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Statistical Analysis Plan

Study title:	Evaluation of Safety and Pharmacodynamics of OP0201 compared to placebo in healthy adults
Study code:	EudraCt: 2016-003667-19
Indication:	Eustachian tube function
Investigational intervention:	OP0201
Comparator:	HFA 134a (placebo)
Sponsor (or representative):	University of Cologne Albertus-Magnus-Platz 50923 Cologne Germany
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Development phase (if appropriate):	Phase I
Principal investigator:	[redacted]
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Content

1 Background

This version of the statistical analysis plan (SAP) is based on Trial protocol OP0201-C001, version V04_01. This analysis plan details the analyses that will be performed for Trial OP0201-C001, a phase 1 study.

1.1 Trial objective

The primary objective of the trial is to assess the safety and tolerability of a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults.

The secondary objectives are to assess tympanic impedance of the Eustachian tube following a single intranasal dose of OP0201 compared to a single intranasal dose of placebo in healthy adults. Additionally, the study will explore whether a single intranasal dose of OP0201 compared to a single intranasal dose of placebo modulates ear pain during the hypobaric/hyperbaric atmospheric pressure chamber assessment in healthy adults [trial protocol, Section 1.3, 1.4].

1.2 Trial design

The Phase I clinical trial has a double-blind, randomized, placebo-controlled, single-dose, 2-period cross-over design. Period 1 is defined as the time from study drug administration on Day 1 until just before study drug administration on Day 8. Period 2 is defined as the time from study drug administration on Day 8 until the end of the study.

The trial will be conducted at a single center with 16 subjects being randomized in a 1:1 ratio to one of two treatment sequences: Sequence 1 (placebo-OP0201) or Sequence 2 (OP0201-placebo). Each subject will participate in the trial for up to 37 days and will have up to 3 scheduled visits and 2 telephone follow-up visits.

There will be a screening period from Day -28 to Day -1. On Day 1 subjects will be randomized to a treatment sequence and administered the masked study treatment of the first period. Subjects will be administered the investigational medicinal product (IMP) into each nare as four consecutive sprays, first to the left nare, followed by four consecutive sprays to the right nare until a total of eight sprays have been administered [trial protocol 3.6.1]. On Day 8, subjects will return to the trial site for the cross-over visit where they will receive the opposite masked treatment administered in the same way. Subjects will remain in the clinic for the duration of approximately 4-6 hours at each visit [trial protocol, Section 3.2].

A centralized blocked randomization stratified by gender (male/female) and non-significant baro-challenges (yes/no) will be performed via the internet-based randomization service TENALEA [trial protocol, Section 3.6.5].

Subjects who withdraw from the trial may be replaced at the discretion of the investigator upon consultation with the sponsor [trial protocol, Section 3.4]. If agreed, drop-outs will be replaced with trial subjects who will be assigned to the same treatment sequence as the subject who dropped-out. This will ensure that 16 subjects are treated at Day 1 and administered the cross-over treatment at Day 8 [trial protocol, Section 5].

Sample size

Using a one-sample t-test with a significance level of 0.05 (2-sided) and a power of 0.8, a sample size of 14.3 is required in order to detect an effect size (treatment difference standardized by standard deviation) of 0.8 (STATA Release 14). Since the ear pain and tympanic impedance variables will be analyzed primarily with a non-parametric method (whose asymptotic relative efficiency to the t-test under the assumption of a normal distribution is 0.955 (Conover, 1971)), the final sample size is adjusted to 16 (=ceiling(14.3/0.955)) in total and 8 per sequence.

1.3 Timing of analyses

After completion of the last visit of the last subject, when 16 subjects have completed the trial, data will be cleaned and approved by CTCC according to their Standard Operating Procedures (SOPs). The cleaned data, including the safety data set, will then be transferred to the statistician. The treatment codes will be unblinded and the final analysis will be performed on the basis of this finalized and approved SAP.

2 Analysis populations

2.1 Definitions

Safety population (SP):

All randomized trial subjects who received at least one spray of IMP in either nare. Evaluation in the SP will be “as-treated”.

Per-protocol population (PP):

All randomized subjects who received the two complete doses (4 sprays to each nare) of IMP at Day 1 and Day 8 with valid measures of eustachian tube opening pressure (ETOP) and eustachian

tube opening duration (ETOD) for passive phases 1 and 5 for both ears and for all three measurement times at Visits 2 and 4. Excluded are subjects with major protocol deviations (determined prior to database lock and unblinding of the data).

2.2 Application

Decision on the analysis populations is made prior to database lock and before the study is unblinded.

The primary analysis will be the as-treated analysis of safety in the safety population. Secondary analyses will be the as-treated analysis of ear pain and tympanic impedance measures in the safety and PP populations.

Subjects who never receive any study treatment even though they were randomized are not included in the analysis populations but demographic and screening variables are evaluated separately and AEs and SAEs will be listed.

2.3 Major protocol violations / Withdrawals

Withdrawals are included in the safety population if they fulfill the criteria as defined in the analysis populations. Drop-outs will be listed.

3 Visit windows

For data that are summarized on a per visit basis, the visit windows used for analysis are defined as follows:

Visit	Day	Analysis Visit Window	Case Report Form (Visit Name)
1	Days -28 to -1	Days -28 to -1	Screening
2	Day 1	Day 1 prior to 0 minutes	Baseline Day 1
2	Day 1	Day 1	Treatment Day 1
3	Day 2	Day 2 - 7	Phone Call Day 2
4	Day 8	Day 8 prior to 0 minutes	Baseline Day 8
4	Day 8	Day 8	Treatment Day 8
5	Day 9	Day 9 to actual exit date	Phone Call Day 9

For data that are summarized within a visit for tympanic impedance and ear pain assessments only, the visit windows used for analysis are defined as follows:

Visit	Day	Analysis Visit Window for Tympanic Impedance and Ear Pain assessments ONLY	Case Report Form (Visit Name)
2	Day 1	-90 to 0 minutes	Baseline Day 1
2	Day 1	0 to 75 minutes	1st Assessment
2	Day 1	76 to 150 minutes	2nd Assessment
4	Day 8	-90 to 0 minutes	Baseline Day 8
4	Day 8	0 to 75 minutes	1st Assessment
4	Day 8	76 to 150 minutes	2nd Assessment

All data collected within the analysis windows defined in the table above is considered to be valid for analysis even if data are collected outside the windows defined in the protocol. Data documented as unscheduled visits in the database will still be considered for analysis, if the time

of measurement lies within the time frame defined above (analysis windows). If more than one data entry is documented for one variable, one person in one analysis window, the last measurement of this value will enter the analysis.

4 Trial centers

This clinical trial will be carried out at a single center, the Department of Otorhinolaryngology, Head and Neck Surgery, University of Cologne, in Germany. The planned sample size is 16 subjects in the PP population.

5 Analysis variables

The analysis variables are described in the protocol including safety variables [trial protocol 3.7.1], pharmacodynamic variables [trial protocol 3.7.2], efficacy variables [trial protocol 3.7.3] and demography and baseline characteristics [trial protocol 3.7.4].

In addition, the following variables will be analyzed:

- Concomitant medications
- Dose (no. of sprays)
- Reason for withdrawal

6 Data conventions

6.1 Handling of missing values and outliers

6.1.1 Missing values

Missing values will not be substituted, unless stipulated explicitly.

If agreed between the investigator and the Sponsor, subjects who drop out will be replaced with a new subject who will be assigned to the same treatment sequence as the subject who dropped-out.

Values that are classified as ‘not done’, ‘not assessable’, or ‘not evaluable’ will be treated as missing in summary tables.

6.1.2 Outliers

For the main analysis of quantitative variables, non-parametric methods, which are less sensitive to outliers, will be used.

6.2 Baseline and change from baseline

For variables with baseline measures assessed on Day 1 and/or Day 8, the last non-missing measurements prior to study treatment on Day 1 will serve as the baseline values for Day 1 analyses, and the last non-missing measurements obtained on Day 8 prior to study treatment on Day 8 will serve as the baseline value for Day 8 analyses.

Differences for change from baseline analyses will be calculated as post-treatment minus baseline.

6.3 Unscheduled visits

Unscheduled visits may occur due to AEs. Data collected at such an unscheduled visit will be used for categorization of visit windows that correspond with the scheduled visits (see Section 3).

Retests are only valid if they occur in the analysis visit window. The retest data will then be used for categorization of visit windows that correspond with the scheduled visits.

6.4 Units

Metric system units will be used as applicable: kilograms for body weight, centimeters for height and System International (SI) units for laboratory data.

6.5 Rounding

Probability values will be rounded to three decimal places. If the result is a value of 0.000, it will be displayed as <0.001. If the result is a value of 1.000, it will be displayed as >0.999.

6.6 Dictionaries

Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA V21.1) nomenclature. Concomitant medications will be coded using Anatomical-Therapeutic-Chemical (ATC) Classification as denoted in the World Health Organization (WHO) Drug Dictionary (WHODrug Global 2018 Sep 1). Medical history will be coded using MedDRA V21.1 nomenclature.

6.7 Summary statistics

Continuous or discrete data will be presented descriptively by sample size (N), arithmetic mean, standard deviation, median, distribution-free confidence interval for median (Meeker et al., 1991, Section 5.2), 1st quartile, 3rd quartile, minimum, and maximum. Unless otherwise stated, for quantitative variables with a continuous or discrete response range, comparisons between two independent groups will be performed with the Wilcoxon rank-sum test and comparisons between

two dependent groups will be performed with the Wilcoxon signed-rank test. All tests will be 2-sided. P-values < 0.05 are considered to be significant. If confidence intervals (CI) are to be provided for 2-group comparison, the exact 95% 2-sided Hodges-Lehmann intervals will be calculated (Randles and Wolfe, 1979, p. 180).

Categorical data will be presented descriptively by sample size (N), frequency count, and percentage. If a statistical test for 2-group comparison is conducted on categorical data, Fisher's exact test is used. If confidence intervals are presented, exact Clopper-Pearson intervals will be provided.

7 Statistical analyses / methods

The analysis will essentially be descriptive; therefore, the significance level is not adjusted for multiple testing. If not otherwise stated, a p-value < 0.05 is defined as significant. Tests and CIs are 2-sided. CIs are calculated with 95% limits.

Comparisons between treatments in the cross-over setting will be made according to the Grizzle two-stage approach (Grizzle, 1965). The primary cross-over analysis will be non-parametric because the determination of the distribution of variables is difficult with respect to the small sample size and because non-parametric testing accounts for outliers. In a sensitivity analysis, a parametric cross-over analysis will be performed. Treatment comparisons will be carried out without covariates. For tied observations, the average of the ranks they would have had if they would have been slightly different will be used. We assume that the distributions of the values that are to be compared have the same shape, but are possibly shifted in location by some amount. Under this assumption, the Wilcoxon rank-sum test may be interpreted as a test of equality between medians of two populations.

7.1 Cross-over analysis method

We will analyze quantitative variables in a cross-over design with baseline data in both periods. Therefore, the differences between baseline and post-treatment outcomes ('post-minus-baseline values') in each period will be calculated. The analysis is done as proposed by Grizzle (1956) assuming a high correlation between the baseline and post-treatment values (correlation > 0.5) (Meyer, 2017).

First stage

The potential carry-over effect and period effect in a cross-over design will be tested for a significant deviation from 0.

Second stage

The significance of the carry-over effect will guide the choice of the appropriate treatment effect. The carry-over effect is considered to be significant if the p-value is < 0.10. If the carry-over effect is not significantly different from 0, the treatment effect will be derived in terms of the cross-over treatment effect as described below. Otherwise, in presence of a significant carry-over effect, the treatment effect will be derived in terms of the Period 1 only treatment effect.

The two-stage procedure will be done for the per-protocol population only. For the safety population, only the Period 1 only treatment effect will be estimated.

Tests and effect estimates will be derived as follows:

- Test for carry-over effect

The carry-over effect is tested by calculating the intraindividual sum of the post-minus-baseline values of Period 1 and Period 2 and comparing these between study sequences with a Wilcoxon rank-sum test.

- Test for period effect

The period effect is tested by calculating the intraindividual differences of the post-minus-baseline values of Period 1 minus Period 2 for one sequence and Period 2 minus Period 1 for the other sequence and comparing these between study sequences with a Wilcoxon rank-sum test.

- Cross-over treatment effect

For the test of the cross-over treatment effect the intraindividual differences of Period 2 minus Period 1 of the post-minus-baseline values will be calculated and compared between study sequences with a Wilcoxon rank-sum test. Assuming that distributions are of the same shape, we are testing the null hypothesis of equal medians in both sequences, meaning that OP0201 and placebo do not differ in outcome.

To estimate the cross-over treatment effect, firstly, the intraindividual differences of Period 2 minus Period 1 of post-minus-baseline values will be calculated (i.e. OP0201 minus placebo in sequence 1 (placebo-OP0201), placebo minus OP0201 in sequence 2 (OP0201-placebo)). Then, the cross-over treatment effect will be estimated by the Hodges-Lehmann estimate, i.e., the median of all possible pairs of differences between sequence 1 and sequence 2 of the ‘Period 2 minus Period 1’ values and dividing this median by two.

The exact Hodges-Lehmann CI for the cross-over treatment effect will also be provided (Randles and Wolfe, 1979, p. 180).

- Period 1 only treatment effect

To test for a Period 1 only treatment effect, Period 1 will be compared between study sequences with a Wilcoxon rank-sum test. Assuming that distributions are of the same shape, this tests the null hypothesis of equal medians in both sequences, without superimposed carry-over and period-effects. The Period 1 only treatment effect will be estimated by the Hodges-Lehmann estimate, i.e., the median of all possible pairs of differences of Period 1 between two sequences. The exact Hodges-Lehmann CI for the treatment effect will also be provided (Randles and Wolfe 1979, p. 180).

Sensitivity analysis

Additionally, a parametric sensitivity analysis will be performed. Therefore, the two-stage procedure (for the PP population) as well as the evaluation of the Period 1 only treatment effect in the Safety population will be performed with the 2-sample t-test. An F-test of equality of variances will be performed to guide the choice of the appropriate 2-sample t-test. A result of the t-test for unequal variances is provided if the F-test results in a p-value < 0.05. The mean cross-over treatment effect over both periods can be estimated as the mean of the intraindividual differences in sequence 1 minus the mean of the intraindividual differences in sequence 2 divided by two. The mean Period 1 only treatment effect will be estimated as the difference of the means of the post-minus-baseline values of the two sequences in Period 1. Furthermore, 2-sided confidence intervals for the difference between mean effects of OP0201 and placebo based on the Student's t distribution will be provided. A mixed model repeated measures (MMRM) approach may also be used to evaluate and control for effects of period and sequence and to estimate the treatment effect based on all available data (assuming any missing observations are missing at random).

Graphical presentation

As a graphical representation of the cross-over analysis in the per-protocol and safety population, appropriate sequence-by-period plots will be produced for the tympanic impedance variables. The plots will show the median with distribution-free confidence interval (Meeker et al., 1991, Section 5.2) of the change from baseline of a variable for each sequence, period, and assessment.

7.2 Subject disposition

Absolute frequencies of subjects per sequence and period in the safety population will be calculated. Absolute frequencies of subjects per sequence and period in the PP population will be calculated.

Frequencies will be shown in a subject flow diagram. Separately for each sequence, the diagram will present the number of subjects included for screening in total, for randomization, at Day 1 and Day 8 in safety and per-protocol population. Number of and reasons for drop-outs will be included.

A subject disposition and exit status will be presented for each of the analysis populations (SP, PP) by study sequence and for both sequences combined. The tables will include subject disposition: enrolled, completed, and discontinued. The reason for premature discontinuation will be displayed by sequence and overall. A listing will be generated for discontinued subjects with exit reasons, treatment sequence and days since randomization.

7.3 Demography and screening characteristics

Demographic and screening variables will be summarized as randomized by sequence and in total for each population.

Continuous variables (age, weight, height, BMI and nasal cavity length for left and right nares) will be summarized by sequence and compared between sequences by Wilcoxon rank-sum test. BMI will be calculated as weight/height^2 [kg/m^2].

The categorical variables gender and race (White versus non-White) will be presented using frequency tabulations and compared between sequences by Fisher's exact test for each population. The distribution of the individual race groups (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White) will also be displayed.

7.4 Baseline characteristics

Baseline characteristics for the categorical stratification factor non-significant baro-challenges (yes/no) will be summarized by sequence and in total for each population and presented using frequency tabulation without any statistical comparisons.

7.5 Concomitant medication

Medication that was stopped at least one day before Day 1 is considered 'pre-treatment' for Period 1. Medication that started 2 or more days after Day 1 and stopped at least the day before Day 8 is considered 'pre-treatment' for Period 2. All other medications that are ongoing on the day of treatment administration or were started the day or the day after treatment administration will be considered "concomitant" for the respective period. Medications recorded with insufficient exposure dates to determine whether or not they were concomitant will be considered concomitant. If the determination of the period of the concomitant medication is not possible, the concomitant medication is counted for both periods. The frequency and percentage of subjects

receiving concomitant medications will be displayed by treatment and by the preferred term (WHO Drug Dictionary ATC classification). No statistical comparison will be performed.

Prior and concomitant medications will separately be listed by subject, preferred term of medication, dose, sequence, start date, end date, the concomitant medication number and referring AE number, gender, age and indication.

7.6 Concurrent procedure

Concurrent procedures will be listed by subject, sequence, procedure, date, reason, whether the procedure is related to AE/SAE, and the referring AE number. No statistical comparison will be performed.

7.7 Past medical, surgical and ear history

Subjects with medical, surgical, and ear history with onset dates prior to the Screening Visit will be tabulated and presented by primary system organ classification (SOC) and preferred term of MedDRA code for each sequence and overall for each population. No statistical comparison will be performed. A listing of the data will be provided.

7.8 Exposure to study treatment / Compliance

The number and percentage of subjects who comply with 4 sprays per nare as well as those with <4 or >4 sprays per nare for at least one nare will be calculated per day and per sequence for each population. No statistical comparison will be performed. A listing of the originally assigned sequence, treatment, non-significant baro-challenges, start date of study drug administration, start and stop time of study drug administration and total dose right and left will be provided.

7.9 Primary analyses

Safety variables will be evaluated in the safety population.

7.9.1 Adverse events / Serious adverse events

For each subject, AEs will be collected from the time after signed informed consent until all symptoms have subsided, pathological laboratory values have returned to pre-event levels, a plausible explanation is found for the AE/ADR, or the trial subject has died

AEs will be coded from the verbatim text into preferred term and the primary SOC using MedDRA dictionary.

7.9.1.1 Pre-treatment adverse events and serious adverse events

Pre-treatment AEs are defined as the AEs that occur after obtaining informed consent and prior to administration of any study treatment. These AEs will be summarized by primary SOC, preferred term, and severity for each sequence and overall.

No statistical comparisons will be conducted.

A listing of pre-treatment AEs, including SAEs, will be created.

7.9.1.2 Treatment emergent AE and treatment emergent SAE

A treatment-emergent AE (TEAE) will be defined within treatment period as an AE that began or changed in severity or seriousness during or after IMP administration and no later than 24 hours after IMP administration. AEs with insufficient date or time information to determine whether or not they were treatment-emergent will be considered treatment-emergent for a given period if partial start date and time data allows assignment to a specific period; otherwise, the AE will be assigned to the period in which the subject received OP0201.

Treatment emergent AEs and SAEs will be analyzed by treatment sequence and treatment period.

One listing of all TEAEs per subject with sequence and period, AE onset date and time, AE number, description (reported term), preferred term (MedDRA code), severity, causal relationship to study drug (see trial protocol Section 6.2.2, defined as related or not related), seriousness, and resolution will be provided. SAEs will be listed additionally with seriousness criteria.

Summary tables will be generated as follows for subjects with TEAEs:

1) Overall incidence of subjects with TEAEs: This table will summarize by sequence*period and overall incidence of any AE, treatment-related AE, any SAE, treatment-related SAE, drug withdrawn due to an AE, and subject deaths during the study. Incidence of subjects with TEAEs will be summarized as described in 6.7.

2) By maximum severity of preferred term within primary SOC: This table will summarize by sequence*period and in total the incidence rates of the maximum severity of each preferred term within a primary SOC. The primary SOCs and preferred terms within each primary SOC will be sorted by descending AE incidence within the total group (i.e., not separately within each sequence). If a subject has multiple reports coding to the same preferred term within a SOC, the maximum severity for that subject will be used and that subject will be counted only once for that preferred term within that primary SOC.

Note: 'Sequence*period' denotes all combinations of the two study sequences with OP0201 and placebo (sequence 1 with OP0201, sequence 1 with placebo, sequence 2 with OP0201, sequence 2 with placebo).

7.9.1.3 Non-treatment-emergent AE

AEs that are not pre-treatment or treatment-emergent will be classified as non-treatment-emergent AEs and will be summarized by primary SOC, preferred term, and severity for each sequence and overall. They will also be listed with AE onset date and time, AE number, description (reported term), preferred term (MedDRA code), severity, causal relationship to study drug (see trial protocol Section 6.2.2, defined as related or not related), seriousness, and resolution will be provided. SAEs will be listed additionally with seriousness criteria.

7.9.2 Otoscopy

Otoscopy will be collected seven times during the trial: at Screening, Day 1 (Baseline, and at two post baseline assessment times [0-60 minutes and 90-150 minutes]), and Day 8 (Baseline and two post baseline assessment times [0-60 minutes and 90-150 minutes]).

A listing of the otoscopy data will be provided.

The incidence for the right ear and, separately, for the left ear will be summarized by sequence*period for all categories. 95% CIs will be calculated for the incidence of shifts from Baseline to 0-60 minutes and to 90-150 minutes, from normal to abnormal for the right ear and, separately, for the left ear by sequence*period, as described for categorical variables in Section 6.7. Tables presenting all changes from Baseline to 0-60 minutes and to 90-150 minutes (from/to normal from/to abnormal) for the right and left ear will be given.

7.9.2.1 Contour

Contour has the following categories: normal, retracted, full, bulging, perforated, or not assessable. The categorization in normal/abnormal will be as follows:
normal = normal,
abnormal = retracted, full, bulging, or perforated.

7.9.2.2 Color

Color has the following categories: normal, partly red, completely red, or not assessable. The categorization in normal/abnormal will be as follows:
normal = normal,
abnormal = partly red or completely red.

7.9.2.3 Fluid behind the tympanic membrane (TM)

Fluid has the following categories: no or yes. The categorization in normal/abnormal will be as follows:
normal = no,
abnormal = yes.
If fluid behind the tympanic impedance is 'yes', color will be rated as yellow, translucent, red, blue, black, or not assessable.

7.9.2.4 Translucency

Translucency has the following categories: translucent, semi-opaque, opaque, or not assessable. The categorization in normal/abnormal will be as follows:

normal = translucent

abnormal = semi-opaque or opaque

7.9.2.5 TEED classification

Teed classification has the following categories: TEED 0, TEED 1, TEED 2, TEED 3, or TEED 4. The categorization in normal/abnormal will be as follows:
normal = TEED 0,
abnormal = TEED 1, TEED 2, TEED 3 or TEED 4.

7.9.3 Tympanometry

Tympanometry will be collected at Screening, Day 1 at Baseline and at 150-215 minutes, and Day 8 at Baseline and at 150-215 minutes.

A listing of the tympanometry data will be provided.

The incidence of results for right ear and, separately, for left ear following tympanometry results, referred to as tympanograms, will be summarized as Type A, Type B, and Type C by sequence*period. A shift table will be provided presenting changes from Type A and C to Type A,

B and C from baseline to 150-215 min for the right ear and, separately, for the left ear by sequence*period.

7.9.4 Nasal and epipharynx endoscopy

7.9.4.1 Nasal endoscopy

Nasal Endoscopy will be collected at Screening, Day 1 Baseline and 90-150 minutes, and Day 8 Baseline and 90-150 minutes.

A listing of the nasal endoscopy data will be provided.

The incidence of results for normal and abnormal evaluations will be summarized for the right nare and, separately, for the left nare by sequence*period. In addition, for comparison of treatments 95% CIs will be calculated for the incidence of shifts from Baseline to 90-150 minutes from normal to abnormal for the right nare and, separately, for the left nare by sequence*period, as described in Section 6.7. A table presenting all changes from Baseline to 90-150 minutes (from/to normal from/to abnormal) for the right and left ear will be given.

7.9.4.2 Epipharynx endoscopy

The single measurement of the epipharynx endoscopy will be collected at Screening, Day 1 Baseline and 90-150 minutes, and Day 8 Baseline and 90-150 minutes.

A listing of the epipharynx endoscopy data will be provided.

The incidence of results for normal and abnormal evaluations will be summarized for each time point by sequence*period. In addition, for comparison of treatments 95% CIs will be calculated for the incidence of shifts from Baseline to 90-150 minutes from normal to abnormal by sequence*period, as described in Section 6.7. A table presenting all changes from Baseline to 90-150 minutes (from/to normal from/to abnormal) for the epipharynx will be given.

7.9.5 12-Lead electrocardiography (ECG)

A single 12-lead electrocardiography (ECG) will be performed at Screening to ensure the trial subject meets study eligibility criteria. The screening ECG will not be analyzed.

Triplicate 12-lead ECGs will be performed at Day 1 Baseline and 150-215 minutes, and Day 8 Baseline and 150-215 minutes. The ECGs will be assessed by a central ECG reading center that will determine whether each single ECG is normal or abnormal or not evaluable. This data will be provided directly to IMSB.

12-lead ECG parameters and comments related to 12-lead ECG will be listed. The categories normal and abnormal will be summarized descriptively by sequence*period, as described in Section 6.7.

The triplicate ECG is defined as abnormal if at least one of the single ECGs is abnormal. The triplicate ECG is defined as not evaluable if all single ECGs are not evaluable. Shift tables will be displayed for the categories normal and abnormal. For Day 1 and Day 8, the incidence of shifts from normal to abnormal with 95% CI will be provided as described in Section 6.7.

7.9.6 Physical examination

A physical examination is to be recorded at Screening and at Day 8 150-215 minutes. Categories to be examined are general appearance, overall status of skin, head, neck, trunk, eyes, heart and lungs (e.g. breathing sounds), abdomen, extremities, lymph nodes and 'other'. Each category is assessed as normal or abnormal or not done.

A listing of the physical examination data will be provided. Furthermore, a listing of physical examinations assessed as 'other' will be provided.

For Screening and the Day 8 150-215 minutes assessment, for each of the physical examination categories, the incidence of normal and abnormal will be summarized by sequence. 95% CIs will be calculated for the incidence of shifts from Screening to 150-215 minutes on Day 8, from normal to abnormal by sequence, as described for categorical variables in Section 6.7. Tables presenting all changes from Screening to 150-215 minutes on Day 8 (from/to normal from/to abnormal) will be given.

7.9.7 Vital signs

Systolic and diastolic blood pressure (mmHg), pulse rate (beats/min), respiratory rate (breaths/min) and body temperature (^{0}C) are to be recorded at Screening, Day 1 Baseline and 150-215 minutes, and Day 8 Baseline and 150-215 minutes.

A listing of the vital signs data will be provided.

All variables will be summarized descriptively by sequence*period, as described in Section 6.7. Additionally, for each of these variables, the change from Baseline to 150-215 minutes will be presented descriptively by sequence*period as described in Section 6.7.

7.9.8 Clinical laboratory evaluations

Laboratory data will be analyzed for Screening and Day 8 150-215 minutes, and will include hematology, blood serum chemistry variables, and urinalysis (as described in Section 3.7.1 of the trial protocol).

The original results of the clinical laboratory tests will be exported in a separate dataset by the local clinical laboratory of the University Hospital Cologne. Based on this dataset, laboratory results for Screening and Day 8 150-215 minutes will be summarized by visit and sequence as continuous variables (described in Section 6.7), as well as the change between the two assessments.

The following laboratory values will not be shown in the data export of the laboratory if they are zero. Therefore, missing lab values will be replaced by zero:

Basophils (%)

Eosinophils (%)

Lymphocytes (%)

Monocytes (%)

Neutrophils (%)

White blood cell count (WBC)

Listings of hematology, biochemistry and urinalysis data (normal/abnormal) will be provided as well as comments of original laboratory parameters.

A listing with all abnormal laboratory values indicating clinical significance will be provided.

Shift tables will be displayed for the categories decreased, normal, increased. For Day 8, the incidence of shifts from normal or not clinically significantly abnormal to abnormal and clinically significant with 95% CI will be provided as described in Section 6.7.

7.9.8.1 Hematology

Hematology variables include hemoglobin [g/dL], % hematocrit, red blood cell count (RBC), white blood cell count (WBC), % neutrophils, % lymphocytes, % monocytes, % basophils, % eosinophils, platelet count, partial thromboplastin time and prothrombin time.

7.9.8.2 Biochemistry

Chemistry variables include sodium, potassium, chloride, magnesium, phosphorus, calcium, creatinine, urea nitrogen, uric acid, total bilirubin, direct bilirubin, alkaline phosphatase (total), GOT

(glutamic-oxaloacetic transaminase), GPT (glutamicpyruvic transaminase), GGT (gamma glutamyltransferase), albumin, total protein.

7.9.8.3 General urinalysis

Urinalysis variables include pH, protein, ketones, bilirubin, blood, nitrite, urobilinogen, specific gravity.

Urine microscopy is performed if needed and reported as a comment. Comments will be listed by sequence and visit.

7.9.8.4 Urine drug screen

A urine drug screen is obtained only at the Screening Visit. No analysis and no listing is planned for this variable.

7.10 Secondary analyses

The secondary analyses aim to analyze the treatment effect for ear pain and continuous tympanic impedance variables. The analysis sets for statistical tests are based on the Safety and PP populations. All effects (treatment, carry-over, and period effects) of the cross-over analysis will be tested according to Section 7.1 in the PP population. However, it is reasonable to assume that for most subjects not included in the PP population a meaningful cross-over difference cannot be calculated. Therefore, the treatment comparison in the safety population (as-treated) will only be based on Period 1.

In the following sections regarding tympanic impedance variables, phase 1+5 connotes that for every subject the mean value of phase 1 and phase 5 is calculated and analyzed.

For ear pain and continuous tympanic impedance variables, descriptive tables as described in Section 6.7 will be provided for all designated time points by sequence*period.

The results of the cross-over analysis will be presented in tables providing p-values regarding treatment (cross-over (for PP population only) and period 1 only (for both PP and Safety populations), carry-over (for PP population only), and period effect (for PP population only), as well as estimated effect sizes along with CIs for treatment effects.

7.10.1 Ear pain

Subjects will score ear pain immediately prior to (Baseline Day 1 and Day 8) and during the hyperbaric chamber evaluation on Day 1 and Day 8, at 0-60 minutes and 90-150 minutes, respectively. Ear pain will be scored by the subjects separately for the left and right ear using an

11-point, whole number, numeric rating scale where 0 = no pain and 10 = worst pain imaginable. A total of three assessments per subject will occur during each hyperbaric chamber evaluation; at the end of phase 1 (during phase 2), at the end of phase 3 (during phase 4) and at the end of phase 5 after completion of the pressure chamber evaluation.

The ear pain data will be listed.

The analysis of ear pain will focus on change of pain from Baseline to assessment for Day 1 and Day 8, for phase 1 (0-60min & 90-150min), phase 3 (0-60min & 90-150min), phase 5 (0-60min & 90-150min). The change of ear pain from Baseline to assessment will be described as the change of the sum of pain of right and left ear, and in addition as the change in worst reported pain across both ears. Thereby, worst reported pain across both ears is defined as the worst pain of either the right or the left ear determined separately for Baseline and the respective assessment. These measures will be summarized as described in Section 6.7 for continuous variables by sequence*period and analyzed for treatment effect in the cross-over design using Grizzle's two-stage procedure (Grizzle, 1965; for PP population) and based on the first period only (for Safety population) according to Section 7.1. Hodges-Lehmann point estimate and 95% 2-sided confidence interval for the median of treatment effect differences between active and placebo will be provided. Furthermore, tables providing the prevalence of pain score >0 in each phase at baseline, i.e. prior to treatment on Day 1 or 8, will be displayed by sequence*period. Additionally, incidence of change from Baseline with three categories (1) 'no change', (2) 'worsening' (i.e., higher score than baseline), and (3) 'improvement' (i.e., lower score than baseline) will be produced to describe change from Baseline for right and left ear separately.

7.10.2 Tympanic impedance measures

Data is collected using study specific software that produces a subject specific pressure profile of five phases that are displayed as 'pressure curves'. All measurements followed a standard protocol of decompression (1 bar–0.8 bar (phase 1) and 1.2 bar–1 bar (phase 5)) and compression (0.8 bar–1.2 bar (phase 3)). In phases 2 and 4 the pressure is kept constant. During phases of decompression and phases of compression, the impedance is recorded on both sides (right and left tympanic membranes) constantly (each displayed as a curve). For this purpose, a size-adjusted rubber earplug is fitted in the ear canal. A 226 Hz tone is delivered by a loudspeaker in the direction of the tympanic membrane. Reflections of the acoustic signal are detected and transmitted to study specific computer software for analysis.

Figure 7-1 Standard Pressure Profile for Phases in Pressure Chamber

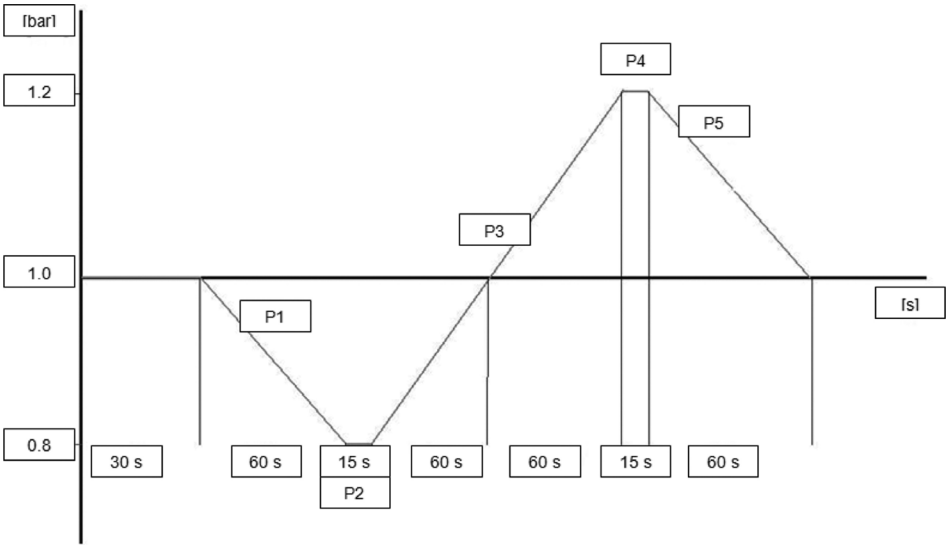

Figure 7-1 represents the standard pressure profile for phase 1 (P1), phase 3 (P3) and phase 5 (P5) in the hypobaric/hyperbaric pressure chamber. X axis: time (seconds). P1 to P5: Phase 1 to Phase 5. Y axis: pressure level (bar) in relation to atmospheric pressure.

7.10.2.1 Eustachian tube opening pressure (ETOP)

Eustachian tube opening pressure (ETOP) is calculated as the absolute value of the difference between the “beginning” atmospheric pressure and the pressure at which the Eustachian tube opens each time during that phase. Thus, the first ETOP value is determined by comparing the opening pressure with the actual beginning atmospheric pressure in the respective phase. However, an accurate value for the ET opening pressure can only be determined for the first measurement. Every following opening can only be measured in comparison to the "closing pressure of the prior opening". ETOP change from baseline is calculated as defined in Section 6.2.

ETOP change will be analyzed for phase 1 and phase 5 by using only the first ETOP. For phase 3, the average of all ETOP changes will be analyzed.

The ETOP data will be listed.

All outcomes will be analyzed as change from Baseline of the respective Period for left and right ear separately, and also for the mean of both ears. Additionally, the mean change from Baseline of phases 1+5 will be calculated for left ear, right ear, and mean of both ears. These measures will be summarized as described in Section 6.7 for continuous variables by sequence*period.

Furthermore, change from Baseline of ETOP of phase 1, 3, and 5 for right, left and mean of both ears and mean change from Baseline of ETOP of phase 1+5 for right ear, left ear and mean of both ears for 0-60 and 90-150 minutes at Day 1 and 8 will be analyzed for treatment effect in the cross-over design using Grizzle's two-stage procedure (Grizzle, 1965; for PP population) and based on the first period only (for Safety population) (see Section 7.1). Hodges-Lehmann point estimate and 95% 2-sided confidence interval for the median of treatment effect differences between active and placebo will be provided. For these analyses appropriate sequence-by-period plots will be produced (see Section 7.1).

7.10.2.2 Eustachian tube opening duration (ETOD)

Eustachian tube opening duration (ETOD) is the period of time between the opening of the Eustachian tube and the subsequent closing of the Eustachian tube. ETOD will be analyzed for phase 1 and phase 5 by using only the first ETOD. For phase 3, the average of all ETOD measurements will be analyzed.

The ETOD data will be listed.

Change from Baseline of ETOD of phase 1, 3, and 5 for right, left and mean of both ears and mean change from Baseline of ETOD of phase 1+5 for right ear, left ear and mean of both ears for assessments at 0-60 and 90-150 minutes at Day 1 and 8 will be presented according to Section 6.7 for continuous variables by sequence*period. Furthermore, change from Baseline of ETOD will be analyzed for treatment effect in the cross-over design using Grizzle's two-stage procedure (Grizzle, 1965; for PP population) and based on the first period only (for Safety population) according to Section 7.1. Hodges-Lehmann point estimate and 95% 2-sided confidence interval for the median of treatment effect differences between active and placebo will be provided. For these analyses appropriate sequence-by-period plots will be produced (see Section 7.1).

7.10.2.3 Eustachian tube opening frequency (ETOF)

ETOF will be analyzed for phase 1, phase 3 and phase 5 as the number of openings per minute in the respective phase.

The ETOF data will be listed.

Change from Baseline of ETOF of phase 1, 3, and 5 for right, left and mean of both ears and mean change from Baseline of ETOF of phase 1+5 for right ear, left ear and mean of both ears for assessments at 0-60 and 90-150 minutes at Day 1 and 8 will be presented according to Section 6.7 for continuous variables by sequence*period. Furthermore, change from Baseline of ETOF will be analyzed for treatment effect in the cross-over design using Grizzle's two-stage procedure

(Grizzle, 1965; for PP population) and based on the first period only (for Safety population) according to Section 7.1. Hodges-Lehmann point estimate and 95% 2-sided confidence interval for the median of treatment effect differences between active and placebo will be provided. For these analyses appropriate sequence-by-period plots will be produced (see Section 7.1).

7.10.2.4 Eustachian tube closing pressure (ETCP)

Eustachian tube closing pressure (ETCP) will be assessed only during phase 1. Closing pressure is the difference of the pressure at a minimum after a tube opening to the initial pressure at the beginning of the decompression phase. The ETCP will be the mean of the first 5 local minima in phase 1.

The ETCP data will be listed.

Change from Baseline of ETCP of phase 1 for right, left and mean of both ears for assessments at 0-60 and 90-150 minutes at Day 1 and 8 will be presented according to Section 6.7 for continuous variables by sequence*period. Furthermore, change from Baseline of ETCP will be analyzed for treatment effect in the cross-over design using Grizzle's two-stage procedure (Grizzle, 1965; for PP population) and based on the first period only (for Safety population) according to Section 7.1. Hodges-Lehmann point estimate and 95% 2-sided confidence interval for the median of treatment effect differences between active and placebo will be provided. For these analyses appropriate sequence-by-period plots will be produced (see Section 7.1).

7.11 Further evaluations

Subjects who were enrolled and randomized but did not receive trial treatment are not included in the analysis populations. Pre-treatment AEs and SAEs and reasons for drop-out will be listed. Demographic tables will be provided for age, gender, race, and prior history of baro-challenges when flying or diving (yes/no). Unscheduled visits that were not analyzed (i.e.,were either outside the analysis windows or were one of multiple visits within the analysis windows that did not replace scheduled visits) will be listed for all data.

7.12 Planned subgroup analyses

A descriptive subgroup analysis will explore differences according to the stratification parameters gender (male/female) and the subject self-reported information on prior history of baro-challenge when flying or diving (yes/no). All descriptive tables (all time points) will be provided for males and females and separately for the two groups of subjects with different prior history of baro-challenges

when flying or diving (yes/no) if more than 5 subjects belong to each group for the following outcomes:

- Severe AEs, SAEs, clinically significant lab values, vital signs, physical examination and ECG results
- Ear pain
- Tympanic impedance parameters

In addition, for ear pain, descriptive tables as described in Section 6.7 for the subgroup with baseline pain >0 (in right or left ear) will be presented for right ear, left ear and worst of right and left ear by sequence*period.

8 Deviations from the protocol

Protocol	**SAP**
p-value ≤ 0.05 defined as significant	p-value < 0.05 defined as significant

9 Interpretation of results

In the cross-over analysis, the test result of the carry-over effect will guide the choice of the proper estimation of treatment effect based on both periods vs. Period 1 only (see Section 7.1). Because of the small sample size, a careful interpretation of the results is essential.

10 Data problems

Not expected. Will be recorded in a separate document, if problems occur.

11 Software

The analyses will be programmed in SAS 9.4.

12 References

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13 Appendices

13.1 List of abbreviations

ADR	Adverse drug reaction
AE	Adverse Event
ATC	Anatomical-Therapeutic-Chemical
CI	Confidence Interval
CTCC	Clinical Trial Centre Cologne
ECG	Electrocardiography
ET	Eustachian Tube
ETCP	Eustachian tube closing pressure
ETOD	Eustachian tube opening duration
ETOF	Eustachian tube opening frequency
ETOP	Eustachian tube opening pressure
GGT	Gamma glutamyltransferase
GOT	Glutamic-oxaloacetic transaminase
GPT	Glutamicpyruvic transaminase
IMP	Investigational Medicinal Product
IMSB	Xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx Biology University of Cologne
MedDRA	Medical Dictionary for Regulatory Activities
PP	Per-Protocol
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SI	System International
SOC	System Organ Classification

SOP	Standard Operating Procedure
SP	Safety Population
TEAE	Treatment-Emergent Adverse Event
TLG	Table / listing / graph
TM	Tympanic membrane
WHO	World Health Organization