CLINICAL STUDY PROTOCOL
Laboratorios SALVAT, S.A.

Study phase: III

Protocol No. CIFLOT3-16IA01

Eudra CT Nº: Not applicable

Drug product: Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025%

Formulation: Otic solution

A Phase III, Multicenter, Randomized, Double-Blind Clinical Trial to Assess the Efficacy and Safety of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Otic Solution Compared to Ciprofloxacin 0.3% Otic Solution and to Fluocinolone acetonide 0.025% Otic Solution in the Treatment of Acute Otitis Externa (AOE).

"CifloteX study"

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Current version: 2.0 (Amendment 1)

Date of current Protocol: 26th February 2018

Previous versions:

Version	Date
Final	24 April 2017

Ethics statement: This study will be conducted according to the protocol and in compliance with the Good Clinical Practice guidelines of the International Conference on Harmonization, the Declaration of Helsinki, and applicable regulatory requirements.

SPONSOR'S SIGNATURE PAGE

Sponsor:	Laboratorios SALVAT, S.A.
Clinical Protocol Number:	CIFLOT3-16IA01
Drug Name:	Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Otic Solution
Protocol Title:	A Phase III, Multicenter, Randomized, Double-Blind Clinical Trial to Assess the Efficacy and Safety of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Otic Solution Compared to Ciprofloxacin 0.3% Otic Solution and to Fluocinolone acetonide 0.025% Otic Solution in the Treatment of Acute Otitis Externa (AOE).
Short title:	Ciprofloxacin plus Fluocinolone acetonide in Acute Otitis Externa ("CifloteX")
Date of Modified Protocol:	26th February 2018
Date of Original Protocol:	24th April 2017

Approved by:

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Enrique Jiménez, MD
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Laboratorios SALVAT, S.A.

Date

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Sponsor:	Laboratorios SALVAT, S.A.
Clinical Protocol Number:	CIFLOT3-16IA01
Drug Name:	Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Otic Solution
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Short title:	Ciprofloxacin plus Fluocinolone acetonide in Acute Otitis Externa ("CifloteX")
Date of Modified Protocol:	26th February 2018
Date of Original Protocol:	24th April 2017

All documentation that has been supplied to me by Laboratorios SALVAT, S.A. (the Sponsor) and/or the Sponsor's designee concerning this study, and that has not been previously published, will be kept in the strictest confidence. This documentation includes, but is not limited to, the study protocol, the Investigator's Brochure, and Case Report Forms (CRF) or Electronic Data Capture (EDC).

The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board / Independent Ethics Committee (IRB/IEC). No changes will be made to the study protocol without the prior written approval of the Sponsor or their designee and the IRB/IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and do agree to abide by all the conditions and instructions contained in this protocol.

_____ _____

Signature (Principal Investigator) Date

_____

Printed Name

1. PROTOCOL SYNOPSIS

Sponsor:	Laboratorios SALVAT, S.A
EudraCT number:	Not applicable
Title:	A Phase III, Multicenter, Randomized, Double-Blind Clinical Trial to Assess the Efficacy and Safety of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Otic Solution Compared to Ciprofloxacin 0.3% Otic Solution and to Fluocinolone acetonide 0.025% Otic Solution in the Treatment of Acute Otitis Externa (AOE).
Short title	Ciprofloxacin plus Fluocinolone acetonide in Acute Otitis Externa
Acronym	CifloteX
Study Number:	CIFLOT3-16IA01
Study Phase:	III
Study Centers:	Multi-centre study in approximately 50 sites in the US.
Objectives:	To evaluate the efficacy and safety of topical Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution. The primary endpoint is to demonstrate therapeutic superiority of Ciprofloxacin plus Fluocinolone acetonide relative to Ciprofloxacin alone and to Fluocinolone acetonide alone for therapeutic cure rate (clinical + microbiological cure) at End of Treatment, in patients suffering from AOE. The principal secondary endpoint is to demonstrate therapeutic superiority of Ciprofloxacin plus Fluocinolone acetonide relative to Ciprofloxacin alone and to Fluocinolone acetonide alone for time to end of pain.
Study Design:	Randomized, parallel-group, double blind, active-controlled, multicentre study in patients suffering from AOE. This study will compare the efficacy and safety of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution to that of Ciprofloxacin 0.3% otic solution and to Fluocinolone acetonide 0.025% otic solution, when administering one vial twice daily during 7 days.
Control Drug, Dosage, and Route of Administration:	Patients will be randomized to three arms and will receive Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution, or Ciprofloxacin 0.3% otic solution, or Fluocinolone acetonide 0.025% otic solution twice a day during 7 days.
Patient Population:	Patients 6 months of age or older with diagnosis of AOE. A total of 500 patients will be randomized in a 2:2:1 ratio (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution : Ciprofloxacin 0.3% otic solution : Fluocinolone acetonide 0.025% otic solution), with the aim of including 375 evaluable patients (150 Ciprofloxacin plus Fluocinolone acetonide, 150 Ciprofloxacin and 75 Fluocinolone acetonide).

Eligibility Criteria:	**Inclusion Criteria:** • Male or female, 6 months of age and over. • Patients with diagnosis of uncomplicated AOE of less than 21 days (3 weeks) in one or both ears, defined as at least 2 for otalgia (0-3 scale) and 2 for edema (0-3 scale) and 1 for otorrhea (0-3 scale). • Patients with a Brighton Grading of II or III • Patients with otorrhea of sufficient volume for sampling for microbiological culture (sample must be taken at baseline visit prior to the first study dose). • Willingness to refrain from swimming through end of the study. • For adult patients, ability to understand and provide written informed consent For pediatric patients, signed informed consent from patient's legally authorized representative; also, if the patient is capable of providing assent, signed assent from the patient as applicable according to local regulations. • Females who are not pregnant, not lactating and are not planning a pregnancy during the study. All females of childbearing potential will be able to participate only if they have a negative urine pregnancy at screening and if they agree to use adequate birth control methods to prevent pregnancy throughout the study.

	Exclusion Criteria: • Previous episode of AOE within 4 weeks prior to enrollment, or 2 or more episodes of AOE within 6 months prior to enrollment. • Tympanic membrane perforation (including tympanostomy tubes) • Current diagnosis of diabetes mellitus, psoriasis, otitis media, malignant otitis externa, surgical mastoid cavities, canal stenosis, cholesteatoma, exostosis, or tumors of either ear. • Current diagnosis of fungal (otomycosis) or viral infection of either ear. • Current diagnosis of dermatitis of the affected ear or surrounding area. • Current presence of any other infection of the ears or other medical condition that might adversely impact the safety of the study participants or confound the study results. • Known or suspected hypersensitivity or history of adverse reaction to quinolones, corticosteroid or any component of the study medication. • Use of any systemic antibacterial or corticosteroid within two weeks prior to enrollment (nasal or inhaled corticosteroids at stable doses at least 30 days prior to enrollment are allowed) • Use of any topical or otic antibiotic, steroidal or antifungal agent in the affected ear(s) within 1 week prior to enrollment, or any other topical or otic product in the affected ear(s) within 1 day prior to enrollment. • Concurrent use of systemic anti-inflammatory agents, including biologic response modifiers. (Analgesics without anti-inflammatory properties, such as acetaminophen, are allowed during the study.) • Previous participation in this trial. • Participation in another clinical trial within the previous 30 days. • Any condition or situation likely to cause the patient (or, if applicable, the patient's primary caregiver) to be unable or unwilling to comply with study treatment or attend all study visits. • Any condition (i.e significant acute or chronic medical, neurological, psychiatric illness…) in the patient or parent/guardian that, in the judgment of the principal investigator, could compromise patient safety, limit the patient's ability to complete the study, and/or compromise the objectives of the study.

Study Evaluation:	**Visit 1 (Baseline; Day 1)** ▪ Informed Consent (and assent form when applicable) ▪ A urine pregnancy test for females of childbearing potential will be performed at Visit 1 prior to randomization. ▪ Assessment of trial eligibility: inclusion/exclusion criteria ▪ Collection of general patient information including demographics ▪ Physical examination ▪ Vital signs examination (Temperature, blood pressure and pulse) ▪ Clinical assessment 1.Brighton grading 2. Signs of AOE in both ears ✓ Edema (absent, mild, moderate, severe) ✓ Otorrhea (absent, mild, moderate, severe) 3. Symptoms of AOE in both ears: ✓ Otalgia (absent, mild, moderate, severe) ▪ Collection of culture specimen. If bilateral AOE is present on exam, culture specimen collection should be performed only on the ear chosen for evaluation. ▪ Debridement of the ear(s) (after collection of the culture specimen). ▪ Dispensing study medication and administration of first dose ▪ Distribution of patient diary Throughout the study, patients or caregivers should record pain severity at least twice daily (prior to dosing) using a proper pain scale according to patient's age. ▪ Adverse events assessment ▪ Concomitant medication use **Visit 2 (Day 3+1)** Visit 2 will consist of a telephone call made on Day 3 or Day 4 of study treatment. At this "phone visit", the study staff appropriately trained and delegated will ask about study medication compliance, progress of otitis symptoms and signs, adverse events, and concomitant medications according to the EDC. Patients who report no improvement in otitis symptoms will be asked to come in for a visit as soon as possible. At this visit, the Investigator will choose one of the two following options for the patient:

	1. Continue the patient in the study. The present visit will be recorded as an unscheduled visit. The patient should continue taking study medication without interruption. A debridement of the ear and placement of otowick may be needed 2. Discontinue study medication. The visit will be recorded as the Visit 3, the End of Treatment visit. The patient will be withdrawn from the study and may be treated with other medication as appropriate at investigator's discretion. The clinical outcome for the patient will be recorded as Treatment Failure. If a patient has unilateral AOE at baseline but develops AOE of the contralateral ear subsequently during the study, then at Visit 2 or at an unscheduled visit prior to Visit 3,, the investigator or designee should obtain a second kit number with the same medication through the IWRS to treat the contralateral ear. **Visit 3 (End of Treatment [EOT]; Day 8+2)** ▪ Vital signs examination (Temperature, blood pressure and pulse) ▪ Clinical assessment: 1. Brighton grading 2. Signs of AOE in both ears: ✓ Edema (absent, mild, moderate, severe) ✓ Otorrhea (absent, mild, moderate, severe) 3. Symptoms of AOE in both ears: ✓ Otalgia (absent, mild, moderate, severe) 4. Overall clinical outcome (resolved, improved, not changed, worsened) ▪ Collection of culture specimen (if otorrhea is present) If bilateral AOE is present on exam, culture specimen collection should be performed only on the ear chosen for evaluation. If patient has received rescue medication prior to this visit, the culture specimen will be not collected because patient will be considered treatment failure. ▪ Debridement of the ear(s) (after collection of the culture specimen)., if applicable ▪ Review of patient diary ▪ Concomitant medication use ▪ Adverse events assessment ▪ Collection of remaining study medication

	Visit 4 (Test of Cure [TOC]; Day 15+2) ▪ Vital signs examination (Temperature, blood pressure and pulse) ▪ Clinical assessment: 1. Brighton grading 2. Signs of AOE in both ears: ✓ Edema (absent, mild, moderate, severe) ✓ Otorrhea (absent, mild, moderate, severe) 3. Symptoms of AOE in both ears: ✓ Otalgia (absent, mild, moderate, severe) 4. Overall clinical outcome (resolved, improved, not changed, worsened) ▪ Collection of culture specimen (if otorrhea is present) If bilateral AOE is present on exam, culture specimen collection should be performed only on the ear chosen for evaluation. ▪ If patient has received rescue medication prior to this visit, the culture specimen will be not collected because patient will be considered treatment failure. ▪ Debridement of the ear(s) (after collection of the culture specimen), if applicable ▪ Concomitant medication ▪ Adverse events assessment ▪ Review and return of patient diary ▪ Completion of end of study form

Efficacy Assessments:	**Primary endpoint:** • To demonstrate superiority of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution relative to Ciprofloxacin 0.3% otic solution and to Fluocinolone acetonide 0.025% otic solution with respect to therapeutic cure rate (clinical + microbiological cure) at EOT. Clinical + microbiological cure will be considered achieved if edema, otalgia and otorrhea are resolved with no further requirement of antimicrobial therapy and bacteriological response is Eradication or Presumed Eradication. **Secondary endpoints:** <u>Principal secondary endpoint:</u> • Time to end of ear pain (TEOP) is the interval (in days) between the first dose of the study medication and the time when the ear pain in the evaluable ear ended. This time will be calculated on the basis of the patient diary entries.. End of ear pain is defined as ending on the first day (morning or evening) on which there is no use of analgesic, the diary pain score is zero and remains at zero for all subsequent visits until the end of the study. <u>Other secondary endpoints:</u> • Sustained microbiological cure • Clinical cure at Visit 3 and Visit 4 • Microbiological cure at Visit 3 (and Visit 4 • Therapeutic cure (clinical+microbiological cure) at Visit 4 • Changes in Brighton grading at Visit 3 and Visit 4 • Changes in otorrhea at Visit 3 and Visit 4 • Changes in edema at Visit 3 and Visit 4 • Changes in otalgia assessed by the investigator at Visit 3 and Visit 4
Safety Assessments:	Adverse events (AE) will be recorded throughout the study. Vital signs assessments will be carried out. A urine pregnancy test for females of childbearing potential will be performed at Visit 1.
Study Medication Compliance:	Compliance will be assessed by a review of the patient diary. The number of doses the patient actually took during the treatment period will be divided by the number of doses the patient was expected to take during that period. The resulting ratio will be multiplied by 100% to determine percent compliance. Patients will be considered "compliant" if their percent compliance is between 80% and 120%.

Sample Size calculation:	We have not been able to find an accurate estimation of study's main variable (therapeutic cure) in previous investigations with Ciprofloxacin 0.3% otic solutions in the treatment of AOE. Rates of patients cured, with different definitions from ours, have been estimated within a range between 60% and 70%. Moreover, in two comparative trials testing the same combination (same product) versus Ciprofloxacin alone in the treatment of AOMT, the percentage of therapeutic cure in patients with *Pseudomonas aeruginosa* and *Staphylococcus aureus* was around 50%. For the calculation of the sample size for this study, the assumption is made that the Ciprofloxacin alone group will have a therapeutic cure rate of 62%, and that the combination will increase it by a relative 25%, which would represent a therapeutic cure rate of 78%. Assuming a two-sided significance level of 5%, and a statistical power of 80%, the required number of patients in each group would be 150. The randomization schedule is defined as 2:2:1 (Ciprofloxacin plus Fluocinolone acetonide / Ciprofloxacin / Fluocinolone acetonide), so the total number of evaluable patients to be recruited is 375 (150 : 150 : 75). In this study, evaluable patients are those with a positive microbiological culture at baseline (MITT population). From previous studies in AOE, the rate of patients included with a negative culture at baseline is approximately 25%, and therefore the number of patients to be finally recruited to obtain 375 evaluable patients would be 500.

Statistical Methods:	For the main objective of the study, the statistical analysis of the primary endpoint will be performed in the MITT-PA/SA population (pathogen positive subset of the CITT population which includes all patients who receive study medication and has culture positive for *P. aeruginosa* and/or *S. aureus* at baseline in the evaluable ear). In order for the requirements of the combination rule to be satisfied, both comparisons of the combination to the components alone must show the combination to be statistically significantly better than the component alone using a two-sided 0.05 significance level. Therapeutic cure at EOT will be compared between treatment groups by using the log rank test stratified on age (6 months to younger than 18 years old, 18 years and older). For the superiority analysis of time to end of pain, survival functions for the time to end of pain for combination versus each product alone will be estimated by using the Kaplan-Meier method. The mean/median per each arm will be summarized and the p-values from the log-rank test will be at the 0.05 two-sided significance level. Other secondary efficacy endpoints: Comparisons between the treatment groups will be performed by using the chi-squared or the Cochran-Mantel-Haenszel test, as appropriate. Safety endpoints will be summarized by treatment group using summary statistics or frequency counts, as appropriate.

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List of Abbreviations

ABR	Auditory Brainstem Response
AE	Adverse event
AEMPS	Agency of Medicines and Medical Devices
AOMT	Acute Otitis Media with tympanostomy tubes
AOE	Acute Otitis Externa
CEIm	Ethic Committee of Investigational medicines
CI	Confidence interval
CIPRO	Ciprofloxacin 0.3%
CIPRO+FLUO	Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025%
CITT	Clinical intent-to-treat
CPMP	Committee for Proprietary Medicinal Products
CPP	Clinical Per-Protocol
CRF	Case Report Form
CSR	Clinical Study Report
ECT	Effective Concomitant Therapy
EDC	Electronic Data Capture
EMA	European Medicines Agency
EOT	End-of-treatment
FDA	Food and Drug Administration
FLUO	Fluocinolone acetonide 0.025%
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent-to-treat
IUD	intrauterine device
IWRS	Interactive Web Response System
LC/MS/MS	Liquid chromatography/tandem mass spectrometry

LDPE	Low-density polyethylene
LLNA	Local Lymph Node Assay
LOQ	Limit of quantification
MPP	Microbiological Per-Protocol
MITT	Microbiological intent-to-treat
[redacted]	[redacted]
OE	Otitis externa
PK	Pharmacokinetic
PP	Per-protocol
SAE	Serious adverse event
TEAE	Treatment-emergent adverse event
TEOP	Time-to-end of pain
tid	Three times a day
TOC	Test-of-cure
TSSS	Total signs/symptoms score
VAS	Visual analog scale
US	United States

1. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

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2. INTRODUCTION

2.1 Background

Otitis externa is an inflammatory process that involves the external auditory canal and is usually caused by bacterial infection. The most common factor leading to infection is excessive moisture in the ear canal, which interferes with the canal's natural defenses against infection.1 Otitis externa is one of the most common otic conditions seen by general practitioners and ear, nose and throat specialists. It occurs in 4 of every 1,000 adults and children in the United States (US) each year.2 The most common causative microorganisms are *Pseudomonas aeruginosa* and *Staphylococcus aureus*.3,4,5,6

Topical antibiotics are the first-line treatment of choice for otitis externa.7 Topical application enhances efficacy by bringing the antibiotic into direct contact with the infected area1,8 reduces the risk of adverse events (AEs) associated with systemic antibiotic therapy, and may help prevent the development of resistance to antibiotics by the pathogen.7

SALVAT currently markets a number of Ciprofloxacin drug products worldwide including Cetraxal (Ciprofloxacin 0.3% otic solution or Ciprofloxacin 0.2% otic solution) and Cetraxal Plus (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution) throughout the world for the treatment of Acute Otitis Externa (AOE). SALVAT received its first NDA approval on May 1, 2009 for the single-entity Cetraxal (Ciprofloxacin 0.2% otic solution) for the treatment of AOE. SALVAT has recently received NDA approval (NDA 20821) April 29, 2016 for OTOVEL (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution in single use vials) for the treatment of Acute Otitis Media with Tympanostomy Tubes (AOMT). OTOVEL was launched in the US in August 2016.

OTOVEL is a sterile, preservative-free otic solution of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025%, supplied in single-use vials. Each vial/application delivers 0.75 mg Ciprofloxacin and 0.0625 mg Fluocinolone acetonide.

The fixed combination of Ciprofloxacin plus Fluocinolone acetonide in a multidose presentation (Cetraxal Plus) received the first approval in 2002 and it is currently marketed in over 40 countries including the European, and is indicated for the local treatment of AOE of bacterial origin.

Ciprofloxacin is a fluoroquinolone antibiotic with broad-spectrum antibacterial activity. The primary mode of action of the fluoroquinolones is inhibition of the bacterial gyrase enzyme.9 Thus, Ciprofloxacin inhibits the synthesis of bacterial nucleic acids. The quinolone antibiotics have been widely used in the treatment of systemic infections (e.g., pneumonia, urinary tract infections, and skin infections) and localized infections (e.g., otitis externa and conjunctivitis). To date, the only topical quinolone preparations that have been approved in the US for otitis externa are ciprofloxacin plus hydrocortisone, ciprofloxacin plus dexamethasone, ofloxacin and finafloxacin.

Topical application of 0.3% quinolone antibiotic drops will result in high local antibiotic concentrations that will be active against the bacterial pathogens associated with otitis externa. Ciprofloxacin is active against Gram-negative bacteria and, at the high local

concentrations present in the auditory canal after topical administration, against Gram-positive bacteria as well. Most bacterial strains that cause otitis externa are highly susceptible to Ciprofloxacin. [6,8,9,10]

Ciprofloxacin is a well-established drug that has been used for many years for the treatment of AOE. It is used worldwide for the treatment of systemic and topical infections.

Ciprofloxacin-corticosteroid combinations are already in use for different otitis conditions. Combinations of ciprofloxacin with hydrocortisone (CIPRO HC Otic)[12,13] and with dexamethasone (CIPRODEX)[14,15] have been approved in the US for treatment of AOE in children 1 year and older and 6 months and older, respectively.

Fluocinolone acetonide, like other topical corticosteroids, has anti-inflammatory, anti-pruritic, and vasoconstrictive properties. The addition of a corticosteroid to otic antibiotic preparations aids in the resolution of the inflammatory response accompanying bacterial infections. Multiple single-entity and combination Fluocinolone acetonide drug products have been approved by the FDA in concentrations ranging from 0.01 to 0.025%.

Currently, in the US there are several approved Fluocinolone acetonide-containing products. Fluocinolone acetonide 0.01%, otic oil drops (DermOtic Oil)[16] is approved for the treatment of chronic eczematous external otitis in adults and pediatric patients 2 years and older. Clinical efficacy was demonstrated in a single, placebo-controlled study in 154 patients (adults and children ≥2 years) administered 5 drops per ear of Fluocinolone acetonide oil twice daily for 7 days. At the end of treatment DermOtic oil was superior to placebo in resolving the signs and symptoms of eczematous external otitis.

The Sponsor has developed Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution for use in the treatment of both AOE and AOMT. The efficacy of this product in AOMT has been assessed in 2 separate studies. The studies demonstrated superiority (in terms of time to cessation of otorrhea) of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution over Ciprofloxacin alone and over Fluocinolone acetonide alone in the treatment of AOMT. The product under the brand of OTOVEL has been approved by FDA for the treatment of AOMT and it is currently marketed in US.

The Sponsor's clinical development program for Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution in AOE includes 1 multicenter phase III study of safety and efficacy (CIFLOT III/00-01) which was conducted in 2000-2001 under good clinical practice (GCP) and supported by the marketing experience.

The study was a phase III, multicenter, randomized, parallel-group, double blind clinical trial designed to study the clinical efficacy and safety of the combination of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution compared with Ciprofloxacin 0.3% otic solution in the treatment of diffuse otitis externa (OE) in 590 subjects aged 7 years and older.

A total of 296 subjects were randomized to the experimental product Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% (CIPRO+FLUO group), and 294 were randomized to the active control, Ciprofloxacin 0.3% (CIPRO group). Four to 6 drops of

Ciprofloxacin 0.3% or Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% were instilled in the affected ear canal three times daily (tid) for 8 days (valid range 7 to 10 days, inclusive). The primary efficacy endpoint was clinical efficacy, which was defined as a combination of clinical and bacteriological cure. Secondary clinical endpoints included the investigator's subjective impression of clinical efficacy at End of Treatment (EOT), subject's evaluation of pain intensity, and Time to end of pain (TEOP).

In the intent-to-treat (ITT) population (n=296 CIPRO+FLUO, n=294 CIPRO), the clinical efficacy was 89.2% (264/296) for subjects in the CIPRO+FLUO group, compared to 82.0% (241/294) for the CIPRO group, with a difference of 7.22% in favor of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025%. The 90% confidence interval (CI) for the difference between proportions was 2.48% to 11.95%; since the 90% CI excluded the predefined limit of -10%, Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% was demonstrated to be non-inferior to Ciprofloxacin in the ITT population. Similar results were observed in the Valid (per protocol [PP]) population.

No statistically significant differences were observed between groups for mean TEOP. Both treatments significantly reduced the intensity of symptoms (otalgia, edema, and otorrhea).

Since the release of the original clinical study report (CSR) for the CIFLOT III/00-01 study on 14 November 2001, 2 addenda have been released, following completion of additional analyses.

- The first addendum (Addendum 1, 14 August 2006), presents the results of a superiority analysis. While the study was designed to demonstrate non-inferiority of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% to Ciprofloxacin, the data reported in the CSR suggested that the combination might actually have been superior to Ciprofloxacin alone. A superiority analysis was conducted, which confirmed the superiority of the combination over Ciprofloxacin alone.
- A second addendum (Addendum 2, 26 May 2010) describes results of analyses conducted to address recommendations from the International Conference on Harmonisation (ICH) Topic E9 "Statistical Principles for Clinical Trials,"[17] the European Medicines Agency (EMA) guideline "Guideline on the Evaluation of Medicinal Products Indicated for Treatment of Bacterial Infections",[18] and the Committee for Proprietary Medicinal Products (CPMP) guidance "Points to Consider on Switching Between Superiority and Non-Inferiority".[19] This included: analyses in 4 newly defined populations (including a microbiological intent-to-treat [MITT] population, comprising subjects with positive microbiological cultures at Baseline); redefining the primary efficacy endpoint as proportion of subjects with clinical cure at end-of-treatment (EOT) in the clinical intent-to-treat population (CITT, all dosed subjects); and converting AOE symptom scores evaluated on a visual analog scale (VAS) to a 4-point categorical scale.

Both addenda support the claim for superiority of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% over Ciprofloxacin alone for the treatment of AOE.

2.2 Rationale for the Study

In December 2014, Rosenfeld and authors from the American Academy of Otolaryngology-Head and Neck Surgery published evidence-based clinical practice guidelines for acute otitis externa (AOE) for primary care and specialist clinicians, which was an update of their earlier guidelines.[20,21]

Strong recommendations from the guidelines were as follows:

- Systemic antimicrobials: Clinicians should not prescribe systemic antimicrobials as initial therapy for diffuse, uncomplicated AOE unless there is extension outside the ear canal or the presence of specific host factors that would indicate a need for systemic therapy.
- Pain management: The clinician should assess patients with AOE for pain and recommend analgesic treatment based on the severity of pain.

In addition, it was recommended that clinicians should use topical preparations for initial therapy of diffuse, uncomplicated AOE.

Topical treatments for AOE include antibiotics (aminoglycosides, polymyxin B, a quinolone, or combinations of these), steroids (eg, hydrocortisone or dexamethasone), and low-pH antiseptics (eg, acetic acid). For uncomplicated AOE the use of a topical antimicrobial (antibiotic or antiseptic), with or without steroid, is highly effective.

Rosenfeld et al (2014) described findings of 3 meta-analyses that supported the use of topical treatment for AOEs. In each meta-analysis, it was concluded that topical treatment is effective, but with no meaningful differences between class of drug, quinolone/non-quinolone, or mono- versus combination treatment, with or without steroid.

In general, and as recommended by the American Academy of Otolaryngology-Head and Neck Surgery guidelines,[20] the selection of topical treatment can be based largely on physician's experience; factors can include cost, adherence to therapy and adverse events. Adherence to therapy is increased when drops are easy to apply and require fewer applications per day.

SALVAT is currently marketing the proposed combination Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution in 10 mL multidose preparation in over 40 foreign countries for the treatment of AOE. Since its 2002 launch, SALVAT has successfully marketed over 4 million units of the referenced product worldwide. There have been no marketplace recalls or field corrections.

SALVAT received the NDA approval on April 29, 2016 for the OTOVEL Otic solution (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% sterile and preservative-free solution in single dose vials) for the treatment of AOMT.

This study is being conducted to support an application for approval to market OTOVEL in the US for the indication of AOE. The reference (comparator) drugs in this study, Ciprofloxacin 0.3% alone otic solution, and Fluocinolone acetonide 0.025% alone otic

solution, are expected to provide a lower efficacy rate when compared with the combination.

2.3 Risk-Benefit Assessment

Ciprofloxacin is in worldwide use for treatment of systemic and topical infections. SALVAT currently markets a number of Ciprofloxacin drug products worldwide including Cetraxal Ótico (Ciprofloxacin 0.3% otic solution) and Cetraxal Plus (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution) throughout the world. SALVAT received its first NDA approval on May 1, 2009 for the single-entity Cetraxal (Ciprofloxacin otic solution) 0.2% for the treatment of AOE and the NDA approval of OTOVEL (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025%) on April 29, 2016 for the treatment of AOMT.

Nonclinical studies have been conducted to assess the ototoxic potential of ciprofloxacin. Nonclinical studies have shown that ciprofloxacin otic solution has little or no ototoxic effect in monkeys[22] or guinea pigs.[22,23,24,25] Damage to isolated cochlear outer hair cells from chinchillas was less with ciprofloxacin 0.3% than with other antibiotic preparations[26].

Three new nonclinical studies with Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% formulation have been done, two of them confirm that the intended formulation could be classified as non-irritant: "Evaluation of the acute dermal irritant and/or corrosive effect of Cetraxal Plus after a single dose administration to female New Zealand White rabbits", non-sensitizer ("Local Lymph Node Assay (LLNA)"). The third study ("A 28-DAY subacute Ototoxicity study of Cetraxal Plus otic solution in Guinea Pigs") showed that Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% did not produce mortality, and were without effect on clinical observations, body weights, physical examinations, otoscopic examinations, macroscopic evaluations, ossicle mobility, cytocochleograms, or middle ear assessments. Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% as well as the Vehicle Control did not produce adverse effects due to the incidence of the findings on the physical examinations, otoscopic examinations, ossicle mobility, cytocochleograms, or middle ear assessments. While no Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Day 29 average of auditory brainstem response (ABR) thresholds exceeded 15 dB, there was a trend for threshold elevation at each test frequency. A mild to moderate threshold was observed in the female group at 10 and 20 kHz. Although this suggests a sensorineural hearing loss caused by the treatment, in the context of the overall assessment where no other ototoxicity changes were observed, the relevance of this isolated finding is low (or limited).

The Neomycin positive control produced expected toxicity in the middle ear, as detailed in the macroscopic observations, ossicles mobility, cytocochleograms, auditory brainstem responses, and middle ear assessments.

Pharmacokinetic determinations of serum Ciprofloxacin were measured following application of Ciprofloxacin-containing otic solution (0.2%, 0.3%, or 0.5%) in pediatric and adult patients. Subjects had either otorrhea with a perforated tympanic membrane, chronic suppurative otitis media, tympanostomy tubes or otitis externa. In nearly all cases,

systemic Ciprofloxacin levels were below detectable limits, indicating that negligible absorption of Ciprofloxacin occurs following otic application at 0.2%-0.5%.

Ciprofloxacin in combination with a corticosteroid (dexamethasone) has been approved for treatment of AOMT in children.

Ciprofloxacin and Ciprofloxacin plus Fluocinolone acetonide otic solution are medications of proven efficacy and safety for the treatment of bacterial otitis externa.

In the CIFLOT III/00-01 study, Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% (CIPRO+FLUO) vs Ciprofloxacin 0.3% (CIPRO) conducted by the Sponsor, both products were considered to have a favorable safety profile. One non-treatment-related serious adverse event (SAE; mild perichondritis) was reported in 1 subject in the CIPRO+FLUO group. In addition, 7 AEs were reported in 7 subjects (1.2%): 2 (1.0%) in the CIPRO+FLUO group and 5 (1.7%) in the CIPRO group. Both non-serious events reported in the CIPRO+FLUO group (tympanic membrane perforation) were mild in severity and were not related to study treatment. Among the 5 AEs reported in the CIPRO group, 2 were considered to be related to study treatment (mild skin eczema in the ear and mild dizziness) and 3 were not related (mild dyspepsia, moderate laryngeal edema, and moderate vertigo). No treatment-related SAEs were reported during this study.

Fluocinolone acetonide is a low to medium potency corticosteroid according to FDA classification. There is evidence that corticosteroids improve otorrhea outcomes in children and AOM in animal models, and reduce the formation of granulation tissue.

Fluocinolone acetonide is a well-known compound that is frequently used as a cream, gel, lotion, ointment for skin topical administration,[27] and as an intravitreal implant[28] or as an oil in ear drops.[29,16] In these different topical routes of administration, plasma concentrations or pharmacokinetics of the compound could not be reported because the systemic exposure to the product was negligible. As a product administered topically into the ear, at or near the intended site of action, systemic absorption of Fluocinolone acetonide is expected to be negligible. This was confirmed by the pharmacokinetics results obtained in the SALVAT clinical studies CIFLOTIII/10IA02 and CIFLOTIII/10IA04.

Two identically designed multicenter, randomized, double-blind clinical trials were conducted to assess the efficacy and safety of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution compared to Ciprofloxacin 0.3% otic solution and to Fluocinolone acetonide 0.025% otic solution in the treatment of AOMT in pediatric patients. Pharmacokinetic analysis of Fluocinolone acetonide and Ciprofloxacin in plasma samples was conducted for the subgroup of patients in these studies. Analysis of plasma samples was performed using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method with a limit of quantification (LOQ) for ciprofloxacin and/or Fluocinolone acetonide in plasma samples of 1 µg/L. In Study CIFLOTIII/10IA02, blood samples were taken in a subgroup of 16 patients at Visit 1 (baseline) and Visit 3 (1-2 hours after the last dose following 7 days of twice-daily dosing) to determine the plasma levels of Ciprofloxacin and/or Fluocinolone acetonide. Only 1 sample, drawn from the patient who received a double dose of the drug due to bilateral AOMT, showed a detectable concentration of ciprofloxacin in plasma (2.998 µg/L) after 7 days of treatment, and no

detectable concentrations of Fluocinolone acetonide in plasma were observed. The patient did not present any treatment-emergent adverse events (TEAE).

In Study CIFLOTIII/10IA04, pharmacokinetic (PK) analysis of Fluocinolone acetonide and Ciprofloxacin in plasma samples was conducted for a subgroup of 14 patients. No detectable concentrations of Fluocinolone acetonide in plasma were observed after 7 days of treatment, and no ciprofloxacin results were reported.

Results of non-clinical studies and the long clinical experience with Ciprofloxacin and Fluocinolone acetonide confirm that this combination product has low potential for both ototoxicity and systemic toxicity. Therefore, these data indicate that the proposed clinical study presents an acceptable risk to patients and the potential for adverse events is very low.

3. OBJECTIVES

3.1 Primary Objective

To demonstrate superiority of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution relative to Ciprofloxacin 0.3% otic solution and to Fluocinolone acetonide 0.025% otic solution with respect to therapeutic cure rate (clinical + microbiological cure) at EOT.

Clinical + microbiological cure will be considered achieved if edema, otalgia and otorrhea are resolved with no further requirement of antimicrobial therapy and bacteriological response is Eradication or Presumed Eradication.

3.2 Secondary Objectives

Principal secondary endpoint:

The principal secondary endpoint is "Time to end of ear pain". This time will be calculated on the basis of the patient diary entries..

End of ear pain is defined as the first day (morning or evening) on which there is no use of analgesics, the diary pain score is zero and remains zero for all subsequent visits until the end of the study.

Throughout the study, patient or caregiver should record ear pain severity twice daily (morning and evening prior to dosing) in the diary using a proper pain scale according to patient's age until the end of study (see Appendix 3):

- Patients younger than 7 years old will use the FLACC scale (it will be assessed by parents or caregivers).
- Patients from 7 years old to younger than 13 years old will record the pain using the Wong Baker Faces Pain Scale.
- And patients 13 years old and older will record the pain using a VAS scale.

The time to end of pain is the interval (in days) between the first dose of study medication and the time when the ear pain in the evaluable ear ended (pain was absent and remains absent until the end of the study without using analgesic). This time will be calculated on the basis of the patient diary entries.

If ear pain in the evaluable ear continues at the end of the study, the time to end of pain variable will be recorded as the length of time between the time of the first dose of study medication and the last time point when a pain measurement was recorded.

Patients who take rescue medication, discontinue or are lost to follow up, and for whom the pain persists at the time of the last observation, will be censored at maximum length (Day 17) in those cases when the diary information is not available.

Other secondary endpoints:

- Sustained microbiological cure
- Clinical cure at Visit 3 and Visit 4
- Microbiological success at Visit 3 and Visit 4
- Therapeutic cure (clinical+microbiological cure) at Visit 4
- Changes in Brighton grading at Visit 3 and Visit 4
- Changes in otorrhea at Visit 3 and Visit 4
- Changes in edema at Visit 3 and Visit 4
- Changes in otalgia assessed by the investigator at Visit 3 and Visit 4
- Adverse events

4. OVERALL DESIGN AND PLAN OF THE STUDY

4.1 Overview

This is a randomized, parallel-group, double-blinded, active-controlled, multicenter study comparing Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution with Ciprofloxacin 0.3% otic solution or Fluocinolone acetonide 0.025% otic solution in the treatment of acute diffuse otitis externa in children, adolescents, and adults. A diagram of the study design is shown in Figure 1, and the schedule of observations and procedures is shown in Table 1 (in Section 8.1).

Patients selected for the study will be male or female, 6 months of age and older, with uncomplicated acute diffuse otitis externa in at least 1 ear. At Visit 1 (Day 1), patients who have signed the Informed Consent Form (or had it signed by their legally authorized representative) and met the study entry criteria will be randomized in a 2:2:1 ratio to either the investigational treatment, Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution, or the comparator treatment, Ciprofloxacin 0.3% otic solution or Fluocinolone acetonide 0.025% otic solution.

At these visits, signs and symptoms of otitis will be assessed and a sample of ear exudates for microbiology culture will be collected before onset of treatment and debridement by suction of the ear(s). Patients or their caregivers will be taught to administer study medication and will be instructed to apply it twice daily for 7 days. Patients will receive a 7-day supply of study medication. Patients with bilateral otitis externa will receive the same treatment in both ears. Patients will be instructed to refrain from swimming during the study treatment period and preferably until the final study visit is completed. Patients will also be advised to use a shower cap or neoprene band when bathing. At visit 1, a urine pregnancy test will be performed in females of childbearing potential. A patient diary will be provided to the patient or caregivers, who should complete it until the end of the study.

Visit 2 will consist of a telephone call made on Day 3 or Day 4 of study treatment to check on each patient's clinical progress through a questionnaire. Patients who report no improvement in otitis symptoms will be asked to come in for a visit as soon as possible. At this visit, the Investigator will choose one of the two following options for the patient:

1. Continue the patient in the study. The present visit will be recorded as an unscheduled visit. The patient should continue taking study medication without interruption.
2. Discontinue study medication. The present visit will be recorded as Visit 3, the End of Treatment (EOT) visit. The patient will be withdrawn from the study and may be treated with other medication as appropriate. The clinical outcome for the patient will be recorded as Treatment Failure.

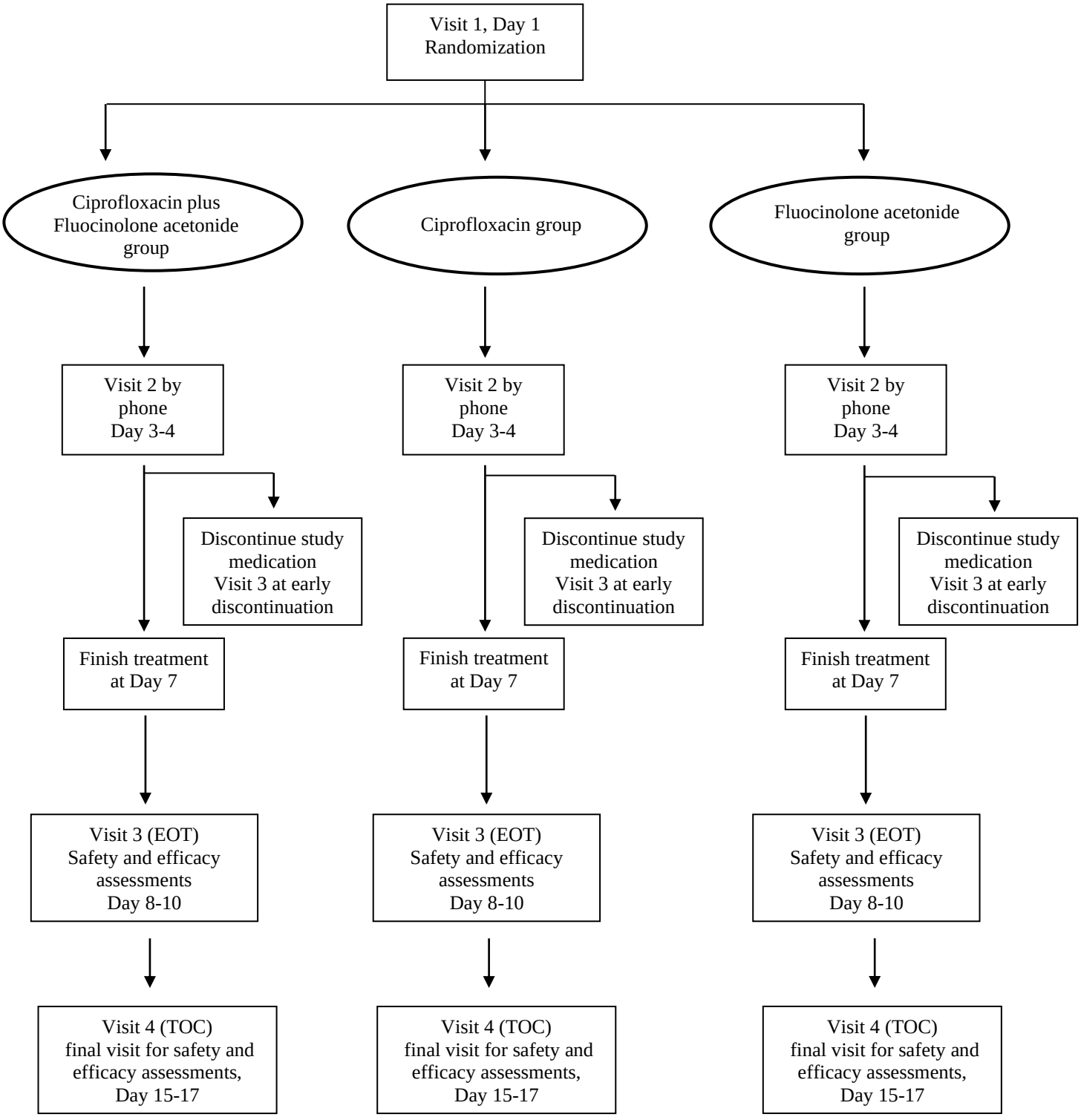
Patients will attend Visit 3 (EOT) at approximately Day 8-10, or within 2 days after premature discontinuation. All patients (except those who have been withdrawn from the study or have withdrawn their consent as described in Section 5.3) will attend Visit 4 (TOC) at Day 15-17. At these visits, symptoms of otitis will be assessed and ear exudate, if

present, will be cultured. The primary efficacy endpoint will be Therapeutic cure (Clinical cure + Microbiological Cure) at End of Treatment.

Visit 4 will be the "Test of Cure" visit. Safety assessments will also be performed at Visit 4. Upon completing Visit 4, patients will have completed their participation in the study.

If a patient receives any otic or systemic treatment administered for reasons associated with otitis externa of the evaluable ear during the study (after Visit 1 but before End-of-study visit), the treatment will be considered rescue medication and the patient will be assessed as treatment failure.

Figure 1 Diagram of Study Design



4.2 Justification for Study Design

The design of this study follows US Food and Drug Administration (FDA) recommendations. The study should include a comparison between Ciprofloxacin plus Fluocinolone acetonide and its individual components to establish the contribution of each component to the combination. Moreover, superiority of the combination over each component should be demonstrated.

Otic formulations containing Ciprofloxacin 0.3% or Ciprofloxacin 0.2% in combination with a corticosteroid have been approved and are marketed in the US to treat AOE.

This study will use the combination of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025%, recently approved by FDA for the treatment of AOMT. This combination has demonstrated in over 40 countries to be safe and effective for the treatment of AOE; the Ciprofloxacin concentration of 0.3% remains within the drug exposure range that has been determined to be safe and effective to treat AOE.

One vial, approximately 0.25 mL, of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution will be sufficient to produce high local concentrations within the external auditory canal ear. This amount of Ciprofloxacin has been demonstrated to be effective and well tolerated based on the results observed in previous studies using this concentration. The comparator drugs in this study, Ciprofloxacin 0.3% otic solution and Fluocinolone acetonide 0.025% otic solution, have not been approved for the treatment of patients with AOE but are requested by the FDA to assess the contribution of each component to the efficacy and safety of the combination. Comparator products have been manufactured specifically for this study maintaining the double blind study design.

The products (investigational product and comparators) will be supplied in blue translucent single-use vials of the same characteristics. The vials will be wrapped in a foil pouch to protect from the light. Fourteen (14) vials will be placed into a pouched and heat-sealed foil which will have a label attached. The same label will be applied to all foil pouches to keep the content blinded as much as possible; the only difference among the labels will be the kit number. The foil pouch will be packaged in a carton box that will also have a label with the kit number.

5. STUDY POPULATION

5.1 Inclusion Criteria

1. Male or female, at least 6 months of age;
2. Patients with diagnosis of uncomplicated acute diffuse otitis externa of less than 21 days (3 weeks) duration, with a total symptom score of at least 5 as defined under Efficacy Parameters, in at least 1 ear. The individual symptom scores, as defined in Sections 7.1.1.1, 7.1.1.3, and 7.1.1.4, must be:
    - Otalgia: at least 2 (moderate or severe);
    - Edema: at least 2 (moderate or severe);
    - Otorrhea: at least 1 (mild, moderate or severe), with sufficient quantity of otorrhea to allow culture.
3. Patients with Brighton grading of II or III in at least 1 ear, as defined in Appendix 2.
4. Patients with otorrhea of sufficient volume for sampling for microbiological culture (sample must be taken at baseline visit prior to the first study dose).
5. Willingness to refrain from swimming through end of the study.
6. For adult patients, ability to understand and provide written informed consent

   For pediatric patients, signed informed consent from patient's legally authorized representative; also, if the patient is capable of providing assent, signed assent from the patient as applicable according to local regulations.
7. Females who are not pregnant, not lactating and are not planning a pregnancy. All females of childbearing potential (those who are not premenstrual, not postmenopausal or not surgically sterile) will be able to participate only if they have a negative urine pregnancy test prior to randomization, and if they agree to use adequate birth control methods to prevent pregnancy throughout the study.

   Adequate birth control methods included topical, hormonal-oral, implantable or injectable contraceptives; mechanical-spermicide in conjunction with a barrier such as condom or diaphragm; intrauterine device (IUD); or surgical sterilization of partner (must be ≥ 6 months post vasectomy). For non-sexually active females, abstinence was regarded as an adequate method of birth control; however, if the patient became sexually active during the study, she must have agreed to use adequate birth control methods as defined above for the remainder of the study.

5.2 Exclusion Criteria

1. Previous episode of AOE within 4 weeks prior to enrollment, or 2 or more episodes of AOE within 6 months prior to enrollment.
2. Tympanic membrane perforation (including tympanostomy tubes).
3. Current diagnosis of diabetes mellitus, psoriasis, otitis media, malignant otitis externa, surgical mastoid cavities, canal stenosis, cholesteatoma, exostosis, or tumors of either ear.
4. Current diagnosis of fungal (otomycosis) or viral infection of either ear.
5. Current diagnosis of dermatitis of the affected ear or surrounding area.
6. Current presence of any other infection of the ears or other medical condition that might adversely impact the safety of the study participants or confound the study results.
7. Known or suspected hypersensitivity or history of adverse reaction to quinolones, corticoids or any component of the study medication.
8. Use of any systemic antibacterial or corticosteroids within two weeks prior to enrollment (nasal or inhaled corticosteroids at stable doses at least 30 days prior to enrollment are allowed)
9. Use of any topical or otic antibiotic, steroidal or antifungal agent in the affected ear(s) within 1 week prior to enrollment, or any other topical or otic product in the affected ear(s) within 1 day prior to enrollment.
10. Concurrent use of systemic anti-inflammatory agents, including biologic response modifiers. (Analgesics without anti-inflammatory properties, such as acetaminophen, are allowed during the study)
11. Previous participation in this trial.
12. Participation in another clinical trial within the previous 30 days.
13. Any condition or situation likely to cause the patient (or, if applicable, the patient's primary caregiver) to be unable or unwilling to comply with study treatment or attend all study visits.
14. Any condition (i.e significant acute or chronic medical, neurologic, or psychiatric illness…) in the patient or parent/guardian that, in the judgment of the principal investigator, could compromise patient safety, limit the patient's ability to complete the study, and/or compromise the objectives of the study.

5.3 Withdrawal, Premature Discontinuation of Study Medication, and Replacement of Patients

Patients must be withdrawn from the study if they withdraw consent to participate. Such withdrawal may occur at any time during the study. Patients are not required to state their reasons for withdrawing consent.

Patients who do not experience improvement in symptoms may prematurely discontinue treatment with study medication. This protocol includes an assessment of whether a patient who experiences no improvement at Visit 2 should discontinue study treatment, as described in Section 8.2.2. However, a patient may discontinue study treatment at the Investigator's discretion at any time during the study treatment period.

Patients may be required to discontinue study treatment after discussion with the Sponsor and/or Investigator for any of the following reasons:

- Adverse event(s);
- At the discretion of the Investigator;
- Violation of eligibility criteria; or
- Deviation from the treatment plan specified in the protocol (e.g., incorrect administration of study medication, failure to attend study visits).

In all cases, the reasons for premature discontinuation of study medication must be recorded in the Electronic Data Capture (EDC) and in the patient's medical records. If there is more than one reason for premature discontinuation of study medication, one reason will be listed in the EDC as the primary reason.

If possible, each patient who prematurely discontinues study treatment should report to the study site for an End of Treatment visit (Visit 3), as described in Section 8.2.3.

Patients who withdraw consent or discontinue study medication prematurely will not be replaced.

5.4 Planned Sample Size and Study Sites

Planned enrollment is 500 children, adolescents and adults. Patients will be randomized in a 2:2:1 ratio (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution : Ciprofloxacin 0.3% otic solution : Fluocinolone acetonide 0.025% otic solution), with the aim of including 375 evaluable patients.

Patients will be stratified at enrollment so that approximately 50% of those enrolled will be younger than 18 years old and approximately 50% will be 18 years old and older. It is expected that approximately 80% of enrolled patients will complete the study.

About 50 study sites in US will recruit patients. Enrollment will be competitive, i.e., each study site will continue to enroll patients until recruiting the total of 375 evaluable patients. No study site may enroll more than 50 patients.

5.5 Patient Identification and Randomization

Patients will be randomized at Visit 1, after signing informed consent (and assent form, when applicable) and meeting eligibility criteria. A block randomization procedure will be conducted through an Interactive Web Response System (IWRS) which will assign a patient number, which will be used to identify the patient during screening and, if applicable, throughout the duration of the study. Patients who do not meet the inclusion and exclusion criteria will be registered as screen failures through the IWRS.

For patients who have bilateral otitis externa, one ear will be considered the evaluable ear, and it will be randomized to a treatment group. If one ear has a higher total symptom score (as defined in Section Overall Clinical Outcome7.1.1.5) than the other, the ear with the higher score will be the evaluable one. If the symptom score is identical in both ears, the investigator will select the evaluable ear. In either case, the non-evaluable ear will receive the same treatment as the evaluable ear.

Patients will be stratified at enrollment by age group. Age will be classified into 2 groups:

- Younger than 18 years old, and
- 18 years old and older.

It is planned that the total number of patients in the study will be evenly divided between the 2 age groups. Stratification is being performed to ensure adequate representation of each age group in the study and not for statistical considerations.

6. STUDY MEDICATION

6.1 Identity

Investigational Medication

Chemical name	Ciprofloxacin hydrochloride and Fluocinolone acetonide
Generic name	Ciprofloxacin HCl (as 0.3% base) plus Fluocinolone acetonide 0.025% Otic Solution
Trade name	OTOVEL™
Dosage form	Auricular solution, sterile
Manufacturer	[redacted] for Laboratorios SALVAT, S.A.
Description	Otic solution in single-dose low-density polyethylene (LDPE) blue translucent vials containing 0.25 mL deliverable volume

Reference Medication

Generic name	Ciprofloxacin HCl (as 0.3% base) or Fluocinolone acetonide 0.025%
Trade name	Not applicable
Dosage form	Auricular solution, sterile
Manufacturer	[redacted] for Laboratorios SALVAT, S.A.
Description	Otic solution in single-dose low-density polyethylene (LDPE) blue translucent vials containing 0.25 mL deliverable volume

6.2 Administration

Study medication will be self-administered by the patient or administered by the patient's caregiver. At Visit 1, a study staff member will instruct the patient or caregiver in how to open the vials and administer study medication, and will supervise the patient or caregiver during administration of the first dose.

The method of administration for the investigational medication and the comparator medication will be the same: instillation of one vial in the affected ear canal(s) twice a day (approximately every 12h, morning and evening) for 7 consecutive days. Ear wicks or sponges may be used at the Investigator's discretion. Use of ear wicks or sponges must be documented in the EDC.

Study medication will be supplied in single-dose vials containing 0.25 mL deliverable volume per vial. A patient kit will contain 1 pouch of 14 blue translucent vials (2 strips of 7 vials). Patients with bilateral otitis externa will receive 2 kits. The patient or caregiver will administer the contents of 1 vial,twice daily to the affected ear(s) for seven days. The Ciprofloxacin and/or Fluocinolone acetonide dosage will be the same for patients of all ages. Each vial will be an intact unit, and the caregiver will twist off the top of the vial to administer the study medication. The solution should be warmed by holding the vial in the hand for one or two minutes to avoid dizziness which may result from the instillation of a

cold solution. The patient should lie with the affected ear upward, and then the medication should be instilled. The outer ear lobe should then be gently pulled upward and outward. This will allow the ear drops to flow down into the ear canal. This position should be maintained for 1 minute. Repeat, if necessary, for the opposite ear.

The planned maximum exposure to the active ingredient is approximately 1.5 mg of Ciprofloxacin and 0.125 mg of Fluocinolone acetonide per day (approximately 10.5 mg of Ciprofloxacin and 0.875 mg of Fluocinolone acetonide over a 7-day period). This quantity will be twice as much in case of bilateral disease.

6.3 Packaging, Labeling, and Storage

The primary packaging of study medication will be performed by [redacted], who will supply the investigational medication as pouches, 14 single-dose vials per pouch, as described in Section 6.2. [redacted] will send the pouches directly to [redacted]. Each individual vial will contain 0.25 mL deliverable volume of Ciprofloxacin 0.3% and/or Fluocinolone acetonide 0.025% otic. The vial is made from LDPE and is manufactured via a blow-fill-seal process. This process seals the vials directly after manufacture and contains the solution within a unit-dose vial that is intact until the user twists off the cap to administer the medication.

[redacted] will serve as the drug distributor to study sites and will label the pouches and cartons ensuring that all study medication kits will be identical in appearance to ensure blinding is adhered to, then pack the kits into boxes, for shipment to each study site that has been approved to receive study medication.

All study medication supplies must be stored in accordance with the manufacturer's instructions (2-25ºC or 35.6-77ºF). Until dispensed to the patients, study medication will be stored in a secure area, accessible to authorized personnel only.

6.4 Blinding and Breaking the Blind

All study medication products (test and comparators) will have the same packaging and labels.

The boxes in which the study medication is packaged, shipped, and dispensed will be identical in appearance. When patients return their used and unused study medication containers to the study site, they will be encouraged to bring them in the original cartons.

Microbiological samples will be processed by a central laboratory under blinded conditions. The central laboratory will be blinded to the treatment assignment of the patient from whom the sample was taken.

Unblinding of study treatment assignment should be an unusual occurrence, and should be done only in case of an emergency when knowledge of the actual treatment becomes medically necessary to affect treatment options. For example, if a patient is worsening, especially if there are worsening systemic signs or symptoms, then, especially because one of the treatment arms does not contain an antibiotic, it may be important to know if treatment with a topical and/or systemic antibiotic might be a reasonable treatment option. The investigator will be able to unblind the study treatment at any time through IWRS.

The treatment unblinding information will be accessible by right-clicking the patient in the "List of patients" tab. A first message will inform the investigator of the unblinding instructions to follow. In order to complete the unblinding process, the following information will be required: the user's password, the patient code concerned by the unblinding request, the patient's birth date and the reason for unblinding.
In this way, the only person unblinded will be the investigator. The investigator should only share this information with people who need to know for the further treatment of the patient and should not be shared with SALVAT or [redacted]'s personnel.

6.5 Drug Accountability

[redacted] will provide each Investigator with sufficient amounts of the investigational medication (Ciprofloxacin plus Fluocinolone acetonide otic solution) and the comparator medication (Ciprofloxacin otic solution and Fluocinolone acetonide otic solution). The Investigator will confirm receipt of all kits of study medication using the IWRS and will document receipt in the written study files.

The Investigator will dispense the study medication only to patients included in this study and following the procedures described in this study protocol. Each patient will be given only the study medication carrying his/her number. Each dispensing will be documented in the EDC.

Patients and their caregivers will be asked to keep all used and unused study medication vials, and containers and return them to a study staff member at Visit 3. If patients or caregivers forget to bring in containers at Visit 3, they must bring them in no later than Visit 4.

All supplies must be accounted for at the end of the study. The Investigator is responsible for ensuring that accurate and adequate records are maintained. These records will include lot numbers, quantities received, dates received, dates dispensed, etc., for the investigational medication (Ciprofloxacin and Fluocinolone acetonide otic solution) and the comparator medications (Ciprofloxacin otic solution and Fluocinolone acetonide otic solution). The Investigator must ensure that all used and unused supplies are returned to [redacted]. The exact number of vials used in the study must be noted, regardless of whether the study was completed or terminated prematurely. At the time of return, the Investigator must verify that all used and unused supplies of study medication have been returned by the patient and that no remaining supplies are in the patient's possession.

6.6 Compliance

At Visit 1, the patient or caregiver will be provided with a patient diary and instructed to record each dose of study medication on the diary. At Visit 2, the patient or caregiver will be asked if he or she is using study medication and filling out the patient diary according to directions. Patient diary will be reviewed at Visit 3 to determine study medication compliance as described in Section 10.1.8.

6.7 Concomitant Medications

Any medication the patient takes other than the study medication specified in the protocol is considered a concomitant medication. This includes prescribed medications, over-the-counter medications, herbal remedies, vitamins, supplements etc. All treatments must be recorded in the EDC in the concomitant medication page.

At Visit 1, patients will be asked what medications they are currently taking or have taken during the last 30 days. At Visits 2, 3, and 4, patients will be asked what concomitant medications they are currently taking or have taken since the last visit, including medications for pain.

6.7.1 Prohibited Concomitant Medications

The following medications will be prohibited during the study (unless the patient has prematurely discontinued study treatment):

- Any investigational drug;
- Any topical or systemic therapy for treatment of otitis externa or otitis media;
- Any systemic antifungal drug;
- Any antibiotic drug other than study medication;
- Any systemic corticosteroid, except nasal or inhaled corticosteroids at stable doses (at least 30 days prior to enrollment)
- Any systemic drug (prescription or over the counter) with anti-inflammatory properties (including biologic response modifiers, nonsteroidal anti-inflammatory drugs such as ibuprofen, naproxen, and cyclooxygenase-2 inhibitors); acetylsalicylic acid for cardiovascular prevention at doses of 325mg per day or less is allowed
- Any compound, agent or substance that is applied to the evaluable external ear or instilled in the evaluable ear canal, other than study medication.

6.7.2 Rescue Medication

Concomitant medications will be considered rescue medication if they meet both of the following criteria:

- Any otic or systemic treatment administered for signs/symptoms of otitis externa of the evaluable ear (Note – topical antibiotics applied in the non-evaluable ear are NOT considered to be rescue medication)
- Started after Visit 1 but before End-of-study visit (Note – antibiotics given at or after End-of-study visit are NOT considered rescue medications)

6.7.3 Recommended Pain Medication

Patients who are taking anti-inflammatory agents are able to be included in the study, but they must stop the anti-inflammatory treatment (including ibuprofen) at enrolment.

For patients who require analgesic medications for otalgia, the recommended medication is acetaminophen (paracetamol). The use of acetaminophen should never been considered as an initial treatment for otalgia, thus will only be administered after confirming that the study medication is not sufficient for pain relief.

7. VARIABLES AND METHODS

7.1 Efficacy Parameters

7.1.1 Clinical Efficacy Parameters

7.1.1.1 Brighton grading

Brighton grading will be assessed in both ears at Visits 1, 3 and 4. For consistency, the same individual should perform the assessments at all 3 visits, if possible. Brighton grading will be assesses as:

- Grade 0: Normal
- Grade I: Tympanic membrane seen. Canal erythematous
- Grade II: Debris in ear canal. Tympanic membrane often obscured by debris.
- Grade III: Edematous ear canal. Tympanic membrane obscured by edematous ear canal. No systemic illness.
- Grade IV: Edematous ear canal. Perichondritis (pinna cellulitis). Systemic illness.

7.1.1.2 Pain (Otalgia)

Otalgia in both ears will be assessed at Visits 1, 3, and 4. The investigator will ask the patient (or caregiver if patient is too young) to assess his or her level of pain on the day of the visit. If the patient has taken analgesic medication, he or she will be asked to assess the level of pain before taking the analgesic. For consistency, the assessment of otalgia should always be performed before the ear is examined. Assessments will be on the scale described in Blanch 2000.[30] Otalgia will be assessed as:

- Severe (3) if it interferes with activities of daily living;
- Moderate (2) if it causes discomfort but does not interfere with activities of daily living;
- Mild (1) if there is awareness of pain but not much discomfort
- Absent (0) if there is total absence of pain.

At Visits 3 and 4, pain will be considered resolved if the pain level is 0. Otalgia will be considered improved if the pain level is lower than in the previous visit.

Moreover, patients will complete a diary twice daily in which they will record assessments of ear pain, pain medication use.

Throughout the study, patient or caregiver should record pain severity at least twice daily (prior to dosing). For patients under 7 years old, caregiver will complete the FLACC scale. Patients from 7 years old to younger than 13 years old will record the pain using the Wong Baker Faces Pain Scale. And patients 13 years old and older will record the pain using a VAS scale (see Appendix 3).

7.1.1.3 Edema

Edema in both ears will be assessed at Visits 1, 3, and 4 by the investigator. For consistency, the same individual should perform the assessments at all 3 visits, if possible. Edema will be assessed as:

- Severe (3) if the tympanic membrane is not visible because of swelling;
- Moderate (2) if the tympanic membrane is partially visible;
- Mild (1) if there is some swelling but the tympanic membrane is fully visible;
- Absent (0) if there is no visible swelling.

At Visits 3 and 4, edema will be considered resolved if the edema level is 0. Edema will be considered improved if the edema level is lower than in the previous visit.

7.1.1.4 Otorrhea

Otorrhea in both ears will be assessed at Visits 1, 3, and 4 by the investigator. For consistency, the same individual should do the assessments at all 3 visits if possible. Otorrhea will be assessed as:

- Severe (3),
- Moderate (2),
- Mild (1),
- Absent (0).

At Visits 3 and 4, otorrhea will be considered resolved if the otorrhea level is 0. Otorrhea will be considered improved if the otorrhea level is lower than in the previous visit.

7.1.1.5 Overall Clinical Outcome

Overall Clinical Outcome is based on the Total signs/symptoms score (TSSS), which is calculated by the sum of otalgia score + edema score + otorrhea score. Patients will be allocated to one of the following categories for Overall Clinical Outcome:

1. Clinical Cure: TSSS is 0, as defined in Sections 7.1.1.2, 7.1.1.3 and 7.1.1.4.
2. Clinical Improvement: TSSS is different than 0 but lower than the previous visit, as defined in Sections 7.1.1.2, 7.1.1.3 and 7.1.1.4.
3. Clinical Failure: TSSS does not meet the definitions of Clinical Cure or Clinical Improvement, as defined in Sections 7.1.1.2, 7.1.1.3 and 7.1.1.4.
4. Indeterminate: Discontinued (for reasons other than Clinical Failure) or lost to follow-up.

7.1.1.6 Time to end of pain

Time to end of ear pain, defined as the interval (in days) between first dose of study medication and the first day (morning or evening) on which there is no use of analgesics, the diary pain score is zero and the score remains at zero for all subsequent visits until the end of the study.

Patients or caregivers will complete a diary twice daily in which they will record assessments of ear pain and pain medication use, including the time and date of the assessment.

The patient diary information will be used to ascertain the time point (morning or evening) and date at which the ear pain in the evaluable ear ended without use of analgesics.

7.1.2 Microbiological Efficacy Parameters

Microbiological cultures of ear discharge will be taken at Visit 1; and in addition at visits 3 and 4 when discharge is present. The otorrhea sample will be taken prior to the debridement of the affected ear(s). A central laboratory will provide microbiological sampling kits with standardized instructions for sample preparation. The entrance to the ear canal will be swabbed with a cotton swab to remove any debris that could cause contamination. The ear canal may be irrigated if that is standard practice at the study site; irrigation must be documented in the EDC. An ACT II Transport Tube, as provided in the microbiological sampling kit, will be gently rubbed against the sides of the ear canal to obtain a suitable specimen for culture. Samples will be shipped to the central laboratory for processing. The central laboratory will be blinded to the treatment assignment of the patient from whom the sample was taken.

The central laboratory will use standardized clinical microbiological laboratory procedures (aerobic cultures only) to identify bacteria by species. Bacterial abundance will be assessed on a scale of 1+ to 4+, per standard laboratory grading (score=0 if the culture is sterile). To be considered a pathogen, the cultured organism must be present at a level of 1+ or higher.

The following species are most likely to be pathogens: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Streptococcus* (primarily beta-hemolytic strains), and Gram-negative enteric bacilli (e.g., *Enterobacter*, *Proteus*, and *Klebsiella* species).

The following bacteria are generally colonizers and will not be categorized as pathogens: alpha-hemolytic streptococci, coagulase-negative staphylococci, *Bacillus* species, *Corynebacterium* species, *Lactobacillus* species, and *Proprionobacterium* species. Fungi and yeasts will not be considered as pathogens.

The culture results will be classified in the following groups:

- Negative: culture does not show any growth
- Positive: culture shows microorganism growth
    - Positive with non- pathogens: microorganism are generally colonizers and /or are not categorized as pathogens
    - Positive with pathogens:
        - Target pathogen: *P. aeruginosa* and/or *S. aureus*

- Non-target pathogens: pathogens different to *P. aeruginosa* and/or *S. aureus*

For any patient with a culture positive for a pathogen at Visit 1, bacteriologic response at Visits 3 will be classified as:

- Eradication if the culture does not show growth of any pathogen;
- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Improvement or Clinical Cure as defined in Section 7.1.1.5;
- Persistence if any pathogen cultured at Visit 1 is still present;
- Presumed Persistence if the Overall Clinical Outcome is Clinical Failure but no culture result is available from Visit 3;
- Superinfection if a pathogen not present at Visit 1 is now present (presence of a nonpathogenic organism will not be considered Superinfection); or

  Indeterminate if none of the above definitions are met and the bacteriologic response cannot be determined.

For any patient with a culture positive for a pathogen at Visit 1, bacteriologic response at Visits 4 will be classified as:

- Eradication if the culture does not show growth of any pathogen;
- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Improvement or Clinical Cure as defined in Section 7.1.1.5;
- Persistence if any pathogen cultured at Visit 1 is still present;
- Presumed Persistence if the Overall Clinical Outcome is Clinical Failure but no culture result is available from Visit 4;
- Recurrence if there is reappearance of the pathogen eradicated or presumably eradicated at Visit 3
- Superinfection if a pathogen not present at Visit 1 and Visit 3 is now present (presence of a nonpathogenic organism will not be considered Superinfection);
- Reinfection if there is isolation of a new pathogen different from the one eradicated or presumably eradicated at Visit 3;or
- Indeterminate if none of the above definitions are met and the bacteriologic response cannot be determined.

If Overall Clinical Outcome at Visit 3 is Clinical Failure (whether or not the patient discontinues prematurely) and no bacterial culture is performed at Visit 3, the bacteriologic response at Visit 3 will be Presumed Persistence. If Overall Clinical Outcome at Visit 4 is Clinical Failure and no bacterial culture is performed at Visit 4, the bacteriologic response at Visit 4 will be Presumed Persistence.

Microbiological outcome will be determined for each patient for Visit 3 and for Visit 4 after microbiological culture results are made available to the Sponsor or designee.

7.1.3 Therapeutic response (Overall Clinical Outcome + Microbiological Outcome)

1. Therapeutic cure: TSSS (otalgia+edema+otorrhea) of "0" and bacteriological response eradication or presumed eradication.
2. Therapeutic failure:
    - Positive culture for pathogen or,
    - negative culture or presumed eradication with TSSS (otalgia+edema+otorrhea)>0

Overall Clinical Outcome + Microbiological Outcome will be determined for each patient for Visit 3 and at Visit 4 after microbiological culture results are made available to the Sponsor or designee.

7.1.4 Primary Efficacy Endpoint

Based on the definitions in Sections 7.1.3, the primary efficacy endpoint will be the proportion of patients with Therapeutic cure at Visit 3 in the MITT_PA/SA population (pathogen positive subset of the CITT population which includes all patients who received study medication and had culture positive for *P. aeruginosa* and/or *S. aureus* at baseline in the evaluable ear).

7.1.5 Secondary Efficacy Endpoints

Based on the definitions in Sections 7.1.1 and 7.1.3, the secondary efficacy endpoints will be:

<u>Principal secondary endpoint:</u>

- Time to end of ear pain.

  End of ear pain is defined as the interval between the first dose of study medication and the first day (morning or evening) on which there is no use of analgesics, the diary pain score is zero and the score remains at zero for all subsequent visits (until end of the study)

  This time will be calculated on the basis of the patient diary entries.

<u>Other secondary endpoints:</u>

- Sustained microbiological cure
- Clinical cure at Visit 3 and Visit 4
- Microbiological success at Visit 3 and Visit 4
- Therapeutic cure (clinical cure +microbiological cure) at Visit 4
- Changes in Brighton grading at Visit 3 and Visit 4
- Changes in otorrhea at Visit 3 and Visit 4
- Changes in edema at Visit 3 and Visit 4
- Changes in otalgia assessed by the investigator at Visit 3 and Visit 4

7.2 Safety Parameters

Safety will be assessed by AEs and vital signs. In addition, a urine pregnancy test for females of childbearing potential will be performed at Visit 1.

7.2.1 Adverse Events

7.2.1.1 Definitions

An adverse event (AE) is any untoward medical event that occurs in a patient or subject who has received an investigational product, and does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the product.

An illness present at entry to the study is considered a pre-existing condition and will not be considered an AE. However, if a pre-existing condition worsens during the study, this may be considered an AE. Pre-existing conditions will be documented in the EDC. If pre-existing signs and symptoms of AOE (otalgia, edema and otorrhea) worsen during the study, this will be considered a treatment failure instead of an AE. If a patient has unilateral AOE at baseline and it becomes bilateral during the study, then the AOE in the contralateral ear will not be considered as an adverse event. All AEs, including intercurrent illnesses, that occur during the study (i.e., between study enrollment and Visit 4) must be reported and documented as described below.

7.2.1.2 Assessment of Adverse Event

Volunteered, observed, and elicited AEs will be recorded. This includes AEs the patient reports spontaneously, those the Investigator observes, and those the elicited in response to questions from the study staff. Patients will be asked open-ended questions, such as “How have you been feeling since your last visit?”, at each study visit. If a patient or patient’s caregiver reports any pain or illness during or after administration of the eardrops (i.e itching), the pain or illness experienced by the patient should be recorded as an AE.

Each AE will be assessed by the Investigator with regard to the following categories.

7.2.1.2.1 Serious Adverse Event

International Conference on Harmonization (ICH) Guidelines and federal regulations define a serious adverse event (SAE) as any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening. This means that the patient is at risk of death at the time of the event; it does not mean that the event hypothetically might have caused death if it were more severe;
- Requires or prolongs patient hospitalization;
- Results in persistent or significant disability or incapacity; or

- Is a congenital anomaly or birth defect.

Important medical events that may not be immediately life-threatening or result in death or hospitalization but that may jeopardize the patient or require intervention to prevent one of the above outcomes usually should also be considered serious.

7.2.1.2.2 Severity

The severity of each AE must be assessed and recorded on the Adverse Events page of the EDC as mild, moderate, or severe.

- Mild: An AE that does not interfere with usual activities;
- Moderate: An AE that interferes with usual activities; or
- Severe: An AE that is intense or debilitating and interferes with usual activities.

7.2.1.2.3 Relationship to Study Medication

The relationship of each AE to study medication must be assessed and recorded on the Adverse Events page of the EDC as one of the following:

- Not related;
- Possibly related; or
- Probably or definitely related.

7.2.1.3 Recording Adverse Events

All AEs that occur during the study must be recorded in the Adverse Events page of the EDC. All Adverse Event EDC entries should contain a brief description of the event, date and time of onset, duration, intensity, treatment required, relationship to study medication, action taken with regard to study medication, outcome, and whether the event is classified as serious.

7.2.1.4 Reporting Serious Adverse Events

The Investigator must report any SAE that occurs during the study (i.e., between study entry and Visit 4), regardless of relationship to study medication, to [redacted] within 24 hours of discovering the event. In addition, if the investigator learns of an SAE occurring after the last follow-up visit, but within 30 days after the last administration of the study drug, he/she should immediately report the event to the Safety Officer at [redacted].

Directions for reporting an SAE appear below.

SERIOUS ADVERSE EVENT REPORTING INSTRUCTIONS

[redacted] INTERNATIONAL

Clinical Trials Safety Center

Email for Reporting SAEs: [redacted]

Fax: [redacted]

Telephone: [redacted] (for urgent issues/questions during business hours)

1. **Fax or email the SAE report form and any supporting documentation to the Safety Center within 24 hours of becoming aware of the event.**
2. **Telephone the Safety Center if you have an urgent issue or cannot fax or email the SAE report form. Provide the Safety Center personnel with the Investigator's name, your name, the telephone number where you can be reached, your study site number, the protocol number and title, and the patient number for an urgent concern/issue.**
3. **Provide the Safety Center personnel with the Investigator's name, your name, the telephone number where you can be reached, your study site number, the protocol number and title, and the patient number.**

The Investigator and the Sponsor (or Sponsor's designee) will review each SAE report and evaluate the relationship of the SAE to study medication. Based on the Investigator's and Sponsor's assessment of the SAE, a decision will be made concerning the need for further action. The primary consideration governing further action is whether new findings affect the safety of patients participating in the clinical study. If the discovery of a new SAE related to the study medication raises concern over the safety of continued administration of the study medication to patients, the Sponsor (or Sponsor's designee) will take immediate steps to notify the FDA/other regulatory authority and all Investigators participating in clinical studies of the study medication.

Further action that may be required includes the following:

- Modification of the protocol, including the addition of investigators;
- Discontinuation or suspension of the study;
- Modification of the existing consent form and informing current study participants of new findings; or
- Addition of any newly identified study medication-related AEs to the list of expected AEs.

7.2.1.5 Follow-Up of Adverse Events

The Investigator must continue to follow all SAEs and those non-serious events assessed as possibly, probably, or definitely related to the study medication until they resolve or until the Investigator assesses them as chronic or stable. The follow-up information must be reported to the Sponsor. This follow-up may extend after the end of the study.

7.2.1.6 Reporting Safety Information

The Investigator must promptly report to his or her Institutional Review Board (IRB) or Independent Ethics Committee (IEC) all unanticipated problems involving risks to patients. This includes death from any cause and all SAEs assessed as possibly, probably, or definitely related to the study medication, and any other events required to be reported by the IRB/IEC.

7.2.1.7 Protocol Deviations Due to an Emergency or Adverse Event

In the case of an emergency or AE, departures from the protocol may be necessary. Such protocol deviations will be determined as allowable on a case-by-case basis. The Investigator or other physician in attendance in such an emergency must contact the Medical Monitor as soon as possible to discuss the circumstances of the emergency. The Medical Monitor and the Investigator will confer to decide whether the patient should continue to receive study medication. All protocol deviations and the reasons for such deviations must be noted in the EDC.

7.2.2 Physical Examination

Physical examinations will be performed at Visit 1. A directed examination of the ears (external inspection, examination of the external auditory canal, and otoscopic visualization of the tympanic membrane), head, nose and oropharynx will be performed.

Vital signs (temperature, blood pressure, and pulse) will be assessed at Visits 1, 3 & 4.

7.2.3 Pregnancy Test

A urine pregnancy test will be performed at Visit 1 for female patients of childbearing potential (females who have had menarche and are not premenstrual, not postmenopausal or not surgically sterile). This test will be performed at the study site or at the local laboratory used by the site. A negative result is required before the patient can be randomized.

Moreover, females must have agreed to use adequate birth control methods to prevent pregnancy throughout the study. Adequate birth control methods included topical, hormonal-oral, implantable or injectable contraceptives; mechanical-spermicide in conjunction with a barrier such as condom or diaphragm; intrauterine device (IUD); or surgical sterilization of partner (must be $\geq$ 6 months post vasectomy). For non-sexually active females, abstinence was regarded as an adequate method of birth control; however, if the patient became sexually active during the study, she must have agreed to use adequate birth control methods as defined above for the remainder of the study.

8. STUDY CONDUCT

8.1 Schedule of Observations

A schedule of observations and assessments to be performed during the study is provided in Table 1.

Table 1 Schedule of Observations

Evaluation	Visit 1 Screening/ Study Entry	Visit 2 By Telephone	Visit 3 End of Treatment	Visit 4 Post-Treatment Follow-Up
Study Day	**1**	**3-4**	**8-10 or within 2 days of early termination**	**15-17**
Informed Consent (and Assent Form when applicable)	X			
Inclusion/exclusion criteria	X			
Medical history	X			
Concurrent symptoms/conditions	X			
Urine pregnancy test[a]	X			
Physical examination	X			
Vital signs[b]	X		X	X
Brighton grading	X		X	X
Ear pain	X		X	X
Edema	X		X	X
Otorrhea	X		X	X
Overall Clinical Outcome			X	X
Microbiological culture of ear discharge[c]	X		X	X
Register patient visit through IWRS	X	X	X	X
Randomization through IWRS	X			
Dispense study medication and explain its use	X	X[d]		
Collect used and unused study medication containers			X[e]	
Dispense patient diary and explain its use	X			
Inquire about otitis symptoms		X[f]		
Review patient diary			X	X
Concomitant medications	X	X	X	X
Adverse events	X	X	X	X

[a] For female patients of childbearing potential.

[b] To include temperature, blood pressure, and pulse rate.

[c] If no discharge is present, no attempt to culture will be made.

[d] If patient with unilateral AOE at baseline becomes bilateral prior to Visit 3 a resupply study medication kit (with the same medication) will be dispensed[e] If patients forget to bring in containers at Visit 3, they must bring them in no later than Visit 4.

[f] If patients report no improvement in otitis symptoms, they will be asked to come in for a visit as soon as possible. At this visit, they may prematurely discontinue study treatment (in which case the visit will be recorded as Visit 3, the End of Treatment visit) or continue study treatment (in which case the visit will be recorded as an unscheduled visit).

8.2 Observations by Visit

8.2.1 Visit 1

Patients who may be eligible to participate in the study will be offered the opportunity to participate. The study will be explained to them and questions about the study will be answered. Patients who elect to participate will sign the Informed Consent Form (or have it signed by their legally authorized representative), and patients who are below the age of majority but able to give assent will sign the Assent Form, before any study procedures may be performed. The IWRS will assign a patient number, which will be used to identify the patient during screening and, if applicable, throughout the duration of the study. Patients who do not meet the inclusion and exclusion criteria will be registered as screen failures through the IWRS. Patients who have provided consent and met the inclusion and exclusion criteria will be enrolled and randomized through the IWRS.

Medical history, concurrent symptoms and conditions, and concomitant medications will be recorded, and a physical examination (as described in Section 7.2.2) will be performed. Female patients of childbearing potential will have a urine pregnancy test.

Brighton grading, otalgia, edema, and otorrhea will be evaluated as described in Appendix 2, and Sections 7.1.1.1, 7.1.1.3, and 7.1.1.4. A microbiological culture of ear discharge will be taken as described in Section 7.1.2 prior to the debridement.

The patient or caregiver will be given a medication kit containing a 7-day supply of study medication. The blind will be maintained as described in Section 6.4. A member of the study staff will instruct the patient or caregiver on how to open the vials and administer study medication, and will supervise the patient or caregiver during administration of first dose.

The patient or caregiver will be provided with a patient diary and be trained on its use. A member of the study staff will instruct the patient or caregiver in how to fill out the patient diary.

Patients will be instructed to refrain from swimming during the study treatment period and preferably until the final study visit is completed. Patients will also be advised to use a shower cap or neoprene head band when bathing.

If any AEs occur during Visit 1, they will be recorded in the Adverse Events page of the EDC.

8.2.2 Visit 2 (Phone Visit)

A study staff member will telephone each patient or caregiver on Day 3 or Day 4 of treatment. The information obtained at this "phone visit" will be recorded in the Visit 2 page of the EDC. Study staff will ask about and record any AEs and any changes in concomitant medications since Visit 1. Study staff will also ask about study medication compliance. Patients or caregivers will also be asked if otitis symptoms are improving or worsening. A specific questionnaire to obtain all these information will be used.

Patients who report no improvement in otitis symptoms will be asked to come in for an unscheduled visit as soon as possible. At this visit, the Investigator will choose one of the two following options for the patient:

1. Continue the patient in the study. The present visit will be recorded as an unscheduled visit. The patient should continue taking study medication without interruption. A debridement of the ear and placement of otowick may be needed.
2. Discontinue study medication. The present visit will be recorded as Visit 3, the End of Treatment visit. The patient will be withdrawn from the study and may be treated with other medication (standard treatment) as appropriate at investigator's discretion. The clinical outcome for the patient will be recorded as Treatment Failure.

If a patient has unilateral AOE at baseline, but develops AOE of the contralateral ear subsequently during the study, then at Visit 2 or at unscheduled visit prior to Visit 3, the investigator or designee will obtain a second kit number (with the same medication) through the IWRS to treat the contralateral ear. This information will be included in the Comments page and Resupply or replacement page of the EDC, and will not be considered as an AE.

8.2.3 Visit 3

Visit 3 will take place between Day 8 and Day 10, or within 2 days of early discontinuation of study treatment.

Adverse events and concomitant medications will be recorded and vital signs will be assessed.

Brighton grading, otalgia, edema, and otorrhea will be evaluated as described in Appendix 2, Sections 7.1.1.1, 7.1.1.2, 7.1.1.3 and 7.1.1.4. Overall Clinical Outcome will be evaluated by the Investigator as described in Section 7.1.1.5. A microbiological culture of ear discharge (if discharge is present) will be taken as described in Section 7.1.2.

If patient took rescue medication (see Section 6.7.2) prior to this visit, the ear discharge will not be collected, because patient will be considered Treatment Failure.

Used and unused study medication containers will be collected from the patient or caregiver by a member of the study staff.

The patient diary will be reviewed to determine study medication compliance as described in Section 6.6.

8.2.4 Visit 4

Visit 4 will take place between Day 15 and Day 17. All patients (except those who have been withdrawn from the study or have withdrawn their consent as described in Section 5.3) are expected to attend Visit 4 (TOC).

Adverse events and concomitant medications will be recorded, and vital signs will be assessed.

Brighton grading, otalgia, edema, and otorrhea will be evaluated as described in Appendix 2, Sections 7.1.1.1, 7.1.1.2, 7.1.1.3, and 7.1.1.4. Overall Clinical Outcome will be evaluated

by the Investigator as described in Section 7.1.1.5. A microbiological culture of ear discharge (if discharge is present) will be taken as described in Section 7.1.2.

If patient took rescue medication (see Section 6.7.2) prior to this visit, the ear discharge will not be collected, because patient will be considered Treatment Failure.

Upon completing Visit 4, the patient will have completed his or her participation in the study.

8.2.5 Unscheduled Visits

Unscheduled visits may be performed at any time, at the Investigator's discretion. Patients who come in for an unscheduled visit should bring their study medication and patient diary.

8.3 Study Termination

If the Sponsor or their designee, the Investigator, or the Medical Monitor discovers conditions arising during the study that indicate the study should be halted, the study may be terminated. Conduct of the study may also be terminated at a particular study site while the study continues at other sites. Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study;
- Failure of the Investigator to enter patients at an acceptable rate;
- Insufficient adherence to protocol requirements; or
- A decision on the part of the Sponsor to suspend or discontinue development of the study medication.

9. DATA HANDLING AND RECORD KEEPING

9.1 Data Quality Assurance

The Sponsor (or Sponsor's designee) will conduct a site visit to verify the qualifications of each Investigator, inspect the site facilities, and inform the Investigator of his or her responsibilities and the procedures for ensuring adequate and correct documentation.

A training extensive site initiation visit will be held to introduce Investigators and their personnel to the study protocol, EDC, procedures, and regulatory requirements.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded in the EDC for this study must be consistent with the patient's source documentation (e.g., medical records).

During the course of the study, a monitor will make site visits to review protocol compliance, compare EDC with individual patients' medical records, assess study medication accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. Entries in the EDC will be verified against source documents. The review of medical records will be performed in a manner that ensures patient confidentiality is maintained.

Regulatory authorities of certain countries, the IRB/IEC, and/or the Sponsor's or designee's Clinical Quality Assurance Group may perform source data checks and/or on-site audit inspections. Investigators and their institutions will provide direct access to source data and documents to these authorities. The Investigator assures the Sponsor and their designee of the necessary support at all times.

9.2 Case Report Forms and Source Documentation

Electronic Data Capture (EDC) and patient diary will be used in this study. The Sponsor or designee will provide a password to each staff member who has the authorization to implement the EDC.

The electronic data for each patient will be checked against source documents at the study site by the site monitor. Any information recorded directly into the EDC (i.e., information for which there is no prior written or electronic record) will be considered source data. The patient diary will be considered source data. A copy of the patient diary and the EDC with audit trail and final data of the EDC including data management changes will be placed in the Investigator's study file.

A Screening Log will be maintained, listing all patients screened, including patients who do not qualify for enrollment.

Instances of missing data will be discussed with the Investigator for resolution. If necessary, data query forms will be generated and sent to the site for further clarifications or corrections. The Sponsor or designee will perform a quality control review on the database. If a self evident correction document is generated during the study, the document must be approved and signed by each principal investigator.

9.3 Archiving Study Records

Essential documents should be retained for a minimum of 25 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region or at least 25 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable laws. The Sponsor or designee must be notified in writing if the Investigator moves or if the storage location of documents is changed.

10. STATISTICAL METHODS

10.1 General Statistical Methods

All data collected in the database will be presented in the data listings.

Continuous data will be summarized by treatment group using descriptive statistics (n, mean, standard deviation, standard error of the mean, median, minimum, and maximum). Categorical data will be tabulated by treatment group and category.

In order for the requirements of the combination rule to be satisfied, both comparisons of the combination to the components alone must show the combination to be statistically significantly better than the component alone using a two-sided 0.05 significance level.

Unless otherwise noted, all analyses and summaries will be based on the microbiological intent-to-treat (MITT), the pathogen positive subset (MITT-PA/SA) and microbiological per-protocol (MPP) populations, which will be the primary populations for efficacy analysis. Efficacy analyses will also be conducted on the Clinical intent-to-treat (CITT) and per-protocol (PP) populations. Safety summaries will be based on the Safety population. These populations are defined in Section 10.1.4.

10.1.1 Sample Size

Planned enrollment is 500 children, adolescents and adults. Patients will be stratified at enrollment so that approximately 50% of those enrolled will be younger than 18 years old and approximately 50% will be over 18 years old or older. Stratification is being performed to ensure adequate representation of each age group in the study and not for statistical considerations.

We have not been able to find an accurate estimation of study's main variable (therapeutic cure) in previous investigations with Ciprofloxacin 0.3% otic solutions in the treatment of AOE. Rates of patients cured, with different definitions from ours, have been estimated within a range between 60% and 70%. Moreover, in two comparative trials testing the same combination (same product) versus Ciprofloxacin alone in the treatment of AOMT, the percentage of therapeutic cure in patients with *Pseudomonas aeruginosa* and *Staphylococcus aureus* was around 50%.

For the calculation of the sample size for this study, the assumption is made that the Ciprofloxacin alone group will have a therapeutic cure rate of 62%, and that the combination will increase it by a relative 25%, which would represent a therapeutic cure rate of 78%. Assuming a two-sided significance level of 5%, and a statistical power of 80%, the required number of patients in each group would be 150. The randomization schedule is defined as 2:2:1 (Ciprofloxacin plus Fluocinolone acetonide : Ciprofloxacin : Fluocinolone acetonide), so the total number of evaluable patients to be recruited is 375 (150 : 150 : 75).

In this study, evaluable patients are those with a positive pathogen microbiological culture at baseline (MITT population). From previous studies in AOE, the rate of patients included with a negative culture at baseline is approximately 25%, and therefore the number of patients to be finally recruited to obtain 375 evaluable patients would be 500.

10.1.2 Interim Analyses

No interim analyses are planned.

10.1.3 Missing, Unused, and Spurious Data

Patients with missing efficacy data or indeterminate outcomes will be considered as treatment failures for efficacy analyses.

For the primary endpoint, patients who discontinued for lack of efficacy or rescue medication use will be considered as treatment failure.

If the "ear pain" continues at the end of the study, the TEOP will be recorded as the length of time between the time of the first dose of study medication and the last time point when a pain measurement was recorded.

Patients who take rescue medication, discontinue or are lost to follow up, and for whom the pain persists at the time of the last observation, will be censored at maximum length (Day 17) in those cases when the diary information is not available.

To explore the effect of the handling of the missing data and assess the robustness of the efficacy results, a complete case analysis will be carried out (i.e. all efficacy analyses will be repeated on actual values i.e. without imputation of the missing data) as sensitivity analysis.

All data, whether summarized or not, will be presented in the data listings.

Spurious data, such as unscheduled visit collections, laboratory tests not specified in the protocol, or Investigator comments, will be presented in the data listings. These data will be excluded from the summary tables.

10.1.4 Analysis Populations

There will be 5 populations defined for this study: Safety, Clinical intent-to-treat (CITT), Microbiological intent-to-treat (MITT), Clinical per-protocol (CPP), and Microbiological per-protocol (MPP).

The **Safety population** will include all patients who received any study medication.

The **CITT population** will include all patients who were randomized.

The **MITT population** will include all CITT patients whose Visit 1 microbiological culture yields 1 or more pathogens, as defined in Section 7.1.2.

The **MITT-PA/SA population** will include the pathogen positive subset of the CITT population which includes all patients who received study medication and had culture positive for *P. aeruginosa* and/or *S. aureus* at baseline in the evaluable ear.

The **CPP population** will include all CITT patients who:

- Satisfied all inclusion and exclusion criteria;
- Did not receive any prohibited concomitant medications;
- Did not have any other major protocol violations;

- Completed Visit 3 and Visit 4 (unless the patient was deemed a clinical failure at an earlier visit than Visit 4); and
- Had compliance rates between 80% and 120% as defined in Section 10.1.8 (patients who are deemed Clinical Failures are to be included if they had compliance rates between 80% and 120% during the first 3 days of study treatment).

The **MPP population** will include all CPP patients whose Visit 1 microbiological culture yields 1 or more pathogens and who had microbiological results (when patient has material to culture) from Visit 3 and/or Visit 4 unless the patient was deemed a clinical failure at an earlier visit than Visit 4.

For the CITT and MITT populations, the treatment group of a patient will be determined by the treatment group to which the patient was randomized.

For the Safety, CPP and MPP populations, the treatment group of a patient will be determined by the treatment the patient received, not necessarily the group to which he or she was randomized. The Safety population will be used for all safety analyses.

The primary populations for efficacy analyses will be the MITT population.

The populations to be used for each of the secondary efficacy endpoints will be detailed in the Statistical Analysis Plan.

10.1.5 Patient Disposition

The numbers of patients in each treatment group who completed the study and who terminated early will be tabulated. For patients who terminated early, primary and secondary reasons for termination will be tabulated. Patients who were excluded from each of the study populations defined in Section 10.1.4, and their reasons for exclusion, will be listed.

10.1.6 Demographics and Baseline Characteristics

Continuous demographic and baseline characteristics, such as age, will be summarized by treatment group with descriptive statistics (n, mean, standard deviation, standard error of the mean, median, minimum, and maximum). Categorical demographic and baseline characteristics such as gender and race will be tabulated (frequency and percent) by treatment group and category. Demographic and baseline characteristic tables will be presented for all study populations.

10.1.7 Protocol Deviations

Protocol deviations are defined as violations from the procedures outlined in the protocol. **Major** protocol deviations are protocol violations likely to affect the study results and leading to the exclusion of the patient from the CPP.

Once the database has been completed and considered as “clean”, a data blind review will be conducted before the database lock in order to identify all major protocol violations and assign patients into each of the analysis sets as defined in section 10.1.4.

Patients with protocol deviations will be presented in the data listings. Protocol violations will be tabulated by treatment group and violation.

10.1.8 Compliance with Study Medication

Compliance will be assessed by a review of the patient diary. The number of doses the patient actually took during a given period will be divided by the number of doses the patient was expected to take during that period. The resulting ratio will be multiplied by 100% to determine percent compliance. Percent compliance during the treatment period and during the first 3 days of the treatment period will be calculated and listed for each patient.

The percent compliance during the first 3 days is needed to identify the clinical failures whom will remain in the CPP population, as mentioned in Section 10.1.4.

Patients will be considered "compliant" if their percent compliance is between 80% and 120%. The proportion of patients who were compliant and non-compliant during the treatment period will be tabulated by treatment group.

10.1.9 Concomitant Medications

Concomitant medications will be tabulated by treatment group and by type of pain treatment. If the concomitant medications analysis by type of pain treatment shows difference between treatment groups, subgroup analyses by type of pain treatment might be carried out on efficacy results as post-hoc analyses.

10.1.10 Efficacy Analyses

Only assessments from the evaluable ear will be used for the efficacy analyses.

10.1.10.1Primary efficacy endpoint

Primary analysis of the primary endpoint

The primary efficacy endpoint will be the therapeutic cure at Visit 3 in the MITT-PA/SA population.

In order for the requirements of the combination policy to be satisfied, both comparisons of the combination to the components alone must show the combination to be statistically significantly better than the component alone using a two-sided 0.05 significance level.

The number and percentage of patients with therapeutic cure at Visit 3 will be summarized by treatment group. The proportion of patients with a therapeutic cure will be compared between the treatment groups by using a chi-squared test.

Efficacy analyses of the primary endpoint will also be conducted on the MITT, MPP, CITT and CPP populations.

10.1.10.2 Secondary efficacy endpoints

Only analysis of the principal secondary endpoint will be confirmatory. Analysis of the other secondary efficacy endpoints will be supportive only. Since each confirmatory comparison (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution over Ciprofloxacin 0.3% otic solution alone and over Fluocinolone acetonide 0.025% otic solution alone for the primary endpoint, and for the principal secondary endpoint) should be

statistically significant at a two-sided 0.05 significance level, no adjustment for multiple comparisons/multiplicity will be made.

Principal secondary endpoint

Time to end of ear pain

Patients or caregivers will complete a diary twice daily (prior to dosing) in which they will record assessments of ear pain and pain medication use, including the time and date of the assessment.

Ear pain is defined as ending on the first day (morning or evening) on which there is no use of analgesics, the diary pain score is zero and the score remains at zero for all subsequent visits until the end of the study.

The patient diary information together with investigator assessment will be used to ascertain the time point (morning or evening) and date at which the ear pain in the evaluable ended without use of analgesics. The time to end of ear pain is the interval (in days) between the first dose of study medication and the time when the ear pain in the evaluable ended without use of analgesics.

If ear pain in the evaluable ear continued to the end of the study, the TEOP will be recorded as the length of time between the time of the first dose of study medication and the last time point when a pain measurement was recorded.

Patients who took rescue medication, discontinued or were lost to follow up, and for whom the pain persisted at the time of the last observation, will be censored at maximum length (Day 17) in those cases when the diary information is not available.

The null hypothesis is that there is no difference in time to end of pain between the combination and the components alone. The alternative hypothesis is that there is a difference in time to end of pain between the combination and the components alone. The comparison of the treatment groups will be made by using the log-rank test stratified on age (6 months to younger than 18 years old versus 18 years and older), and a difference will be claimed if the null hypothesis can be rejected at the two-sided 0.05 level.

Other secondary endpoints

Sustained Microbiological cure

For any patient with a culture positive for a pathogen at Visit 1, bacteriological response at Visits 3 will be classified as Eradication, Presumed Eradication, Persistence, Presumed Persistence, Superinfection or Indeterminate and bacteriological response at Visit 4 will be classified as Eradication, Presumed Eradication, Persistence, Presumed Persistence, Recurrence, Superinfection, Reinfection or Indeterminate (as defined in Section 7.1.2).

The number and percentage of patients with each response will be summarized by visit and treatment group. The proportion of patients with sustained microbiological cure i.e. with a response of Eradication or Presumed Eradication at both Visit 3 and Visit 4 will be compared between the Ciprofloxacin containing products (Ciprofloxacin 0.3% plus

Fluocinolone acetonide 0.025% otic solution and Ciprofloxacin 0.3%) and the Fluocinolone acetonide 0.025% otic solution treatment groups by using chi-squared test.

Overall clinical outcome at Visit 3 and Visit 4

The Investigator will make the assessment of Clinical Outcome for each patient at Visit 3 and at Visit 4. Clinical Outcome will be classed as Clinical cure or Clinical failure (as defined in Section 7.1.1.5).

The number and percentage of patients with each outcome will be summarized by visit and treatment group. For each visit, the proportion of patients with a response of Clinical success will be compared between the treatment groups by using a chi-squared test.

Overall clinical outcome will also be summarized by bacterial species identified as "target OTIC organism" (*P. aeruginosa and S. aureus*).

Microbiological outcome at Visit 3 and Visit 4

The proportion of patients with a response of Eradication or Presumed Eradication at Visit 3 and Visit 4 will be compared between the Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution and the Fluocinolone acetonide 0.025% otic solution and between Ciprofloxacin 0.3% otic solution and Fluocinolone acetonide 0.025% otic solution treatment groups by using a chi-squared test.

Therapeutic cure at Visit 4

The number and percentage of patients with therapeutic cure at Visit 4 will be summarized by treatment group. The proportion of patients with a therapeutic cure will be compared between the treatment groups by using a chi-squared test.

Change in Brighton grading at Visit 3 and Visit 4

Brighton grading will be assessed by the Investigator at Visits 1, 3 and 4, as defined in Section 7.1.1.1.

The number and percentage of patients with each grade will be summarized by visit and treatment group.

For the 3-level response, the proportion of patients with a Brighton grading of "0" will be considered as Resolved. It will be considered as "Improved" at Visit 3 and Visit 4 if the grade is lower than the previous visit. Brighton grading will be considered as "Not Improved" otherwise. Furthermore if Brighton grading is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If it is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using a Chi-square test.

Change in otorrhea at Visit 3 and Visit 4

Otorrhea will be assessed by the Investigator at Visits 1, 3 and 4. Otorrhea will be assessed as Severe, Moderate, Mild or Absent (as defined in Section 7.1.1.4). Otorrhea will be considered resolved at Visits 3 and 4 if the eardrum edema is assessed as Absent.

The number and percentage of patients with each outcome (Severe, Moderate, Mild or Absent) will be summarized by visit and treatment group. For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using a Chi-square test.

For the 3-level response, otorrhea will be considered as "Improved" at Visit 3 and Visit 4 if otorrhea is assessed by the investigator as "Mild" and was assessed as "Severe" or "Moderate" at Visit 1 (Day 1) or if otorrhea was assessed by the investigator as "Moderate" at Visit 3 or Visit 4 and was assessed as "Severe" at Visit 1. Otorrhea will be considered as "Not Improved" otherwise. Furthermore if otorrhea is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If otorrhea is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

Changes in edema at Visit 3 and Visit 4

Edema will be assessed by the Investigator at Visits 1, 3 and 4. Edema will be assessed as Severe, Moderate, Mild or Absent (as defined in Section 7.1.1.3). Edema will be considered resolved at Visits 3 and 4 if the eardrum edema is assessed as Absent. Edema will be considered improved at Visits 3 and 4 if the edema is assessed as Mild.

The number and percentage of patients with each outcome (Severe, Moderate, Mild or Absent) will be summarized by visit and treatment group. In addition, the number and percentage of patients with eardrum edema considered resolved, improved or not improved will be summarized at Visits 3 and 4 by treatment group. For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using a Chi-square test.

For the 3-level response, edema will be considered as "Improved" at Visit 3 and Visit 4 if the edema is assessed by the investigator as "Moderate" and was assessed as "Severe" at Visit 1. Edema will be considered as "Not Improved" otherwise.
Furthermore if edema is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If edema is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

Changes in pain at Visit 3 and Visit 4

Pain will be assessed at Visits 1, 2, 3 and 4. Pain will be assessed as Severe, Moderate, Mild, Absent or Unable to assess (as defined in Section 7.1.1.2). Pain will be considered resolved at 3 and 4 if the pain is assessed as Absent. Pain will be considered improved at Visits 3 and 4 if the pain is assessed as Mild.

The number and percentage of patients with each outcome (Severe, Moderate, Mild or Absent) will be summarized by visit and treatment group. In addition, the number and percentage of patients with pain considered resolved, improved or not improved will be summarized at Visits 2, 3 and 4 by treatment group. For Visits 2, 3 and 4, the proportion of patients with a response of Resolved will be compared between the treatment groups by using a chi-squared test.

For the 3-level response, pain will be considered as "Improved" at Visit 3 and Visit 4 if the pain is assessed by the investigator as "Moderate" and was assessed as "Severe" at Visit 1. Pain will be considered as "Not Improved" otherwise.
Furthermore if pain is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If pain is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

Additional analysis

Antimicrobial susceptibility

The number of pathogens isolated along with the Minimal Inhibitory Concentration required to inhibit the growth of the bacteria tested (MIC) range values, the Minimal Inhibitory Concentration required to inhibit the growth of 50% of the bacteria tested (MIC_{50}) and the Minimal Inhibitory Concentration required to inhibit the growth of 90% of the bacteria tested (MIC_{90}) will be summarized by treatment group at Visit 3 (EOT) and Visit 4 (TOC) for each bacterial species identified as "target OTIC organism" (*P. aeruginosa and S. aureus*).

Antimicrobial susceptibility against Ciprofloxacin and other antibiotics tested (as shown in Table 2) was presented as follows:

Table 2 Antibiotics tested for susceptibility results by organism

Drug	Organism	
	S. aureus	***P. aeruginosa***
Ciprofloxacin	✓	✓
Ofloxacin	✓	✓
Azithromycin	✓	
Amoxicillin	✓	
Amoxicillin/Clavunate	✓	
Cefuroxime	✓	
Trimethoprim/Sufamethoxazole	✓	✓
Methicillin	✓	

10.1.11 Safety Analyses

All safety analyses will be based on the Safety population.

Safety will be assessed by reports of AEs and vital signs findings. Adverse events will be tabulated by body system, preferred term, and treatment group. Tabulations of AEs by body system, preferred term, treatment group, and severity, and by body system, preferred term, treatment group, and relationship to study medication, will also be provided. Listings of AEs, SAEs, deaths, and AEs leading to discontinuation will be produced. Adverse events will be tabulated for the following subsets:

1. All AEs;
2. Adverse events leading to discontinuation (from the study and from the study medication);
3. Adverse events resulting in death;
4. SAEs; and
5. Adverse events related to study medication.

Patients reporting the same AE more than once will be counted only once for this event in the AE summary table. For tabulation by severity, the AE with the greatest severity reported by the patient will be included in the table. Similarly, for tabulation by relationship to study medication, the AE with the closest relationship to study medication will be included in the table.

Summary statistics will be provided for vital signs.

10.2 Changes in Statistical Methods

Any deviation(s) from the original Statistical Analysis Plan will be described and justified in the final study report.

11. ETHICS, LEGAL, AND ADMINISTRATIVE ASPECTS

11.1 Good Clinical Practice

The procedures described in this study protocol are designed to ensure that the Sponsor and Investigator abide by the principles of the Good Clinical Practice guidelines of the ICH and of the Declaration of Helsinki (shown in Appendix 1). The study also will be performed in keeping with local legal requirements. The Investigator's signature on this protocol constitutes acceptance of these guidelines.

11.2 Informed Consent

Before being admitted to the study, each patient, or patient's legally authorized representative, will provide informed consent according to the regulatory and legal requirements of the participating country. Also, patients under the age of majority but capable of providing assent will provide assent, as applicable. The Investigator will not undertake any procedure specifically required for the clinical study until valid consent (and assent, if applicable) has been obtained. The terms of the consent (and assent, if applicable) and when it was obtained must be documented in the EDC. One or two original Informed Consent Forms (and Assent Form, if applicable) must be signed and dated by the individual administering consent according to local rules and regulations. The original signed Informed Consent Form (ICF) will be retained by the Investigator as part of the study records.

The Principal Investigator or Sub-Investigator will provide one original signed ICF or a copy to the patient or legal representative, according to local rules and regulations.

Should a protocol amendment be made, the ICF and Assent Form may be revised to reflect the changes in the protocol.

If the ICF and Assent Form are revised, it is the responsibility of the Investigator to ensure that the amended documents are reviewed and approved by the IRB/IEC, and that it is signed by all patients currently in the study and all patients subsequently entered in the study (or by their legally authorized representatives).

11.3 Approval of Study Protocol

Before the start of the study, the study protocol and other appropriate documents will be submitted to the IRB/IEC. Written approval from the IRB/IEC must be obtained before any patients are screened. The study protocol and other appropriate documents will be submitted to other authorities as required by local legal requirements.

11.4 Amending the Protocol

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from all persons who approved the original protocol, and receive IRB/IEC approval prior to implementation. Following approval, the protocol amendment(s) will be submitted to the Investigational New Drug application, and other applications, if any, under which the study is being conducted. Administrative changes (minor changes that do not affect the patient benefit/risk ratio) may be made by the Sponsor without any further approvals.

All amendments and administrative changes will be distributed to all protocol recipients with instructions to append them to the protocol.

11.5 Confidentiality

All study findings and documents will be regarded as confidential. The Investigator and members of his/her research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating patients must be maintained. Patients will be identified on EDC and other documents submitted to the Sponsor or designee by their patient number, and/or birth date, or in such other way as may be required by local data protection regulations. Documents not to be submitted to the Sponsor or designee that identify the patient (e.g., the signed Informed Consent Form, the initials) must be maintained in confidence by the Investigator.

11.6 Liability and Insurance

The Sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements, without prejudice to the liability insurance corresponding to the Investigator, the persons instructed by the Investigator, and the hospital, practice, or institute in which they are employed. The civil liability of the Investigator, the persons instructed by him, and the hospital, practice, or institute in which they are employed and the liability of the Sponsor in respect of financial loss due to personal injury and other damage that may arise as a result of the performance of this study are governed by the applicable law.

11.7 Publication Policy

The clinical study report will be a presentation of the pooled results, which will be prepared by the Sponsor with the assistance of some or all of the investigators.

An Investigator shall not publish any data (poster, abstract, paper, etc.) without having obtained written approval from the Sponsor prior to submission for publication or presentation.

The above policy applies to information from a prematurely discontinued or other non-completed study as well as from a completed study. Results from Investigators shall not be made available to any third party by the investigating team outside the publication procedure as described above.

The Sponsor will not quote from publications by Investigators in its scientific information and/or promotional material without full acknowledgment of the source (i.e., author and reference).

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13. APPENDICES

Appendix 1
Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

PREAMBLE

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

GENERAL PRINCIPLES

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

RISKS, BURDENS AND BENEFITS

16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

VULNERABLE GROUPS AND INDIVIDUALS

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

    All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

RESEARCH ETHICS COMMITTEES

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

PRIVACY AND CONFIDENTIALITY

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

INFORMED CONSENT

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult

family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is

available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

USE OF PLACEBO

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

POST-TRIAL PROVISIONS

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

Appendix 2
The Brighton Grading

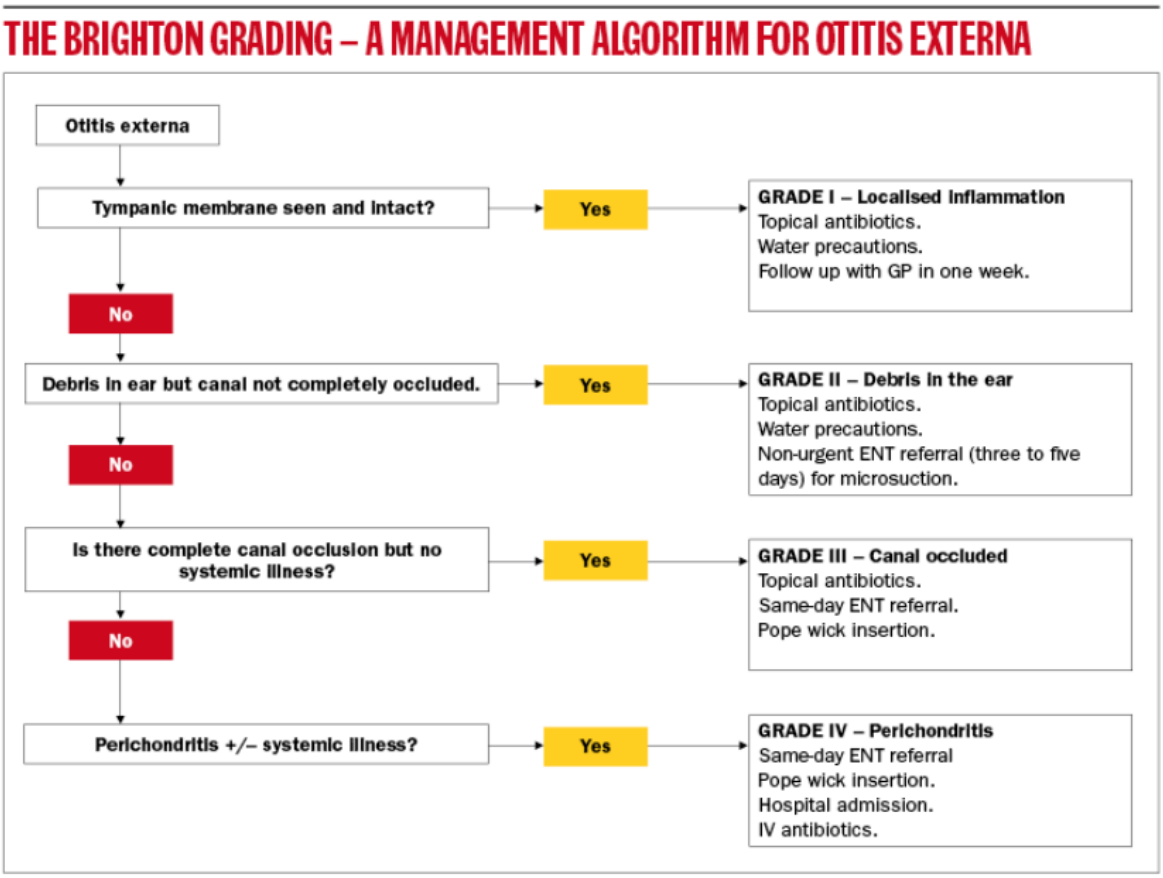
Patients present with a varying severity of signs and symptoms due to the spectrum of otitis externa disease. In order to classify disease to allow appropriate management we suggest Investigators will follow the Brighton grading scheme to classify the otitis externa:

Grade I is characterized by localized inflammation and some pain but usually no hearing loss. The tympanic membrane can be seen and underlying secondary pathology excluded.

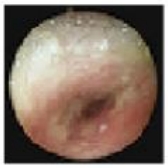
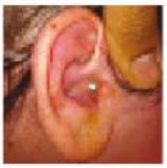
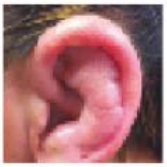
In grade II there is debris in the ear canal but it is not completely occluded. Debris may obstruct the view of the tympanic membrane.

In grade III the ear canal is edematous, occluded and often completely closed. The tympanic membrane cannot be seen.

Grade IV is characterized by edema and the tympanic membrane is obscured. There is also perichondritis with pinna cellulitis, and the patient will be systemically unwell (fever, pain, signs of sepsis).



THE BRIGHTON GRADING SCHEME

BRIGHTON GRADE	VIEW	DESCRIPTION
Grade I		● Tympanic membrane seen. ● Canal erythematous.
Grade II		● Debris in ear canal. ● Tympanic membrane often obscured by debris.
Grade III		● Oedematous canal. ● Tympanic membrane obscured by oedematous ear canal. ● No systemic illness. ● Shows Pope wick in situ.
Grade IV		● Oedematous ear canal. ● Perichondritis (pinna cellulitis). ● Systemic illness.

Appendix 3

Pain Scales – Pain assessment tools by age

A. FLACC SCALE (for children < 7 years old)

FLACC stands for face, legs, activity, crying and consolability. The FLACC pain scale was developed to help medical observers to assess the level of pain in children who are too young to cooperate verbally. It can also be used in adults who are unable to communicate.

The FLACC questionnaire will be completed before the drug administration considering:

If child is awake: observe for 1 to 5 minutes or longer. Observe legs and body uncovered. Reposition patient or observe activity. Assess body for tenseness and tone. Initiate consoling intervention if needed.

If child is asleep: observe for 5 minutes or longer. Observe body and legs uncovered. If possible, reposition the patient. Touch the body and assess for tenseness and tone.

The FLACC scale is based on observations made regarding the patient's face, the position of their legs, their actions, and whether they are calm or consolable. Zero to two points are assigned for each of these 5 areas of observation.

For each category (face, legs, activity, cry, consolability), the more appropriate statement corresponding to the child's symptoms will be checked.

FACE
□ (0)- No particular expression or smile □ (1)- Occasional grimace or frown, withdrawn, disinterested □ (2)- Frequent to constant frown, clenched jaw, quivering chin
LEGS
□ (0)- Normal position or relaxed □ (1)- Uneasy, restless, □ (2)- Kicking or legs drawn up
ACTIVITY
□ (0)- Lying quietly, normal position, moves easily □ (1)- Squirming, shifting back/forth, tense □ (2)- Arched, rigid, or jerking
CRY
□ (0)- No cry, awake or asleep □ (1)- Moans or whimpers, occasional complaint □ (2)- Crying steadily, screams or sobs, frequent complaints
CONSOLABILITY
□ (0)- Content, relaxed □ (1)- Reassured by occasional touching, hugging, or "talking to," distractible □ (2)- Difficult to console or comfort

Face

- Score 0 if the patient has a relaxed face, makes eye contact, shows interest in surroundings.
- Score 1 if the patient has a worried facial expression, with eyebrows lowered, eyes partially closed, cheeks raised, mouth pursed.
- Score 2 if the patient has deep furrows in the forehead, closed eyes, an open mouth, deep lines around nose and lips.

Legs

- Score 0 if the muscle tone and motion in the limbs are normal.
- Score 1 if patient has increased tone, rigidity, or tension; if there is intermittent flexion or extension of the limbs.
- Score 2 if patient has hypertonicity, the legs are pulled tight, there is exaggerated flexion or extension of the limbs, tremors.

Activity

- Score 0 if the patient moves easily and freely, normal activity or restrictions.
- Score 1 if the patient shifts positions, appears hesitant to move, demonstrates guarding, a tense torso, pressure on a body part.

- Score 2 if the patient is in a fixed position, rocking; demonstrates side-to-side head movement or rubbing of a body part.

Cry

- Score 0 if the patient has no cry or moan, awake or asleep.
- Score 1 if the patient has occasional moans, cries, whimpers, sighs.
- Score 2 if the patient has frequent or continuous moans, cries, grunts.

Consolability

- Score 0 if the patient is calm and does not require consoling.
- Score 1 if the patient responds to comfort by touching or talking in 30 seconds to 1 minute.
- Score 2 if the patient requires constant comforting or is inconsolable.

Whenever feasible, behavioral measurement of pain should be used in conjunction with self-report. When self-report is not possible, interpretation of pain behaviors and decisions regarding treatment of pain require careful consideration of the context in which the pain behaviors are observed.

Interpreting the Behavioral Score

Each category is scored on the 0–2 scale, which results in a total score of 0–10.

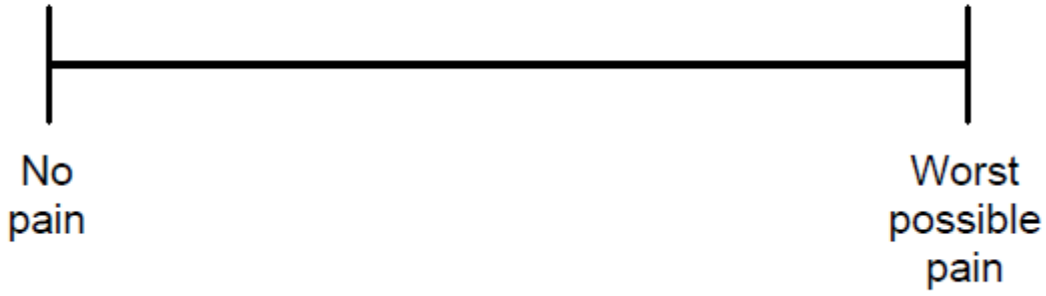
The overall score is recorded as follows:
0 = Relaxed and comfortable
1-3 = Mild discomfort
4-6 = Moderate pain
7-10 = Severe discomfort/pain

By recording the FLACC score periodically, the medical personnel can gain some sense of whether the patient's pain is increasing, decreasing or stable.

B. Wong Baker Faces Pain Scale (for children ≥7 years old to <13 years old)

The Wong Baker Faces Pain Scale combines pictures and numbers to allow pain to be rated by the user. It can be used in children over the age of 3, and in adults, but in the study will be used only by patients from 7 years old to younger than 13 years old. The faces range from a smiling face to a sad, crying face. A numerical rating is assigned to each face, of which there are 6 total.

C. Visual Analogue Scale (VAS) (for patients ≥13 years old)



Perhaps one of the most commonly used pain scales in healthcare, the numerical rating scale offers the individual in pain to rate their pain score. It is designed to be used by those over the age of 9. In the numerical scale, the user has the option to verbally rate their scale from 0 to 10 or to place a mark on a line indicating their level of pain. 0 indicates the absence of pain, while 10 represents the most intense pain possible.

The Numerical Rating Pain Scale allows the healthcare provider to rate pain as mild, moderate or severe, which can indicate a potential disability level.

Appendix 4

Microbiology specimen collection & Transport

Proper specimen collection is extremely critical for successful isolation and identification of infectious organisms.

Collection Procedure

1. Cleanse the area surrounding the infection with a swab containing cleaning solution to remove any contaminating material or flora that may have collected in the ear canal.
2. The ear canal may be irrigated if that is standard practice at the study site.
3. Remove the outer packaging outside of the clean area (always wear gloves). Take the package containing the sterile tube and swab into the clean area.
4. Gently rub against the sides of the ear canal to obtain a suitable specimen for culture. Rotate the swab in the area containing the exudate.
5. After sample collection, remove the A.C.T. ® II cap and quickly insert the swab deep into the agar.
6. Break the swab shaft evenly with the lip of the tube.
7. Replace the cap and quickly tighten to minimize exposure to air.
8. Label appropriately with labels provided in Subject Laboratory Booklet
9. Maintain A.C.T. ® II tube at room temperature.

NOTE: Debridement of the ear is permitted after otorrhea sample collection.

Specimen shipping Procedure

1. Ship on the day of collection at ambient temperature to [redacted] central laboratory.
2. If shipment is delayed, keep the specimen at room temperature and ship on the next business day. The specimen must be received at the central laboratory within 72 hours after collection.

Laboratorios SALVAT, S.A.

CiflotеX

A Phase III, Multicenter, Randomized, Double-Blind Clinical Trial to Assess the Efficacy and Safety of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% Otic Solution Compared to Ciprofloxacin 0.3% Otic Solution and to Fluocinolone acetonide 0.025% Otic Solution in the Treatment of Acute Otitis Externa (AOE).

Statistical Analysis Plan

[redacted]

SPONSOR SIGNATURE PAGE

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14/NOV/2018
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[redacted] SIGNATURE PAGE

Signatures below confirm that the review process has been completed in accordance with **SOP-GDO-WW-019**.

This document has been approved and signed electronically on the final page by the following:

	Signatory
Author	**Kathleen Heslin** Project Role: Biostatistics Lead

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LIST OF ABBREVIATIONS

AE	Adverse Event
AOE	Acute Otitis Externa
AOMT	Acute Otitis Media with tympanostomy tubes
ATC	Anatomical Therapeutic Chemical
CI	Confidence Interval
CITT	Clinical Intent-to-Treat
CM	Concomitant Medication
CMH	Cochran-Mantel-Haenszel
CPP	Clinical Per-Protocol
eCRF	Electronic Case Report Form
ECT	Effective Concomitant Therapy
EOT	End of Treatment
FLACC	Face, Legs, Activity, Cry, Consolability
ICH	International Conference on Harmonization
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimal Inhibitory Concentration
MIC_{50}	Minimal Inhibitory Concentration required to inhibit the growth of 50% of the bacteria tested
MIC_{90}	Minimal Inhibitory Concentration required to inhibit the growth of 90% of the bacteria tested
MITT	Microbiological Intent-to-Treat
MITT-PA/SA	Pathogen Positive Microbiological Intent-to-Treat
MPP	Microbiological Per-Protocol
NDA	New Drug Application
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TEOP	Time to End of Ear Pain
TOC	Test of Cure
TSSS	Total Signs/Symptoms Score
US	United States
VAS	Visual Analog Scale
WHO	World Health Organization

1 INTRODUCTION

Otitis externa is an inflammatory process that involves the external auditory canal and is usually caused by bacterial infection. The most common factor leading to infection is excessive moisture in the ear canal, which interferes with the canal's natural defenses against infection. Otitis externa is one of the most common otic conditions seen by general practitioners and ear, nose and throat specialists. It occurs in 4 of every 1,000 adults and children in the United States (US) each year. The most common causative microorganisms are Pseudomonas aeruginosa and Staphylococcus aureus.

Topical antibiotics are the first-line treatment of choice for otitis externa. Topical application enhances efficacy by bringing the antibiotic into direct contact with the infected area, avoiding the risk of adverse events (AEs) associated with systemic antibiotic therapy, and may help prevent the development of resistance to antibiotics by the pathogen.

SALVAT is currently marketing the proposed combination Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution in 10 mL multidose preparation in over 40 foreign countries for the treatment of Acute Otitis Externa (AOE). Since its 2002 launch, SALVAT has successfully marketed over 4 million units of the referenced product worldwide. There have been no marketplace recalls or field corrections to date.

SALVAT received the New Drug Application (NDA) approval on April 29, 2016 for the OTOVEL Otic solution (Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% sterile and preservative-free solution in single dose vials) for the treatment of Acute Otitis Media with tympanostomy tubes (AOMT).

This study is being conducted to support an application for approval to market Ciprofloxacin plus Fluocinolone acetonide in the US for the indication of AOE. The reference (comparator) drugs in this study, Ciprofloxacin 0.3% alone otic solution, and Fluocinolone acetonide 0.025% alone otic solution, are expected to provide a lower efficacy rate when compared with the combination.

The planned analyses identified in this statistical analysis plan (SAP) may be included in the clinical study report (CSR), regulatory submissions, or future manuscripts. Also, post-hoc exploratory analyses not necessarily identified in this SAP may be performed to further examine study data. Any post-hoc, or unplanned, exploratory analyses performed, if included, will be clearly identified as such in the final CSR.

This SAP is written in accordance with principles described in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline E9 and is designed to guide the statistical analysis of Study CIFLOT3-16IA01. This SAP is based upon the following study documents:

- Study Protocol, Version v2.0 (Amendment 1) (February 26, 2018)
- Electronic Case Report Form (eCRF), Version Final (March 15, 2018)

In the event of future amendments to the protocol, this SAP will be modified as necessary to account for changes relevant to the statistical analysis.

The purpose of the SAP is to specify the statistical analysis in more detail than stated in the clinical study protocol and to ensure that the data listings, summary tables, and figures which will be produced, and the statistical methodologies that will be applied, are complete and appropriate to assess study objectives and reporting goals.

2 STUDY OBJECTIVES

The primary objective is to demonstrate superiority of Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution relative to Ciprofloxacin 0.3% otic solution and to Fluocinolone acetonide 0.025% otic solution with respect to therapeutic cure rate (clinical + microbiological cure) at end of treatment (EOT).

Clinical + microbiological cure will be considered achieved if edema, otalgia and otorrhea are resolved with no further requirement of antimicrobial therapy and bacteriological response is Eradication or Presumed Eradication.

The principal secondary endpoint is "Time to end of ear pain" (TEOP). This time will be calculated on the basis of the patient diary entries.

Other secondary endpoints:

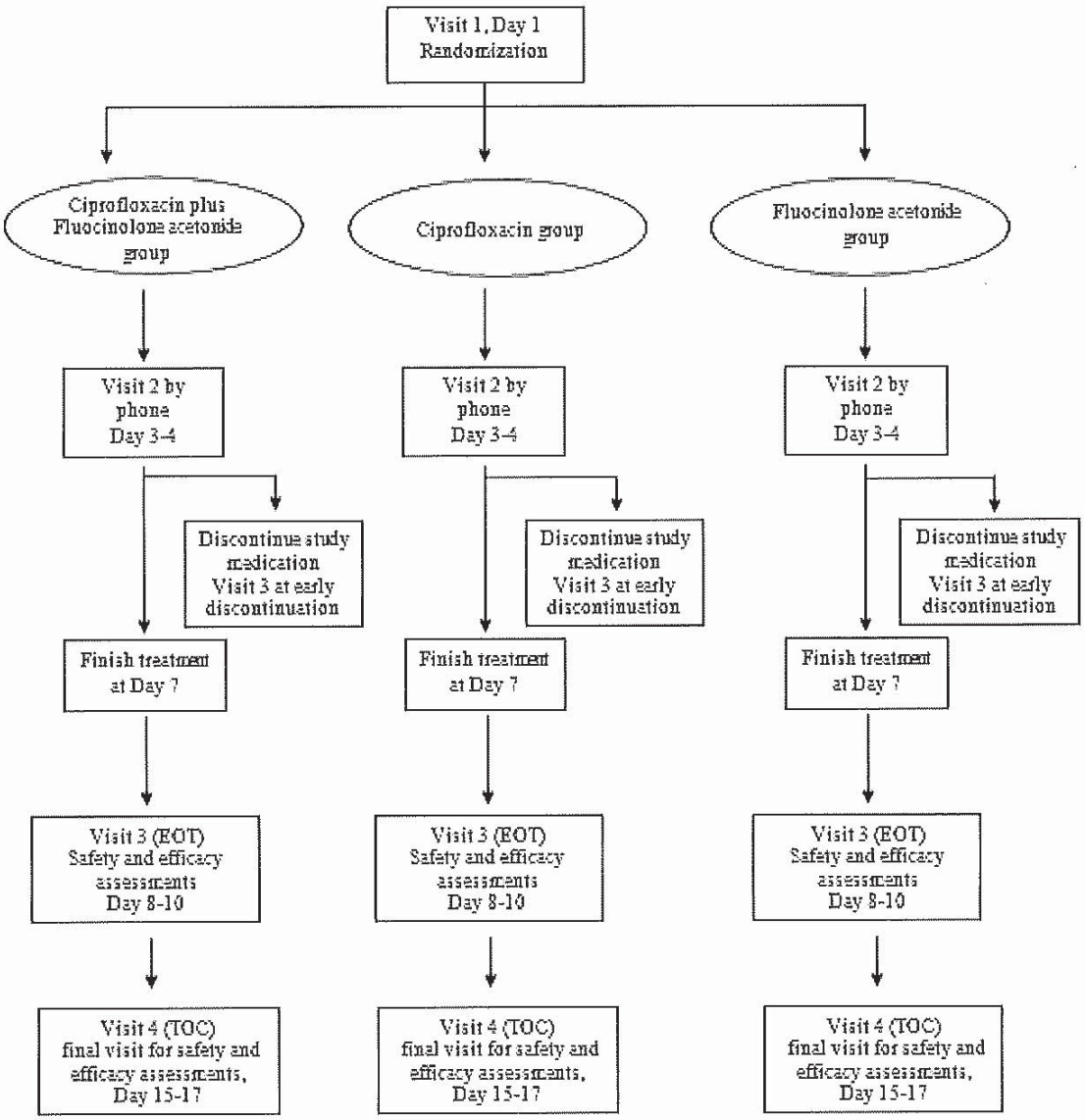
- Sustained microbiological cure
- Clinical cure at Visit 3 and Visit 4
- Microbiological cure at Visit 3 and Visit 4
- Therapeutic cure (clinical+microbiological cure) at Visit 4
- Changes in Brighton grading at Visit 3 and Visit 4
- Changes in otorrhea at Visit 3 and Visit 4
- Changes in edema at Visit 3 and Visit 4
- Changes in otalgia assessed by the investigator at Visit 3 and Visit 4
- Adverse events

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a Phase III, randomized, parallel-group, double-blinded, active-controlled, multicenter study comparing Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution with Ciprofloxacin 0.3% otic solution or Fluocinolone acetonide 0.025% otic solution in the treatment of AOE in children, adolescents, and adults. A diagram of the study design is shown in Figure 1.

Figure 1 Diagram of Study Design



A schedule of study procedures and evaluations is provided in Appendix 1.
Efficacy will be assessed by the proportion of patients with Therapeutic cure at Visit 3.

Five hundred (500) patients selected for the study will be male or female, 6 months of age and older, with uncomplicated AOE in at least 1 ear, with the aim of including 375

evaluable patients. At Visit 1 (Day 1), patients who have signed the Informed Consent Form (or had it signed by their legally authorized representative) and met the study entry criteria will be randomized in a 2:2:1 ratio to either the investigational treatment, Ciprofloxacin 0.3% plus Fluocinolone acetonide 0.025% otic solution, or the comparator treatment, Ciprofloxacin 0.3% otic solution or Fluocinolone acetonide 0.025% otic solution.

The method of administration for the investigational medication and the comparator medication will be the same in all age groups: instillation of one vial in the affected ear canal(s) twice a day (approximately every 12h, morning and evening) for 7 consecutive days. Ear wicks or sponges may be used at the Investigator's discretion.

There are no interim analyses planned for this study.

Details of the study design are given in the study protocol.

3.2 Efficacy and Safety Variables

The primary efficacy variable is therapeutic response at Visit 3. Therapeutic response is the combined overall clinical outcome and the overall microbiological outcome.

The secondary efficacy variables include:

- Time to end of ear pain
- Sustained microbiological cure
- Overall Clinical Outcome at Visits 3 and 4
- Microbiological response at Visits 1, 3, and 4
- Therapeutic response at Visit 4
- Brighton grading at Visits 1, 3, and 4
- Otorrhea at Visits 1, 3, and 4
- Otalgia at Visits 1, 3, and 4
- Edema at Visits 1, 3, and 4

The safety variables include AEs reported throughout the study, vital signs (temperature, blood pressure, and heart rate) at Visits 1, 3, and 4, and physical examination at Visit 1.

4 STATISTICAL METHODS

4.1 Data Quality Assurance

All tables, figures and data listings to be included in the report will be independently checked for consistency, integrity and statistical rigor in accordance with [redacted]'s standard operating procedures.

4.2 General Presentation Considerations

Unless otherwise indicated, 'Baseline' will be defined as the last available pre-treatment assessment. For this study, entry visit, or "Visit 1/Day 1" when screening, randomization and drug dispensing occur, will be considered as baseline. 'End of Study' will be defined as the last available post-treatment assessment. 'Treatment Day' will be calculated relative to the date of randomization (i.e. Visit 1 Day 1) using formula: Treatment Day = Assessment Date - Randomization Date + 1.

Continuous data will be summarized in terms of the mean, standard deviation (SD), median, minimum, maximum and number of observations, unless otherwise stated. The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean and median will be reported to one more decimal place than the raw data recorded in the database. The SD will be reported to two more decimal places than the raw data recorded in the database. In general, the maximum number of decimal places reported shall be four for any summary statistic.

Categorical data will be summarized in terms of the number of patients providing data at the relevant time point (n), frequency counts and percentages.

Percentages will be presented to one decimal place. Percentages will not be presented for zero counts. Percentages will be calculated using n as the denominator. If sample sizes are small, the data displays will show the percentages, but any textual report will describe frequencies only.

Changes from baseline in categorical data will be summarized using shift tables where appropriate. For continuous variables, actual values and changes from baseline will be summarized using descriptive statistics by treatment group and visit.

Unless otherwise stated, all statistical tests are two-sided and will be performed at 5% significance level. P-values less than 0.001 will be presented as "<0.001", and p-values greater than 0.999 will be presented as ">0.999". Otherwise p-values, in general, will be presented to three decimal places. Confidence intervals (CI) will be presented with the same decimal place as the corresponding point estimates.

All report outputs will be produced using SAS® version 9.3 or a later version in a secure and validated environment. Details of report outputs including programming specifications will be detailed in the supporting mock TLF shells document.

4.3 Study Patients

4.3.1 Disposition of Patients

Information on the disposition of all patients who enter the study will be provided, from screening to study completion.

The following patient data will be presented by treatment group, including overall categories:

- The number of patients screened (overall only)
- The number of patients who were screen failures (overall only)
- The number of patients randomized
- The number of patients randomized with pathogen positive culture
- The number of patients randomized with positive culture for *P. aeruginosa* and/or *S. aureus*
- The number and percent of patients who were treated/not treated
- The number of patients in the Clinical Per-Protocol (CPP) population
- The number of patients in the Microbiological Per-Protocol (MPP) population
- The number and percent who completed the study
- The number and percent of patients who withdrew from study drug

Percentages of patients will be based on the number of patients randomized as 100%. The number and percentage of patients who withdrew (early terminated) during the study will be presented by reason for study discontinuation. The number and percentage of patients who withdrew from study medication will be presented by reason for study medication discontinuation.

In addition, patient listings will be provided for patients who discontinued the study early (post-randomization) with reason for discontinuation. Screen failed patients will also be listed with reason for screen failure.

4.3.2 Protocol Deviations

Protocol deviations are defined as violations from the procedures outlined in the protocol. Major protocol deviations are defined as those deviations from the protocol likely to have an impact on the perceived efficacy and/or safety of study treatments and leading to the exclusion from the CPP population and the MPP population. The impact of major protocol deviations on the efficacy and/or safety results will be investigated by assessing the robustness of the study results and conclusions to the choice of analysis population, both including and excluding data potentially affected by major protocol deviations.

Major protocol deviations and any action to be taken regarding the exclusion of patients or affected data from specific analyses are defined in the IWRS Specifications.

Once the database has been completed and considered as "clean," a data blind review will be conducted before the database lock in order to identify all major protocol violations and assign patients into each of the analysis sets as defined in Section 4.4.

Patients with protocol deviations will be presented in the data listings. Protocol violations will be tabulated by treatment group and violation.

4.4 Analysis Populations

There will be 6 populations defined for this study: Safety, Clinical intent-to-treat (CITT), Microbiological intent-to-treat (MITT), MITT-PA/SA, CPP, and MPP.

The **Safety population** will include all patients who received any study medication.

The **CITT population** will include all patients who were randomized.

The **MITT population** will include all CITT patients whose Visit 1 microbiological culture yields 1 or more pathogens, as defined in Section 4.7.2.

The **MITT-PA/SA population** will include the pathogen positive subset of the CITT population which includes all patients who received study medication and had culture positive for *P. aeruginosa* and/or *S. aureus* at baseline in the evaluable ear.

The **CPP population** will include all CITT patients who:

- Satisfied all inclusion and exclusion criteria;
- Had analysis results from Visit 1 otorrhea sample;
- Did not receive any prohibited concomitant medications. Note if a patient receives prohibited medication on or after receiving rescue medication and does not have any other major protocol deviations, the patient will be included in the CPP;
- Did not have any other major protocol violations;
- Completed Visit 3 and Visit 4 (unless the patient was deemed a clinical failure at an earlier visit than Visit 4);
- Had compliance rates between 80% and 120% as defined in Section 4.6 (patients who are deemed Clinical Failures are to be included if they had compliance rates between 80% and 120% during the first 3 days of study treatment); and
- Received at least 4 doses of study medication during the first 72 hours.

The **MPP population** will include all CPP patients whose Visit 1 microbiological culture yields 1 or more pathogens and who had microbiological results (when patient has material to culture) from Visit 3 and/or Visit 4 unless the patient was deemed a clinical failure at an earlier visit than Visit 4.

For the CITT, MITT and MITT-PA/SA populations, the treatment group of a patient will be determined by the treatment group to which the patient was randomized.

For the Safety, CPP and MPP populations, the treatment group of a patient will be determined by the treatment the patient received, not necessarily the group to which he or she was randomized. If a patient receives only Fluocinolone acetonide for the duration of the study, they will be summarized in the Fluocinolone acetonide treatment group. If a patient receives only Ciprofloxacin for the duration of the study, they will be summarized in the Ciprofloxacin treatment group. Otherwise, patients will be summarized in the Ciprofloxacin plus Fluocinolone acetonide treatment group. The Safety population will be used for all safety analyses.

Unless otherwise noted, all efficacy analyses and summaries will be based on the MITT-PA/SA population, which will be the primary population for efficacy analysis. Efficacy analyses will also be conducted on the CITT, MITT, CPP and MPP populations. Safety summaries will be based on the Safety population.

4.5 Demographic and Other Baseline Characteristics

4.5.1 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for all study populations by treatment group. Summaries will include descriptive statistics for continuous and categorical measures. Patient characteristics to be presented include:

- Age
- Age Group
  - 6 months to <18 years, ≥18 years
  - 6 months to <7 years, 7 years to <13, ≥13 years
  - 6 months to <12 years, 12 years to <18 years, ≥18 years
- Gender
- Race
- Ethnicity
- Baseline Pathogen
- Ear wick placement

Age will be calculated as the time in years between a patient's birth date and the date of informed consent.

Patient listings of demographic and baseline characteristics will be provided.

4.5.2 Medical History

General medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.0. A summary of medical history by system organ class

(SOC) and preferred term (PT) will be presented as frequencies and percentages for each treatment group and overall.

Medical history will also be listed. The listing will be sorted by treatment group, patient identification number, SOC, PT, and reported term.

A separate listing will be presented for AOE history.

4.5.3 Prior and Concomitant Medications

Prior and concomitant medications (CMs) will be coded using the World Health Organization (WHO) Drug dictionary version Q4 2016 (B2 Enhanced, Dec 1st, 2016). Medications and all information collected will be reported via the eCRF.

For any recorded medication other than study medications, medication start and stop dates will be compared to the date of first dose of study medication to allow medications to be classified as either prior only, both prior and concomitant, or concomitant only.

Medications that start and stop prior to the first dose of study medication will be classified as prior only. If a medication starts before the first dose of study medication and stops on or after the first dose of study medication then the medication will be classified as both prior and concomitant. Medications will be classified as concomitant only if they started on or after the first dose of study medication.

Partial date rules for flagging prior and concomitant medications will follow the rules detailed in the AEs. In case of missing dates, a medication will be considered as concomitant only.

A summary of CMs will be presented as frequencies and percentages by Anatomical Therapeutic Chemical (ATC) Class 1 and preferred term for each treatment group and overall.

CMs will be also tabulated by treatment group and type of pain treatment. Each CM will be categorized into one of 5 categories:

1. Acetaminophen (Paracetamol)
2. Ibuprofen
3. Medication Containing Codeine
4. Other Pain Treatment
5. Other Medication Than Pain Treatment

The proportion of patients that took each type of pain treatment will be compared between treatment groups using Pearson's Chi Squared tests for independence. If the concomitant medications analysis by type of pain treatment shows difference between treatment groups, subgroup analyses by type of pain treatment will performed on efficacy results as post-hoc

analyses. If less than 80% of the expected counts are less than 5 or at least one category has no patients in a treatment group, then Fisher's exact test will be used.

A listing of CMs and prior medications will also be provided.

CMs will be considered rescue medication if they meet both of the following criteria:

- Any otic or systemic treatment administered for signs/symptoms of otitis externa of the evaluable ear (Note – topical antibiotics applied in the non-evaluable ear are NOT considered to be rescue medications)
- Started after Visit 1 but before End-of-study visit (Note – antibiotics given after End-of-study visit are NOT considered rescue medications)

The number of patients taking rescue medications will be tabulated by treatment group. A listing will also be provided.

Effective concomitant therapy (ECT) will be defined as any antibacterial or antiseptic treatment administered for reasons not associated with acute otitis externa.

The number of patients taking ECTs will be tabulated by treatment group.

Type of pain treatment and medications that qualify as rescue medications or ECTs will be determined during the blinded data review before database lock.

4.6 Treatment Compliance

Study medication compliance will be assessed during the first 3 days of the treatment period, during the treatment period (first 7 days), and during the overall study.

Treatment Compliance is calculated as:

$$\frac{\text{Number of doses taken}}{\text{Expected number of doses taken}} \times 100\%$$

The number of doses taken is the number of doses the patients actually took during a given period.

The number of expected doses taken is the number of doses the patient was expected to take during that period. It is calculated based on the patient's duration of participation in the study. If a patient completes treatment, 6 doses are expected in the first 3 days of treatment. For the treatment period, 14 doses are expected. If a subject does not complete treatment in the treatment period (first 7 days), then the expected number of doses for the overall study will be twice the number of days that the patient took study medication. If a patient discontinues early, the expected number of doses is based on the time of early discontinuation.

Percent compliance will be summarized by treatment group. Compliance will also be summarized by treatment group and age group (6 months to <18 years and ≥18 years).

Compliance will be listed for each patient.

Patients will be considered "compliant" if their percent compliance is between 80% and 120%. The proportion of patients who were compliant and non-compliant during the treatment period and during the first 3 days of the treatment period will be tabulated by treatment group.

4.7 Efficacy Evaluation

4.7.1 Hypothesis

This study is designed to test for superiority. The primary analyses will compare the combination treatment group with each component alone using the MITT-PA/SA population. The null hypotheses will be that there is no difference between Ciprofloxacin plus Fluocinolone acetonide and Ciprofloxacin alone and that there is no difference between Ciprofloxacin plus Fluocinolone acetonide and Fluocinolone acetonide alone. The alternative hypothesis will be that there is a difference. Symbolically, this is expressed as follows:

$$Ho: p_{\text{Ciprofloxaci plus Fluocinolone acetonide}} = p_{\text{Ciprofloxacin}}$$
$$Ha: p_{\text{Ciprofloxaci plus Fluocinolone acetonide}} \neq p_{\text{Ciprofloxacin}}$$

and

$$Ho: p_{\text{Ciprofloxaci plus Fluocinolone acetonide}} = p_{\text{Fluocinolone acetonide}}$$
$$Ha: p_{\text{Ciprofloxaci plus Fluocinolone acetonide}} \neq p_{\text{Fluocinolone acetonide}}$$

where p is the proportion of patients with therapeutic cure.

A two-sided chi-square (or χ^2) test with α=0.05 will be used to test this hypothesis and conduct the between-treatment comparison. This approach will also be used for other (secondary) efficacy endpoints as described in Section 2.

The principal secondary analysis will also compare the combination treatment group with each component alone. The null hypothesis is that there is no difference in time to end of pain between the combination and the components along. The alternative hypothesis is that there is difference in time to end of pain between the combination and the components alone.

The comparison will be made using the log-rank test stratified on age (6 months to <18 years versus ≥18 years) with α=0.05.

Only assessments in the evaluable ear will be used for efficacy analyses.

4.7.1.1 Handling of Dropouts or Missing Data

Patients with missing efficacy data or indeterminate outcomes will be considered as treatment failures for efficacy analyses.

For the primary endpoint, patients who discontinued for lack of efficacy or rescue medication use will be considered as treatment failure.

Patients who take rescue medication, discontinue or are lost to follow up, and for whom the pain persists at the time of the last observation, will be censored at maximum length (Day 17) in those cases when the diary information is not available.

To explore the effect of the handling of the missing data and assess the robustness of the efficacy results, a complete case analysis will be carried out (i.e. all efficacy analyses will be repeated on actual values i.e. without imputation of the missing data) as sensitivity analysis on the MITT population.

4.7.1.2 Multiple Comparisons/Multiplicity

No adjustment for multiple comparisons is planned for the efficacy endpoints being monitored.

Safety results, including the summaries of AEs and changes in vital signs data, will be interpreted on clinical grounds. No formal statistical hypothesis testing will be performed.

4.7.1.3 Interim Analyses

No interim analyses are planned.

4.7.1.4 Examination of Subgroups

To assess the effects of various demographic and baseline characteristics on treatment outcome, subgroup analyses for the primary endpoint, therapeutic outcome at Visit 3, and the principal secondary endpoint, TEOP, will be performed based on the following subgroups:

- Age Group

  - 6 months to <18 years, ≥18 years
  - 6 months to <7 years, 7 years to <13, ≥13 years
  - 6 months to <12 years, 12 years to <18 years, ≥ 18 years
- Ear wick placement: yes, no

Descriptive summaries and analyses will be provided for each endpoint by treatment group and subgroup for the MITT and MITT-PA/SA populations.

For the primary endpoint, the Cochran-Mantel-Haenszel (CMH) test will be used, stratifying on subgroup. Differences in response rates with the corresponding 95% CIs will be reported for each subgroup level. The proportionsfor each subgroup level will be shown with the p-value from the Breslow Day test for homogeneity of the odds ratios.

For the principal secondary endpoint, each age subgroup comparison will be made using a Cox proportional hazards model with factors for treatment group, age group, and the age group-by-treatment interaction. For the ear wick placement subgroup, the comparison will be made using a Cox proportional hazards model stratified on age (6 months to <18 years versus ≥18 years) with factors for treatment group, ear wick placement, and the ear wick placement-by-treatment interaction. The estimated hazard ratios together with their associated 95% CI and two-sided p-value will be shown for each subgroup.

Subgroup analyses may also be performed based on concomitant medication type if necessary as outlined in Section 4.5.3.

4.7.2 Primary Efficacy Variable – *Therapeutic Response*

The primary endpoint for the assessment of efficacy is therapeutic response at Visit 3 in the MITT-PA/SA population. Efficacy analyses of the primary endpoint will also be conducted on the MITT, MPP CITT and CPP populations.

Therapeutic cure is the combined clinical and microbiological cure. Clinical and microbiological cure will be considered achieved if edema, otalgia, and otorrhea are resolved with no further requirement of antimicrobiological therapy and bacteriological response is Eradication or Presumed Eradication.

Overall Clinical Outcome is based on the Total Signs/Symptoms Scale (TSSS), which is calculated by the sum of otalgia score + edema score + otorrhea score. Clinical cure is a TSSS equal to 0.

Otalgia is the level of pain in the ear and is measured on a scale of 0 to 3. It will be assessed as:

- Severe (3) if it interferes with activities of daily living;
- Moderate (2) if it causes discomfort but does not interfere with activities of daily living;
- Mild (1) if there is awareness of pain but not much discomfort

- Absent (0) if there is total absence of pain.

Edema is the level of swelling in the ear and is measured on a scale of 0 to 3. It will be assessed as:

- Severe (3) if the tympanic membrane is not visible because of swelling;
- Moderate (2) if the tympanic membrane is partially visible;
- Mild (1) if there is some swelling but the tympanic membrane is fully visible;
- Absent (0) if there is no visible swelling.

Otorrhea is the discharge from the ear and is measured on a scale of 0 to 3. It will be assessed as:

- Severe (3),
- Moderate (2),
- Mild (1),
- Absent (0).

Bacteriological response will be categorized for each patient based on the microbiological culture results. Positive results will be categorized into three groups: target pathogen, non-target pathogen, and non-pathogen. For analysis purposes, patients with a target pathogen or non-target pathogen will be considered to be culture positive for a pathogen. Negative results and non-pathogen positive results will be considered to be culture negative for a pathogen. For any patient with a culture positive for a pathogen at Visit 1, bacteriologic response at Visits 3 will be classified as:

- Eradication if the culture does not show growth of any pathogen;
- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Improvement or Clinical Cure as defined above;
- Persistence if any pathogen cultured at Visit 1 is still present;
- Presumed Persistence if the Overall Clinical Outcome is Clinical Failure but no culture result is available from Visit 3;
- Superinfection if a pathogen not present at Visit 1 is now present (presence of a nonpathogenic organism will not be considered Superinfection); or
- Indeterminate if none of the above definitions are met and the bacteriologic response cannot be determined.

For any patient with a culture positive for a pathogen at Visit 1, bacteriologic response at Visits 4 will be classified as:

- Eradication if the culture does not show growth of any pathogen;
- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Improvement or Clinical Cure as above;
- Persistence if any pathogen cultured at Visit 1 is still present;
- Presumed Persistence if the Overall Clinical Outcome is Clinical Failure but no culture result is available from Visit 4;

- Recurrence if there is reappearance of the pathogen eradicated or presumably eradicated at Visit 3;
- Superinfection if a pathogen not present at Visit 1 and Visit 3 is now present (presence of a nonpathogenic organism will not be considered Superinfection);
- Reinfection if there is isolation of a new pathogen different from the one eradicated or presumably eradicated at Visit 3; or
- Indeterminate if none of the above definitions are met and the bacteriologic response cannot be determined.

For any patient with a negative culture or a culture positive for a non-pathogen at Visit 1, bacteriologic response at Visits 3 will be classified as:

- Presumed Eradication if the culture does not show growth of any pathogen or if there is no material to culture and the Overall Clinical Outcome is Clinical Improvement or Clinical Cure as defined above;
- Presumed Persistence if the Overall Clinical Outcome is Clinical Failure but no culture result is available from Visit 3;
- Superinfection if a pathogen not present at Visit 1 is now present; or
- Indeterminate if none of the above definitions are met and the bacteriologic response cannot be determined.

For any patient with a negative culture or a culture positive for a non-pathogen at Visit 1, bacteriologic response at Visits 4 will be classified as:

- Presumed Eradication if the culture does not show growth of any pathogen or if there is no material to culture and the Overall Clinical Outcome is Clinical Improvement or Clinical Cure as defined above;
- Presumed Persistence if the Overall Clinical Outcome is Clinical Failure but no culture result is available from Visit 4;
- Recurrence if there is no material to culture, the Overall Clinical Outcome is Clinical Failure, and the Overall Clinical Outcome at Visit 3 is Clinical Cure;
- Superinfection if a pathogen not present at Visit 1 and Visit 3 is now present;
- Indeterminate if none of the above definitions are met and the bacteriologic response cannot be determined.

The above rules will be used for microbiological outcome summaries for the patients in the CITT and CPP populations that have no pathogen at Visit 1, since microbiological response cannot be calculated without any pathogen at Visit 1.

If Overall Clinical Outcome at Visit 3 is Clinical Failure (whether or not the patient discontinues prematurely) and no bacterial culture is performed at Visit 3, the bacteriologic response at Visit 3 will be Presumed Persistence. If Overall Clinical Outcome at Visit 4 is Clinical Failure and no bacterial culture is performed at Visit 4, the bacteriologic response at Visit 4 will be Presumed Persistence.

Therapeutic cure: TSSS (otalgia+edema+otorrhea) of 0 and bacteriological response of eradication or presumed eradication

Therapeutic failure:

- Positive culture for pathogens (independent of TSSS) or
- Negative culture or presumed eradication with TSSS>0

If a patient took rescue medication prior to Visit 3, the response will be considered a failure at Visit 3. Similarly, if patient took rescue medication prior to Visit 4, the response will be considered a failure at Visit 4.

Therapeutic response will be summarized by treatment group and visit in terms of proportion of cures and failures at each visit.

A Pearson's Chi Squared test for independence will be used to test the hypotheses states in Section 4.7.1 that there is no difference between the combined treatment and each individual component at Visits 3 and 4. If less than 80% of the expected counts are less than 5 or at least one category has no patients in a treatment group, then Fisher's exact test will be used. Differences in response rates with the corresponding 95% CIs will be reported. Sensitivity analyses to investigate the impact of the choice of method for handling missing data and the choice of analysis population will be performed as described in Section 4.7.1.3.

The homogeneity of the treatment effect for a number of important subgroups will be investigated as described in Section 4.7.1.4.

A by-patient listing of the therapeutic response will be provided.

4.7.3 Secondary Efficacy Variables

Time to End of Ear Pain

Ear pain will be defined as ending on the first day (morning or evening) on which there is no use of analgesics, the diary pain score is zero and the score remains at zero for all subsequent visits until the end of the study. This clinical efficacy variable from the patient's perspective is the assessment of pain as recorded in the patient diary.

Throughout the study, patient or caregiver should record ear pain severity twice daily (morning and evening prior to dosing) in the diary using a proper pain scale according to patient's age until the end of study:

- Patients younger than 7 years old will use the Face, Legs, Activity, Cry, Consolability (FLACC) scale (it will be assessed by parents or caregivers).
- Patients from 7 years old to 13 years old will record the pain using the Wong Baker Faces Pain Scale.

- And patients 13 years old and older will record the pain using a Visual Analog Scale (VAS) scale.

The patient diary information together with investigator assessment will be used to ascertain the time point (morning or evening) and date at which the ear pain in the evaluable ear ended without use of analgesics.

Time to end of ear pain is defined as the interval (in days) between first dose of study medication and the first day (morning or evening) on which the ear pain in the evaluable ear ended (pain was absent and remains absent until the end of the study without using analgesic). This time will be calculated on the basis of the patient diary entries using a proper pain scale according to patient's age.

If a patient experiences an end to ear pain in the evaluable ear, then the TEOP will be recorded as the length of time between the first dose of study treatment and the time point entered in the field:

TEOP= date and time point of end of ear pain – date and time point of first dose of study medication

- If ear pain in the evaluable ear continued to the end of the study, the TEOP will be recorded as the length of time between the time of the first dose of study medication and the last time point when a pain measurement was recorded. For statistical purposes, such observations will be considered "censored".

TEOP= date and time point of last ear pain measurement – date and time of first dose of study medication

- In patients with treatment failure (including rescue medication with otic or systemic antibiotics), the TEOP will be censored at the maximum value (17 days).
- Patients who discontinue or are lost to follow up, and for whom the pain persisted at the time of the last observation, will be censored at maximum length (17 days) in those cases when the diary information is not available.

The null hypothesis is that there is no difference in time to end of pain between the combination and the components along. The alternative hypothesis is that there is difference in time to end of pain between the combination and the components alone. The comparison will be made using the log-rank test stratified on age (6 months to <18 years versus ≥18 years). The p-value will be shown for each comparison between the combination and individual component. The median TEOP will be calculated using the Kaplan Meier method and will be presented with its 95% CI for each treatment group.

Kaplan Meier plots will also be shown by treatment group and strata.

Sensitivity analysis of "time to end of pain" will be performed by using the full information as assessed from patient diaries without regard for the patient's need for analgesic therapy.

Sustained Microbiological Outcome

For any patient with a culture positive for a pathogen at Visit 1, bacteriological response at Visits 3 will be classified as Eradication, Presumed Eradication, Persistence, Presumed Persistence, Superinfection or Indeterminate and bacteriological response at Visit 4 will be classified as Eradication, Presumed Eradication, Persistence, Presumed Persistence, Recurrence, Superinfection, Reinfection or Indeterminate, as defined in Section 4.7.2.

The number and percentage of patients with each response will be summarized by visit and treatment group. The proportion of patients with sustained microbiological cure i.e. with a response of Eradication or Presumed Eradication at both Visit 3 and Visit 4 will be compared between the treatment groups by using the same chi-squared test as the primary efficacy endpoint.

If a patient took rescue medication prior to Visit 4, he/she will be considered to not have achieved sustained microbiological cure.

A by-patient listing of sustained microbiological outcome will be provided.

Overall Clinical Outcome

Overall Clinical Outcome is based on the TSSS, which is calculated by the sum of otalgia score + edema score + otorrhea score. Patients will be allocated to one of the following categories for Overall Clinical Outcome:

1. Clinical Cure: TSSS is 0
2. Clinical Improvement: TSSS is different than 0 but lower than the previous visit
3. Clinical Failure: TSSS does not meet the definitions of Clinical Cure or Clinical Improvement.
4. Indeterminate: Discontinued (for reasons other than Clinical Failure) or lost to follow-up

Overall clinical outcome will be summarized by treatment group and visit in terms of proportion in each category at each visit.

Responses will be categorized into two groups for analysis. Clinical improvement, Clinical Failure, and indeterminate will be collapsed into a single category: Clinical Failure. Overall clinical outcome will be analyzed using the same method as the primary efficacy endpoint. A Pearson's Chi Squared test for independence will be used to test that there is no difference between the combined treatment and each individual component at Visits 3 and 4.

If a patient took rescue medication prior to Visit 3, the response will be considered a clinical failure at Visit 3. Similarly, if a patient took rescue medication prior to Visit 4, the response will be considered a clinical failure at Visit 4.

A by-patient listing of overall clinical outcome will be provided.

Microbiological Outcome

Microbiological Outcome is described in Section 4.7.2.

Microbiological outcome will be summarized by treatment group and visit in terms of proportion in each category at each visit.

If a patient took rescue medication prior to Visit 3, the response will be considered a failure at Visit 3. Similarly, if a patient took rescue medication prior to Visit 4, the response will be considered a failure at Visit 4.

Responses will be summarized in terms of a 3-level response. Responses of Eradication or Presumed Eradication will be categorized at favorable. Responses of Persistence, Presumed Persistence, Superinfection, Reinfection, and Recurrence will be categorized at unfavorable. Indeterminate responses will be categorized as indeterminate. The proportion of patients with a favorable response at Visit 3 and Visit 4 will be compared between the Ciprofloxacin plus Fluocinolone acetonide and the Fluocinolone acetonide and between Ciprofloxacin and Fluocinolone acetonide treatment groups by using the same chi-squared test as the primary efficacy endpoint.

Microbiological outcome will also be presented by pathogen (*P. aeruginosa*, *S. aureus*, *T. otitidis*, and Other). The per-pathogen microbiological outcome will be derived for each pathogen identified at baseline based on the following classification:

- Eradication: elimination of pathogen identified in Visit 1 culture evidenced by the absence of that pathogen in a subsequent culture
- Presumed Eradication: elimination of pathogen identified in Visit 1 culture evidenced by the absence of any purulent discharge from which to obtain a subsequent culture in patients whose signs and/or symptoms of infection has improved or resolved
- Persistence: continued presence of pathogen identified in Visit 1 culture evidenced by the isolation of that pathogen in a subsequent culture
- Presumed Persistence: continued presumed presence of pathogen identified in Visit 1 culture when; the isolation of that pathogen in a subsequent culture was not performed, or a culture result was not available, in a patient with persistent or worsening signs and/or symptoms of infection
- Recurrence: if there is a reappearance of a pathogen (originally isolated at Visit 1) at Visit 4 eradicated or presumably eradicated at Visit 3

- Indeterminate: if none of the above definitions are met and the outcome cannot be determined

Note that new pathogens identified after Visit 1 do not have a per-pathogen outcome, the presence of a new pathogen after Visit 1 will be included in the overall microbiological response only.

A by-patient listing of microbiological outcome will be provided.

Brighton Grading

Brighton grading will be assessed at Visits 1, 3 and 4. For consistency, the same individual should perform the assessments at all 3 visits, if possible. Brighton grading will be assessed as:

- Grade 0: Normal
- Grade I: Tympanic membrane seen. Canal erythematous
- Grade II: Debris in ear canal. Tympanic membrane often obscured by debris.
- Grade III: Edematous ear canal. Tympanic membrane obscured by edematous ear canal. No systemic illness.
- Grade IV: Edematous ear canal. Perichondritis (pinna cellulitis). Systemic illness.

The number and percentage of patients with each grade will be summarized by visit and treatment group.

Grades will also be summarizes in terms of a 3-level and a 2-level response. For the 3-level response, the proportion of patients with a Brighton grading of "0" will be considered as Resolved. It will be considered as "Improved" at Visit 3 and Visit 4 if the grade is lower than the previous visit. Brighton grading will be considered as "Not Improved" otherwise. Furthermore if Brighton grading is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If it is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

If a patient took rescue medication prior to Visit 3, the response will be considered not resolved at Visit 3. Similarly, if a patient took rescue medication prior to Visit 4, the response will be considered not resolved at Visit 4.

For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using the same chi-squared test as the primary efficacy endpoint.

A by-patient listing of Brighton grading data will be provided.

Otalgia

Otalgia will be assessed by the Investigator at Visits 1, 3, and 4. Otalgia will be assessed as Severe, Moderate, Mild, or Absent as defined in Section 4.7.2.

The number and percentage of patients with each outcome (Severe, Moderate, Mild or Absent) will be summarized by visit and treatment group.

Assessments will also be summarized in terms of a 3-level and a 2-level response. For the 3-level response, pain will be considered resolved at Visits 3 and 4 if the pain is assessed as Absent. Pain will be considered as "Improved" at Visit 3 and Visit 4 if pain is assessed by the investigator as "Mild" and was assessed as "Severe" or "Moderate" at Visit 1 (Day 1) or if pain was assessed by the investigator as "Moderate" at Visit 3 or Visit 4 and was assessed as "Severe" at Visit 1. Pain will be considered as "Not Improved" otherwise. Furthermore if pain is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If pain is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

If a patient took rescue medication prior to Visit 3, the response will be considered not resolved at Visit 3. Similarly, if a patient took rescue medication prior to Visit 4, the response will be considered not resolved at Visit 4.

For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using the same Chi-squared test as the primary efficacy endpoint.

A by-patient listing of otalgia data will be provided.

Edema

Edema will be assessed by the Investigator at Visits 1, 3 and 4. Edema will be assessed as Severe, Moderate, Mild, or Absent as defined in Section 4.7.2.

The number and percentage of patients with each outcome (Severe, Moderate, Mild or Absent) will be summarized by visit and treatment group.

Assessments will also be summarized in terms of a 3-level and a 2-level response. For the 3-level response, edema will be considered resolved at Visits 3 and 4 if the eardrum edema is assessed as Absent. Edema will be considered as "Improved" at Visit 3 and Visit 4 if edema is assessed by the investigator as "Mild" and was assessed as "Severe" or "Moderate" at Visit 1 (Day 1) or if edema was assessed by the investigator as "Moderate" at Visit 3 or Visit 4 and was assessed as "Severe" at Visit 1. Edema will be considered as "Not Improved" otherwise. Furthermore, if edema is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If edema is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

If a patient took rescue medication prior to Visit 3, the response will be considered not resolved at Visit 3. Similarly, if a patient took rescue medication prior to Visit 4, the response will be considered not resolved at Visit 4.

For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using the same Chi-squared test as the primary efficacy endpoint.

A by-patient listing of edema data will be provided.

Otorrhea

Otorrhea will be assessed by the Investigator at Visits 1, 3 and 4. Otorrhea will be assessed as Severe, Moderate, Mild, or Absent as defined in Section 4.7.2.

The number and percentage of patients with each outcome (Severe, Moderate, Mild or Absent) will be summarized by visit and treatment group.

Assessments will also be summarized in terms of a 3-level and a 2-level response. For the 3-level response, otorrhea will be considered resolved at Visits 3 and 4 if the otorrhea is assessed as Absent. Otorrhea will be considered as "Improved" at Visit 3 and Visit 4 if otorrhea is assessed by the investigator as "Mild" and was assessed as "Severe" or "Moderate" at Visit 1 (Day 1) or if otorrhea was assessed by the investigator as "Moderate" at Visit 3 or Visit 4 and was assessed as "Severe" at Visit 1. Otorrhea will be considered as "Not Improved" otherwise. Furthermore if otorrhea is considered "Not Improved" or "Improved" in the 3-level response then it will be identified as "Not Resolved" in the 2-level response. If otorrhea is considered "Resolved" in the 3-level response then it will also be considered "Resolved" in the 2-level response.

If a patient took rescue medication prior to Visit 3, the response will be considered not resolved at Visit 3. Similarly, if a patient took rescue medication prior to Visit 4, the response will be considered not resolved at Visit 4.

For Visits 3 and 4, the proportion of patients with a response of Resolved and Not Resolved will be compared between the treatment groups by using the same Chi-squared test as the primary efficacy endpoint.

A by-patient listing of otorrhea data will be provided.

Ear Pain Scales

Ear pain scales will be assessed by the patient twice a day in the patient's Diary Card to calculate the TEOP. The ear pain scales assessed at Visit 3 and Visit 4 will be categorized as follows:

- Disappeared if there is no ear pain
- Improved if the response is better than the response at Visit 1
- No change if the response is the same as the response at Visit 1
- Worsened if the response is worse than the response at Visit 1
- Missing if there is no response

The number and percentage of patients with each outcome will be summarized by visit and treatment group for each pain scale and for all pain scaled combined. The proportion of patients with the responses will be compared between the treatment groups by using the same Chi-squared test as the primary efficacy endpoint.

4.8 Safety Evaluation

All safety analyses will be based on the Safety population as defined in Section 4.4.

4.8.1 Extent of Exposure

Exposure as measured by duration of treatment will be summarized by treatment group. Extent of exposure will be calculated for the treatment period.

Days of exposure will be calculated for each patient: (date of last dose of treatment drug – date of first dose + 1).

The duration of study participation will be calculated as (the latest of (last visit/follow-up date or study termination date) – randomization date + 1.

Exposure data will be listed.

4.8.2 Adverse Events

Adverse events will be coded using the MedDRA Version 20.0. Adverse events will be tabulated by body system, preferred term, and treatment group.

Treatment–emergent adverse events (TEAEs) will be tabulated for the following subsets:

1. All TEAEs;
2. Related TEAEs;
3. Serious adverse events (SAEs);
4. Related Serious TEAEs;
5. Adverse events leading to study discontinuation;
6. Adverse events leading to discontinuation of study medication;
7. Adverse events resulting in death;

Tabulations of AEs by body system, preferred term, treatment group, and severity, and by body system, preferred term, treatment group, and relationship to study medication will also be provided.

The data will be displayed as number of subjects experiencing the AE, percentage of subjects, and number of AEs. All AE summaries will provide the number of patients reporting at least 1 AE and the total number of events reported.

General Rules for Adverse Events

1. An AE will be considered as treatment-emergent if it begins on or after the first study drug dosing or that worsens in severity after at least 1 dose of study drug has been administered. In case of insufficient information to determine if the event occurred before, during or after study drug dosing, the AE will be considered as treatment-emergent.

   The imputation method for the handling of missing or partial dates will be as follows:
   Start dates

   - If the day is missing, but month and year are present and the same as the month and year of the first dose of study drug, then day will be set to the same day as the start of study drug. Otherwise, this will be imputed as the first day of the month.
   - If the month is missing, but year is present and the same as the year of the first dose of study drug, then month will be set to the same month as the start of study drug. Otherwise, this will be imputed as January.
   - If the year is missing, the event will be considered as treatment emergent and the start date will be imputed as the date of first treatment.

   Stop dates

   - If the day is missing, this will be imputed as the last day of the month, or the last day of participation in the study if the AE end month is the same as the last date of participation in the study.
   - If the month is missing, this will be imputed as December, or the last month of participation in the study if the AE end year is the same as the last date of participation in the study
   - If the year is missing, the AE will be considered as ongoing and no imputation of the date will occur.
2. Patients will be classified as having withdrawn from the study due to an AE if the patient had a study drug action taken recorded as "permanently discontinued" on the Adverse Events page of the eCRF or patient had the study completion item recorded as 'No' with 'Adverse Event' as the primary reason for discontinuation on the study completion page of the eCRF.

3. AEs that occur before the first dose of study treatment will not be reported/summarized but will be listed as non-TEAE.
4. If a patient experiences more than one AE in a particular SOC, they will only be included once in the count for the SOC but will appear in the count for each appropriate PT within the SOC (unless it is the same PT).
5. AEs related to study drug tables will include only those AEs with a relationship to study drug of 'related', 'probable', 'possible', or if there is a missing relationship on the AE page of the eCRF.
6. For AE causality, when there is more than one AE of the same PT, the most related will be considered in the summary tables by causality.
7. For AE severity, when there is more than one AE of the same PT, the worst severity will be considered in the summary tables by severity.
8. If severity is missing, the event will be considered serious.

The AEs will be ordered by decreasing frequency, then alphabetically, for total patients for each SOC and PT within an SOC.

TEAEs will also be summarized by age group.

An overall summary of AEs will be provided by treatment group and overall. The summary will include incidences for the following:

- Any TEAE
- Any Treatment Related TEAE
- Any SAE
- Any Treatment Related SAE
- Any TEAE leading to Study Discontinuation
- Any TEAE leading to Permanent Study Medication Discontinuation
- Any TEAE resulting in death
- Severe TEAE
- Severe related TEAE

A by-patient listing of all adverse events (including non-treatment-emergent events) will be provided. This listing will be presented by treatment group and will include: center, patient identifier, age, sex, race, AE (SOC, PT, and verbatim term), date of onset, date of resolution, duration, severity, seriousness, seriousness criteria, action taken, outcome and causality.

Patients reporting the same AE more than once will be counted only once for this event in the AE summary table. For tabulation by severity, the AE with the greatest severity reported by the patient will be included in the table. Similarly, for tabulation by relationship to study medication, the AE with the closest relationship to study medication will be included in the table.

4.8.3 Deaths, Serious Adverse Events, and Other Significant Adverse Events

An overview of AEs, including the number and percentage of patients who died, reported SAEs, and discontinued due to AEs will be provided. The incidence of treatment emergent SAEs will be presented by SOC, PT, and treatment group.

Listings of all SAEs and deaths as well as AEs leading to discontinuation will be provided.

4.8.4 Vital Signs, Physical Findings and Other Observations Related to Safety

4.8.4.1 Vital Signs

Summaries of blood pressure (systolic and diastolic), temperature, and heart rate will be presented by visit and treatment group. Summary statistics will be produced for both observed and change values from baseline for each parameter.

If there are multiple records of vital sign measurements at a visit, the last record will be used.

Unscheduled visits and repeat measurements will be excluded from the summaries, but included in listings. Missing data will be maintained as missing.

4.8.4.2 Physical Examination

Physical examination abnormalities at Visit 1 will be listed.

4.8.4.3 Pregnancy Test

Pregnancy Test results at Visit 1 for female patients of childbearing potential will be listed.

4.9 Antimicrobial Susceptibility

The number of pathogens isolated along with the Minimal Inhibitory Concentration required to inhibit the growth of the bacteria tested (MIC)will be summarized by treatment group at Visit 3 (EOT) and Visit 4 (TOC) for each bacterial species identified as "target OTIC organism" (*P. aeruginosa and S. aureus*).

Antimicrobial susceptibility against Ciprofloxacin and other antibiotics tested (as shown in Table 4-1) was presented as follows:

Table 4-1 Antibiotics tested for susceptibility results by organism

Drug	Organism	
	S. aureus	*P. aeruginosa*
Ciprofloxacin	✓	✓
Ofloxacin	✓	✓
Azithromycin	✓	
Amoxicillin	✓	
Amoxicillin/Clavunate	✓	
Cefuroxime	✓	
Trimethoprim/Sulfamethoxazole	✓	✓
Methicillin	✓	

4.10 Determination of Sample Size

Planned enrollment is 500 children, adolescents and adults. Patients will be stratified at enrollment so that approximately 50% of those enrolled will be younger than 18 years old and approximately 50% will be over 18 years old or older. Stratification is being performed to ensure adequate representation of each age group in the study and not for statistical considerations.

We have not been able to find an accurate estimation of study's main variable (therapeutic cure) in previous investigations with Ciprofloxacin 0.3% otic solutions in the treatment of AOE. Rates of patients cured, with different definitions from ours, have been estimated within a range between 60% and 70%. Moreover, in two comparative trials testing the same combination (same product) versus Ciprofloxacin alone in the treatment of AOMT, the percentage of therapeutic cure in patients with *Pseudomonas aeruginosa* and *Staphylococcus aureus* was around 50%.

For the calculation of the sample size for this study, the assumption is made that the Ciprofloxacin alone group will have a therapeutic cure rate of 62%, and that the combination will increase it by a relative 25%, which would represent a therapeutic cure rate of 78%. Assuming a two-sided significance level of 5%, and a statistical power of 80%, the required number of patients in each group would be 150. The randomization schedule is defined as 2:2:1 (Ciprofloxacin plus Fluocinolone

acetonide:Ciprofloxacin:Fluocinolone acetonide), so the total number of evaluable patients to be recruited is 375 (150 : 150 : 75). It is expected that approximately 80% of enrolled patients will complete the study.

In this study, evaluable patients are those with a positive pathogen microbiological culture at baseline (MITT population). From previous studies in AOE, the rate of patients included with a negative culture at baseline is approximately 25%, and therefore the number of patients to be finally recruited to obtain 375 evaluable patients would be 500.

4.11 Changes in the Conduct of the Study or Planned Analysis

Changes in the conduct of the study may be instituted through a protocol amendment. Planned analyses will be revised as appropriate. Changes will be finalized prior to database lock.

5 REFERENCES

Guidelines for Industry: Statistical Principles for Clinical Trials (E9), International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, February 1998.

Appendix 1 Schedule of Observations

Table 2 Schedule of Observations

Evaluation	Visit 1 Screening/ Study Entry	Visit 2 By Telephone	Visit 3 End of Treatment	Visit 4 Post-Treatment Follow-Up
Study Day	1	3-4	8-10 or within 2 days of early termination	15-17
Informed Consent (and Assent Form when applicable)	X			
Inclusion/exclusion criteria	X			
Medical history	X			
Concurrent symptoms/conditions	X			
Urine pregnancy test[a]	X			
Physical examination	X			
Vital signs[b]	X		X	X
Brighton grading	X		X	X
Ear pain	X		X	X
Edema	X		X	X
Otorrhea	X		X	X
Overall Clinical Outcome			X	X
Microbiological culture of ear discharge[c]	X		X	X
Register patient visit through IWRS	X	X	X	X
Randomization through IWRS	X			
Dispense study medication and explain its use	X	X[d]		
Collect used and unused study medication containers			X[e]	
Dispense patient diary and explain its use	X			
Inquire about otitis symptoms		X[f]		
Review patient diary			X	X
Concomitant medications	X	X	X	X
Adverse events	X	X	X	X

[a] For female patients of childbearing potential.
[b] To include temperature, blood pressure, and pulse rate.
[c] If no discharge is present, no attempt to culture will be made.
[d] If patient with unilateral AOE at baseline becomes bilateral prior to Visit 3 a resupply study medication kit (with the same medication) will be dispensed[e]
If patients forget to bring in containers at Visit 3, they must bring them in no later than Visit 4.
[f] If patients report no improvement in otitis symptoms, they will be asked to come in for a visit as soon as possible. At this visit, they may prematurely discontinue study treatment (in which case the visit will be recorded as Visit 3, the End of Treatment visit) or continue study treatment (in which case the visit will be recorded as an unscheduled visit).