
- If both patient eyes met criteria (i)-(iii), and the baseline-designated fellow eye yielded baseline cultures at or above threshold for an additional species not present at or above threshold in the study eye, then the additional bacterial species

isolated at or above threshold from that patient's fellow eye was also included in tabulations of species-specific study eyes for that species.

- Note that all tabulations of baseline bacterial pathogens using the species-specific study eye designation thus included isolates from a patient's fellow eye only if that species was not present at or above threshold in that patient's study eye. Therefore, the species-specific study eye designation ensured that each bacterial species was counted only once per patient in any tables or summaries presenting an analysis by species.

In summary, the study eye and fellow eye designations were used to evaluate data at the eye level, whereas the species-specific study eye and species-specific fellow eye designations were used to evaluate microbial data at the species level.

7.2 Results From Individual Studies

This section summarizes the results from the 3 independent safety and efficacy trials (Studies 373, 433, and 434) conducted with besifloxacin ophthalmic suspension in patients with bacterial conjunctivitis.

The primary efficacy endpoints were clinical resolution and microbial eradication at Visit 3 (Day 8 or 9) for Study 373 and Visit 2 (Day 5 \pm 1) for Studies 433 and 434. Clinical resolution in Study 373 was defined as absence of the following 3 clinical signs/indices: conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection. Clinical resolution in Studies 433 and 434 was defined as the absence of conjunctival discharge and bulbar conjunctival injection. In all studies, microbial eradication was defined as the absence of all accepted ocular bacterial species that were present at or above threshold levels at baseline.

To appropriately compare results from Studies 373, 433, and 434, additional analyses were conducted on Study 373 data for clinical resolution and microbial eradication using a definition for clinically diagnosed bacterial conjunctivitis (baseline-designated study eye) comparable to that used in Studies 433 and 434. For these additional analyses, the definition of baseline-designated study eye and analyses of clinical resolution are based on 2 clinical signs (conjunctival discharge and bulbar conjunctival injection, as used in Studies 433 and 434), whereas the original definition of study eye and analyses for Study 373 are based on 3 clinical signs (conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection).

7.2.1 Study 373

7.2.1.1 Patient Disposition

Disposition of patients in Study 373 is shown in Figure 7. A total of 269 patients were randomized at 35 sites in the US to receive besifloxacin ophthalmic suspension (n = 137) or vehicle (n = 132). All patients who were randomized to treatment, received at least 1 dose of study medication, and had baseline cultures indicating pathogenic bacterial levels, were included in the ITT population. Overall, 118 of 269 randomized patients (44%) had culture-

confirmed acute bacterial conjunctivitis at baseline (Visit 1) and were eligible for inclusion in the ITT, culture-confirmed population; 60 (50.8%) of these patients were randomized to besifloxacin ophthalmic suspension and 58 (49.2%) to vehicle. Of these, 2 patients in the vehicle treatment group withdrew from the study prior to Visit 2 (Day 4 ± 1).

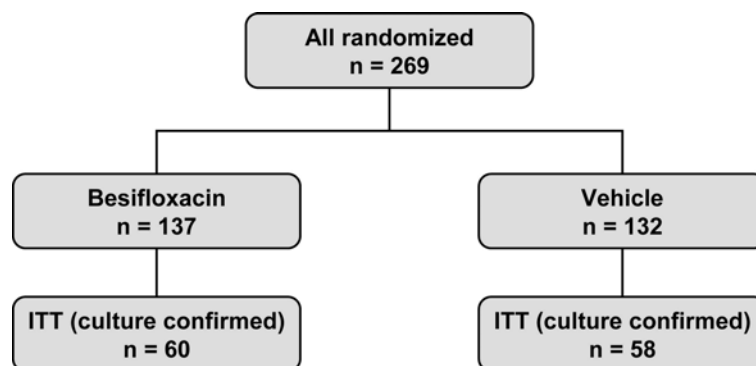


Figure 7. Disposition of Patients in Study 373

7.2.1.2 Demographics

Demographics for randomized and culture-confirmed patients in Study 373 are summarized in Table 17. Overall, patient demographics were comparable between the besifloxacin ophthalmic suspension and vehicle treatment groups in the randomized and culture-confirmed populations. Patients in the randomized and culture-confirmed populations were mainly female and Caucasian with mean ages of 34.2 years and 31.7 years, respectively.

Table 17. Demographics for Randomized and Culture-Confirmed Patients—Study 373

Demographics	Randomized		Culture Confirmed	
	Besifloxacin (n = 137)	Vehicle (n = 132)	Besifloxacin (n = 60)	Vehicle (n = 58)
Mean age (SD), years	33.3 (22.3)	35.1 (22.4)	28.7 (23.3)	34.7 (24.0)
Distribution of age categories, n (%)				
<2 years	2 (1.5)	1 (0.8)	2 (3.3)	1 (1.7)
2 to 19 years	44 (32.1)	37 (28.0)	26 (43.3)	19 (32.8)
20 to 59 years	71 (51.8)	71 (53.8)	24 (40.0)	27 (46.6)
≥60 years	20 (14.6)	23 (17.4)	8 (13.3)	11 (19.0)
Gender, n (%)				
Male	51 (37.2)	56 (42.4)	25 (41.7)	27 (46.6)
Female	86 (62.8)	76 (57.6)	35 (58.3)	31 (53.4)
Race, n (%)				
Caucasian	116 (84.7)	106 (80.3)	48 (80.0)	47 (81.0)
Asian	2 (1.5)	2 (1.5)	2 (3.3)	0
Black or African-American	6 (4.4)	11 (8.3)	1 (1.7)	6 (10.3)
Other	13 (9.5)	13 (9.8)	9 (15.0)	5 (8.6)

7.2.1.3 Results

INCIDENCE OF BASELINE PATHOGENS

The range of baseline pathogens that were encountered in Study 373 is shown in Table 18. The majority of isolates consisted of *Haemophilus* spp., streptococci, staphylococci, and coryneform bacteria. These organisms are fairly common to what would be expected in any study of bacterial conjunctivitis.

**Table 18. Baseline Pathogens With Incidence \geq 1% in Species-Specific Study Eyes Across All Treatment Groups—Study 373
Besifloxacin vs Vehicle**

Organism	Incidence, ^a n (%)
<i>H. influenzae</i>	46 (31.7)
<i>S. pneumoniae</i>	40 (27.6)
<i>S. aureus</i>	20 (13.8)
<i>S. epidermidis</i>	7 (4.8)
<i>S. oralis</i>	4 (2.8)
<i>S. mitis</i> group ^b	3 (2.1)
CDC coryneform group G	2 (1.4)
<i>Serratia marcescens</i>	2 (1.4)
<i>Stenotrophomonas maltophilia</i>	2 (1.4)
<i>Haemophilus parainfluenzae</i>	2 (1.4)

^a Among 145 species-specific study eye pathogens at baseline (Visit 1).

^b In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

CLINICAL RESOLUTION

Results for clinical resolution (based on the absence of 2 and 3 clinical signs/indices) at Visit 2 (Day 4 \pm 1) and Visit 3 (Day 8 or 9) are summarized in Table 19 and illustrated in Figure 8A and Figure 8B for the ITT, culture-confirmed population.

At Visit 3 (primary efficacy endpoint), when the last non-missing observation from Visit 2 or later was carried forward, a statistically significantly greater percentage of patients in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group experienced clinical resolution (based on absence of 3 clinical signs—conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection) (61.7% vs 35.7%; $p = 0.0013$, Cochran-Mantel-Haenszel [CMH] adjusted for center effects). In addition, to better compare these results to Studies 433 and 434 and data from other recent fluoroquinolone development programs, clinical resolution in the baseline-designated study eye was analyzed based on the absence of 2 clinical signs (conjunctival discharge and bulbar conjunctival injection). When missing values and discontinued patients were imputed as clinical resolution failures, a statistically significant greater rate of clinical

resolution was observed in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group at Visit 3 (73.3% vs 43.1%; $p = 0.0014$, exact Pearson chi-squared test value not adjusted for center effects, or $p = 0.0004$, CMH adjusted for center effects).

For the secondary efficacy endpoint, clinical resolution at Visit 2 (Day 4 \pm 1), no statistically significant difference was observed between the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group based on an analysis of the absence of 3 clinical signs (Table 19 and Figure 8A) or 2 clinical signs (Table 19 and Figure 8B).

Table 19. Clinical Resolution by 2 or 3 Indices at Visit 2 (Day 4 \pm 1) and Visit 3 (Day 8 or 9) (ITT, Culture Confirmed)—Study 373

Clinical Resolution (3 indices) ^a	Visit 2 (Day 4 \pm 1)		<u>Primary Endpoint</u> Visit 3 (Day 8 or 9)	
	Besifloxacin (N = 60)	Vehicle (N = 56)	Besifloxacin (N = 60)	Vehicle (N = 56)
Yes, n (%)	14 (23.3)	8 (14.3)	37 (61.7)	20 (35.7)
No, n (%)	46 (76.7)	48 (85.7)	23 (38.3)	36 (64.3)
p value ^c	0.2434/0.3144		0.0058/0.0013	
Clinical Resolution (2 indices) ^b	Besifloxacin (N = 60)	Vehicle (N = 58)	Besifloxacin (N = 60)	Vehicle (N = 58)
Resolution, n (%)	20 (33.3)	10 (17.2)	44 (73.3)	25 (43.1)
Non-resolution, ^d n (%)	40 (66.7)	48 (82.8)	16 (26.7)	33 (56.9)
p value ^c	0.0574/0.0691		0.0014/0.0004	
95% CI ^e	(0.21, 31.97)		(12.26, 48.20)	

CI = Confidence interval.

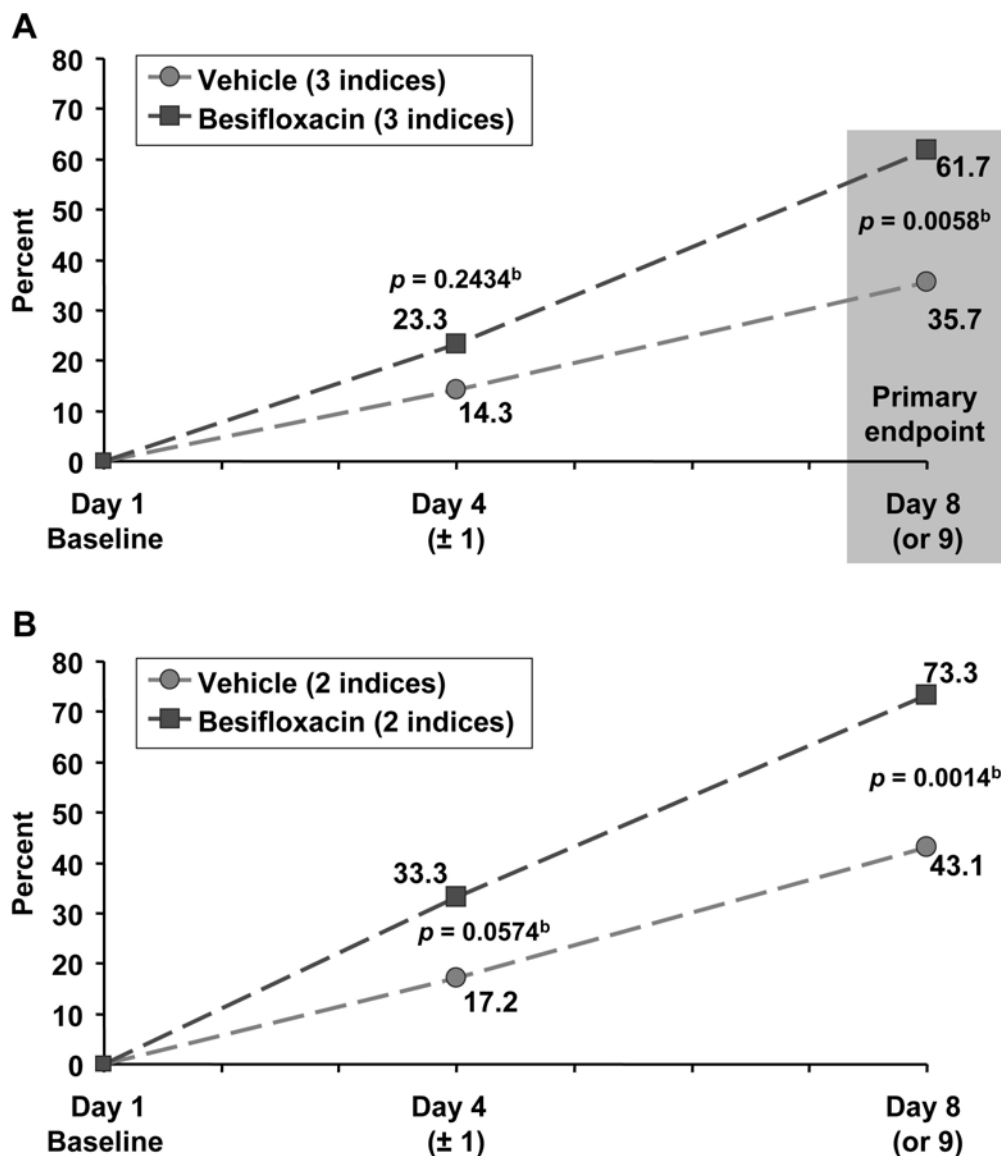
^a Clinical resolution defined as the absence of ocular discharge, bulbar conjunctival injection, and palpebral conjunctival injection, based on the original analyses.

^b Clinical resolution defined as the absence of ocular discharge and bulbar conjunctival injection, based on the additional analyses.

^c p values from exact Pearson chi-squared test/CMH test stratified by center, respectively.

^d Non-resolution refers to any score other than 'resolution.' Missing or discontinued patients imputed as 'non-resolution.'

^e Difference calculated as besifloxacin minus vehicle. Positive values favor besifloxacin.



^a Ocular discharge, bulbar conjunctival injection, and palpebral conjunctival injection.

^b Exact Pearson chi-squared test p value.

Figure 8. (A) Clinical Resolution by 3 Indices^a at Visit 2 (Day 4 ± 1) and Visit 3 (Day 8 or 9) (ITT, Culture Confirmed)—Study 373; (B) Clinical Resolution by 2 Indices at Visit 2 (Day 4 ± 1) and Visit 3 (Day 8 or 9) (ITT, Culture-confirmed)—Study 373

MICROBIAL ERADICATION

Results for microbial eradication at the eye level (eradication of all baseline pathogens) at Visit 2 (Day 4 ± 1) and Visit 3 (Day 8 or 9) are summarized for the ITT, culture-confirmed population in Table 20 and illustrated in Figure 9. At Visit 3 (primary efficacy endpoint), when the last non-missing post-baseline observation was carried forward, a statistically significant greater percentage of patients in the besifloxacin ophthalmic suspension

treatment group versus vehicle treatment group experienced microbial eradication (90.0% vs 69.1%; $p = 0.0092$, exact Pearson chi-squared test; $p = 0.0041$, CMH adjusted for center effects). For the secondary efficacy endpoint, microbial eradication at Visit 2 (Day 4 \pm 1), a significantly greater rate of microbial eradication was observed in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group (90.0% vs 51.8%; $p < 0.0001$, exact Pearson chi-squared test; $p < 0.0001$, CMH adjusted for center effects) (Table 20 and Figure 9).

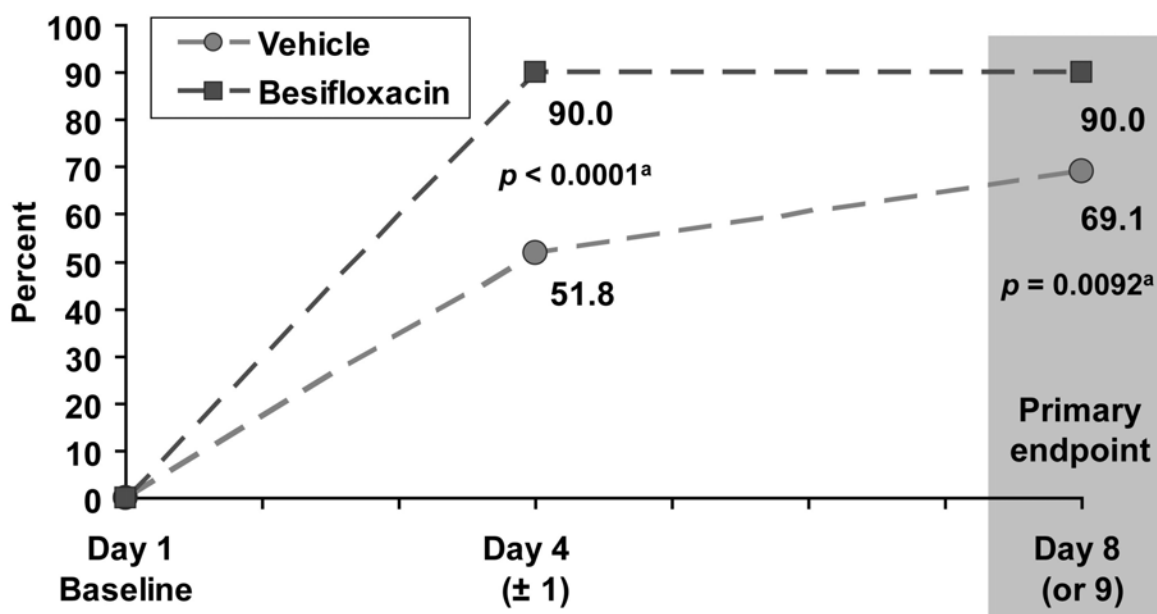
Table 20. Microbial Eradication at Visit 2 (Day 4 \pm 1) and Visit 3 (Day 8 or 9) (ITT, Culture Confirmed)—Study 373

Microbial Eradication	Visit 2 (Day 4 \pm 1)		Primary Endpoint Visit 3 (Day 8 or 9)	
	Besifloxacin (N = 60)	Vehicle (N = 54)	Besifloxacin (N = 60)	Vehicle (N = 55)
Yes, n (%)	54 (90.0)	28 (51.8)	54 (90.0)	38 (69.1)
No, n (%)	6 (10.0)	26 (48.1)	6 (10.0)	17 (30.9)
p value ^a	<0.0001/<0.0001		0.0092/0.0041	

CI = Confidence interval.

^a p values from exact Pearson chi-squared test/CMH test stratified by center, respectively.

Note: Depending on the number of bacterial species at or above threshold at Day 1, each patient may present multiple scores.



^a Exact Pearson chi-squared test p value.

Figure 9. Microbial Eradication at Visit 2 (Day 4 \pm 1) and Visit 3 (Day 8 or 9) (ITT, Culture-confirmed)—Study 373

MICROBIAL ERADICATION OF BASELINE PATHOGENS

Microbial eradication at Visit 3 (Day 8 or 9) by baseline pathogens is shown in Table 21 for besifloxacin versus vehicle. The species-specific eradication data show the broad-spectrum nature of besifloxacin and the high rates of eradication regardless of the Gram-stain characteristics of the organisms.

Table 21. Microbial Eradication at Visit 3 (Day 8 or 9) by Baseline Species-Specific Study Eye Isolates With Incidence $\geq 1\%$ in Study 373—Besifloxacin vs Vehicle

Pathogen	Isolates eradicated/ encountered (%)	
	Besifloxacin	Vehicle
Gram-positive isolates	41/47 (87)	22/40 (55)
Gram-negative isolates	28/29 (97)	22/29 (76)
<i>H. influenzae</i>	24/25 (96)	17/21 (81)
<i>S. aureus</i>	9/10 (90)	4/10 (40)
<i>S. epidermidis</i>	3/3 (100)	1/4 (25)
<i>S. pneumoniae</i>	19/24 (79)	8/16 (50)
<i>S. oralis</i>	2/2 (100)	2/2 (100)
CDC coryneform group G	2/2 (100)	0

LACK OF FLUOROQUINOLONE RESISTANCE DEVELOPMENT DURING STUDY 373

A total of 47 pathogens isolated at or above threshold at Visit 2 or Visit 3 (11 besifloxacin treated, 36 vehicle treated) were determined by PFGE analysis to be genetically concordant. MIC testing of all 47 genetically concordant isolate pairs indicated that susceptibility of Visit 2 or Visit 3 isolates did not increase by more than 2-fold for any of the tested fluoroquinolones, including besifloxacin.

VIRAL TEST RESULTS

In this study, 8 of 269 randomized patients had positive viral cultures at baseline. Two were included in the bacterial culture-confirmed population, and both were treated with besifloxacin vehicle. For 1 patient, the baseline bacterial pathogen was eradicated at Visit 3 and the conjunctivitis was improved but not resolved. For the second patient the bacterial pathogen was eradicated at Visit 2 but the conjunctivitis was unchanged. No data for this patient was available at Visit 3.

7.2.1.4 Efficacy Conclusions for Study 373

In patients with culture-confirmed bacterial conjunctivitis, the primary efficacy endpoints of clinical resolution and bacterial eradication at Visit 3 (Day 8 or 9) were achieved in a significantly greater percentage of patients who received besifloxacin ophthalmic suspension versus vehicle. These findings were observed based on both the original analysis with clinical resolution defined as the absence of 3 clinical signs (conjunctival discharge, bulbar and palpebral conjunctival injection) and the additional analysis with clinical resolution defined as the absence of 2 clinical signs (conjunctival discharge and bulbar conjunctival injection). Furthermore, besifloxacin showed potent antimicrobial activity against a wide range of organisms.

7.2.2 Study 433

7.2.2.1 Patient Disposition

Disposition of patients in Study 433 is shown in Figure 10. A total of 957 patients were randomized and received at least 1 dose of study drug at 58 sites in the United States; 473 received besifloxacin ophthalmic suspension and 484 received vehicle. These patients comprised the Safety Population. Of the 957 randomized patients, 874 (91.3%) completed the study; 442 (93.4%) patients treated with besifloxacin ophthalmic suspension and 432 (89.3%) patients treated with vehicle. A total of 83 (8.7%) patients discontinued from the study; 31 (6.6%) treated with besifloxacin ophthalmic suspension and 52 (10.7%) patients treated with vehicle. The difference in the discontinuation rates was statistically significant ($p = 0.0219$, Fisher's exact test). Lack of efficacy in the vehicle treatment group was the largest contributor to this difference.

A total of 40.8% (390/957) randomized patients had culture-confirmed bacterial conjunctivitis. These patients comprised the mITT population (199 randomized to besifloxacin ophthalmic suspension and 191 randomized to vehicle). Of the 390 randomized patients, 364 (93.3%) completed the study; 191 (96.0%) patients randomized to besifloxacin ophthalmic suspension and 173 (90.6%) patients randomized to vehicle. A total of 26 (6.7%) patients in the mITT population discontinued from the study: 8 (4.0%) patients randomized to besifloxacin ophthalmic suspension and 18 (9.4%) patients to the vehicle treatment groups. There was a statistically significant difference between treatment groups in the number of patients who withdrew from the study ($p = 0.0414$, Fisher's exact test).

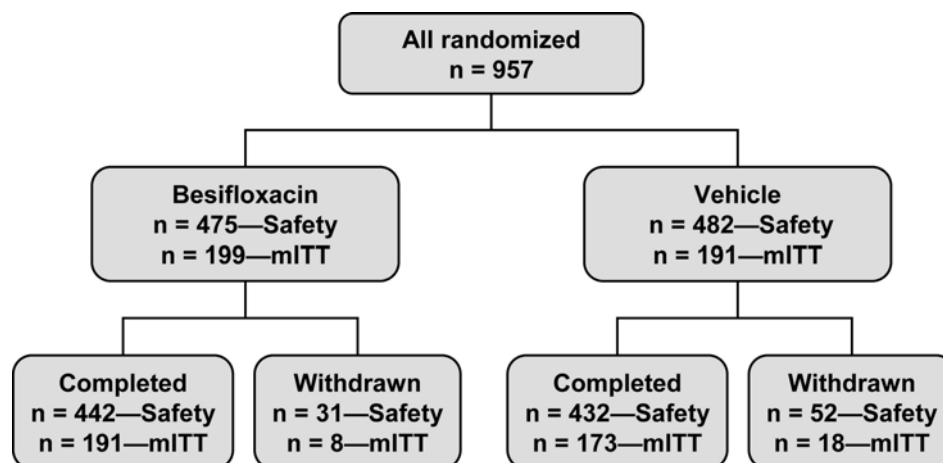


Figure 10. Disposition of Patients in Study 433

7.2.2.2 Demographics and Patient Characteristics

Demographics for as-randomized and culture-confirmed patients in Study 433 are summarized in Table 22. Overall, patient demographics were comparable between the besifloxacin ophthalmic suspension and vehicle treatment groups in the as-randomized and culture-confirmed populations. Patients in the as-randomized and culture-confirmed populations were mainly female (62.9% and 60.8%, respectively) and Caucasian (66.7% and 64.4%, respectively) with mean ages of 27.3 years and 23.3 years, respectively.

Table 22. Demographics for As-Randomized and Culture-Confirmed Patients—Study 433

Demographics	As Randomized		Culture Confirmed	
	Besifloxacin (n = 475)	Vehicle (n = 482)	Besifloxacin (n = 199)	Vehicle (n = 191)
Mean age (SD), years	27.3 (21.8)	27.3 (21.7)	22.2 (22.4)	24.4 (24.0)
Distribution of age categories, n (%)				
<2 years	21 (4.4)	20 (4.1)	17 (8.5)	13 (6.8)
2 to 19 years	196 (41.3)	196 (40.7)	97 (48.7)	96 (50.3)
20 to 59 years	212 (44.6)	226 (46.9)	70 (35.2)	63 (33.0)
≥60 years	46 (9.7)	40 (8.3)	15 (7.5)	19 (9.9)
Gender, n (%)				
Male	173 (36.4)	182 (37.8)	75 (37.7)	78 (40.8)
Female	302 (63.6)	300 (62.2)	124 (62.3)	113 (59.2)
Race, n (%)				
Caucasian	312 (65.7)	312 (64.7)	125 (62.8)	126 (66.0)
Asian	10 (2.1)	7 (1.5)	3 (1.5)	5 (2.6)
Black or African-American	44 (9.3)	46 (9.5)	18 (9.0)	18 (9.4)
Other	109 (22.9)	117 (24.3)	53 (26.6)	42 (22.0)

7.2.2.3 Results

INCIDENCE OF BASELINE PATHOGENS

Comparable to Study 373, a wide range of baseline pathogens were encountered in Study 433. *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *S. epidermidis* were observed most frequently in this and other studies, and these formed the primary basis of the microbiological analysis of the organisms encountered (Table 23).

Table 23. Baseline Pathogens With Incidence \geq 1% in Species-Specific Study Eyes Across All Treatment Groups—Study 433 Besifloxacin vs Vehicle

Organism	Incidence, ^a (%)
<i>S. pneumoniae</i>	140 (29.2)
<i>H. influenzae</i>	129 (26.9)
<i>S. aureus</i>	55 (11.5)
<i>S. epidermidis</i>	34 (7.1)
<i>S. mitis</i> group ^b	29 (6.0)
CDC coryneform group G	9 (1.9)
<i>Brevibacterium</i> spp. ^c	6 (1.2)
<i>Streptococcus</i> spp. ^c	6 (1.2)
<i>S. salivarius</i>	5 (1.0)

^a Among 480 species-specific study eye pathogens at baseline (Visit 1).

^b In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

^c Species name could not be determined.

CLINICAL RESOLUTION

Results for clinical resolution by 2 clinical signs/indices (conjunctival discharge and bulbar conjunctival injection) at Visit 2 (Day 5 \pm 1) and Visit 3 (Day 8 or 9) are summarized in Table 24 and illustrated in Figure 11 for the mITT, culture-confirmed, as-randomized population. At Visit 2 (primary efficacy endpoint), when missing values and discontinued patients were imputed as clinical resolution failures, a statistically significantly greater percentage of patients in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group had clinical resolution (45.2% vs 33.0%; $p = 0.0169$, exact Pearson chi-squared test value not adjusted for center effects or $p = 0.0084$, CMH adjusted for center effects).

At Visit 3, when missing values and discontinued patients were imputed as clinical resolution failures, a statistically significantly greater percentage of patients in the besifloxacin ophthalmic suspension treatment group versus vehicle treatment group experienced clinical resolution (84.4% vs 69.1%; $p = 0.0005$, exact Pearson chi-squared test value not adjusted for center effects or $p = 0.0011$, CMH adjusted for center effects).

Table 24. Clinical Resolution by 2 Indices at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture Confirmed, As Randomized)—Study 433

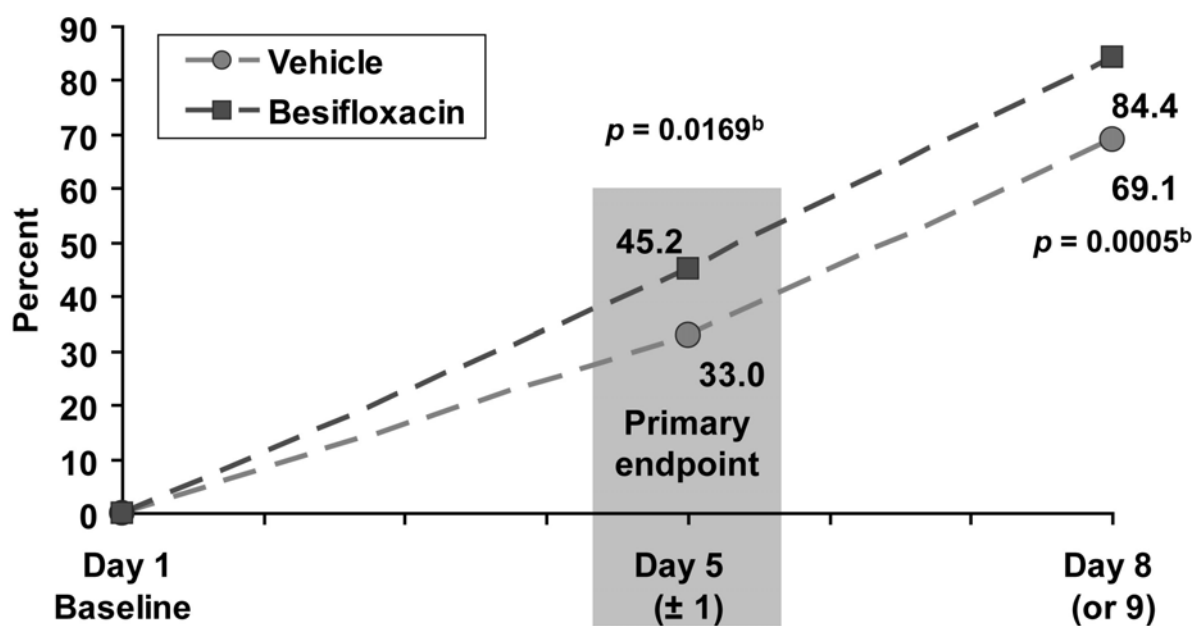
Clinical Resolution (2 indices) ^a	Primary Endpoint Visit 2 (Day 5 ± 1)		Visit 3 (Day 8 or 9)	
	Besifloxacin (N = 199)	Vehicle (N = 191)	Besifloxacin (N = 199)	Vehicle (N = 191)
Yes, n (%)	90 (45.2)	63 (33.0)	168 (84.4)	132 (69.1)
No, n (%)	109 (54.8)	128 (67.0)	31 (15.6)	59 (30.9)
<i>p</i> value ^b	0.0169/0.0084		0.0005/0.0011	
95% CI ^c	(2.52, 21.97)		(6.92, 23.70)	

CI = Confidence interval.

^a Ocular discharge and bulbar conjunctival injection.

^b *p* values from exact Pearson chi-squared test/CMH test stratified by center, respectively.

^c Difference calculated as besifloxacin minus vehicle. Positive values favor besifloxacin.



^a Ocular discharge (pus) and bulbar conjunctival injection (redness).

^b Exact Pearson chi-squared test *p* value.

Figure 11. Clinical Resolution by 2 Indices^a at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Randomized)—Study 433

MICROBIAL ERADICATION

Results for eradication of baseline bacterial infection at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) are summarized in Table 25 and illustrated in Figure 12. At Visit 2 (primary efficacy endpoint), when missing values and discontinued patients were imputed as microbial eradication failures, the percentage of patients in the besifloxacin ophthalmic suspension treatment group who had microbial eradication was statistically significantly greater compared with the vehicle treatment group (91.5% vs 59.7%; $p < 0.0001$, exact Pearson chi-squared test value not adjusted for center effects or CMH adjusted for center effects). This benefit of besifloxacin ophthalmic suspension over vehicle in eradicating baseline bacterial infection was maintained at Visit 3 (88.4% vs 71.7%; $p < 0.0001$, exact Pearson chi-squared test value not adjusted for center effects or CMH adjusted for center effects).

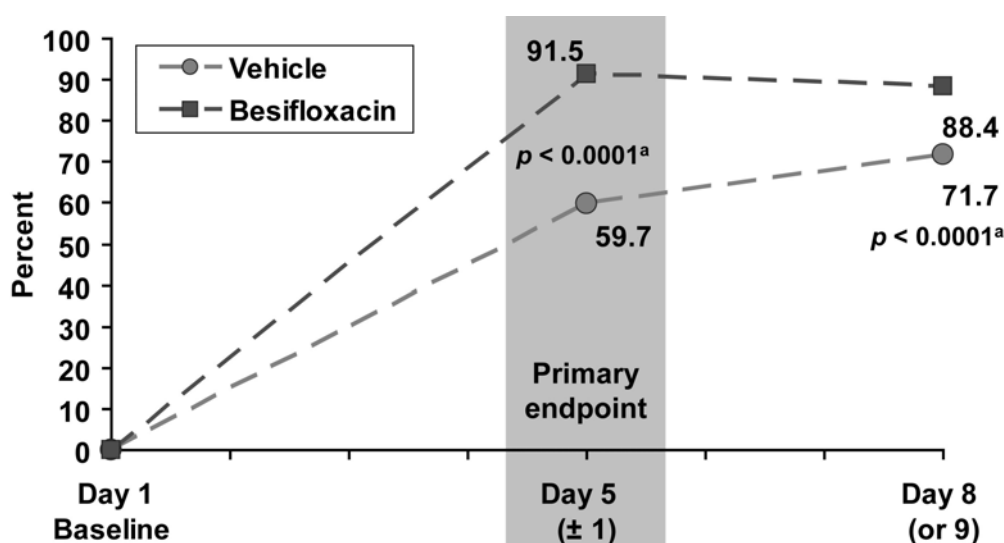
Table 25. Microbial Eradication at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Randomized)—Study 433

Microbial Eradication (missing or discontinued patients imputed as 'no')	Primary Endpoint Visit 2 (Day 5 ± 1)		Visit 3 (Day 8 or 9)	
	Besifloxacin (N = 199)	Vehicle (N = 191)	Besifloxacin (N = 199)	Vehicle (N = 191)
Yes, n (%)	182 (91.5)	114 (59.7)	176 (88.4)	137 (71.7)
No, n (%)	17 (8.5)	77 (40.3)	23 (11.6)	54 (28.3)
p value ^a	<0.0001/<0.0001		<0.0001/<0.0001	
95% CI ^b	(23.25, 40.29)		(8.79, 24.64)	

CI = Confidence interval; CMH = Cochran-Mantel-Haenszel.

^a p values from exact Pearson chi-squared test/CMH test stratified by center, respectively.

^b Difference calculated as besifloxacin minus vehicle. Positive values favor besifloxacin.



^a Exact Pearson chi-squared test p value.

Figure 12. Microbial Eradication at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Randomized)—Study 433

MICROBIAL ERADICATION OF BASELINE PATHOGENS

Microbial eradication at Visit 3 (Day 8 or 9) is shown by baseline pathogens in Table 26 for besifloxacin versus vehicle. Comparable to Study 373, these results show a broad-spectrum nature of the microbial eradication with besifloxacin, very high eradication rates independent of the Gram-stain characteristics, and high eradication rates for the most prevalent organisms encountered.

Table 26. Microbial Eradication at Visit 2 (Day 5 ± 1) by Baseline Species-Specific Study Eye Isolates With Incidence ≥ 1% in Study 433—Besifloxacin vs Vehicle

Pathogen	Isolates eradicated/ encountered (%)	
	Besifloxacin	Vehicle
Gram-positive isolates	159/173 (92)	97/155 (63)
Gram-negative isolates	69/78 (89)	50/74 (68)
<i>H. influenzae</i>	55/63 (87)	43/66 (65)
<i>S. aureus</i>	23/24 (96)	13/31 (42)
<i>S. epidermidis</i>	17/18 (94)	11/16 (69)
<i>S. pneumoniae</i>	66/73 (90)	40/67 (60)
<i>S. mitis</i> group ^a	6/7 (86)	10/12 (83)
CDC coryneform group G	7/7 (100)	1/2 (50)
<i>S. salivarius</i>	3/3 (100)	2/2 (100)

^a In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

LACK OF FLUOROQUINOLONE RESISTANCE DEVELOPMENT DURING STUDY 433

A total of 122 pathogens isolated at or above threshold at Visit 2 or Visit 3 (29 besifloxacin treated, 93 vehicle treated) were determined by PFGE analysis to be genetically concordant. MIC testing of all 122 genetically concordant isolate pairs indicated that susceptibility of Visit 2 or Visit 3 isolates did not increase by more than 2-fold for any of the tested fluoroquinolones, including besifloxacin.

VIRAL TEST RESULTS

In this study, 73 of 957 randomized patients had positive viral cultures at baseline. Nine were included in the bacterial culture-confirmed population; 2 of these were treated with vehicle and 7 were treated with besifloxacin. For the patients treated with vehicle, the baseline bacterial pathogen was eradicated for only 1 patient at Visit 3 and the conjunctivitis was improved but not resolved. Among the 7 patients treated with besifloxacin, baseline bacterial pathogens were eradicated in 6 cases at Visit 2.

Conjunctivitis was resolved in 3 of the patients by Visit 2 and all were resolved by the final visit.

7.2.2.4 Efficacy Conclusions for Study 433

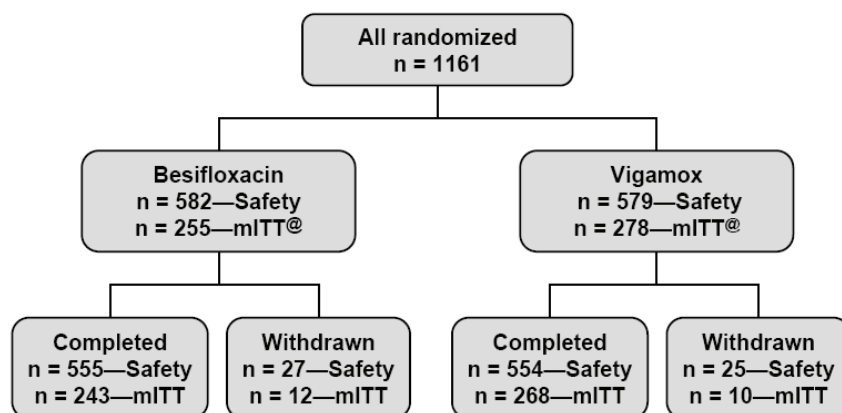
Overall, in patients with culture-confirmed bacterial conjunctivitis, results from the primary efficacy endpoints, clinical resolution and bacterial eradication by Visit 2 (Day 5 \pm 1 day), demonstrated that besifloxacin ophthalmic suspension had efficacy outcomes that are significantly superior to those observed with vehicle. Furthermore, besifloxacin showed potent antimicrobial activity against a wide range of organisms.

7.2.3 Study 434

7.2.3.1 Patient Disposition

Disposition of patients in Study 434 is shown in Figure 13. A total of 1161 patients were randomized and received at least 1 dose of study drug in this study conducted at 73 sites in the United States and 11 sites in Asia; 1005 patients randomized at US sites and 156 patients at Asian sites. Of the 1161 randomized patients, 582 received besifloxacin ophthalmic suspension and 579 received Vigamox. These patients comprised the safety population. Of the 1161 patients, 1109 (95.5%) completed the study; 555 (95.4%) patients randomized to besifloxacin ophthalmic suspension and 554 (95.7%) to Vigamox. A total of 52 (4.5%) patients in the safety population withdrew from the study; 27 (4.6%) in the besifloxacin ophthalmic suspension treatment group and 25 (4.3%) in the Vigamox treatment group.

A total of 45.9% (533/1161) randomized patients had baseline culture results in at least 1 eye indicating bacteria levels at or above threshold for any accepted ocular species. These patients comprised the mITT, culture-confirmed population (255 besifloxacin ophthalmic suspension and 278 Vigamox). Of the 533 patients, 511 (95.9%) completed the study; 243 (95.3%) patients randomized to besifloxacin ophthalmic suspension and 268 (96.4%) to Vigamox. A total of 22 (4.1%) patients in the mITT population withdrew from the study; 12 (4.7%) and 10 (3.6%) patients in the besifloxacin ophthalmic suspension and Vigamox treatment groups, respectively. The most common reasons for patient discontinuation included adverse events (AEs) and patients being lost to follow-up.



@ In the culture-confirmed (mITT), as-treated population, 252 patients were treated with besifloxacin and 281 patients were treated with Vigamox.

Figure 13. Disposition of Patients in Study 434

7.2.3.2 Demographics and Patient Characteristics

Demographics for randomized and culture-confirmed patients in Study 434 are summarized in Table 27. Overall, patient demographics were comparable between the besifloxacin ophthalmic suspension and Vigamox treatment groups in the randomized and culture-confirmed populations. Patients treated with besifloxacin or Vigamox in the culture-confirmed population were mainly female (56.7% and 50.5%, respectively) and Caucasian (69.8% and 70.1%, respectively) with mean ages of 31.6 years and 38.3 years, respectively.

Table 27. Demographics for Randomized and Culture-confirmed Patients—Study 434

Demographics	Randomized		Culture Confirmed ^a	
	Besifloxacin (n = 582)	Vigamox (n = 579)	Besifloxacin (n = 252)	Vigamox (n = 281)
Mean age (SD), years	34.1 (23.5)	36.1 (24.7)	31.6 (26.2)	38.3 (27.7)
Distribution of age categories, n (%)				
<2 years	22 (3.8)	15 (2.6)	18 (7.1)	12 (4.3)
2 to 19 years	166 (28.5)	171 (29.5)	90 (35.7)	78 (27.8)
20 to 59 years	295 (50.7)	273 (47.2)	97 (38.5)	113 (40.2)
≥60 years	99 (17.0)	120 (20.7)	47 (18.7)	78 (27.8)
Gender, n (%)				
Male	250 (43.0)	256 (44.2)	109 (43.3)	139 (49.5)
Female	332 (57.0)	323 (55.8)	143 (56.7)	142 (50.5)
Race, n (%)				
Caucasian	385 (66.2)	391 (67.5)	176 (69.8)	197 (70.1)
Asian	87 (14.9)	89 (15.4)	34 (13.5)	44 (15.7)
Black or African-American	73 (12.5)	63 (10.9)	27 (10.7)	27 (9.6)
Other	37 (6.4)	36 (6.2)	15 (6.0)	13 (4.6)

^a As-treated population

7.2.3.3 Results

INCIDENCE OF BASELINE PATHOGENS

Comparable to Studies 373 and 433, a wide range of baseline pathogens were encountered. Again, organisms with the highest incidence included *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *S. epidermidis*, as well as various other streptococci, staphylococci, and corynebacteria (Table 28).

Table 28. Baseline Pathogens With Incidence \geq 1% in Species-Specific Study Eyes Across All Treatment Groups—Study 434 Besifloxacin vs Vigamox

Organism	Incidence, ^a n (%)
<i>H. influenzae</i>	169 (24.2)
<i>S. pneumoniae</i>	122 (17.5)
<i>S. aureus</i>	115 (16.5)
<i>S. epidermidis</i>	70 (10.0)
<i>S. mitis</i> group ^b	33 (4.7)
CDC coryneform group G	18 (2.6)
<i>S. oralis</i>	10 (1.4)
<i>Aerococcus viridans</i>	8 (1.1)
<i>C. pseudodiphtheriticum</i>	7 (1.0)
<i>S. lugdunensis</i>	7 (1.0)
<i>Moraxella catarrhalis</i>	7 (1.0)
<i>Streptococcus</i> sp. ^c	7 (1.0)

^a Among 699 species-specific study eye pathogens at baseline (Visit 1).

^b In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

^c Species name could not be determined.

CLINICAL RESOLUTION

Results for clinical resolution by 2 clinical signs/indices (conjunctival discharge and bulbar conjunctival injection) at Visit 2 (Day 5 \pm 1) and Visit 3 (Day 8 or 9) are summarized in Table 29 and illustrated in Figure 14. At Visit 2 (primary efficacy endpoint), when missing values and discontinued patients were imputed as clinical resolution failures, besifloxacin ophthalmic suspension was non-inferior to Vigamox for clinical resolution based on the 95% confidence interval (CI) of the difference (58.3% vs 59.4%, respectively; 95% CI, -9.48%, 7.29%), and there was no statistically significant difference in clinical resolution between the 2 treatment groups ($p = 0.8601$, exact Pearson chi-squared test $p = 0.6520$, CMH adjusted for center effects).

At Visit 3, when missing values and discontinued patients were imputed as clinical resolution failures, besifloxacin ophthalmic suspension was non-inferior to Vigamox for clinical resolution based on the 95% CI of the difference (84.5% vs 84.0%, respectively; 95% CI, -5.67%, 6.75%), and there was no statistically significant difference in clinical resolution between the 2 treatment groups $p = 0.9055$, exact Pearson chi-squared test, or $p = 0.5014$, CMH adjusted for center effects).

Table 29. Clinical Resolution by 2 Indices at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Treated)—Study 434

Clinical Resolution (2 indices) ^a	Primary Endpoint Visit 2 (Day 5 ± 1)		Visit 3 (Day 8 or 9)	
	Besifloxacin (N = 252)	Vigamox (N = 281)	Besifloxacin (N = 252)	Vigamox (N = 281)
Yes, n (%)	147 (58.3)	167 (59.4)	213 (84.5)	236 (84.0)
No, n (%)	105 (41.7)	114 (40.6)	39 (15.5)	45 (16.0)
p value ^b	0.8601/0.6520		0.9055/0.5014	
95% CI ^c	(-9.48, 7.29)		(-5.67, 6.75)	

CI = Confidence interval; CMH = Cochran-Mantel-Haenszel.

^aOcular discharge and bulbar conjunctival injection

^b p values from exact Pearson chi-squared test/CMH test stratified by center, respectively.

^cDifference calculated as besifloxacin minus Vigamox. Positive values favor besifloxacin.

Note: Percentages are based on the number of patients indicated in the column heading (culture-confirmed as-treated population).

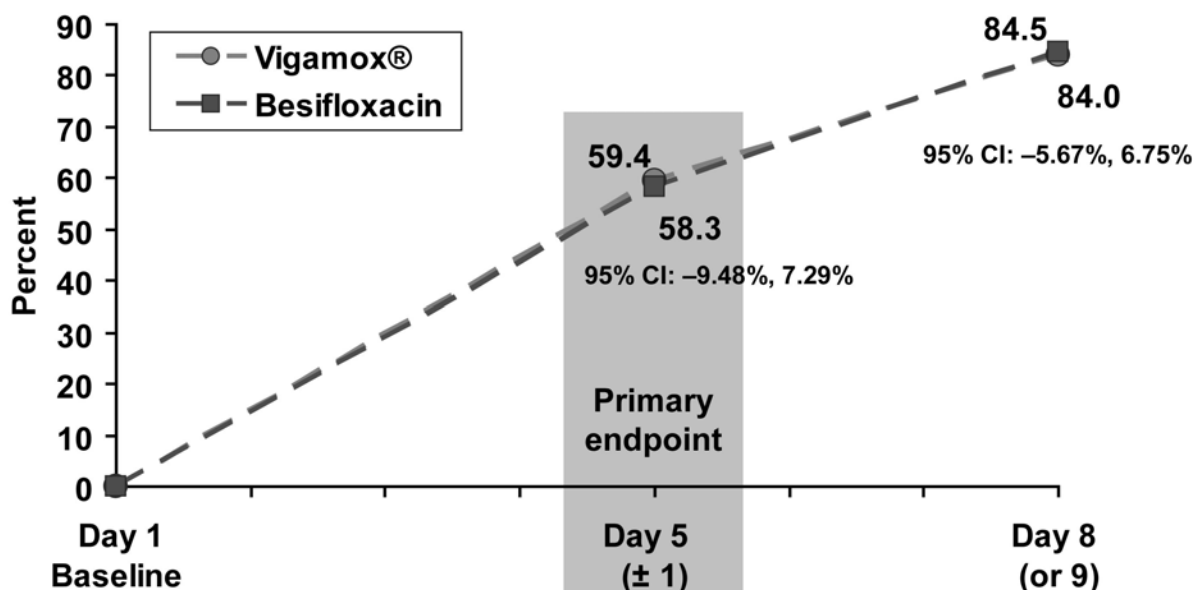


Figure 14. Clinical Resolution by 2 Indices at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Treated)—Study 434

MICROBIAL ERADICATION

Results for eradication of baseline bacterial infection at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) are summarized in Table 30 and illustrated in Figure 15. At Visit 2, when missing values and discontinued patients were imputed as microbial eradication failures, besifloxacin ophthalmic suspension was non-inferior to Vigamox for microbial eradication based on the 95% CI of the difference (93.3% vs 91.1%, respectively; 95% CI, -2.44%, 6.74%), and there was no statistically significant difference between the 2 treatment groups ($p = 0.4217$, exact Pearson chi-squared test or $p = 0.1238$, CMH adjusted for center effects).

At Visit 3, when missing values and discontinued patients were imputed as microbial eradication failures, besifloxacin ophthalmic suspension was non-inferior to Vigamox for microbial eradication based on the 95% CI of the difference (87.3% vs 84.7%, respectively; 95% CI, -3.32%, 8.54%), and there was no statistically significant difference in microbial eradication between the 2 treatment groups ($p = 0.4544$, exact Pearson chi-squared test or $p = 0.0608$, CMH adjusted for center effects).

Table 30. Microbial Eradication at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Treated)—Study 434

Microbial Eradication (missing or discontinued patients imputed as 'no')	Primary Endpoint Visit 2 (Day 5 ± 1)		Visit 3 (Day 8 or 9)	
	Besifloxacin (N = 252)	Vigamox (N = 281)	Besifloxacin (N = 252)	Vigamox (N = 281)
Yes, n (%)	235 (93.3)	256 (91.1)	220 (87.3)	238 (84.7)
No, n (%)	17 (6.7)	25 (8.9)	32 (12.7)	43 (15.3)
p value ^a	0.4217/0.1238		0.4544/0.0608	
95% CI ^b	(-2.44, 6.74)		(-3.32, 8.53)	

CI = Confidence interval; CMH = Cochran-Mantel-Haenszel.

^a p values from exact Pearson chi-squared test/CMH test stratified by center, respectively.

^b Difference calculated as besifloxacin minus Vigamox. Positive values favor besifloxacin.

Note: Percentages are based on the number of patients indicated in the column heading (culture-confirmed, 'as-treated' population).

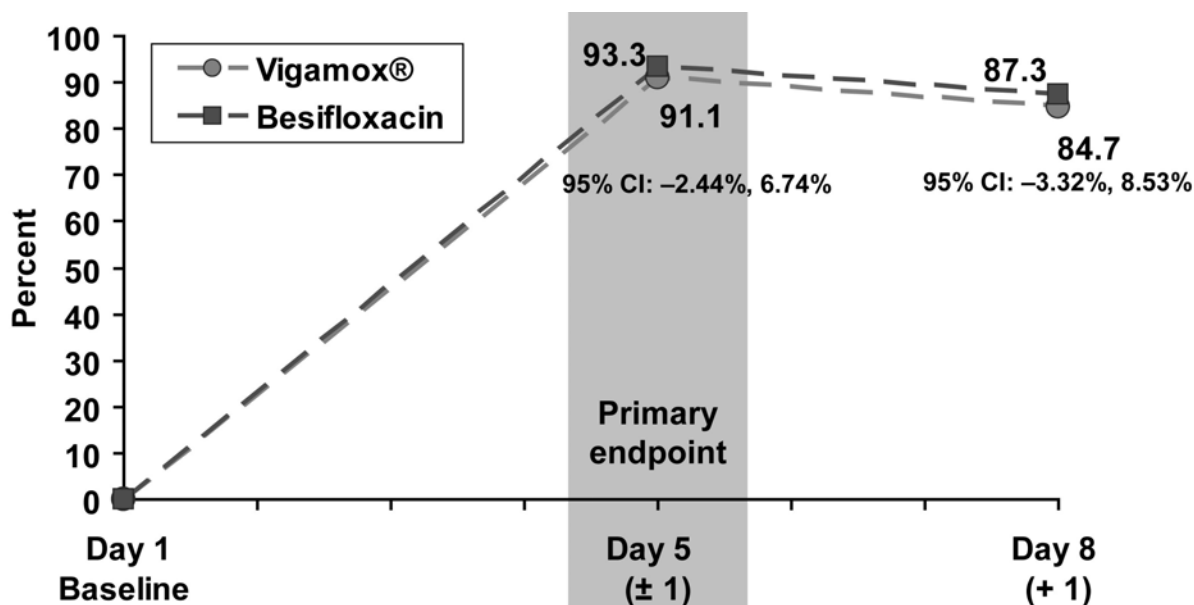


Figure 15. Microbial Eradication at Visit 2 (Day 5 ± 1) and Visit 3 (Day 8 or 9) (mITT, Culture-confirmed, As Treated)—Study 434

MICROBIAL ERADICATION OF BASELINE PATHOGENS

Microbial eradication at Visit 3 (Day 8 or 9) is shown by baseline pathogens in Table 31 for besifloxacin versus Vigamox. Comparable to the other 2 studies, these results show a broad-spectrum nature of the microbial eradication with besifloxacin, very high eradication rates independent of the Gram-stain characteristics, and high eradication rates for the most prevalent organisms encountered.

Table 31. Microbial Eradication at Visit 2 (Day 5 ± 1) by Baseline Species-Specific Study Eye Isolates With Incidence ≥ 1% in Study 434—Besifloxacin vs Vigamox

Pathogen	Isolates eradicated/encountered (%)	
	Besifloxacin	Vigamox
Gram-positive isolates	209/227 (92)	219/244 (90)
Gram-negative isolates	98/102 (96)	120/126 (95)
<i>H. influenzae</i>	75/79 (95)	85/90 (94)
<i>S. aureus</i>	50/59 (85)	48/56 (86)
<i>S. epidermidis</i>	27/29 (93)	36/41 (88)
<i>S. pneumoniae</i>	53/56 (95)	60/66 (91)
<i>S. mitis</i> group ^a	10/11 (91)	13/14 (93)
CDC coryneform group G	6/7 (86)	11/11 (100)
<i>S. oralis</i>	6/6 (100)	3/4 (75)
<i>C. pseudodiphtheriticum</i>	5/5 (100)	2/2 (100)
<i>S. lugdunensis</i>	4/4 (100)	3/3 (100)

^a In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

LACK OF FLUOROQUINOLONE RESISTANCE DEVELOPMENT DURING STUDY 434

A total of 65 pathogens isolated at or above threshold at Visit 2 or Visit 3 (26 besifloxacin treated, 39 Vigamox treated) were determined by PFGE analysis to be genetically concordant. MIC testing of all 65 genetically concordant isolate pairs indicated that susceptibility of Visit 2 or Visit 3 isolates did not increase by more than 2-fold for any of the tested fluoroquinolones, including besifloxacin.

VIRAL TEST RESULTS

In this study, 67 of 1161 randomized patients had positive viral cultures at baseline. Seventeen were included in the bacterial culture-confirmed population; 8 of these were treated with Vigamox and 9 were treated with besifloxacin. Two of the 17 patients, both treated with Vigamox, had positive bacterial cultures at follow-up visits, 1 at Visit 2 and 1 at Visit 3. Of the 8 patients treated with Vigamox, 3 patients had resolution of the baseline conjunctivitis at Visit 2 and 5 had resolution at Visit 3. Of the 9 patients treated with besifloxacin, 4 had resolution of the baseline conjunctivitis at Visit 2, and 6 out of 7 patients that returned for Visit 3 had resolution.

7.2.3.4 Efficacy Conclusions for Study 434

In patients with culture-confirmed bacterial conjunctivitis, results for the primary efficacy endpoints, clinical resolution and microbial eradication at Visit 2 (Day 5 \pm 1), demonstrated that besifloxacin ophthalmic suspension was non-inferior to Vigamox, suggesting that treatment of bacterial conjunctivitis with besifloxacin ophthalmic suspension will produce efficacy outcomes that are similar to those observed when treating with Vigamox.

7.3 Clinical Microbiology

7.3.1 Integrated Summary of Species-Specific Microbiological Eradication

In this section, only key microbial efficacy data from the integrated analyses of Studies 373, 433, and 434 are presented. For the species-specific microbiological eradication, baseline pathogens with levels at or above threshold were analyzed as treated.

In the original analyses for Study 373, data were analyzed using the baseline-designated study eye (ie, clinically diagnosed bacterial conjunctivitis based on 3 clinical signs—conjunctival discharge, bulbar conjunctival injection and palpebral conjunctival injection), and unlike analyses for Studies 433 and 434, no data were analyzed using a species-specific study eye designation. To facilitate comparison of the results between studies, additional analyses were completed to the final report for Study 373, using a species-specific study eye designation for the summary of clinical and microbial outcome for each Gram-positive and each Gram-negative bacterial species. In these additional analyses, the definition of the species-specific study eye was the same as that used for clinical Studies 433 and 434.

7.3.1.1 Statistical Analysis and Data Tabulation Implications

All integrated analyses are based on the culture-confirmed (mITT) study population (n = 1041), which includes all ITT patients from Study 373 and all mITT patients from Studies 433 and 434. The mITT study population included all patients in the study population for whom baseline cultures in at least 1 eye indicated bacteria levels at or above threshold for any accepted ocular species.

The integration of the microbiological data across the three studies included the integration of Visit 2 data (Day 4 ± 1 day for Study 373 and Day 5 ± 1 day for Studies 433 and 434) and the integration of Visit 3 (Day 8 or 9 for Studies 373, 433, and 434). In these integrated analyses, the primary endpoint visit is Visit 2 and the secondary endpoint visit is Visit 3, which is consistent with the analyses of individual Studies 433 and 434 but is different from the analyses of Study 373 where Visit 3 was considered the primary endpoint visit. Microbiological eradication was identically defined among the three studies.

7.3.1.2 Overall Analysis of Studies 373, 433, and 434

Results from species-specific study eyes at Visit 2 or 3 in the culture-confirmed (mITT) as-treated population (or the equivalent ITT population in Study 373) will be described. *In vitro* susceptibilities to besifloxacin and other antibacterial agents were determined for all isolates regardless of treatment group.

7.3.1.3 Incidence of Key Organisms at Baseline

The baseline distribution of key pathogens across Studies 373, 433, and 434 is shown in Table 32. In total, 1324 bacterial isolates were reported; Study 373 contributed 145 isolates, Study 433 contributed 480 isolates, and Study 434 contributed 699 isolates. Study 434 included 95 isolates from Asian sites, accounting for 7.2% (95/1324) of isolates from all three studies or 13.6% (95/699) of isolates from Study 434. The contribution of isolates per treatment group was as follows: besifloxacin, 656 (49.5%) isolates; Vigamox, 370 (27.9%) isolates; and vehicle, 298 (22.5%) isolates. This ratio was also observed in most cases at the species level. The besifloxacin treatment arm was included in all 3 studies being integrated, the vehicle treatment arm was part of Studies 373 and 433, and the Vigamox treatment arm was part of Study 434 only. Asian sites were part of Study 434 only.

Of the 1324 bacterial isolates, 886 (66.9%) were Gram-positive, while the remaining 438 (33.1%) were Gram-negative. The most frequently isolated organisms across all 3 studies were *Haemophilus influenzae* (344 isolates, 26.0%), *Streptococcus pneumoniae* (302 isolates, 22.8%), *Staphylococcus aureus* (190 isolates, 14.4%), *Staphylococcus epidermidis* (111 isolates, 8.4%), *Streptococcus mitis* group (65 isolates, 4.9%), CDC coryneform group G (29 isolates, 2.2%), and *Streptococcus oralis* (18 isolates, 1.4%) (Table 32).

Table 32. Baseline Pathogens With Incidence \geq 1% in Species-Specific Study Eyes—Studies 373, 433, 434 Combined

Organism	Incidence, n (%)			
	Study 373 (N = 145)	Study 433 (N = 480)	Study 434 (N = 699)	Total (N = 1324)
<i>H. influenzae</i>	46 (31.7)	129 (26.9)	169 (24.2)	344 (26.0)
<i>S. pneumoniae</i>	40 (27.6)	140 (29.2)	122 (17.5)	302 (22.8)
<i>S. aureus</i>	20 (13.8)	55 (11.5)	115 (16.5)	190 (14.4)
<i>S. epidermidis</i>	7 (4.8)	34 (7.1)	70 (10.0)	111 (8.4)
<i>S. mitis</i> group ^a	3 (2.1)	29 (6.0)	33 (4.7)	65 (4.9)
CDC coryneform group G	2 (1.4)	9 (1.9)	18 (2.6)	29 (2.2)
<i>S. oralis</i>	4 (2.8)	4 (0.8)	10 (1.4)	18 (1.4)

^a In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

7.3.1.4 Antibacterial Susceptibility of Baseline Pathogens

Susceptibility testing of clinical trial isolates was performed for besifloxacin and comparator test agents. Isolates cultured in Studies 373, 433, and 434 yielded besifloxacin susceptibility patterns similar to those observed in the nonclinical studies. A total of 1324 isolates were recovered from patients at baseline (Visit 1) in the culture-confirmed (mITT), as-treated population species-specific study eye across all treatment groups. Overall MIC₅₀/MIC₉₀ values for the 1324 isolates of all species from all treatment groups combined were 0.06/0.25 µg/mL for besifloxacin and 0.125/0.5 µg/mL for moxifloxacin. MIC₉₀ values were slightly higher in isolates from Asia: 1 µg/mL for besifloxacin and 2 µg/mL for moxifloxacin. Of the 1324 bacterial isolates, 886 (66.9%) were Gram-positive, while the remaining 438 (33.1%) were Gram-negative (Table 33). The besifloxacin MIC₅₀/MIC₉₀ values were 0.06/0.25 µg/mL for Gram-positive bacteria and 0.03/0.5 µg/mL for Gram-negative bacteria.

As discussed previously, patients' fellow eyes could contribute species-specific study eye isolates if that species was not already present in that patient's study eye. Table 33 outlines the contribution of fellow eyes to the number of species-specific study eye isolates.

Table 33. Distribution of Baseline Species-Specific Pathogens Across Baseline-Designated Study Eyes and Baseline-Designated Fellow Eyes in the Culture-Positive, As-Treated Population

Organism	Baseline-Designated Fellow Eye/All Species-Specific Study Eye Isolates (%)			
	Besifloxacin	Vigamox	Vehicle	Overall
All species	40/656 (6.1)	18/370 (4.9)	15/298 (5.0)	73/1324 (5.5)
Gram-positive	34/447 (7.6)	15/244 (6.1)	14/195 (7.2)	63/886 (7.1)
Gram-negative	6/209 (2.9)	3/126 (2.4)	1/103 (1.0)	10/438 (2.3)
<i>H. influenzae</i>	2/167 (1.2)	2/90 (2.2)	1/87 (1.1)	5/344 (1.5)
<i>S. aureus</i>	7/93 (7.5)	0/56 (0)	0/41 (0)	7/190 (3.7)
<i>S. epidermidis</i>	6/50 (12.0)	6/41 (14.6)	4/20 (20.0)	16/111 (14.4)
<i>S. pneumoniae</i>	1/153 (0.7)	0/66 (0)	0/83 (0)	1/302 (0.3)

MIC DISTRIBUTION FOR BESIFLOXACIN AND COMPARATOR TEST AGENTS FOR SELECTED SPECIES FROM COMBINED BESIFLOXACIN, VEHICLE, AND VIGAMOX TREATMENT GROUPS

The antibacterial susceptibility profile of each Visit 1 bacterial isolate was determined for besifloxacin and other antimicrobials. The integrated MIC range, MIC₅₀ and MIC₉₀ values of selected species for besifloxacin, azithromycin, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, and ofloxacin are provided in Table 34.

Table 34. *In Vitro* Activity of Besifloxacin and Comparators Against Key Organisms—Studies 373, 433, and 434 Species-Specific Study Eye Isolates

Organism	Phenotype	N	MIC (µg/mL)	Test Agent						
				BESI	AZITH	CIPRO	GATI	LEVO	MOXI	OFLOX
All	All	1324	Range	0.008 - 8	0.008 - >8	≤0.004 - >8	≤0.004 - >8	≤0.004 - >8	≤0.004 - >8	0.008 - >8
			MIC ₅₀	0.06	2	0.25	0.125	0.25	0.125	0.5
			MIC ₉₀	0.25	>8	2	0.5	1	0.5	2
Gram-positive	All	886	Range	0.008 - 8	0.008 - >8	0.015 - >8	0.008 - >8	0.008 - >8	0.008 - >8	0.008 - >8
			MIC ₅₀	0.06	1	0.5	0.25	0.5	0.125	1
			MIC ₉₀	0.25	>8	4	1	2	0.5	4
Gram-negative	All	438	Range	0.008 - 8	0.015 - >8	≤0.004 - >8	≤0.004 - 8	≤0.004 - 8	≤0.004 - 8	0.015 - >8
			MIC ₅₀	0.03	2	0.015	0.015	0.03	0.03	0.03
			MIC ₉₀	0.5	>8	0.125	0.25	0.125	0.25	0.25
CDC coryneform group G		29	Range	0.008 - 2	0.06 - >8	0.03 - 8	0.03 - 8	0.06 - >8	0.03 - >8	0.125 - >8
			MIC ₅₀	0.015	0.125	0.06	0.06	0.06	0.03	0.125
			MIC ₉₀	0.125	>8	0.5	0.5	1	0.25	2
<i>Corynebacterium pseudodiphtheriticum</i>		8	Range	0.015 - 0.25	0.125 - >8	0.03 - 1	0.06 - 0.5	0.06 - 1	0.03 - 0.5	0.125 - 2
			MIC ₅₀	0.25	>8	0.5	0.5	0.5	0.25	1
			MIC ₉₀	--	--	--	--	--	--	--
<i>Corynebacterium striatum</i>		8	Range	0.015 - 0.25	0.06 - >8	0.015 - 8	0.015 - 2	0.03 - 4	0.015 - 2	0.125 - >8
			MIC ₅₀	0.015	0.125	0.03	0.03	0.06	0.03	0.125
			MIC ₉₀	--	--	--	--	--	--	--
<i>Haemophilus influenzae</i>	All	344	Range	0.008 - 0.5	0.015 - >8	≤0.004 - 1	≤0.004 - 0.5	≤0.004 - 1	0.008 - 1	0.015 - 2
			MIC ₅₀	0.03	2	0.015	0.015	0.03	0.03	0.03
			MIC ₉₀	0.06	4	0.015	0.03	0.03	0.06	0.06
<i>Moraxella lacunata</i> ^a		9	Range							
			MIC ₅₀							
			MIC ₉₀							
<i>Staphylococcus aureus</i>	All	190	Range	0.008 - 8	0.06 - >8	0.06 - >8	0.03 - >8	0.03 - >8	0.03 - >8	0.125 - >8
			MIC ₅₀	0.03	2	0.5	0.125	0.25	0.06	0.5
			MIC ₉₀	0.5	>8	>8	4	8	2	>8

Table 34. *In Vitro* Activity of Besifloxacin and Comparators Against Key Organisms—Studies 373, 433, and 434 Species-Specific Study Eye Isolates (continued)

Organism	Phenotype	N	MIC (µg/mL)	Test Agent						
				BESI	AZITH	CIPRO	GATI	LEVO	MOXI	OFLOX
<i>Staphylococcus epidermidis</i>	All	111	Range	0.03 - 4	0.5 - >8	0.125 - >8	0.06 - >8	0.125 - >8	0.06 - >8	0.25 - >8
			MIC ₅₀	0.06	1	0.25	0.125	0.25	0.125	0.5
			MIC ₉₀	0.5	>8	>8	2	8	4	>8
<i>Staphylococcus hominis</i>		9	Range	0.03 - 0.5	1 - >8	0.125 - 8	0.06 - 2	0.125 - 4	0.06 - 1	0.25 - 8
			MIC ₅₀	0.06	4	0.125	0.125	0.125	0.06	0.25
			MIC ₉₀	--	--	--	--	--	--	--
<i>Staphylococcus lugdunensis</i>		8	Range	0.06 - 0.5	0.015 - >8	0.25 - 8	0.25 - 2	0.25 - 2	0.125 - 2	0.5 - 4
			MIC ₅₀	0.125	0.5	0.25	0.25	0.25	0.25	1
			MIC ₉₀	--	--	--	--	--	--	--
<i>Streptococcus mitis</i> ^b		20	Range	0.06 - 0.25	0.06 - 8	0.25 - 4	0.25 - 1	0.5 - 2	0.06 - 0.5	1 - 4
			MIC ₅₀	0.125	2	1	0.5	1	0.125	2
			MIC ₉₀	0.125	4	2	1	1	0.25	2
<i>Streptococcus mitis</i> group		45	Range	0.03-1	0.3 - >8	0.06 - >8	0.06 - 2	0.125 - >8	0.03 - 2	0.25 - >8
			MIC ₅₀	0.125	2	1	0.5	1	0.125	2
			MIC ₉₀	0.25	8	4	0.5	2	0.25	4
<i>Streptococcus oralis</i>		18	Range	0.015 - 0.25	0.06 - >8	0.03 - 4	0.03 - 1	0.125 - 2	0.015 - 0.5	0.125 - 4
			MIC ₅₀	0.125	4	2	0.5	1	0.25	2
			MIC ₉₀	0.25	>8	4	1	2	0.25	4
<i>Streptococcus pneumoniae</i>	All	302	Range	0.03 - 0.25	0.06 - >8	0.125 - >8	0.125 - 1	0.125 - 2	0.06 - 1	0.5 - 4
			MIC ₅₀	0.06	0.125	0.5	0.25	0.5	0.125	1
			MIC ₉₀	0.125	>8	1	0.5	1	0.125	2
<i>Streptococcus salivarius</i>		9	Range	0.06 - 0.25	0.06 - >8	1 - 2	0.5 - 2	1 - 2	0.125 - 1	2 - 4
			MIC ₅₀	0.125	8	2	0.5	1	0.25	2
			MIC ₉₀	--	--	--	--	--	--	--

^a No MIC values could be determined using standard test methods.

^b In this table, *S. mitis* and *S. mitis* group are listed separately.

The same improved efficacy of besifloxacin was observed for the multi-drug resistant strains. In fact, in this case with no exceptions, besifloxacin was more active than or equal to the competitor drugs.

Clinical isolates of *S. aureus* and *S. epidermidis* were grouped according to their susceptibility to oxacillin and ciprofloxacin. Table 35 shows the MIC data for besifloxacin and comparator antimicrobial agents for those isolates.

Table 35. In Vitro (MIC₉₀) Activity versus Resistant Staphylococcal Isolates—Studies 373, 433, and 434

Pathogen	N	MIC ₉₀ (µg/mL)			
		Besifloxacin	Moxifloxacin	Gatifloxacin	Azithromycin
<i>S. aureus</i>					
MSSA-CS	144	0.06	0.125	0.25	>8
MRSA-CS ^a	9	0.06 ^a	0.06 ^a	0.25 ^a	>8 ^a
MSSA-CR	17	2	8	>8	>8
MRSA-CR	17	4	>8	>8	>8
<i>S. epidermidis</i>					
MSSE-CS	50	0.06	0.125	0.25	>8
MRSE-CS	27	0.06	0.125	0.25	>8
MSSE-CR	10	1	8	8	>8
MRSE-CR	24	4	>8	>8	>8

CR = Ciprofloxacin resistant; CS = Ciprofloxacin susceptible.

^a Due to limited isolates, highest MIC value is given.

7.3.1.5 Analysis on Organism-by-Organism Basis for Key Organisms

Overall, 86 bacterial conjunctival pathogenic species were isolated at baseline at or above threshold from species-specific study eyes and identified during the conduct of Studies 373, 433, and 434. Within the besifloxacin ophthalmic suspension treatment group, organisms with > 10 isolates (in order of prevalence) included *H. influenzae*, *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *S. mitis* group, CDC coryneform group G, and *Streptococcus oralis*. These species were termed Key Organisms.

Globally (US and Asia sites combined), microbial eradication rates for all species combined were 92.2% in the besifloxacin treatment group, 61.4% in the vehicle treatment group, and 91.6% in the Vigamox treatment group at Visit 2. At Visit 3, the corresponding numbers were 88.4%, 72.5%, and 85.7%, respectively (Table 36).

Table 36. Integrated Species-Specific Microbial Eradication Rates in Culture-confirmed, As-Treated Population (Global)

Organism	Besifloxacin		Vehicle		Vigamox	
	Visit 2	Visit 3	Visit 2	Visit 3	Visit 2	Visit 3
All species	606/656 (92.2%)	580/656 (88.4%)	183/298 (61.4%)	216/298 (72.5%)	339/370 (91.6%)	317/370 (85.7%)
Gram-positive	412/447 (92.2%)	392/447 (87.7%)	114/195 (58.5%)	140/195 (71.8%)	219/244 (89.8%)	211/244 (86.5%)
Gram-negative	193/209 (92.3%)	188/209 (90.0%)	69/103 (67.0%)	76/103 (73.8%)	120/126 (95.2%)	106/126 (84.1%)
CDC coryneform group G	15/16 (93.8%)	15/16 (93.8%)	1/2 (50.0%)	2/2 (100.0%)	11/11 (100.0%)	11/11 (100.0%)
<i>C. pseudodiphtheriticum</i>	6/6 (100.0%)	6/6 (100.0%)	0/0 0	0/0 0	2/2 (100.0%)	2/2 (100.0%)
<i>C. striatum</i>	5/5 (100.0%)	5/5 (100.0%)	0/0 0	0/0 0	2/3 (66.7%)	3/3 (100.0%)
<i>H. influenzae</i>	152/167 (91.0%)	148/167 (88.6%)	56/87 (64.4%)	64/87 (73.6%)	85/90 (94.4%)	79/90 (87.8%)
<i>M. lacunata</i>	5/5 (100.0%)	4/5 (80.0%)	2/3 (66.7%)	3/3 (100.0%)	1/1 (100.0%)	1/1 (100.0%)
<i>S. aureus</i>	81/93 (87.1%)	78/93 (83.9%)	16/41 (39.0%)	20/41 (48.8%)	48/56 (85.7%)	46/56 (82.1%)
<i>S. epidermidis</i>	47/50 (94.0%)	44/50 (88.0%)	11/20 (55.0%)	15/20 (75.0%)	36/41 (87.8%)	32/41 (78.0%)
<i>S. hominis</i>	5/6 (83.3%)	6/6 (100.0%)	1/2 (50.0%)	1/2 (50.0%)	1/1 (100.0%)	1/1 (100.0%)
<i>S. lugdunensis</i>	5/5 (100.0%)	5/5 (100.0%)	0/0 0	0/0 0	3/3 (100.0%)	2/3 (66.7%)
<i>S. mitis</i> group ^a	17/19 (89.5%)	16/19 (84.2%)	10/12 (83.3%)	10/12 (83.3%)	13/14 (92.9%)	13/14 (92.9%)
<i>S. oralis</i>	10/11 (90.9%)	8/11 (72.7%)	2/3 (66.7%)	2/3 (66.7%)	3/4 (75.0%)	3/4 (75.0%)
<i>S. pneumoniae</i>	142/153 (92.8%)	132/153 (86.3%)	47/83 (56.6%)	61/83 (73.5%)	60/66 (90.9%)	57/66 (86.4%)
<i>S. salivarius</i>	5/5 (100.0%)	4/5 (80.0%)	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)

Note: Visit 2 was defined as Day 4 ± 1 in Study 373 and as Day 5 ± 1 in Studies 433 and 434; Visit 3 was defined as Day 8 or 9 in all 3 studies.

^a In this analysis, *S. mitis* group includes only isolates identified as *S. mitis* or *S. mitis* group.

Graphic representations of the microbial eradication relative to the besifloxacin MIC distribution are shown in Figures 16 to 19 for *H. influenzae*, *S. aureus*, *S. epidermidis*, and *S. pneumoniae*.

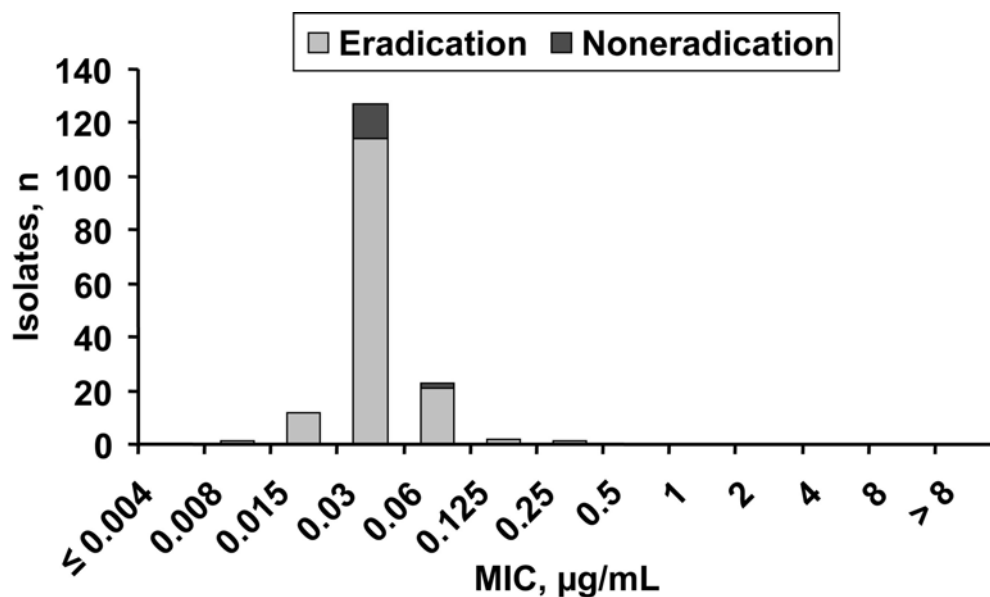


Figure 16. Baseline *H. influenzae* (All Phenotypes) Species-Specific Microbial Eradication in Study Eyes in Studies 373, 433, and 434 (Culture-confirmed, As Treated)

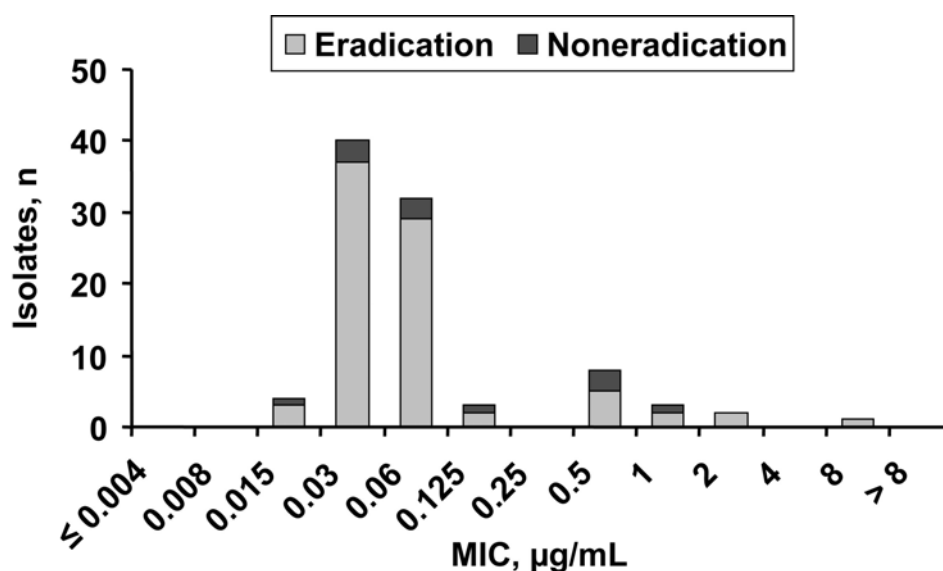


Figure 17. Baseline *S. aureus* (All Phenotypes) Species-Specific Microbial Eradication in Study Eyes in Studies 373, 433, and 434 (Culture-confirmed, As Treated)

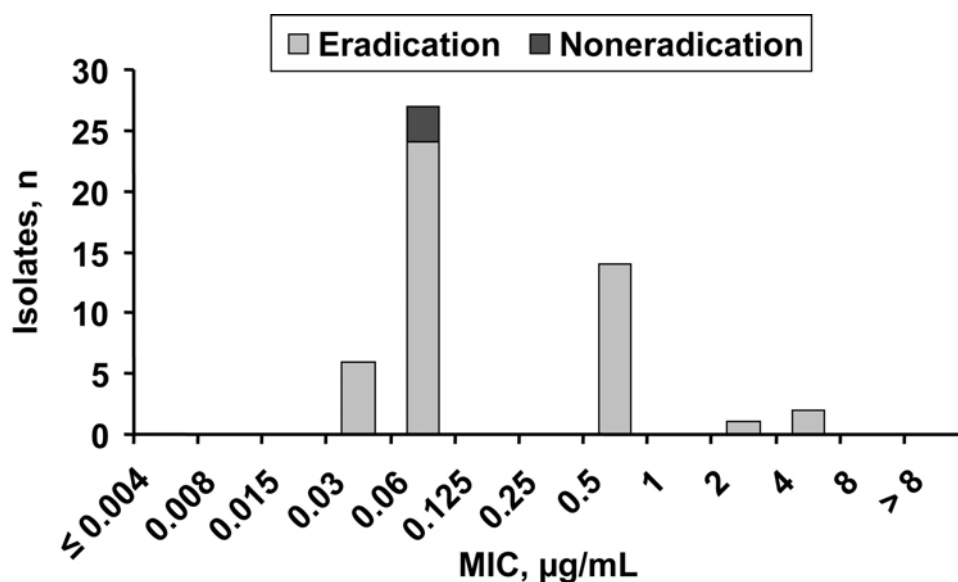


Figure 18. Baseline *S. epidermidis* (All Phenotypes) Species-Specific Microbial Eradication in Study Eyes in Studies 373, 433, and 434 (Culture-confirmed, "As Treated")

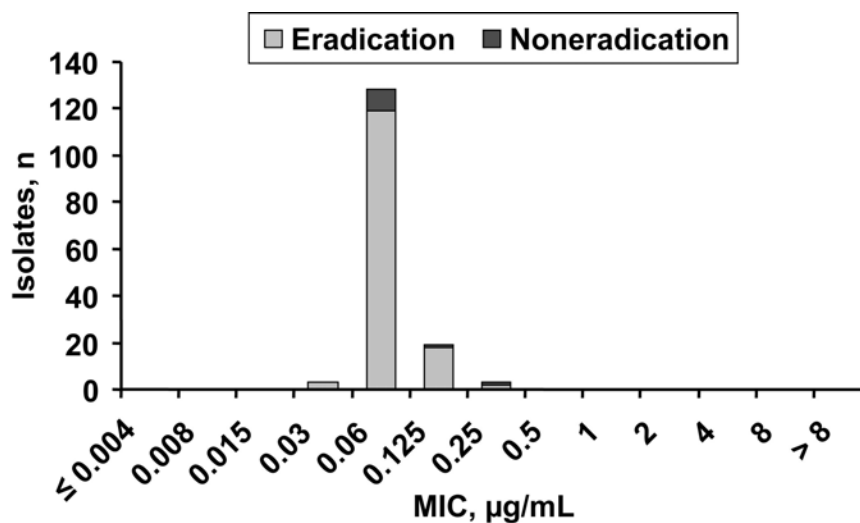


Figure 19. Baseline *S. pneumoniae* (All Phenotypes) Species-Specific Microbial Eradication in Study Eyes in Studies 373, 433, and 434 (Culture-confirmed, As Treated)

Microbial eradication and MIC data for pathogens of ophthalmic significance encountered with low frequency in Studies 373, 433, and 434 are presented in Table 37.

Table 37. Pathogens of Ophthalmic Significance Encountered With Low Frequency in Studies 373, 433, and 434 ^a

Species	N	MIC values	Eradication ^b
<i>Moraxella catarrhalis</i>	3	0.06	100% (3/3)
<i>Neisseria gonorrhoeae</i>	2	0.008, 0.25	100% (2/2)
<i>Neisseria meningitidis</i>	2	0.008, 0.015	100% (2/2)
<i>Neisseria sicca</i>	1	0.125	100% (1/1)
<i>Pseudomonas aeruginosa</i>	4	1, 2, 2, 4	100% (4/4)

^a Besifloxacin treatment group, culture-positive, as-treated population.

^b Microbial eradication by study Visit 2.

Figures 20 and 21 compare the number of isolates from US and Asian sites based on their MIC distributions. Figure 20 depicts data for all Gram-positive isolates, while Figure 21 represents all Gram-negative isolates.

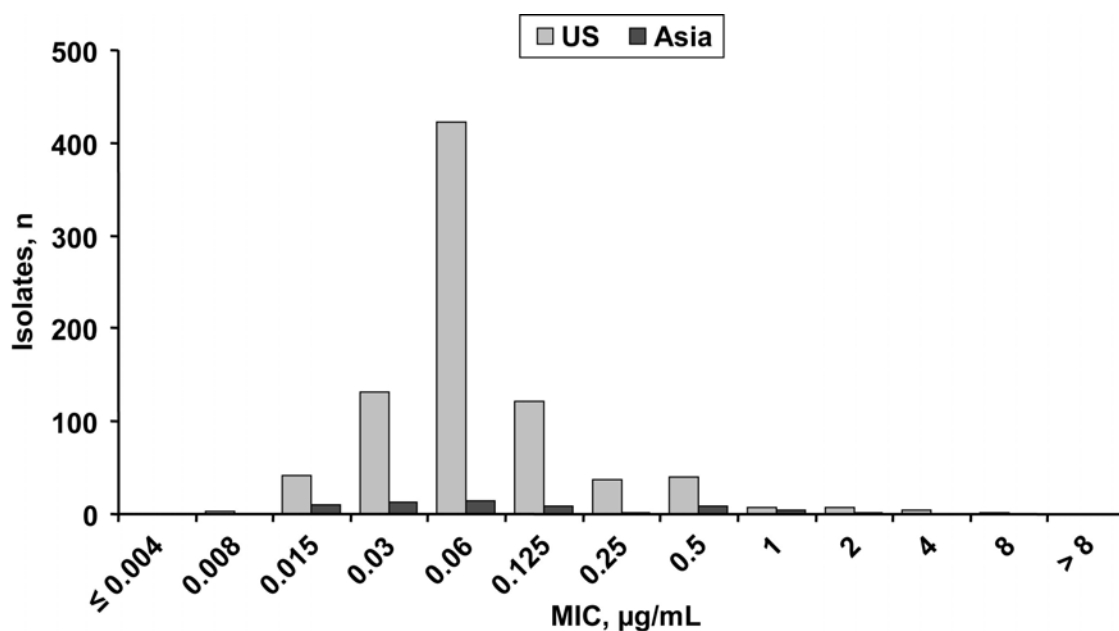


Figure 20. Baseline Distribution of MIC values for Gram-Positive Pathogens in Species-Specific Study Eyes in Patients from US or Asia Treated in Studies 373, 433, and 434 (Culture-confirmed, As-Treated Population)

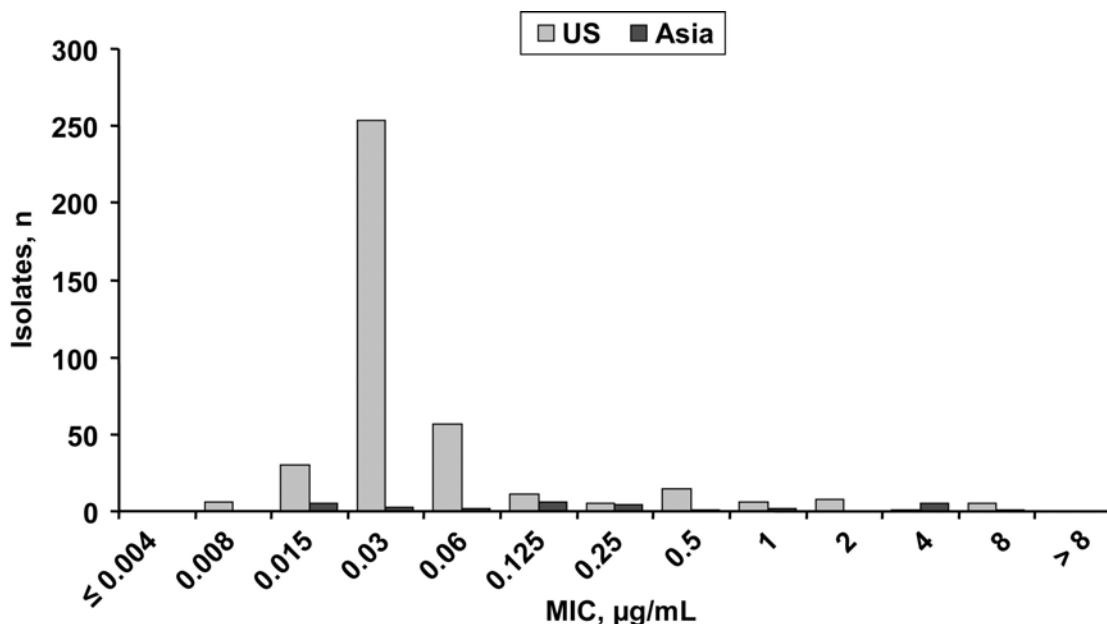


Figure 21. Baseline Distribution of MIC Values for Gram-Negative Pathogens in Species-Specific Study Eyes in Patients From US or Asia Treated in Studies 373, 433, and 434 (Culture-confirmed, As-Treated Population)

7.3.1.6 Species-Specific Microbiological Eradication Failures

Isolate pairs from eyes with the same species at or above threshold at both baseline and follow-up visits were evaluated by Pulsed Field Gel Electrophoresis (PFGE) analysis to (i) distinguish new infections from recurrence and (ii) determine if any microbial eradication failures were correlated with antimicrobial resistance development during the study period. Concordant (similar) PFGE results for 2 clinical isolates suggest that the bacteria are closely related and belong to the same strain. The finding of two concordant isolates at baseline and follow-up with ≥ 4 -fold increase in MIC values would have suggested the development of antimicrobial resistance during the study period; however, this finding was not observed. Strains with discordant PFGE fingerprints are not closely related, suggesting that one strain was replaced by another between baseline and the follow-up visit. In several instances, microbial eradication failures were the result of infection with a discordant strain.

In summary, microbial eradication failures were not a predictor of clinical resolution outcomes. No correlation was observed between bacterial species and microbial eradication failure other than the prevalence of the organism within the overall population of bacterial conjunctivitis isolates. Failures were the result of the persistence of the baseline (Visit 1) strain or re-infection with discordant strains of the same species. Analyses of the antibacterial susceptibility data showed that in no case did baseline strains develop resistance to besifloxacin or other fluoroquinolone test agents during the treatment period. The combined PFGE and susceptibility data did not indicate development of fluoroquinolone resistance for any isolates in the besifloxacin, Vigamox, or vehicle treatment groups across Studies 373, 433, and 434.

7.3.1.7 Summary of Integrated Clinical Microbiological Results

The primary objective of this integrated analysis was to evaluate the clinical microbial efficacy of besifloxacin ophthalmic suspension, 0.6%, compared to either vehicle or Vigamox, administered TID for 5 days in the treatment of bacterial conjunctivitis.

Studies 373, 433, and 434 were large, controlled studies conducted according to Good Clinical Practices. Sites from both the United States and Asia were included in Study 434. In general, the US and Asian sites were similar regarding isolates, phenotypes, and sensitivities.

From a microbiological perspective, the baseline pathogen distribution was similar across the besifloxacin ophthalmic suspension, vehicle, and Vigamox treatment groups.

The relative frequency of organisms isolated at threshold levels or higher from these studies, *H. influenzae*, *S. pneumoniae*, *S. aureus*, and *S. epidermidis*, were similar to previous reports in patients with bacterial conjunctivitis.

Besifloxacin was active against a wide range of organisms, including antimicrobial-resistant strains. Overall, the sensitivities of the pathogens obtained from patients in the besifloxacin ophthalmic suspension treatment group were similar to those obtained from patients in the Vigamox or vehicle treatment groups (these included resistant phenotypes). Furthermore, no besifloxacin or moxifloxacin resistant strains emerged in any of the 3 clinical studies.

In these controlled studies, besifloxacin ophthalmic suspension showed potent antimicrobial activity against a wide range of organisms, similar to the comparator fluoroquinolone. These data indicate that treatment of bacterial conjunctivitis with besifloxacin ophthalmic suspension will produce microbial eradication rates that are similar to those observed when treating with Vigamox and superior to the vehicle control.

7.4 Analysis of Clinical Information Relevant to Dosing Recommendations

In each of the 3 controlled studies (Studies 373, 433, and 434), patients instilled besifloxacin ophthalmic suspension in the affected eye(s) TID for 5 days. Patients were instructed to invert the closed bottle and shake once prior to administering the drug. The 0.6% concentration of besifloxacin and TID dosing is supported by the PK/PD relationship analysis and data from the extensive preclinical and clinical development program.

Studies were conducted to assess the PK/PD relationship of besifloxacin ophthalmic suspension, 0.6%, from PK studies generated in humans along with the *in vitro* microbial efficacy (PD) data (MIC₉₀ values) generated from several prominent microorganisms isolated from patients with bacterial conjunctivitis. In addition, the effect of protein binding on besifloxacin PK/PD ratios also is reported for comparison to address the potential impact of protein binding on the microbial activity of besifloxacin. The results of this modeling exercise demonstrated that topical ocular application of 0.6% besifloxacin ophthalmic suspension results in high therapeutic levels of besifloxacin in human tears, which remained above the MIC₉₀ value for most ocular pathogens up to 24 hours after dosing (mean C_{24h} = 1.60 ± 2.28 µg/g). The PK/PD ratios for these bacteria obtained after a simulated TID dosing scheme demonstrate that the C_{max}/MIC₉₀ and AUC₂₄/MIC₉₀ ratios are high, and substantially

above the target values published for fluoroquinolones (ie, C_{\max}/MIC_{90} ratio of > 10 and AUC/MIC_{90} ratio of > 100 -125 regardless of whether total besifloxacin concentrations or only unbound besifloxacin concentrations are considered. Taken together, these results provide a PK/PD-based rationale that supports the favorable efficacy observed with besifloxacin in the treatment of bacterial conjunctivitis.

7.5 Persistence of Efficacy and/or Tolerance Effects

Bacterial conjunctivitis is an acute, self-limiting disease. In the clinical safety and efficacy trials conducted in support of this application, patients were dosed TID for 5 days with besifloxacin ophthalmic suspension versus vehicle (Studies 373 and 433) or Vigamox (Study 434). Rates of clinical resolution and microbial eradication observed at Visit 2 (Day 4 ± 1 for Study 373 and Day 5 ± 1 for Studies 433 and 434) and Visit 3 (Day 8 or 9 for all studies) provided no evidence of tolerance or resistance.

7.6 Summary of Clinical Efficacy

To support the marketing application of besifloxacin ophthalmic suspension, 0.6%, 3 large, controlled safety and efficacy trials (Studies 373, 433, and 434) were conducted. These studies assessed the clinical and microbial efficacy of besifloxacin ophthalmic suspension compared with vehicle (Studies 373 and 433) or Vigamox (Study 434) for the treatment of bacterial conjunctivitis. Results from these studies demonstrated that besifloxacin ophthalmic suspension administered TID for 5 days was superior to vehicle and non-inferior to Vigamox. The primary efficacy endpoints were met for each of these studies.

Primary endpoint definitions differed between Study 373 and Studies 433 and 434. The primary efficacy endpoints were clinical resolution and microbial eradication at Visit 3 (Day 8 or 9) for Study 373 and clinical resolution and microbial eradication at Visit 2 (Day 5 ± 1) for Studies 433 and 434. The time point for Visit 2 was defined as Day 4 ± 1 for Study 373 and Day 5 ± 1 for Studies 433 and 434; however, the time point for Visit 3 was defined as Day 8 or 9 in all three studies. In addition to the difference in timepoint definitions, the definitions for clinical diagnosis and clinical resolution of bacterial conjunctivitis also differed between Study 373 and Studies 433 and 434. In Study 373, patients were required to present with a minimum of grade 1 for conjunctival discharge and a minimum of grade 1 for either bulbar or palpebral conjunctival injection for a clinical diagnosis of bacterial conjunctivitis. For Studies 433 and 434, a minimum of grade 1 for conjunctival discharge and bulbar conjunctival injection was required for diagnosis of bacterial conjunctivitis. Clinical resolution was defined as the absence of 3 clinical signs (conjunctival discharge, bulbar conjunctival injection, and palpebral conjunctival injection) in Study 373 and 2 clinical signs (conjunctival discharge and bulbar conjunctival injection) in Studies 433 and 434. However, microbial eradication was defined similarly in all 3 studies as the absence of all accepted ocular bacterial species that were present at or above threshold levels at baseline. All patients who were randomly assigned to treatment and had culture-confirmed conjunctivitis were evaluated for the primary endpoints in the ITT analysis in Study 373 or the mITT analysis in Studies 433 and 434.

The difference in the rates of clinical resolution between the besifloxacin ophthalmic suspension and vehicle treatment groups at Visit 2 (Day 4 \pm 1) was not statistically significant in Study 373, whereas the difference in the rates of clinical resolution between the besifloxacin ophthalmic suspension and vehicle treatment groups at Visit 2 (Day 5 \pm 1) in Study 433 was statistically significant. In the original analysis for Study 373, the primary efficacy analysis was conducted at Visit 3 (Day 8 or 9). The apparent difference in clinical resolution rates between these studies can be accounted for in part by the different visit days, Visit 2 being one day earlier in Study 373 (Day 4 \pm 1) compared with Visit 2 in Study 433 (Day 5 \pm 1). This hypothesis is further supported by the fact that the rates of clinical resolution for both the besifloxacin ophthalmic suspension and vehicle treatment groups were higher in Study 433 than in Study 373. In addition, clinical resolution was defined as the absence of 3 clinical signs (conjunctival discharge, bulbar and palpebral conjunctival injection) in Study 373 and the absence of 2 clinical signs (conjunctival discharge and bulbar conjunctival injection) in Study 433. The analysis for Study 373, which defined clinical resolution as the absence of 2 clinical signs at Visit 2 (Day 4 \pm 1) did not result in a statistically significant difference between treatment groups, although the rates of resolution for both treatment groups increased. At Visit 3 (Day 8 or 9), the difference in the rates of clinical resolution between the besifloxacin ophthalmic suspension and vehicle treatment groups was statistically significant for both studies, although overall rates were lower for Study 373 (original analysis) than for Study 433. At this visit, the difference in clinical resolution rates between the studies can be accounted for by the different definitions of clinical resolution between the two studies. When the data for Study 373 were analyzed with clinical outcome derived based on the same 2 clinical signs as for Study 433 (ie, conjunctival discharge and bulbar conjunctival injection), the difference in the rates of clinical resolution between the besifloxacin ophthalmic suspension and vehicle treatment groups at Visit 3 was in favor of besifloxacin and higher than the rates observed for the original analysis of Study 373.

When the rates of clinical resolution are compared between the vehicle-controlled Study 433 and the active-controlled Study 434, the rates of clinical resolution were higher for patients in the besifloxacin ophthalmic suspension treatment group in Study 434 compared with Study 433; however, the microbial eradication rates for patients in the besifloxacin ophthalmic suspension treatment groups were similar in both studies. Differences in the control may have contributed to this finding by introducing an expectation bias in the active-controlled study.

Overall, microbial eradication rates for patients in the besifloxacin ophthalmic suspension treatment group were high at Visit 2 and were sustained through Visit 3 (Day 8 or 9) for both Studies 373 and 433. The difference in eradication rates between the besifloxacin ophthalmic suspension and vehicle treatment groups were statistically significant at both study visits. Despite the difference in Visit day definitions, besifloxacin ophthalmic suspension induced equally high levels of bacterial eradication, demonstrating its efficacy and rapid treatment effect.

8 CLINICAL SAFETY OF BESIFLOXACIN

The safety database for besifloxacin ophthalmic suspension constitutes 4 clinical studies conducted in healthy volunteers (Study C-02-403-001, Study 424, Study ROC2-05-070, and Study 507) and 4 clinical studies conducted in patients with a clinical diagnosis of bacterial conjunctivitis (Study 478, Study 373, Study 433, and Study 434) (Table 9). The integrated safety summary includes patients treated for bacterial conjunctivitis in the controlled safety and efficacy trials (Studies 373, 433, and 434). Adverse events will be presented integrated over these 3 studies, and the rates of AEs associated with besifloxacin treatment will be compared statistically to those observed with vehicle alone. Key comparisons of besifloxacin to Vigamox within Study 434 are also noted. Additional measures that support the safety profile, biomicroscopy, ophthalmoscopy, and VA, will be presented by individual study. Additionally, Study 507, evaluating the corneal endothelial cell density effects of besifloxacin will be presented.

8.1 Integrated Analysis of Safety—Studies 373, 433, and 434

8.1.1 Extent of Exposure

A total of 2387 patients with a clinical diagnosis of bacterial conjunctivitis were exposed to besifloxacin ophthalmic suspension, vehicle, or Vigamox administered TID for 5 days in Studies 373, 433, and 434 (Table 38). Within these studies, 1192 patients received besifloxacin ophthalmic suspension, 0.6%, 616 patients received besifloxacin ophthalmic suspension vehicle, and 579 patients received the comparator drug, Vigamox.

In Studies 373, 433, and 434 combined, a total exposure to besifloxacin ophthalmic suspension of at least 5851 patient-days was observed (Table 38).

Table 38. Patient Exposure to Treatments by Study

Study	Treatment	Patients enrolled	Patients completed, n	Patient-days of exposure
Study 373	Besifloxacin suspension, 0.6%	137	134	680
	Vehicle	132	122	626
Study 433	Besifloxacin suspension, 0.6%	473	442	2304
	Vehicle	484	432	2319
Study 434	Besifloxacin suspension, 0.6%	582	555	2867
	Vigamox	579	554	2846
		2,387	2,239	

8.1.2 Deaths, Serious Adverse Events, and Discontinuation from Study Drug

8.1.2.1 Deaths

There were no deaths reported for any of the studies conducted in this clinical program.

8.1.2.2 Serious Adverse Events

The overall incidence of serious adverse events (SAEs) in the 3 clinical studies was low, with SAEs reported for 2 of 1192 patients (0.2%) treated with besifloxacin ophthalmic suspension, 1 of 616 patients (0.2%) exposed to vehicle, and 1 of 579 patients (0.2%) treated with Vigamox. All SAEs were non-ocular and none was considered to be treatment related (Table 39).

Table 39. Non-Ocular SAEs in Integrated Safety Population

	SAEs	Clinical Study	Details
Besifloxacin (n = 1192)	1	373	39 yr female, dehydration and anemia
	1	434	87 yr male, congestive heart failure
Vehicle (n = 616)	1	433	20 mo male, pneumonia
Vigamox (n = 579)	1	434	48 yr female, acute viral infection
Total	4		

SAE = Serious adverse event; yr = years; mo = months.

8.1.2.3 Discontinuation Due to Adverse Events

Discontinuations due to AEs across Studies 373, 433, and 434 are summarized in Table 40. These results did not suggest safety concerns associated with besifloxacin ophthalmic suspension. In the 3 clinical studies combined, discontinuations due to AEs occurred in 15 of 1192 patients (1.3%) treated with besifloxacin ophthalmic suspension, 7 of 616 patients (1.1%) exposed to vehicle, and 5 of 579 patients (0.9%) treated with Vigamox. These events were considered to be possibly related to treatment in 2 patients (0.2%) treated with besifloxacin ophthalmic suspension, 1 patient (0.2%) exposed to vehicle, and 2 patients (0.4%) treated with Vigamox.

Table 40. Discontinuations Due to Adverse Events—Safety Population (Studies 373, 433, and 434)

	Study 373		Study 433		Study 434	
	Besifloxacin (N = 137)	Vehicle (N = 132)	Besifloxacin (N = 473)	Vehicle (N = 484)	Besifloxacin (N = 582)	Vigamox (N = 579)
Discontinuations due to AEs, n	0	1	4	6	11	5
Treatment-related, n	--	0	1—possibly	1—possibly	1—possibly	2—possibly
Details of related AEs	--	--	Dermatitis	Corneal infiltrates	Photophobia	Iritis & decreased VA; allergy to study drug

AE = Adverse event; VA = Visual acuity.

8.1.3 Non-Serious Adverse Events

Typical of this study population (ie, patients with bacterial conjunctivitis) and route of administration, the most frequently reported AEs were ocular. In Studies 373, 433, and 434, the severity of AEs was predominantly mild. Overall, patients treated in the vehicle group experienced a much higher incidence of ocular AEs compared with those treated with besifloxacin ophthalmic suspension. The ocular events with the highest incidence in patients treated with besifloxacin ophthalmic suspension were conjunctivitis, blurred vision, conjunctivitis bacterial, and eye irritation. These events all occurred at a higher percentage in the vehicle treatment group than in the besifloxacin ophthalmic suspension treatment group.

The incidence of systemic/non-ocular AEs reported in Studies 373, 433, and 434 did not differ between the treatment groups.

8.1.3.1 Treatment-Emergent Non-Ocular Adverse Events

Treatment-emergent (occurring on or after the day of treatment initiation) non-ocular AEs that occurred in $\geq 0.5\%$ of eyes treated with besifloxacin ophthalmic suspension, vehicle, or Vigamox are summarized in Table 41. No statistically significant differences were observed between the besifloxacin ophthalmic suspension and vehicle treatment groups for any non-ocular AEs. In all treatment groups, headache was the most frequently reported non-ocular AE (at the preferred term level). In the besifloxacin ophthalmic suspension treatment group, 21 of 1192 patients (1.8%) reported headaches, which were mostly mild in severity. Of these events, 8 (0.7%) were considered to be treatment related. In the vehicle treatment group, 11 of 616 patients (1.8%) reported headaches, which were mostly mild in severity. Of these events, 2 (0.3%) were considered to be related to vehicle. In the Vigamox treatment group, 9 of 579 patients (1.6%) reported headaches, which were mostly mild in severity. Of these events, 1 was considered to be related to treatment with Vigamox. As expected, due to the low systemic exposure (< 0.5 ng/mL), non-ocular AEs were rare.

Table 41. Treatment-Emergent Non-Ocular Adverse Events Occurring in $\geq 0.5\%$ of All Treated Patients Within Any Treatment Group—Safety Population (Studies 373, 433, and 434)

Preferred Term	Besifloxacin (N = 1192)	Vehicle (N= 616)	Vigamox (N= 579)	<i>p</i> value ^a
Total number of AEs	107	64	45	
Number of patients with ≥ 1 AE	75 (6.3%)	48 (7.8%)	31 (5.4%)	0.2378
Headache	21 (1.8%)	11 (1.8%)	9 (1.6%)	>0.9999
Pharyngolaryngeal pain	8 (0.7%)	5 (0.8%)	3 (0.5%)	0.7725
Pyrexia	6 (0.5%)	4 (0.6%)	1 (0.2%)	0.7424
Cough	4 (0.3%)	4 (0.6%)	1 (0.2%)	0.4562
Pharyngitis streptococcal	3 (0.3%)	3 (0.5%)	1 (0.2%)	0.4159
Upper respiratory tract infection	2 (0.2%)	2 (0.3%)	4 (0.7%)	0.6091

AE = Adverse event.

^a *p* value based on Fisher's exact test, comparing besifloxacin and its vehicle.

Note: Treatment emergent refers to subsequent to the treatment of the study eye. The total number of adverse events counts all adverse events for patients. Patients may have more than one adverse event per body system and preferred term. At each level of patient summarization, a patient was counted once if he/she reported one or more events. Percentages are based on the number of patients who received the indicated treatment.

8.1.3.2 Treatment-Emergent Ocular Adverse Events

For all treated eyes (both study and fellow eyes) in the safety population, treatment-emergent ocular AEs were more common than non-ocular AEs. At least 1 ocular AE occurred in 13.8% (249/1810) of eyes in the besifloxacin ophthalmic suspension treatment group, 19.8% (190/961) of eyes in the vehicle treatment group, and 14.0% (120/855) of eyes in the Vigamox treatment group. Compared with the vehicle treatment group, the besifloxacin ophthalmic suspension treatment group had a significantly lower number of eyes with at least 1 treatment-emergent ocular AE ($p < 0.0001$, Fisher's exact test).

Treatment-emergent ocular AEs that occurred in $\geq 0.5\%$ of eyes treated with besifloxacin ophthalmic suspension, vehicle, or Vigamox are summarized in Table 42. The most prevalent ocular AEs were consistent with the underlying ocular disease being studied. Five ocular AEs (at the preferred term level) were reported at statistically significant different rates (Fisher's exact test, no adjustment for multiple comparisons) between the besifloxacin ophthalmic suspension and vehicle treatment groups; conjunctivitis ($p = 0.0223$), blurred vision ($p = 0.0035$), eye irritation ($p = 0.0187$), and increased lacrimation ($p = 0.0085$) were reported at lower rates in the eyes treated with besifloxacin ophthalmic suspension than vehicle, whereas the incidence of viral conjunctivitis was higher in the eyes treated with besifloxacin ophthalmic suspension versus vehicle ($p = 0.0185$).

Although not provided in the integrated analysis, the only AE that was reported at a statistically significant different rate between treatment groups in Study 434 was eye irritation, which was experienced in 0.3% (3/865) eyes treated with besifloxacin and 1.4%

(12/855) of eyes treated with Vigamox ($p = 0.0201$). Differences in all other AE rates in Study 434, including each of those discussed above, were statistically insignificant.

Table 42. Treatment-Emergent Ocular Adverse Events Occurring in $\geq 0.5\%$ of All Treated Eyes Within Any Treatment Group—Safety Population (Studies 373, 433, and 434)

Preferred term	Besifloxacin (N ^a = 1810)	Vehicle (N ^a = 961)	Vigamox (N ^a = 855)	<i>p</i> value ^b
Total number of AEs	327	258	153	
Number of eyes with ≥ 1 AE	249 (13.8%)	190 (19.8%)	120 (14.0%)	<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 pruritis	18 (1.0%)	18 (1.9%)	3 (0.4%)	0.0761
Conjunctival hemorrhage	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 edema	6 (0.3%)	4 (0.4%)	5 (0.6%)	0.7457
Conjunctival hyperemia	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 hyperemia	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 hyperemia	4 (0.2%)	3 (0.3%)	4 (0.5%)	0.6994
Corneal staining	4 (0.2%)	1 (0.1%)	4 (0.5%)	0.6645
Lacrimation increased	1 (0.1%)	6 (0.6%)	2 (0.2%)	0.0085

AE = Adverse event.

^a N = all treated eyes for the specified treatment group and includes study and fellow eyes.

^b *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 patient 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.

8.1.4 Other Ocular Safety Assessments

Additional safety measures assessed in Studies 373, 433, and 434, included visual acuity, slit lamp examination (biomicroscopy), and ophthalmoscopy. Results from these assessments were not integrated and are presented below by study.

8.1.4.1 Visual Acuity

Pin-holed VA examinations using either a Snellen chart or Lea Symbols were conducted at each visit and recorded in the Snellen format. Visual acuity line changes were calculated for each eye at follow-up visits, and a binary response variable was derived, indicating whether or not there was a VA loss of > 2 lines (ie, clinically significant).

In Study 373, 97.3% of eyes had VA of 20/40 or better at baseline. A clinically significant change in VA (> 2 Snellen line loss) was observed in 1 of 118 patients (0.8%) in the vehicle group versus none of the 132 patients in the besifloxacin treatment group at Visit 3.

In Study 433, 91.9% of eyes had VA of 20/40 or better at baseline. At Visit 3, 2 study eyes (1 study eye in each treatment group) had a > 2 line reduction in pin-holed VA. There was no statistically significant difference between the treatment groups.

In Study 434, 92.3% of eyes had VA of 20/40 or better at baseline. At Visit 3, 4 study eyes (3 in the besifloxacin treatment group and 1 in the Vigamox treatment group) had a > 2 -line reduction in pin-holed VA. There were no statistically significant differences between the treatment groups.

8.1.4.2 Biomicroscopy Examination

Biomicroscopy (slit lamp) examinations of the lids, limbus, conjunctiva, cornea, anterior chamber, lens, and vitreous were conducted at each visit.

In Study 373, treatment-emergent findings (those that increased ≥ 1 grade from baseline) were observed in treated eye(s) of 15 of 135 patients (11.1%) randomized to besifloxacin ophthalmic suspension and 8 of 122 patients (6.6%) randomized to vehicle; the between-group difference was not statistically significant.

In Studies 433 and 434, the biomicroscopy findings were consistent with the condition being treated. The rate of treatment-emergent findings of lid, limbus, conjunctiva, cornea, anterior chamber, lens and vitreous abnormalities, or worsening of these abnormalities, was low ($< 3\%$) and similar between treatment groups for both study eyes and treated fellow eyes.

8.1.4.3 Ophthalmoscopy

The fundus was assessed for pathology. In Study 373, none of the patients had a treatment-emergent finding in a treated eye. In Studies 433 and 434, no pathology was noted for the vast majority ($> 95\%$) of eyes and there were no treatment-emergent findings.

8.2 Corneal Endothelial Cell Density Study

The objective of Study 507 was to assess the corneal endothelial cell density changes in healthy volunteers when besifloxacin ophthalmic suspension, 0.6%, was administered TID for 5 days.

A total of 120 healthy volunteers (240 eyes) were enrolled in this randomized, contralateral eye study at 2 investigative sites in the United States. Eligible healthy volunteers received besifloxacin ophthalmic suspension TID at approximately 6-hour intervals for 5 days in a randomly selected eye, while the fellow eye received no drug treatment. Healthy volunteers returned to the study center for Visit 2 (Day 6) and were exited from the study.

The primary endpoint for this study was the difference in the change from baseline in endothelial cell density between eyes treated with besifloxacin ophthalmic suspension and untreated fellow eyes as measured using specular microscopy. The secondary endpoint was the change from baseline in endothelial cell density within eyes treated with besifloxacin ophthalmic suspension and within untreated fellow eyes.

On the primary efficacy endpoint, no statistically or clinically significant difference was observed in the change from baseline in endothelial cell density between eyes treated with besifloxacin ophthalmic suspension (mean change: 0.58 ± 96.95 cells/mm²) and untreated fellow eyes (mean change: -9.93 ± 97.88 cells/mm²) ($p = 0.269$). Results for the secondary efficacy endpoint also showed no statistically or clinically significant change in endothelial cell density within eyes treated with besifloxacin ophthalmic suspension (mean change: 0.58 ± 96.95 cells/mm², $p = 0.948$) or within untreated fellow eyes (mean change: -9.93 ± 97.88 cells/mm², $p = 0.269$).

Treatment with besifloxacin ophthalmic suspension did not result in any statistically or clinically significant changes from baseline in endothelial cell density within or between treated and untreated eyes (Table 43).

Table 43. Effects of Besifloxacin on Corneal Endothelial Cell Density—Study 507

Treatment	Mean baseline cell density, cells/mm ² (SD)	Change in cell density from baseline to Visit 2, cells/mm ² (SD)
Besifloxacin (n = 120)	2850.7 (334.9)	0.6 (97.0)
No treatment (n = 120)	2859.5 (380.3)	-9.9 (97.9)
<i>p</i> values	0.269 ^a / 0.948 ^b / 0.269 ^c	

SD = Standard deviation.

^a *p* value for the difference in change from baseline between treatment and fellow eye.

^b *p* value for the change from baseline for besifloxacin treated eyes.

^c *p* value for the change from baseline for untreated fellow eyes.

Note: *p* values were calculated from a 2-sided, paired *t*-test, $\alpha = 0.05$.

8.3 Summary of Safety

Overall, a total of 1192 patients in Studies 373, 433, and 434 were exposed to besifloxacin ophthalmic suspension. During the course of these 3 studies, few patients withdrew from treatment as a result of AEs.

Adverse events reported were mostly ocular. Ocular AEs were typical of the study population (ie, patients with bacterial conjunctivitis) and were generally mild in severity and transient in nature. At least 1 ocular AE occurred in 13.8% of eyes in the besifloxacin ophthalmic suspension treatment group, 19.8% of eyes in the vehicle treatment group, and 14.0% of eyes in the Vigamox treatment group. In Studies 373, 433, and 434, no deaths occurred, and the incidence of SAEs was very low in all treatment groups (4 total SAEs). All SAEs were non-ocular and none was considered to be treatment related.

Across all 3 studies, no statistically significant differences were observed between treatment groups for visual acuity, biomicroscopy/slit lamp examination, or ophthalmoscopy.

In addition, ocular and systemic PK studies have demonstrated that besifloxacin ophthalmic suspension has high ocular retention ($\geq 1.6 \mu\text{g/g}$ for at least 24 hours after a single dose), low systemic exposure ($< 0.5 \text{ ng/mL}$), and no effect on corneal endothelial cell density.

9 BENEFIT/RISK SUMMARY

Bacterial conjunctivitis is a common external ocular infection that affects persons of all ages. The condition often presents suddenly in one eye and can readily spread to the fellow eye as a contagious disease. Bacterial conjunctivitis is characterized by marked hyperemia or redness of the eye, and mild to moderate purulent conjunctival discharge. Symptoms often include tearing, itching, and ocular irritation. The disease is generally self-limiting and usually does not cause permanent loss of vision or structural damage; however, intervention with a topical broad-spectrum ocular anti-infective agent is standard of care for providing rapid symptomatic relief, reducing the rate of re-infection, possibly preventing the spread of the infection to others, and most importantly, improving the rate of early clinical remission and overall microbial eradication.

The spectrum of causative pathogens continues to evolve, and the incidence of resistance of these organisms to anti-infectives has been increasing (Cavuoto et al., 2008). Therefore, there is a continued need for development of novel anti-infectives with improved potency and activity against drug-resistant pathogens. Benefits for the clinician and patient offered by besifloxacin ophthalmic suspension, 0.6%, include local treatment for a local disease, convenient dosing (TID), long dwell time on the ocular surface where the disease process is ongoing, broad-spectrum antibacterial activity against a wide variety of pathogens, potent microbial eradication, bactericidal activity, low propensity for resistance development, and a favorable safety profile. The 3 clinical efficacy and safety studies (Studies 373, 433, and 434) conducted to support the NDA (22-308) demonstrated superior outcomes for besifloxacin ophthalmic suspension administered TID for 5 days versus its vehicle for both clinical

resolution and microbial eradication and clinical and microbial outcomes similar to those observed with Vigamox. Besifloxacin ophthalmic suspension administered TID for 5 days showed broad-spectrum eradication for Gram-negative and Gram-positive organisms, potent activity against resistant strains, and improved MIC₉₀ values versus comparator antibacterial agents used to treat bacterial conjunctivitis. These findings, along with evidence supporting the low propensity of besifloxacin ophthalmic suspension for resistance development, demonstrate the benefit of this drug for the treatment of bacterial conjunctivitis.

The risks associated with systemically administered fluoroquinolones have been shown to include QT prolongation, positive genotoxicity, ERG changes, phototoxicity, and adverse effects on joint tissues. The nonclinical development program for besifloxacin was designed to characterize the potential class effects, as well as other potential toxic effects specific to besifloxacin. The profile of besifloxacin showed similarities to profiles reported with other fluoroquinolones but with no cause of concern given its intended topical ophthalmic use. Furthermore, nonclinical studies have demonstrated that systemic effects of besifloxacin are induced only at very high systemic concentrations that could not reasonably be achieved with ocular administration of besifloxacin ophthalmic suspension.

Overall, besifloxacin ophthalmic suspension has been shown to be safe and well tolerated. Clinical studies have demonstrated low systemic exposure following administration of single and multiple doses of besifloxacin ophthalmic suspension and no corneal endothelial cell density changes. Moreover, the incidence of reported AEs was low with no treatment-related SAEs. The incidence of non-ocular and ocular AEs was low. Most non-ocular AEs were unrelated to study drug and the most prevalent ocular AEs were consistent with the underlying ocular disease being studied.

In summary, besifloxacin ophthalmic suspension has been shown to be safe and effective for the treatment of bacterial conjunctivitis in adequate and well-controlled studies. Besifloxacin ophthalmic suspension fits the ideal profile for the treatment of bacterial conjunctivitis because it is a local ocular treatment for a local ocular disease, has convenient dosing that is efficacious, a long dwell time on the ocular surface, broad-spectrum antibacterial activity against a wide variety of pathogens, potent microbial eradication, bactericidal activity, low propensity for resistance development, and a favorable safety profile.

10 CONCLUSION

Besifloxacin ophthalmic suspension, 0.6%, is safe and effective for the treatment of bacterial conjunctivitis and has a favorable benefit/risk profile to support this indication.

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