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Statistical Analysis Plan (SAP)

Sponsor:	Circius Pharma AB Södra långebergsgatan 34-36 436 32 Askim Sweden
Study code:	CIR_001
Study title:	A prospective, Post-Market Clinical Follow-up investigation to verify performance and safety of Otinova® Ear Spray when used in the treatment of external otitis symptoms
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1 LIST OF ABBREVIATIONS

ADE – Adverse Device Effect
AE – Adverse Event
ASADE – Anticipated Serious Adverse Device Effect
BMI – Body Mass Index
CF – Clean File
CI – Confidence Interval
CIP – Clinical Investigational Plan
CRF – Case Report Form
ECG – Electrocardiography
FAS – Full Analysis Set
LOCF – Last Observation Carried Forward
MedDRA – Medical Dictionary for Regulatory Affairs
OE- Otitis Externa, external otitis
PPS – Per Protocol Set
SADE – Serious Adverse Device Effect
SAE – Serious Adverse Event
SAF – Safety Analysis Set
SAP – Statistical Analysis Plan
SAS – Statistical Analysis System
SOC – System Organ Class
STAT - Biostatistician
USADE – Unanticipated Serious Adverse Device Effect
WHO – World Health Organisation

2 INTRODUCTION

This Statistical Analysis Plan (SAP) gives details regarding the statistical analyses and data presentation outlined in the final Clinical Investigation Plan (CIP) for the study "A prospective, Post-Market Clinical Follow-up investigation to verify performance and safety of Otinova® Ear Spray when used in the treatment of external otitis symptoms." dated 22-Feb-2022.

3 CLINICAL INVESTIGATION DETAILS

3.1 Clinical Investigation Objectives

3.1.1 Primary objective

The primary objective is:

- To assess **reduction of swelling, erythema and otorrhea** by Otinova® Ear Spray when used in the treatment of external otitis.

3.1.2 Secondary objectives

The secondary objectives are:

- To assess the **reduction of itching, otalgia and tenderness** by Otinova® Ear Spray when used in the treatment of external otitis
- To investigate **reduction of pathologic microbes (bacteria, fungi, yeast)** in the ear canal by Otinova® Ear Spray when used in the treatment of external otitis
- To assess the **removal of moisture in the ear canal** related to external otitis
- To assess the **volume of the ear canal**
- To assess the **user handling** of Otinova® Ear Spray (ease of use of spray, facilitation of correct application)
- To assess **sleep disruption**
- To assess **use of pain relief medication**
- To assess **use of antibiotics**

3.1.3 Safety objectives

The safety objectives are:

- To assess the **safety** of Otinova® Ear Spray.

3.2 Clinical Investigation Design

3.2.1 General

This is a prospective, open, single-centre, single-arm clinical investigation designed to verify performance and safety of Otinova® Ear Spray when used by subjects with external otitis (otitis externa; OE) symptoms in need of treatment. The outcome will be evaluated and incorporated in the product specific CER and serve as a base for continuous investigations.

Following EC approval, 40 subjects suffering from OE symptoms will be recruited from one site in Sweden and receive treatment with Otinova® Ear Spray per instruction for use (twice daily during the test period, for 7 days). Three visits are planned for each subject, including a screening/baseline visit at the clinic, a follow-up visit at the clinic and a follow-up telephone visit to assess any safety issue. If considered necessary by the investigator, study nurse or subject, additional contacts (e.g., visits/phone calls/video calls) can be arranged.

The overall duration of the investigation is estimated to five months, including a four-month recruitment period. Expected duration of each subject's participation is 14 days. The investigation will be considered complete when the last subject has completed the last visit.

The treatment with the medical device will start at Baseline/Visit 1 if the subject fulfills all eligibility criteria. The treatment will continue for 7 days. Subject will use a diary for the treatments, any unexpected events and/or change in ear pain medications.

3.3 Schedule of clinical investigation procedures

Assessment	Visit 1 Screening/ Baseline	Visit 2 Follow-up	Visit 3 Follow-up
	Day 1	Day 8 (+1 days)	Day 14 (+/- 1 days)
Subject information sheet and informed consent	X		
Subject demographics[1]	X		
Inclusion/Exclusion criteria	X		
Relevant medical and surgical history	X		
History of OE[2]	X		
Pregnancy test (fertile women)	X		
Otoscopic exam incl. score	X	X	
Microbial swab testing	X	X	
Concomitant medication	X	X	X
Adverse event questions		X	X
Device instructions, start of treatment	X		
Diary[3] **instructions**	X		
Diary review and collection		X	
Device collection		X	
Usability questionnaire[4]		X	
End of investigation			X

Notes:
[1] Age, gender, height, weight, BMI
[2] Including previous episodes of OE, duration of diagnosis/age of onset, hearing impairment
[3] Diary for collecting data of device usage, symptoms, ear pain medication use, impact of ear pain on sleep
[4] Usability questionnaire including assessment of ease of use of spray and application

3.4 Number of Subjects

The study population will consist of 40 subjects with OE symptoms.

3.5 Methods of Assigning Subject to Device Groups

Each subject will be its own control, all subjects will receive the same treatment. No randomization will be performed.

3.6 Blinding

Not applicable.

4 STATISTICAL AND ANALYTICAL PLANS

4.1 Sample Size Justification

In order to show that the lower limit of the exact 95% confidence interval for the proportion cured regarding swelling, erythema and otorrhea will be higher than 50% with a probability of 80%, given a 0.75 proportion of cured at significance level 0.05, 33 evaluable subjects are needed. A drop-out rate of 10% is assumed and hence the sample size needs to be a minimum of 37 subjects. In order to account fo drop-outs as well as the inclusion of both children and adults, a sample size of 40 subjects was therefore chosen.

4.2 Definition of Analysis Sets

4.2.1 Safety Analysis Set (SAF)

The SAF will consist of all included subjects who started treatment.

4.2.2 Intention To Treat Analysis Set (ITT)

The ITT will include all subjects included in the SAF and have at least one set of efficacy measurements after treatment with the investigational device.

4.2.3 Per Protocol Set (PP)

The PP set will include all subjects included in the ITT set who:
Do not have any major CIP deviations which will affect the assessment of efficacy.

The final criteria for the PP set, regarding which CIP deviations that warrant exclusions, will be determined at the clean file meeting when all data on CIP deviations are available.

4.2.4 Use of Analysis Sets

The presentation of safety data will be based on the SAF.
Demographics and baseline data will be presented for both ITT and PP.
The primary and secondary analyses will be performed using both the ITT and the PP. The ITT will be considered as the main analysis.

4.3 Multiple Comparisons/Multiplicity

No adjustment for multiplicity of testing will be done. There will be one formal statistical analysis (primary efficacy endpoint) in line with the sample size determination. All other statistical tests will be considered as exploratory.

4.4 Handling of Drop-outs, Missing Data and Outliers

Outliers will be included in summary tables and listings and will not be handled separately. Available data from prematurely withdrawn subjects will be included in the analysis as far as possible. Missing data will not be imputed.

4.5 Adjustment for Confounders

Since the study has only one arm adjustment for confounders is not possible.

4.6 Multicenter Studies

Not applicable.

4.7 Examination of Subgroups

For the following subgroups, proportion cured regarding swelling, erythema and otorrhea with exact 95% CI and proportion improved regarding itching, otalgia and tenderness with exact 95% CI will be calculated:

- Age 5-17
- Age 18 and over.

4.8 Blind Review

Not applicable.

5 Variables

5.1 Baseline Characteristics and Demographics

The following baseline characteristics will be given in total for the ITT and PP population:

- Age
- Gender
- BMI
- Duration of diagnosis
- Age at onset of OE
- Hearing impairment

5.2 Efficacy variables

5.2.1 Primary efficacy variable

Clinical cure – analyzed as proportion of subjects who have all OE signs of swelling, erythema and otorrhea scored as 0 on a scale from 0-2 (0=none, 1=mild/moderate, 2=severe) after 7 days of treatment, based on otoscopic exam.

NOTE: One ear of each subject will be included in the analysis in this clinical investigation. It will be either on Left ear or Right ear.

5.2.2 The secondary efficacy variables

5.2.2.1 OE signs

A sum score will be created for the OE signs swelling, erythema and otorrhea. This sum score will be handled as a continuous variable. Analyzed as change in clinical sum score of clinical signs swelling, erythema and otorrhea from Visit 1 to Visit 2, based on the sum of the three scores (0-6).

5.2.2.2 OE symptoms

Changes of OE symptoms itching, otalgia and tenderness using a score from 0 to 4, where 0 = no symptoms, 1= Mild, 2 = Moderate, 3= Severe and 4 = Very Severe, as reported by the subject in diary. Analyzed as:

- Change in each of the clinical symptoms itching, otalgia and tenderness from Day 1 to Day 7. These variables will be regarded as ordered categorical and will be described by visit and by change (improved, no change and improved).
- Change in clinical sum score of clinical symptoms itching, otalgia and tenderness from Day 1 to Day 7, based on the sum of the three scores (0-12).
- Change in clinical symptoms itching, otalgia and tenderness from Day 1 to Day 3 and Day 5

5.2.2.3 Microbial cure

Prescence of pathologic microbes in the ear canal will be assessed by a microbial swab sample. Two (2) samplings will be performed, the first at Visit 1 and the second sampling at Visit 2. Microbial cure attained if all pre-therapy pathologic microbes are absent after 7 days of treatment. Analyzed as proportion of subjects cured.

5.2.2.4 Moisture in the ear canal

Reduction of moisture in the ear canal, otorrhea, between baseline (Visit 1) and after 7 days of treatment (Visit 2), by otoscopic exam, using a 3-point scale (0 = none, 1 = mild/moderate, 2 = severe), by otoscopic exam. Analyzed as proportion of subjects with signs of otorrhea at baseline on a scale from 0-2 (0=none, 1=mild/moderate, 2=severe) and show an improvement of 1 or more points. Analyzed as proportion of subjects with improvement and unchanged.

5.2.2.5 Volume of ear canal

Change of volume of the ear canal between baseline (Visit 1) and after 7 days of treatment (Visit 2), by otoscopic exam, using a 3-point scale (0 = none, 1 = mild/moderate, 2 = severe). Reduced swelling of the ear canal results in increased volume of the ear canal. Analyzed as proportion of subjects with signs of swelling at baseline on a scale from 0-2 (0=none, 1=mild/moderate, 2=severe) and show an improvement of 1 or more points and unchanged.

5.2.2.6 User handling

Subject experience of user handling of Otinova will be captured at visit 2, through a paper questionnaire to be filled out by the subject. The questionnaire will contain questions regarding ease of use of spray and ease of application. Each question will be answered by the subject by choosing the most correct answer on a scale or replying on a yes or no question. Individual items in questionnaire will be described by numbers and percentages for each answer.

5.2.2.7 Sleep disruption

Analyzed as proportion of subjects with less sleep disruption Day 7 compared to Day 1, reported in subject diary and proportion of parents with less sleep disruption Day 7 compared to Day 1, reported in subject diary.

Analyzed as change in sleep distruption from Day 1 to Day 3, Day 5 and Day 7 for subjects and parents.

5.2.2.8 Use of ear pain medication

Use of pain relief medication for ear pain, as reported in subject diary, replying on a yes or no question, described by numbers and percentages.

5.2.2.9 Use of antibiotics

Use of antibiotics for OE symptoms, as listed in Medication log, described by numbers and percentages.

5.3 Safety Variables check updated for MDR

Adverse Events

For full details on Adverse Events (AEs), Adverse Device Effects (ADEs), Serious Adverse Events

(SAEs), Serious Adverse Device Effects (SADEs) and Unanticipated Serious Adverse Device Effect (USADE), see final version of CIP.

Adverse Device Effect (ADE) *ISO 14155:2020*
Adverse event related to the use of an investigational medical device.

Note 1: This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.

Note 2: This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.

Note 3: This uncludes 'comparator' if the comparator is a medical device.

Adverse Event (AE) *ISO 14155:2020*
Untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device and whether anticipated or unanticipated.

NOTE 1: This definition includes event related to the investigational medical device or the comparator.

NOTE 2: This definition includes events related to the procedures involved.

NOTE 3: For users or other persons, this definition is restricted to events related to investigational medical devices.

Device Deficiency *ISO 14155:2020*
Inadequacy of an investigational medical device related to its identity, quality, durability, reliability, safety or performance. This may include malfunctions, use error, or inadequacy in the information supplied by the manufacturer.

Note 1: Device deficiencies include malfunctions, use errors, and inadequacy in the information supplied by the manufacturer including labelling.

Note 2: This definition includes device deficiencies related to the investigational medical device or the comparator.

Serious Adverse Device Effect (SADE) *ISO 14155:2020*
Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

Serious Adverse Event (SAE) *ISO 14155:2020*
Adverse event that led to any of the following:

a) death,
b) serious deterioration in the health of the subject, users, or other persons as defined by one or more of the following:
    i. A life-threatening illness or injury, or
    ii. A permanent impairment of a body structure or a body function including chronic diseases, or
    iii. In-patient or prolonged hospitalization, or
    iv. medical or surgical intervention to prevent life-threatening illness or injury, or permanent impairment to a body structure or a body function,

c) foetal distress, foetal death, a congenital abnormality, or birth defect including physical or mental impairment.

Note: Planned hospitalization for pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.

Serious health threat *ISO 14155:2020*
Signal from any adverse event or device deficiency that indicates an imminent risk of death or a serious deterioration in the health in subjects, users or other persons, and that requires prompt remedial action for other subjects, users or other persons.

Note: This would include events that are of significant and unexpected nature such that they become alarming as a potential serious health hazard or possibility of multiple deaths occurring at short intervals.

Unanticipated Serious Adverse Device Effect (USADE) safety*ISO 14155:2020*
Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment.

Note: Anticipated Serious Adverse Device Effect (ASADE) is an effect which by its nature, incidence, severity or outcome has been identified in the risk assessment.

6 TREATMENT INFORMATION AND EXTENT OF EXPOSURE

6.1 Active Treatment

Otinova® Ear Spray is intended be used to treat external otitis.

6.2 Extent of Exposure

Exposure to the Otinova® Ear Spray device will be presented by the number of doses per study subject.

6.3 Compliance of Study Product

Compliance the study product will be presented by the total number of times each subject has been treated with Otinova® Ear Spray . In accordance with the CIP (ref 1), Otinova® Ear Spray shall be used 14 times throughout the study period; morning and evening for 7 days. The total treatment time is 7 days.

6.4 Concomitant Medications

Subjects are allowed to continue their regular medication during the study, with exception for medication listed in the exklusion criteria. Concomitant medication data will be listed only.

7 STATISTICS

7.1 General statistical Methodology

This is a single-arm investigation with no comparison group. No randomization will be performed. The objective is to confirm performance and safety in subjects with OE symptoms.
Performance will be measured by proportion of subjects with all signs of the 3 OE parameters swelling, erythema and otorrhea scored as 0 at Visit 2. The sum score will handled as a continuous variable. Continuous variables are described with mean, SD, median and range and categorical variables with numbers and percentages.
Change in dichotomous and ordered categorical variables will be performed with Sign test and change in continuous variables will be performed with Fisher's non-parametric permutation test for

paired observations. Proportions of cured and improved will be given with exact 95% confidence interval.
No adjustment for confounders will be performed due to the single arm design.
The primary analysis, clinical cure of swelling, erythema and otorrhea, will be analyzed as proportions of subjects cured with exact 95% confidence interval. A Sum score will be created for swelling, erythema and otorrhea. This sum score will be handled as a continuous variable. Secondary endpoints of cure and improvement will be analyzed in the same way. All significance tests will be two-sided and conducted at the 5% significance level.

Individual items in questionnaire and diary will be described by numbers and percentages for each answer.

Distribution of number of doses and days will be described.

Demographics and baseline variables will be summarized for the study population.

For the following subgroups, proportion cured regarding swelling, erythema and otorrhea with exact 95% CI and proportion improved regarding itching, otalgia and tenderness with exact 95% CI will be calculated:
• Age 5-17
• Age 18 and over

Supportive figures will be given for primary and selected secondary efficacy variables, visit 1 and visit 2 on the x-axis, categorical variables as vertical bars and continuous variables as box-plots including means on the y-axis.

The incidences of adverse events, and adverse events that can be causally related (ADE) to the device, will be evaluated based on the event and severity. These AEs will be reported as frequencies (n), percentages (%), and mode.

7.2 Primary Efficacy Analysis

Primary statistical analysis will be the estimation with exact 95% confidence interval of proportion of subjects with all signs of swelling, erythema and otorrhea scored as 0 on a scale from 0-2 (0=none, 1=mild/moderate, 2=severe) at visit 2 (after 7 days of treatment) in the ITT-population.
If the lower limit of this 95% exact confidence interval is >50% then the pass criteria for the study is reached.
The distribution of the each of the three components in primary variable, swelling, erythema, otorrhea (0-3) will be given at visit 1 (Baseline), visit 2 (after 7 days of treatment) and change from visit 1 to visit 2.

7.3 Secondary Efficacy Analyses

The secondary efficacy analyses will be the analyses of all secondary variables in section 5.2.2 according to the methods given in section 7.1 "General Statistical Methodology" both on ITT- and on PP-population.

7.4 Pre-planned subgroup analysis

Primary efficacy and selected secondary efficacy variables will be performed on subjects divided into sub-groups Age 5-17 and Age 18 and over.

7.5 Correlations analysis efficacy variables with extent of exposure.

The extent of exposure will be divided into two groups <11, >= 11 doses. These to groups will be compared regarding selected efficacy variables. For dichotomous variables Fisher's exact test will be used, for continuous variables Fisher's non-parametric permutation test will be used and for ordered categorical variables Mantel-Haenszel chi square test will be used. Spearman correlation coefficient will be given for all correlations.

7.6 Exploratory Efficacy Analyses, Correlations with Primary efficacy variable.

For the following baseline variables, age, gender, BMI, Duration of diagnosis, age at onset of OE and hearing impairment, estimation of proportion cured will be given for each subgroup. Continuous variables will be described in tertiles. Odds ratios with 95% confidence interval will be given from univariable logistic regression together with area under the ROC curve.

7.7 Demographic and Baseline variables.

Demographic and Baseline variables will be summarized according to section 7.1 "General Statistical Methodology" on ITT population and on PP population.

7.8 Safety Analyses

7.8.1 Analyses

No statistical analyses are planned for safety parameters.

7.8.2 Presentation

AE/ADE:

The following summaries of AEs and SAEs will be given in total:

- Total number of AEs
- Total number of unique AEs
- Total number of unique, related AEs
- Total number (%) of subjects with at least one AE
- Total number (%) of subjects with at least one related AE
- Total number (%) which had AE as reason for premature discontinuation of study product

Severity, action taken, concomitant therapy started, and subject outcome of the AEs will be given in data listings only. AEs, which were reason for premature discontinuation of study product, will be listed separately.

Depending on the number of AEs reported, the most frequently reported (e.g. in more than 5% of the patients) AEs might be summarized separately.

The total number of SAEs and patients with a least one SAE will always be given. Further summaries of SAEs depending on the number of SAEs observed.

SAE/SADE:

SAEs/SADEs, if any, will be listed only.

8 Changes from the CIP before database lock

- Correlations analysis of selected efficacy variables with extent of exposure. Section 7.5

- Exploratory Efficacy Analyses. Correlations with Primary efficacy variable. Section 7.6

9 Listing of Tables, Figures and Listings

9.1 Listing of Tables

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14.1.2	Protocol Deviations Leading to Exclusion from PP Population
14.1.3.1	Demographics and Baseline Characteristics (ITT)
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14.2.1.1	Primary Efficacy Analysis (ITT)
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14.2.1.3	Primary Efficacy Analysis (PP Population)
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14.2.5	Exploratory Efficacy Analyses. Correlations with Primary efficacy variable. Univariable logistic regression. (ITT Population)
14.3.1.1	Duration of Exposure (Safety Population)
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14.3.2.2	Adverse Events, by System Organ Class and Preferred Term (Safety Population)
14.3.2.3	Adverse Events, by System Organ Class, Preferred Term and Maximum Reported Intensity (Safety Population)
14.3.2.4	Adverse Events, by System Organ Class, Preferred Term and Causality Assessment (Safety Population)
14.3.2.5	Serious Adverse Events, by System Organ Class and Preferred Term (Safety Population)
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9.2 Listing of Figures

Figure Number	Table Title
14.2.1.1-14.2.1.x	Figures of primary efficacy variable visit 2 and the components swelling, erythema and otorrhea by visit. Vertical bars on ITT population.
14.2.2.1-14.2.2.x	Figures of selected secondary variables by visit. Categorical variables as vertical bars and continuous as box-plots.

14.2.3.1-14.2.3.x	Items in the questionnaire will be presented with vertical bars.
14.2.4.1-14.2.4.x	Extent of Exposure (number of doses taken will be given as vertical-bars.

9.3 Listings

Listing number	Listing Title
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16.2.4.3	Prior and Concomitant Medications
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10 REFERENCES

11 APPROVAL

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