STATISTICAL ANALYSIS PLAN

Protocol Title:	A Phase III, Multicenter, Randomized, Double-Blind Clinical Trial to Assess the Efficacy and Safety of Clotrimazole 1% Otic Solution Compared to Placebo for the Treatment of Fungal Otitis Externa (Otomycosis).
Acronym / Protocol Number:	CLEAR-1 / CLOTOT3-16IA01 CLEAR-2 / CLOTOT3-16IA03
Protocol Version / Date:	2.0 (Amendment 1) / 31 October 2019
Investigational Product:	Clotrimazole 1% otic solution (SVT-15652)
Sponsor:	Laboratorios Salvat, S.A. Gall 30-36 08950 - Esplugues de Llobregat - Barcelona - Spain
SAP Version / Date:	2.0 / 15 February 2022

SIGNATURE PAGE

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Protocol Number:	CLOTOT3-16IA01, CLOTOT3-16IA03
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We, the undersigned, have reviewed and approved this Statistical Analysis Plan:

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VERSION HISTORY

Version	Version Date	Description
1.0	07 December 2021	Original signed version
1.1	15 February 2022	• Added data source of mycological results • Updated definition of mycological response • Updated reasons to exclude from MPP • Updated calculation of days of exposure • Updated calculation of overall compliance • Updated definition of TEAE

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
CITT	Clinical intent-to-treat
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOT	End-of-treatment
ITT	Intent-to-treat
IWRS	Interactive Web Response System
LOCF	Last post-baseline observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
MITT	Mycological Intent-to-Treat
MPP	Mycological Per-Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TEAE	Treatment-emergent Adverse Event
TOC	Test-of-cure
TSSS	Total signs/symptoms score
WHO	World Health Organization

1 INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to provide a description of the statistical methods to be implemented for the analysis of data from the study with protocol numbers CLOTOT3-16IA01 (CLEAR-1) version 2.0 (Amendment 1) and CLOTOT3-16IA03 (CLEAR-2) version 2.0 (Amendment 1). The SAP will be finalized prior to database lock. Any deviations from the SAP after database lock will be documented in the final Clinical Study Report (CSR).

2 STUDY OVERVIEW

2.1 Study Objectives

2.1.1 Primary Objective

To demonstrate the superior efficacy of Clotrimazole vs. placebo in the treatment of otomycosis, with respect to the therapeutic cure at test-of-cure (TOC; Visit 4) in the mycological intent-to-treat (MITT) population.

Therapeutic cure is defined as both mycological cure AND clinical cure.

Mycological cure is defined as eradication (culture does not show growth of any fungal pathogen) or presumed eradication (there is no material to culture and the overall clinical outcome is clinical cure)

Clinical cure is defined as a total signs/symptoms score (TSSS) of zero on a 4-point scale for pruritus, otalgia, ear fullness and otorrhea.

No Response is defined as:

- a positive fungal culture (i.e., identification of a fungal pathogen from fungal and/or bacterial cultures) OR
- sum of signs and symptoms >0 on TOC

2.1.2 Secondary Objectives

- Overall clinical cure at Visits 2, 3 and 4.
- Mycological cure at Visits 2, 3 and 4.
- Therapeutic cure at Visit 2 and 3.
- Changes in TSSS at Visits 2, 3 and 4.
- Changes in individual signs and symptoms at Visits 2, 3 and 4.

In the case of bilateral otomycosis the non-selected ear will also be treated and assessed for its clinical evolution.

2.2 Study Design

2.2.1 Overview

This is a randomized, parallel-group, double-blinded, active-controlled, multicenter study comparing Clotrimazole 1% otic solution with Placebo in the treatment of fungal otitis externa (otomycosis) in adults. A diagram of the study design is shown in Figure 1, and the schedule of observations and procedures is shown in Table 1.

Patients selected for the study will be male or female, 18 years of age and older, with uncomplicated otomycosis 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 2:1 ratio to either the investigational treatment, Clotrimazole 1% otic solution, or the Placebo, Saline solution 0.9%.

At these visits, signs and symptoms of otomycosis will be assessed and a sample of ear exudate (otorrhea or debris if otorrhea is not available) for bacterial and mycological cultures will be collected before onset of treatment and debridement. Debridement of the ear will be preferably done by suction. Patients will be taught to administer study medication and will be instructed to apply it twice daily for 14 days. Patients will receive a 14-day supply of study medication. Patients with bilateral otomycosis 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.

Day 1 will be the day when the first dose is administered (important when the first dose is administered the day after the baseline visit).

At Visit 1, a urine pregnancy test will be performed in females of childbearing potential.

Patients who show up before day 8 with no improvement or worsening of their signs/symptoms, and the investigator's decision is to discontinue the patient from the study medication, this will be considered as Visit 2, and all the procedures corresponding to this visit will apply.

Patients will attend Visit 2 on Day 8-10 of study treatment to check on each patient's clinical progress. At this visit, signs and symptoms of otomycosis will be assessed and otorrhea sample will be collected if exudate is present. In the case of patients who report no improvement in otomycosis signs/symptoms at Visit 2 (Day 8-10), the Investigator will choose one of the two following options for the patient:

1. Continue with the study medication. Patient continues in the study and attends visit 3.
2. Discontinue study medication. The Investigator may treat patient with other medication (rescue medication) at their discretion.
    - If investigator prescribes rescue medication, the patient will be withdrawn from the study. The clinical outcome for the patient will be recorded as Treatment failure at the End of Study form.
    - If investigator does not prescribe any rescue medication, the patient will be expected to return for a clinical and safety evaluation at Visit 3.

Non-discontinued patients will attend Visit 3 (EOT: end-of treatment) and Visit 4 (TOC: test-of-cure) at approximately Day 15 and Day 24, respectively. At these visits, signs and symptoms of otomycosis will be assessed and ear exudate, if present, will be collected for bacterial and mycological cultures. The primary efficacy endpoint will be the proportion of patients with Therapeutic cure (Clinical cure + Mycological Cure) at Test of Cure (Visit 4).

In the case of patients who report no improvement in otomycosis signs/symptoms at Visit 3 (Day 15-17), the Investigator will choose one of the two following options for the patient:

1. Continue the patient in the study without any rescue medication until Visit 4 (Day 24-26). The patient will come to Visit 4.
2. Prescribe rescue medication and withdraw the patient from the study. The clinical outcome for the patient will be recorded as Treatment Failure at the End of Study form.

Figure 1 Diagram of Study Design

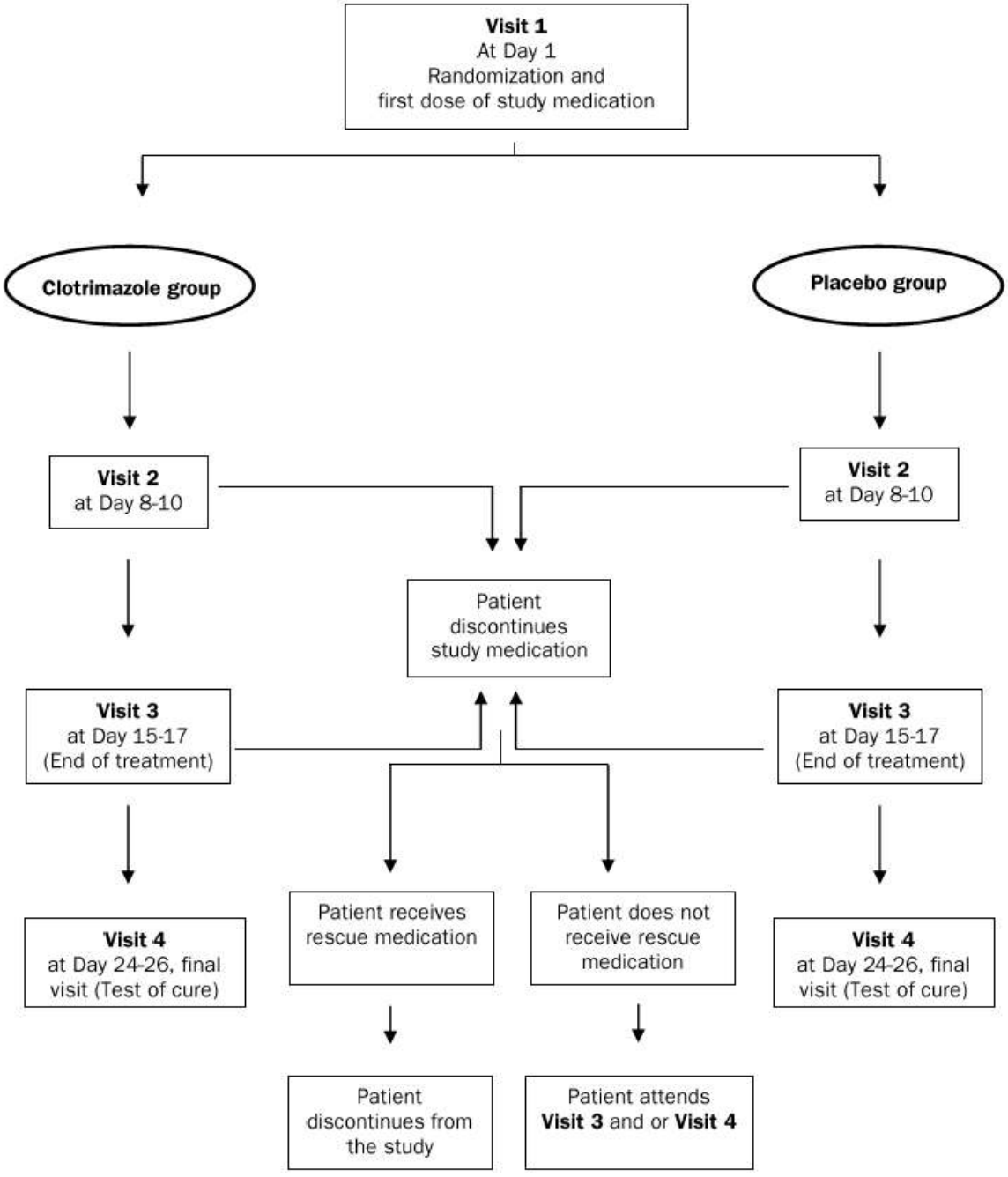

Table 1 Schedule of Observations

Evaluation	Visit 1 Screening (Study Entry)	Visit 2 On treatment	Visit 3 End of Treatment	Visit 4 Test of cure
Study Day	**1**	**8-10**	**15-17**	**24-26**
Informed Consent	X			
Inclusion/exclusion criteria	X			
Medical history	X			
Concurrent symptoms/conditions	X			
Urine pregnancy test[a]	X			
Physical examination[b]	X			
Vital signs[c]	X	X	X	X
Pruritus	X	X	X	X
Otalgia	X	X	X	X
Ear fullness	X	X	X	X
Otorrhea	X	X	X	X
Overall Clinical Outcome		X	X	X
Bacterial and Mycological cultures of ear discharge	X	X[d]	X[d]	X[d]
Debridement of the ear	X	X[d]	X[d]	X[d]
Randomization through IWRS	X			
Dispense study medication and explain its use	X	X[e]		
Provide a visit calendar	X			
Collect used and unused study medication containers			X[f]	
Concomitant medications	X	X	X	X
Adverse events	X	X	X	X

[a] For female patients of childbearing potential.
[b] To include a directed examination of the ears, head, nose and oropharynx.
[c] To include temperature, blood pressure, and pulse rate.
[d] If no discharge (otorrhea or debris) is present, no attempt to culture will be made.
[e] If patient with unilateral otomycosis at baseline becomes bilateral prior to Visit 3 a resupply study medication kit (with the same medication) will be dispensed for the non-evaluable ear.
[f] If patients forget to bring in containers at this visit, they must bring them in no later than Visit 4.

2.2.2 Patient Identification and Randomization

Patients will be randomized at Visit 1, after signing informed consent and meeting eligibility criteria. Randomization will be conducted through an Interactive Web Response System (IWRS) include blocking. 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.

For patients who have bilateral otomycosis, the ear with a higher TSSS (as defined in Section 2.3.1.1.5) will be considered the evaluable one. If the TSSS is identical in both ears, the left ear will be selected as the evaluable ear. In either case, the non-evaluable ear will receive the same treatment as the evaluable ear.

2.2.3 Blinding and Breaking the Blind

All study medication products (test and placebo) 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 carton.

Mycological and bacterial 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. The investigator will be able to break the blind and get the subject's treatment assignment through IWRS at any time, by completing the required information. The investigator should not share the unblinded information with Laboratorios Salvat, S.A. (Salvat) or [redacted] personnel.

2.2.4 Sample Size Determination

Planned enrollment is 191 patients randomized in 2:1 ratio (Clotrimazole 1% otic solution: Placebo) to obtain 150 evaluable patients (100 Clotrimazole 1% and 50 Placebo) for each trial.

Clotrimazole otic solution should be statistically superior to the placebo ($p<0.05$) with regard to the therapeutic cure at the test-of-cure visit, using the MITT population.

Assuming a significance level of 5% and a desired statistical power of 95%, a placebo effect of 30%, and a 2:1 allocation schedule, the total number of patients needed to detect a difference of 30% in the therapeutic cure (100% relative increase) is 150 (100 with clotrimazole and 50 with placebo). The suspected dropout rate due to negative mycological culture at baseline is 21% that leads to a required inclusion of 191 patients (127 for the clotrimazole group and 64 patients for the placebo group).

As CLEAR-1 and CLEAR-2 are twin trials, if one of them is completed before the other, an updated sample size computation will be performed for the latter in order to have a more accurate picture of treatment and control arm success probabilities.

2.3 Study Endpoints

2.3.1 Efficacy Parameters

2.3.1.1 Clinical Efficacy Parameters

2.3.1.1.1 Pruritus

Pruritus in both ears will be assessed by the investigator at all visits. For consistency, the same individual should perform all the assessments at all visits, if possible. Pruritus will be assessed as:

- Severe (3) if pruritus is marked or intense
- Moderate (2) if pruritus is definitely present
- Mild (1) if pruritus is slight
- Absent (0) if there is complete absence of pruritus

At Visits 2, 3 and 4, pruritus will be considered resolved if the level is 0. Pruritus will be considered improved if the level is lower than the previous pruritus assessment.

2.3.1.1.2 Otalgia

Otalgia in both ears will be assessed at all visits. The investigator will ask the patient to assess his or her level of otalgia on the day of the visit. If the patient has taken analgesic medication, he or she will be asked to assess the level of otalgia 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. 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; or
- Absent (0) if there is total absence of pain.

At Visits 2, 3 and 4, otalgia will be considered resolved if the level is 0. Otalgia will be considered improved if the level is lower than the previous otalgia assessment.

2.3.1.1.3 Ear fullness

Ear fullness (aural fullness or ear pressure) in both ears will be assessed by the investigator at all visits. For consistency, the same individual should perform all the assessments at all visits, if possible. Ear fullness will be assessed as:

- Severe (3) if ear fullness, is marked or intense
- Moderate (2) if ear fullness is definitely present
- Mild (1) if ear fullness is slight
- Absent (0) if there is complete absence of ear fullness

At Visits 2, 3 and 4, ear fullness will be considered resolved if the level is 0. Ear fullness will be considered improved if the level is lower than the previous ear fullness assessment.

2.3.1.1.4 Otorrhea

Otorrhea in both ears will be assessed by the investigator at all visits. For consistency, the same individual should perform all the assessments at all visits, if possible. Otorrhea will be assessed as:

- Severe (3): copious discharge that prevents visualization of the tympanic membrane unless the fluid is suctioned
- Moderate (2): the anterior sulcus is full, but the tympanic membrane is still clearly visible
- Mild (1): little fluid accumulating in the anterior tympanomeatal sulcus but the tympanic membrane is still visible
- Absent (0) absence of fluid

At Visits 2, 3 and 4, otorrhea will be considered resolved if the level is 0. Otorrhea will be considered improved if the level is lower than the previous otorrhea assessment.

2.3.1.1.5 Overall Clinical Outcome

Overall Clinical Outcome is based on the TSSS, which is calculated by the sum of pruritus score + otalgia score + ear fullness 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 2.3.1.1.1, 2.3.1.1.2, 2.3.1.1.3 and 2.3.1.1.4.
2. Clinical Improvement: TSSS is different than 0 but lower than the previous visit, as defined in Sections 2.3.1.1.1, 2.3.1.1.2, 2.3.1.1.3 and 2.3.1.1.4.

3. Clinical Failure: TSSS does not meet the definitions of Clinical Cure or Clinical Improvement, as defined in Sections 2.3.1.1.1, 2.3.1.1.2, 2.3.1.1.3 and 2.3.1.1.4.
4. Not changed: TSSS is different than 0 but unchanged compared to the previous visit.
5. Indeterminate: Discontinued (for reasons other than Clinical Failure) or lost to follow up.
6. Not applicable: In case of the non-evaluable ear in patients with unilateral otomycosis or at Visit 2 a new occurrence of otomycosis is determined in the non-evaluable ear and the unilateral otomycosis becomes bilateral.

Patients who take rescue medication (as defined in Protocol Section 6.7.2) will be considered Clinical Failures.

2.3.1.2 Mycological Efficacy Parameters

2.3.1.2.1 Mycological Outcome

Samples of ear discharge will be taken at V1, and in addition at visits V2, V3 and V4, when discharge is present. The otorrhea/debris sample will be taken prior to the debridement of the affected ear. A central laboratory will provide exudate sampling kits with standardized instructions for sample preparation (as defined in the Laboratory manual).

The otorrhea/debris sample will be sent 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 microbiological laboratory procedures to identify fungal and bacterial species. If the culture does not show growth of any fungal pathogen from central laboratory or the local laboratory sent the wrong organism to central laboratory, the mycological result from local laboratory will be used.

Susceptibility testing will be performed on fungal isolates that are present at baseline and TOC for an individual subject according the Clinical and Laboratory Standard Institute methods for clotrimazole and comparator drugs to which the fungal isolates are susceptible.

For any patient with a culture positive for fungi at Visit 1, mycological outcome at Visit 2 will be classified as:

- Eradication if the culture does not show growth of any fungal pathogen;
- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Cure;
- Persistence if any fungal pathogen cultured at Visit 1 is still present;
- Presumed Persistence: if there is no material to culture or sample was not taken or sample was not shipped to lab and the Overall Clinical Outcome is not Clinical Cure;
- Superinfection if a new fungal pathogen not present at Visit 1 is isolated while the initial pathogen is also present (presence of a nonpathogenic organism will not be considered Superinfection);
- Reinfection: if there is isolation of a new fungal pathogen different from the one present at Visit 1 and the initial pathogen is not present; or
- Indeterminate if none of the above definitions are met and the mycological outcome cannot be determined.

For any patient with a culture positive for fungi at Visit 1, mycological outcome at Visit 3 will be classified as:

- Eradication if the culture does not show growth of any fungal pathogen;

- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Cure;
- Persistence if any fungal pathogen cultured at Visit 1 is still present;
- Presumed Persistence: if there is no material to culture or sample was not taken or sample was not shipped to lab and the Overall Clinical Outcome is not Clinical Cure;
- Recurrence: if there is reappearance of the fungal pathogen present at Visit 1 which was eradicated or presumably eradicated at Visit 2;
- Superinfection if a new fungal pathogen not present at Visit 1 is isolated while the initial pathogen is also present (presence of a nonpathogenic organism will not be considered Superinfection);
- Reinfection: if there is isolation of a new fungal pathogen different from the one present at Visit 1 and the initial pathogen is not present; or
- Indeterminate if none of the above definitions are met and the mycological outcome cannot be determined.

For any patient with a culture positive for fungi at Visit 1, mycological outcome at Visit 4 will be classified as:

- Eradication if the culture does not show growth of any fungal pathogen;
- Presumed Eradication if there is no material to culture and the Overall Clinical Outcome is Clinical Cure;
- Persistence if any fungal pathogen cultured at Visit 1 is still present;
- Presumed Persistence: if there is no material to culture or sample was not taken or sample was not shipped to lab and the Overall Clinical Outcome is not Clinical Cure;
- Recurrence: if there is reappearance of the fungal pathogen present at Visit 1 which was eradicated or presumably eradicated at Visit 2 and/or 3;
- Superinfection if a new fungal pathogen not present at Visit 1 is isolated while the initial pathogen is also present (presence of a nonpathogenic organism will not be considered Superinfection);
- Reinfection: if there is isolation of a new fungal pathogen different from the one present at Visit 1 and the initial pathogen is not present; or
- Indeterminate if none of the above definitions are met and the mycological outcome cannot be determined.

Mycological outcome will be determined for each patient for Visit 2, Visit 3 and for Visit 4 after mycological culture results are made available to the Sponsor or designee.

Mycological cure is defined as eradication (culture does not show growth of any fungal pathogen) or presumed eradication (there is no material to culture and overall clinical outcome is clinical cure).

For any patient with non fungal pathogen at Visit 1, mycological response at Visit 2 will be classified as:

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

For any patient with non fungal pathogen at Visit 1, mycological response at Visit 3 will be classified as:

- Presumed Eradication if the culture does not show growth of any fungal pathogen or if there is no material to culture and the Overall Clinical Outcome is Clinical Cure;
- Presumed Persistence if any fungal pathogen cultured at Visit 2 is still present or if the Overall Clinical Outcome is Clinical Failure but no culture result is available;
- Superinfection if a pathogen not present at Visit 1 and Visit 2 is now present;
- Indeterminate if none of the above definitions are met and the mycological response cannot be determined.

For any patient with non fungal pathogen at Visit 1, mycological response at Visit 4 will be classified as:

- Presumed Eradication if the culture does not show growth of any fungal pathogen or if there is no material to culture and the Overall Clinical Outcome is Clinical Cure;
- Presumed Persistence if any fungal pathogen cultured at Visit 2 and 3 is still present or if the Overall Clinical Outcome is Clinical Failure but no culture result is available
- Recurrence if a pathogen isolated at Visit 2, which was not present at Visit 3, reappears at Visit 4
- Superinfection if a pathogen not present at Visit 1, Visit 2 and Visit 3 is now present;
- Indeterminate if none of the above definitions are met and the mycological response cannot be determined.

The above rules will be used for microbiological outcome summaries for the patients in the clinical intent-to-treat (CITT) populations that have no pathogen at Visit 1, since mycological response cannot be calculated without any pathogen at Visit 1.

If Overall Clinical Outcome at Visit 2, 3 or 4 (follow up visits) is Clinical Failure (whether or not the patient discontinues prematurely) and no mycological culture is performed at one of those follow-up visits, the mycological response at that follow-up visit will be Presumed Persistence.

2.3.1.3 Therapeutic response

The therapeutic response is a combined assessment of the Overall Clinical Outcome plus the Mycological Outcome. The following categories are defined:

1. Therapeutic cure: TSSS (pruritus + otalgia + ear fullness + otorrhea) = 0 and mycological outcome eradication or presumed eradication.
2. Therapeutic failure:
   - Positive fungal culture, OR
   - TSSS > 0 regardless of mycological outcome.

2.3.1.4 Primary Efficacy Endpoint

Based on the definitions in Section 2.3.1.1.5, 2.3.1.2 and 2.3.1.3, the primary efficacy endpoint will be the proportion of patients with Therapeutic cure at Test of Cure (Visit 4) in the MITT population.

2.3.1.5 Secondary Efficacy Endpoints

Based on the definitions in Sections 2.3.1.1, 2.3.1.2 and 2.3.1.3, the secondary efficacy endpoints will be:

- Overall clinical cure at Visits 2, 3 and 4.
- Mycological cure at Visits 2, 3 and 4.
- Therapeutic cure at Visit 2 and 3.
- Changes in TSSS at Visits 2, 3 and 4.
- Changes in individual signs and symptoms at Visits 2, 3 and 4.

In the case of bilateral otomycosis the non-selected ear will also be treated and assessed for its clinical evolution.

2.3.2 Safety Parameters

Safety will be assessed by adverse events (AEs) and physical examination. In addition, a urine pregnancy test for females of childbearing potential will be performed at Visit 1.

2.3.2.1 Adverse Events

Adverse events will be recorded throughout the study and at early termination, and will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

An AE is any untoward medical event that occurs in a patient or subject who has received an investigational product, whether or not considered drug related. 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 study 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 Electronic Data Capture (EDC). If pre-existing signs and signs/symptoms of otomycosis (pruritus, otalgia, ear fullness and otorrhea) worsen during the study, this will be considered a treatment failure instead of an AE.

2.3.2.2 Physical Examination

Physical examination will be performed at V1. A directed examination of the ears (external inspection and examination (with or without microscope) of the external auditory canal), head, nose and oropharynx will be performed.

2.3.2.3 Vital Signs

Vital signs (temperature, blood pressure, and pulse) will be assessed at all visits.

2.3.2.4 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).

3 STATISTICAL METHODOLOGY

3.1 General Considerations

3.1.1 Definition of Baseline

For microbiological data, baseline pathogen(s) are determined from the samples collected prior to the first dose of study drug.

For all efficacy and safety endpoints, baseline is defined as the last measurement or assessment prior to the first dose of study drug.

3.1.2 Summary Statistics

Categorical data will generally be summarized with counts and percentages of subjects. The denominator used for the percentage calculation will be clearly defined. Continuous data will generally be summarized with descriptive statistics including n (number of non-missing values), mean, median, standard deviation, minimum, and maximum.

3.1.3 Handling of Dropouts and Missing Data

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

For the primary and secondary endpoints, patients who discontinue for lack of efficacy or rescue medication will be considered as treatment failures.

In cases of missing or incomplete dates (e.g. AE and concomitant medications), the missing component(s) will be assumed as the most conservative value possible. For example, AEs with missing start dates, but with stop dates either overlapping into the treatment period or missing, will be counted as treatment-emergent, taking the worst-case approach. When partial dates are present in the data, both a partial start date and/or a partial stop date will be evaluated to determine whether it can be conclusively established that the AE started prior to the start of study drug or ended prior to the start of study drug. If the above cannot be conclusively established based on the partial and/or present dates, then the AE will be considered as treatment-emergent. Actual data values as they appear in the original electronic Case report form (eCRF) will be presented in the data listings.

Missing values for other variables will not be imputed and only observed values will be used in data analyses and summaries.

3.2 Analysis Populations

There will be 4 populations defined for this study: Safety, CITT, MITT, and Mycological per-protocol (MPP).

3.2.1 Safety Population

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

3.2.2 Clinical intent-to-treat (CITT)

The CITT population will include all patients who were randomized.

3.2.3 Mycological intent-to-treat (MITT)

The MITT population will include all CITT patients who had a positive baseline fungal culture for *Aspergillus spp* and/or *Candida spp*. The primary populations for efficacy analyses will be the MITT population.

3.2.4 Mycological per-protocol (MPP)

The MPP population will include all randomized patient who had positive baseline culture for *Aspergillus spp* and/or *Candida spp* and who:

- Satisfied all inclusion and exclusion criteria;
- Did not receive any prohibited concomitant medications (except if it is considered rescue medication);
- Did not have any other major protocol violations;
- Completed Visit 2;
- Completed Visit 3 and Visit 4 (unless the subject was deemed a clinical failure at an earlier visit than Visit 4);
- Completed Visit 4 within the designated visit window;

- Had compliance rates between 80% and 100% as defined in Section 3.3.7 (patients who are deemed Clinical Failures are to be included if they had compliance rates between 80% and 100% during the first 3 days of study treatment);
- Had mycological results (when subject has material to culture) from Visit 1, Visit 2, Visit 3 and/or Visit 4 unless the subject 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 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.

3.3 Subject Data and Study Conduct

3.3.1 Subject Disposition

Counts and percentages of subjects screened and in each of the analysis populations will be summarized by treatment and in total based on all screened subjects. Reasons for exclusion from each analysis population will also be summarized. Patients who were excluded from each of the study populations defined in Section 3.2, and their reasons for exclusion, will be listed.

Patient disposition by country and by site will also be summarized.

The counts and percentages of subjects completed, and who terminated early, along with the primary reason and secondary reasons for the termination (per study eCRF specifications) will be presented on CITT and MITT population.

3.3.2 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 subject from the MPP.

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 subjects into each of the analysis sets as defined in Section 3.2.

Patients with protocol deviations will be presented in the data listings. Counts and percentages of subjects with protocol deviations by deviation category will be summarized by treatment and in total based on CITT population.

The protocol deviations are considered critical (or very serious) and major (or serious), according to the following definitions:

- Critical or very serious: Deviations affecting / they have adversely affected the rights, safety or welfare of subjects and / or the quality and integrity of data
- Major or serious: Deviations that may affect / have adversely affected the rights, safety or welfare of subjects and / or the quality and integrity of data.

In addition, the protocol deviations related to COVID-19 will be categorized and summarized by treatment and in total based on CITT population separately.

3.3.3 Demographic and Baseline Characteristics

The following demographic and baseline characteristics will be summarized:

- Age (years) and age categories (<65 years, ≥65 years)
- Sex
- Childbearing potential
- Race
- Ethnicity
- Risk factors for otomycosis (exposure to excess moisture, trauma, use of hearing aids, topical otic antibacterial, quantitative or qualitative changes in earwax, other)
- Bilateral otomycosis (yes, no)
- Evaluable ear (left, right)
- Baseline pathogen (yes [fungal only, fungal and bacterial, bacterial only], no)
- Ear wick placement
- Baseline pathogen species

Demographic and baseline characteristics will be summarized with descriptive statistics or counts and percentages of subjects as appropriate by treatment and in total based on Safety, CITT, and MITT Population.

3.3.4 Medical History

Counts and percentages of subjects with medical history will be summarized by event/diagnosis/procedure by treatment and in total on CITT population.

3.3.5 Concomitant Medications

Concomitant medications (including rescue medication) taken during the course of the study or within 30 days prior to Screening are recorded. This also includes prescribed medications, over-the-counter medications and herbal supplements.

Concomitant medications will be coded to anatomical therapeutic chemical (ATC) class and preferred term using the World Health Organization Drug Dictionary Global (WHODrug Global B3, Sept 2019). For summary purposes, medications will be considered prior medications if they stopped prior to the first dose of study drug and concomitant medications if they were taken at any time after the first dose of study drug (i.e. started prior to the first dose of study drug and were ongoing or started after the first dose of study drug).

If a medication has incomplete start or stop dates, dates will be imputed to determine whether a medication should be considered prior or concomitant. If a medication start date is incomplete, the first day of the month will be imputed for missing day and January will be imputed for missing month. If a medication stop date is incomplete, the last day of the month will be imputed for missing day and December will be imputed for missing month. Incomplete start and stop dates will be listed as collected without imputation.

Counts and percentages of subjects taking prior and concomitant medications by ATC class and preferred term will be summarized by treatment and in total based on the CITT population.

The number of patients taking prohibited medications (as defined in the protocol section 6.7.1) will be tabulated by treatment group. A listing will also be provided.

The number of patients taking rescue medications (as defined in the protocol section 6.7.2) will be tabulated by treatment group. A listing will also be provided.

3.3.6 Study Drug Exposure and Compliance

Days of exposure to study drug will be calculated as (date/time of last dose of study drug – date/time of first dose of study drug) / 3600 / 24 + 0.5, rounded to the nearest half day. If time of last dose of study drug or time of first dose of study drug is missing, then days of exposure to study drug will be calculated as (date of last dose of study drug – date of first dose of study drug + 1).

Days of exposure to study drug will be summarized by treatment based on the CITT population with descriptive statistics and with counts and percentages of subjects with exposure in the following categories:

- <1 week (<7 days)
- 1 to <2 weeks (7 - <14 days)
- ≥2 weeks (≥14 days)

Study medication compliance will be assessed during the first 7 day of the treatment period, during the treatment period (first 14 days), and during the overall study.

Percent compliance to the study drug regimen will be calculated as 100 x number of vials actually administered / number of vials expected to be taken. The number of doses administered, the number of used vials returned for evaluable ear, and the number of unused vials returned for evaluable ear are all considered to determine the number of vials actually administered for 14-day treatment period and the overall study. 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, 14 doses are expected in the first 7 days of treatment. For treatment period, 28 doses are expected. If a subject does not complete treatment in the treatment period (first 14 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 to the study drug regimen will be summarized by treatment and age group (<65 years, ≥65 years) based on the Safety, CITT, MITT populations with descriptive statistics and with counts and percentages of subjects with compliance in the following categories:

- <80%
- 80-120%
- >120%

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 7 and 14 days of the treatment period will be tabulated by treatment group.

3.4 Efficacy Assessment

For the efficacy analyses, only the assessments from the evaluable ear will be used.

3.4.1 Primary Efficacy Endpoints

Primary Analysis

The primary endpoint for efficacy will be the proportion of subjects with therapeutic cure at test-of-cure (Visit 4).

- Therapeutic cure is defined as both mycological cure and clinical cure.

- Mycological cure is defined as eradication (culture does not show growth of any fungal pathogen) or presumed eradication (there is no material to culture and the overall clinical outcome is clinical cure).
- Clinical cure is defined as a TSSS of zero on a 4-point scale for pruritus, otalgia, ear fullness and otorrhea.

Therapeutic cure at Visit 4 will be compared between treatment groups by using a chi-square test, under a two-sided 5% significance level. The 95% confidence intervals together with the p-value based on the normal approximation to the binomial distribution will be presented for the between-treatment group differences in the therapeutic cure rate. For each treatment group, the 95% confidence intervals of the therapeutic cure rate using the Clopper-Pearson method[1] will also be presented.

Patients with missing efficacy data or indeterminate outcomes will be considered as treatment failures for efficacy analyses. Patients who discontinue for lack of efficacy or rescue medication will be considered as treatment failures.

For the main objective of the study, the statistical analysis of the primary endpoint will be performed in the MITT population.

Sensitivity Analysis

- The proportion of subjects with therapeutic cure at Visit 4 will be analyzed using the standardized estimator (Steingrimsson, 2017)[2].
- The proportion of subjects with therapeutic cure at Visit 4 will be analyzed using multiple imputation method to define missing data.
- The proportion of subjects with therapeutic cure at Visit 4 will be analyzed using worst case scenario to define missing data, in which missing endpoints for treatment arm patients are set to therapeutic failure and missing endpoints for control arm patients are set to clinical cure.
- The proportion of subjects with therapeutic cure at Visit 4 will be analyzed using the last post-baseline observation carried forward (LOCF) method to define missing data.

Supportive Analysis

The efficacy analyses of the primary endpoint conducted on the MITT population will be repeated on the MPP and CITT populations.

3.4.2 Secondary Efficacy Endpoints

Analysis of the secondary efficacy endpoints will be supportive only:

- Overall clinical cure at Visits 2, 3 and 4.
- Mycological cure at Visits 2, 3 and 4.
- Therapeutic cure at Visits 2 and 3.
- Changes in signs/symptoms at Visits 2, 3 and 4.

  Changes in each sign/symptom (pruritus, otorrhea, ear fullness and otalgia) and in the overall clinical outcome (TSSS) will be analyzed as:

  - 2-level approach: resolved/not resolved.
  - 3-level approach: resolved/improved/not improved

- mean change in rating from the baseline assessment

The proportion of patients with clinical, mycological and therapeutic cure, the 2-level approach for changes in sign/symptoms and the overall clinical outcome (including the change from baseline and the change from the previous visit) will be summarized by visit and treatment group. For each visit, the proportions of patients will be compared between the treatment groups by using a chi-square test, under a two-sided 5% significance level. The 95% confidence intervals together with the p-value based on the normal approximation to the binomial distribution will be presented for the between-treatment group differences. For each treatment group, the 95% confidence intervals using the Clopper-Pearson method will also be presented.

For Visits 2, 3 and 4, the proportion of patients with a response of Resolved and Not Improved will be compared between the treatment groups by using a chi-square test, under a two-sided 5% significance level. The 95% confidence intervals together with the p-value based on the normal approximation to the binomial distribution will be presented for the between-treatment group differences. For each treatment group, the 95% confidence intervals using the Clopper-Pearson method will also be presented.

Patients with missing efficacy data or indeterminate outcomes will be considered as treatment failures for efficacy analyses. Patients who discontinue for lack of efficacy or rescue medication will be considered as treatment failures.

The statistical analysis of the secondary endpoint will be performed in the MITT population and repeated on the MPP and CITT populations.

3.4.2.1 Mycological outcome

The proportion of patients with a response of Eradication or Presumed Eradication at Visit 2, Visit 3 (EOT) and Visit 4 (TOC) will be compared between the Clotrimazole 1% otic solution and the Placebo treatment groups by using a chi-squared test, under a two-sided 5% significance level. The 95% confidence intervals together with the p-value based on the normal approximation to the binomial distribution will be presented for the between-treatment group differences. For each treatment group, the 95% confidence intervals using the Clopper-Pearson method will also be presented.

Mycological 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 2, Visit 3 and Visit 4 will be compared between the Clotrimazole and Placebo treatment groups by using the same chi-squared test as the primary efficacy endpoint.

Mycological outcome will also be presented by pathogen on the MITT population (Candida spp, Aspergillus spp).

3.4.2.2 Changes in signs/symptoms

Counts and percentages of patients with signs/symptoms (pruritus, otalgia, ear fullness and otorrhea) assessed by the investigator as "Absent", "Mild", "Moderate" or "Severe" will be summarized at V1, V2, V3, and V4.

The change from V1 in each sign and symptom will be assessed and classified as follows:

- "Resolved" at V2, V3 and V4 if that sign/symptom is assessed by the investigator as "Absent"
- "Improved" at V2, V3 and V4 if that sign/symptom is assessed by the investigator as:
    - "Mild" and was assessed as "Severe" or "Moderate" at V1 or
    - "Moderate" and was assessed as "Severe" at V1.
- "Remain Absent" at V2, V3 and V4 if the eardrum edema is assessed by the investigator as "Absent" and was assessed as "Absent" at V1
- "Not improved" otherwise

Counts and percentages of patients with a change from V1 in each sign/symptom considered as "Resolved", "Improved" or "Not improved" will be summarized at V2, V3 and V4 and will be compared between treatment groups by using a chi-square test, under a two-sided 5% significance level. The 95% confidence intervals together with the p-value based on the normal approximation to the binomial distribution will be presented for the between-treatment group differences. For each treatment group, the 95% confidence intervals using the Clopper-Pearson method will also be presented.

Counts and percentages of patients with each sign/symptom considered as "Resolved" or "Not resolved" will be summarized at V2, V3, and V4 and will be compared between treatment groups by using a chi-square test, under a two-sided 5% significance level. The 95% confidence intervals together with the p-value based on the normal approximation to the binomial distribution will be presented for the between-treatment group differences. For each treatment group, the 95% confidence intervals using the Clopper-Pearson method will also be presented.

3.4.3 Subgroups

Analyses of primary efficacy variable will be performed for subgroups of patients based on baseline characteristics for the following:

- Age group (<65 years, ≥65 years)
- Sex (male versus female)
- Unilateral/bilateral
- Fungal infection/Fungal and bacterial infection/Bacterial infection

Any additional subgroup may be considered.

3.4.4 Additional analysis

3.4.4.1 Antimycological susceptibility

Antifungal susceptibility against clotrimazole and comparators will be tested. Interpretation of antimycological susceptibility by baseline pathogen will also be provided. Antimycological susceptibility will be summarized by treatment groups based on the MITT population. Antimycological susceptibility will be presented in data listings.

3.5 Safety Assessment

Safety data will be summarized by actual treatment received and in total based on the Safety Population.

3.5.1 Adverse Events (AEs)

AEs will be captured from the date of informed consent through study completion. All AEs will be coded to system organ class and preferred term using MedDRA version 23.0. Treatment-emergent adverse events (TEAEs) are defined as AEs with onset on, or after the first dose of study drug or with onset prior to the first dose of study drug that increase in severity on, or after the first dose of study drug.

An overview of AEs will be provided including counts and percentages of subjects (and event counts) with the following:

- Any TEAEs (overall, by relationship, and by severity)
- Any study drug related TEAEs
- Any serious adverse events (SAEs) (overall and by relationship)
- Any severe TEAEs (overall and by relationship)
- Any moderate TEAEs (overall and by relationship)
- Any mild TEAEs (overall and by relationship)
- Any TEAEs leading to study discontinuation
- Any TEAEs leading to discontinuation of study drug
- Any AEs resulting in death

Patients with multiple events will be counted only once within each category.

The number and percentage of patients reporting a TEAE in each treatment group will be tabulated by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship; by SOC and PT in each of the age groups (<65 years, ≥65 years). Summary tables will be presented by decreasing frequency of SOC in total group and decreasing frequency of PT in total group within SOC. For all analyses of TEAEs, if the same AE (based on PT) is reported for the same patient more than once, the AE is counted only once for that PT.

A list of patients who have SAEs, a list of patients who discontinue from study drug, and a list of death will be provided. All adverse events will be listed.

3.5.2 Physical Examination

Physical examination will be presented in data listings.

3.5.3 Vital Signs

Actual values and change from baseline values for vital signs will be summarized by treatment group for each visit.

3.5.4 Pregnancy Test

Pregnancy test will be presented in data listings.

4 INTERIM ANALYSES

No interim analyses are planned.

5 CHANGES FROM PROTOCOL-SPECIFIED STATISTICAL ANALYSES

The following items were changed between the protocol and the SAP:

- The secondary endpoint of Mycological cure was modified to "Mycological cure at Visits 2, 3 and 4.".
- Definition of mycological response when baseline culture was positive was modified. Protocol states "Presumed persistence if there is no material to culture and the Overall Clinical Outcome is not Clinical Cure". This definition was updated to "Presumed Persistence if there is no material to culture or sample was not taken or sample was not shipped to lab and the Overall Clinical Outcome is not Clinical Cure" in SAP. In protocol, Reinfection was defined as "Reinfection if there is isolation of a new fungal pathogen different from the one eradicated or presumably eradicated" and was modified as "Reinfection if there is isolation of a new fungal pathogen different from the one present at Visit 1 and the initial pathogen is not present" in SAP. Reinfection was also added to the classification of mycological outcome at Visit 2. Superinfection was re-defined as "Superinfection if a new fungal pathogen not present at Visit 1 is isolated while the initial pathogen is also present (presence of a nonpathogenic organism will not be considered Superinfection)" for all visits in SAP.
- The protocol states that one of the inclusion criteria of MPP population is subject who "Completed Visit 2, Visit 3 and Visit 4 (unless the subject was deemed a clinical failure at an earlier visit than Visit 4) with the designated visit window". This was modified as subjects who "Completed Visit 3 and Visit 4 (unless the subject was deemed a clinical failure at an earlier visit than Visit 4)" and "Completed Visit 4 within the designated visit window" in SAP. Visit 2 and Visit 3 out of window were removed from MPP exclusion criteria. Visit 2 not completed was added into MPP exclusion criteria.

6 PROGRAMMING SPECIFICATIONS

Analyses will be performed using SAS® version 9.4. All available data will be presented in subject data listings which will be sorted by subject and visit date as applicable. Detailed Programming Specifications will be provided in a separate document.

APPENDIX A: REFERENCES

1. Alan A. Categorical Data Analysis, 3rd Edition, Wiley, Hoboken, NJ, 2013.
2. Steingrimsson JA, Hanley DF, Rosenblum M. Improving precision by adjusting for prognostic baseline variables in randomized trials with binary outcomes, without regression model assumptions. Contemp Clin Trials 2017; 54:18-24.