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

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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:

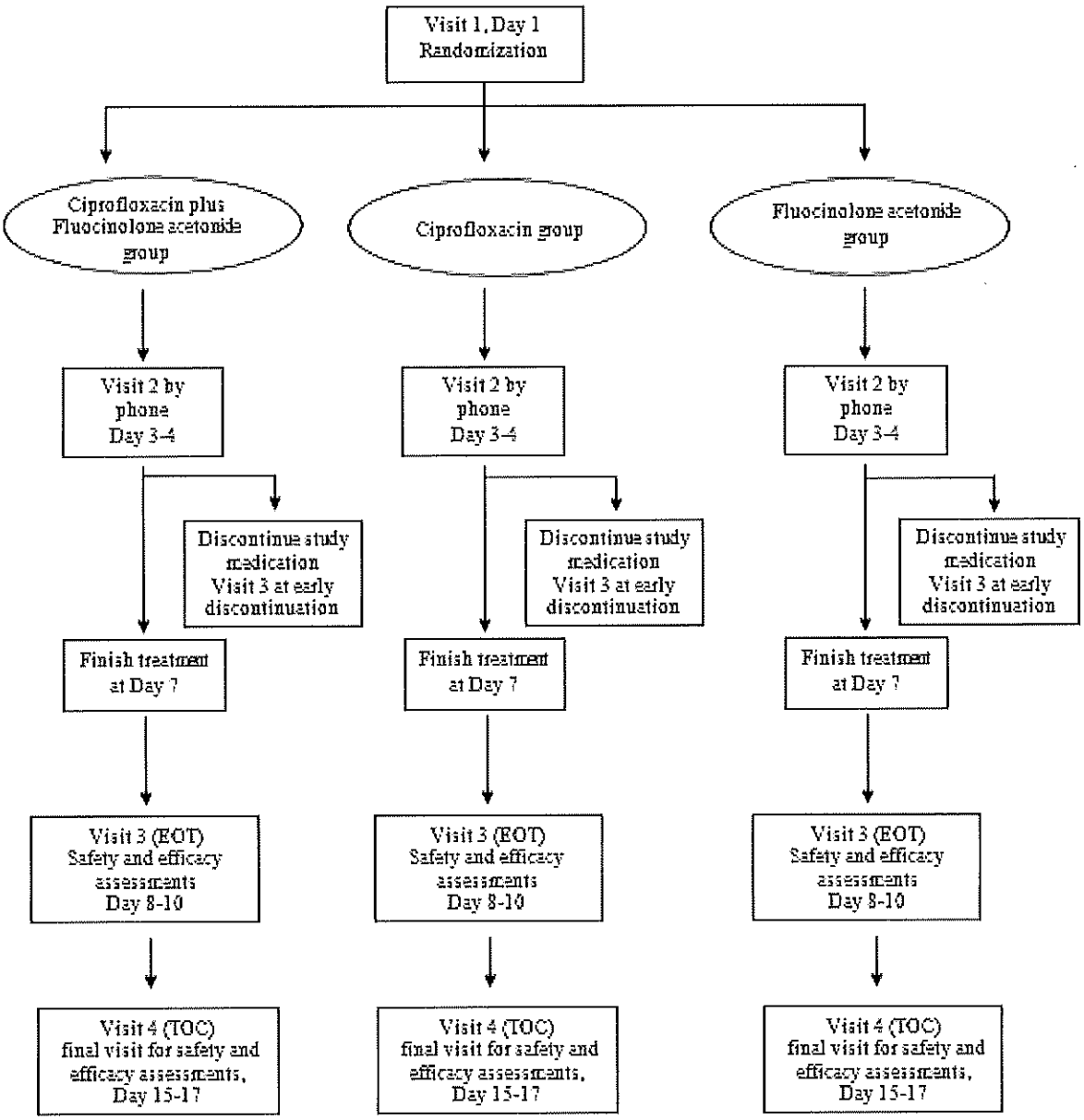
- Sustained microbiological cure
- Clinical cure at Visit 3 and Visit 4
- Microbiological cure at Visit 3 and Visit 4
- Therapeutic cure (clinical+microbiological cure) at Visit 4
- Changes in Brighton grading at Visit 3 and Visit 4
- Changes in otorrhea at Visit 3 and Visit 4
- Changes in edema at Visit 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).