STATISTICAL ANALYSIS PLAN

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A Phase 1/2, Randomized, Placebo-controlled Study to Assess the Safety, Tolerability, Efficacy, and Pharmacokinetics of ASP0598 Otic Solution Following Topical application into the Ear in Subjects with Chronic Tympanic Membrane Perforation (CTMP)

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I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
A-B	Air bone
AE	Adverse Event
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CMQ	Custom MedDRA Query
COVID-19	Coronavirus Disease 2019
CSR	Clinical Study Report
CTMP	Chronic Tympanic Membrane Perforation
CV	Coefficient of Variation
dB	Decibels
DMC	Data Monitoring Committee
EOS	End of Study
FAS	Full Analysis Set
IAP	Interim Analysis Plan
ICE	Intercurrent Event
ICH	International Conference on Harmonization
IRT	Interactive Response Technology
LS	Least Square
MAD	Multiple Ascending Dose
MDE	Multiple Dose Expansion
MedDRA	Medical Dictionary for Regulatory Activities
MH	Mantel–Haenszel
MI	Multiple imputation
mL	Milliliter
mmHg	Millimeters of mercury
MMRM	Mixed Model Repeated Measures
MNAR	Missing Not At Random
PD1-x	Protocol Deviation 1-x
PT	Preferred Term
PTA	Pure Tone Audiometry
ROI	Region of Interest
SAD	Single Ascending Dose
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation

Abbreviations	Description of abbreviations
SE	Standard Error
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TLF	Tables, Listings, and Figures
TMP	Tympanic Membrane Perforation
TVAS	Tinnitus Visual Analog Scale

List of Key Terms

Terms	Definition of Terms
TEAE	An adverse event (AE) observed after starting administration of the study drug through end of study visit. If an AE occurs on Day 1 and the onset check box is marked "Onset after the dose of study drug" or the onset check box is left blank, then the AE will be considered Treatment-Emergent AE.
Drug-related TEAE	Any TEAE with a causal relationship assessed as "yes" by the investigator.
Study Period	Period of time from the first study site initiation date to the last study site completing the study.

1 INTRODUCTION

This Statistical Analysis Plan (SAP) contains technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes procedures for executing the statistical analysis to fulfil the objectives of the study.

The final SAP will be approved prior to database lock. Any changes to the dose escalation phase of the SAP will be approved prior to unblinding the dose escalation phase data for the prescribed interim analysis. Interim analysis plan (IAP) for the dose escalation phase will be prepared separately, and approved prior to unblinding the dose escalation phase.

Changes from the planned analyses in the final SAP that impact the statistical analyses will be documented in the Clinical Study Report (CSR).

2 STUDY OBJECTIVE(S) AND DESIGN

2.1 Study Objective(s)

Primary objective: To evaluate the safety and tolerability of ASP0598 Otic Solution.

Secondary objective: To evaluate the efficacy of ASP0598 Otic Solution.

Exploratory objective:

To evaluate otological parameters that may correlate with efficacy outcome and pharmacokinetics of ASP0598 Otic Solution.

To evaluate the safety of ASP0598 Otic Solution.

2.2 Study Design

Details of the schedule of clinical assessments are available in the protocol.

2.3 Randomization

Dose escalation

Dose escalation will consist of up to 4 cohorts for single ascending dose (SAD) and up to 2 cohorts for multiple ascending dose (MAD). In each cohort of the SAD, a total of 5 subjects will be randomized in a 4:1 ratio of ASP0598 Otic Solution and placebo. In each cohort of the MAD, a total of 8 subjects will be randomized in a 3:1 ratio of ASP0598 Otic Solution and placebo.

For each cohort in SAD and MAD, a simple block randomization schedule will be generated.

Dose expansion

The decision to open the single and multiple dose expansion phases will be based on the safety and efficacy results of the interim analyses after completion of SAD and after completion of MAD, respectively.

Up to 39 subjects will be randomized to each of the single dose and/or multiple dose expansion phases of the study. Based on the interim analysis results,

- If 3 treatment groups are selected, approximately 39 subjects will be randomized in a 1:1:1 ratio of high dose ASP0598 otic solution, low dose ASP0598 otic solution and placebo (13 subjects in each treatment group).
- If 2 treatment groups are selected, approximately 26 subjects will be randomized in a 1:1 ratio of high dose ASP0598 otic solution (13 subjects) or placebo (13 subjects).

Stratification at the time of randomization will be based on the % size of the TMP (≤ 15%, > 15%) and subjects will be balanced equally between the treatment groups.

For all phases subjects may be replaced at the discretion of the sponsor. If replaced, the replacement subject will be given the same treatment group the original subject's randomized treatment group. Randomization will be performed via interactive response technology (IRT) using central allocation. If a subject is assigned a randomization number, but does not receive study drug, the randomization number will not be used again. Specific procedures for randomization through the IRT are contained in the study-specific IRT manual.

3 SAMPLE SIZE

SAD and MAD

Formal sample size calculations were not performed. The dose escalation phase is not intended to show statistical difference between ASP0598 Otic Solution and placebo subjects.

A sample size of 5 per cohort (n = 4 ASP0598 Otic Solution, n = 1 placebo) for SAD and 8 per cohort (n = 6 ASP0598 Otic Solution, n = 2 placebo) for MAD is based on precedent set by other clinical studies of similar nature. The number of subjects and study procedures planned for this clinical study are considered reasonable to achieve the clinical study objectives.

Dose expansion

Assuming the complete closure rate in placebo and ASP0598 higher concentration group are 10% and 80% respectively, eleven (11) subjects per treatment group will provide more than 90% power to detect a statistically significant difference at a 2-sided significance level of 0.05. Considering the drop-out rate of 10%, 13 subjects per treatment group will be enrolled (nQuery Advisor 7.0).

4 ANALYSIS SETS

In accordance with International Conference on Harmonization (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

The determination of whether subjects are included or excluded from the safety and efficacy analysis sets will be made prior to database lock. For the dose escalation phase, the determination will be made prior to finalization of relevant programming activities for the prescribed interim analysis.

4.1 Full Analysis Set

The full analysis set (FAS) will consist of all randomized subjects who receive a dose of

study drug and have baseline value and at least 1 post baseline complete closure assessment during study period. This will be the primary analysis set for efficacy analyses.

In the analyses of FAS, subjects will be presented by randomized (planned) treatment group even if the treatment they received was different.

For study period, post baseline values will not contribute to the FAS as shown below:

- SAD: if the study day of the value is > minimum(study day of end of study visit, 57) + 7 days.
- Single dose expansion and MAD: if the study day of the value is > minimum(study day of end of study visit, 85) + 7 days.
- Multiple dose expansion: if the study day of the value is > minimum(study day of end of study visit, 113) + 7 days.

4.2 Safety Analysis Set

The safety analysis set (SAF) consists of all randomized subjects who receive a dose of study drug. The SAF will be the primary analysis set for the safety data and pharmacokinetic data.

In the analyses of SAF, subjects will be presented by actual treatment received.

For MAD and multiple dose expansion, actual treatment will be the same as planned treatment if the subject receives randomized treatment for the entire treatment duration. Otherwise, actual treatment will be the treatment corresponding to the first treatment actually received.

5 EFFICACY AND SAFETY ENDPOINTS

5.1 Primary Safety Endpoint(s)

- Treatment-emergent adverse events (TEAEs)
    - **TEAE:** an AE observed after starting administration of the study drug through end of study visit. If an AE occurs on Day 1 and the onset check box is marked “Onset after the dose of study drug” or the onset check box is left blank, then the AE will be considered TEAE. Refer to Section 6.9.1 for end day of end of study visit definition.
    - **Drug-related TEAE**: any TEAE with a causal relationship assessed as “yes” by the investigator. If the relationship is missing then it is considered as drug-related.

      Imputed date for AE onset date may be used to determine TEAE (Section 6.9.3.1).

    The investigator will use the following definitions to rate the severity of each AE:

    - Mild
    - Moderate
    - Severe

- Incidence of AEs of special interest will be defined as shown below.
  1. Cholesteatoma or neoplasm
  2. Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness)
  3. Otitis media or otitis externa

  AEs of special interest will be identified using custom MedDRA queries (CMQs). Detailed information about AEs of special interest is provided in Appendices 1, 2 and 3.

- Change from baseline in bone conduction hearing at 1, 2, 4 kHz by Pure Tone Audiometry (PTA)

  A lower value indicates better level of hearing.

  For the change from baseline, a negative value (decrease from baseline) indicates improvement (i.e., better level of hearing).

  Mean bone conduction hearing (dB):

  Arithmetic mean of the values at 1, 2 and 4 kHz. If the value is missing for any of the 3 frequencies, then the mean value will be set to missing.

- Change from baseline in Tinnitus Visual Analog Scale (TVAS)

  The TVAS is a numeric scale and ranges from 0 (not at all strong or loud) to 10 (extremely strong or loud). A lower value indicates less level of discomfort.

  For the change from baseline, a negative value (decrease from baseline) indicates improvement (i.e., less level of discomfort).

  TVAS may be reported for one of the following areas: ear (left, right or bilateral) or other.

  At a time point, if a subject did not experience tinnitus, TVAS will be reported as 0. In this case, the TVAS will be considered as 0 for all two areas.

  At a time point, if a subject experiences tinnitus, the result will be reported with a value ranging from 1 to 10 for one of the areas. In this situation, the TVAS result will be imputed as 0 for the other areas [e.g., if TVAS = 3 for the ear (left) then TVAS will be imputed as 0 for ear (right) and other. if TVAS = 3 for the ear (bilateral) then TVAS will be imputed as 3 for ear (left) and ear (right) and 0 for other].

5.2 Primary Efficacy Endpoints

Not applicable.

5.3 Secondary Efficacy Endpoints

SAD and Single dose expansion

Categorical endpoint:

- Complete closure of Tympanic Membrane Perforation (TMP) at Week 8 for SAD and at Week 12 for single dose expansion

TMP complete closure observed response:

Using the non-missing result of complete closure of TMP, an observed response is

Yes: if a subject with complete closure of TMP,

No: if the above condition is false.

TMP complete closure imputed response at Week 8 for SAD:

Using the non-missing result at Week 8, an imputed response is same as observed response at Week 8.

If a response is missing at Week 8, assign the latest post baseline observed response available prior to Week 8.

TMP complete closure imputed response at Week 12 for single dose expansion (Primary analysis imputation):

For endpoint #1 defined in Section 5.3.2, using the non-missing result at Week 12, an imputed response is

No intercurrent events (ICE) occurs: same as observed response at Week 12,

ICE occurs: assign the latest post baseline observed response available just before the ICE.

TMP complete closure imputed response at Week 12 for single dose expansion (Sensitivity analysis imputation):

For endpoint #1 defined in Section 5.3.2, using the non-missing result at Week 12, an imputed response is

No ICE occurs: same as observed response at Week 12,

ICE occurs: No.

Continuous endpoints:

- Change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 8 for SAD and at Week 12 for single dose expansion

  Ratio of TMP size per total area of tympanic membrane (%):

  [TMP Size ÷ Periphery of the Tympanic Membrane Region of Interest (ROI)] × 100

- Change from baseline in TMP size at Week 8 for SAD and at Week 12 for single dose expansion

  Here TMP size = TMP ROI from imaging data.

MAD and Multiple dose expansion

Categorical endpoint:

- Complete closure of TMP at Week 12 for MAD and at Week 16 for multiple dose

expansion

TMP complete closure observed response:

Using the non-missing result of complete closure of TMP, an observed response is

Yes: if a subject with complete closure of TMP,

No: if the above condition is false.

TMP complete closure imputed response at Week 12 for MAD:

Using the non-missing result at Week 12, an imputed response is same as observed response at Week 12.

If a response is missing at Week 12, assign the latest post baseline observed response available prior to Week 12.

TMP complete closure imputed response at Week 16 for multiple dose expansion (Primary analysis imputation):

For endpoint #1 defined in Section 5.3.2, using the non-missing result at Week 16, an imputed response is

No ICE occurs: same as observed response at Week 16,

ICE occurs: assign the latest post baseline observed response available just before the ICE.

TMP complete closure imputed response at Week 16 for multiple dose expansion (Sensitivity analysis imputation):

For endpoint #1 defined in Section 5.3.2, using the non-missing result at Week 16, an imputed response is

No ICE occurs: same as observed response at Week 16,

ICE occurs: No.

TMP complete closure imputed response at Week 12 for multiple dose expansion:

Using the non-missing result at Week 12, an imputed response is same as observed response at Week 12.

If a response is missing at Week 12, assign the latest post baseline observed response available prior to Week 12.

Continuous endpoints:

- Change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 12 for MAD and at Week 16 for multiple dose expansion
- Change from baseline in TMP size at Week 12 for MAD and at Week 16 for multiple dose expansion

For the change from baseline in the ratio of TMP size per total area of tympanic membrane

and the change from baseline in TMP size, a negative value (decrease from baseline) indicates improvement.

For dose expansion, hypothetical strategy will be applied to analyze the secondary endpoints. Details of ICEs and estimand are shown in Section 5.3.1.

5.3.1 Intercurrent Events

Single dose expansion and Multiple dose expansion

1. **Intercurrent events (ICEs)**: For the subjects discontinued during the study investigational period, the following ICEs may happen:
   - Randomized but never received study drug
   - Study investigational period discontinuation due to
     - AE
     - Lack of efficacy
     - Lost to follow up
     - Protocol deviation
     - Withdrawal by subject
     - Other
   - Death
2. **Treatment regimen under evaluation:** randomized treatment administered as directed per schedule of assessments.

5.3.2 Estimand

Population: defined by the appropriate inclusion/exclusion criteria in the protocol.

Efficacy endpoints:

3. Complete closure of TMP at Week 12 for single dose expansion and Week 16 for multiple dose expansion.
4. Change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 12 for single dose expansion and Week 16 for multiple dose expansion.
5. Change from baseline in TMP size at Week 12 for single dose expansion and Week 16 for multiple dose expansion.

Strategy for addressing ICEs: hypothetical strategy will be used to estimate the outcome at Week 12 for single dose expansion and Week 16 for multiple dose expansion.

Population-level summary:

- **Endpoint #1 (Categorical):** difference in proportion of subjects with complete closure of TMP at Week 12 for single dose expansion and Week 16 for multiple dose expansion between ASP0598 and placebo.
- **Endpoint #2 (Continuous):** difference in mean change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 12 for single dose expansion and Week 16 for multiple dose expansion between ASP0598 and placebo.

- **Endpoint #3 (Continuous):** difference in mean change from baseline in TMP size at Week 12 for single dose expansion and Week 16 for multiple dose expansion between ASP0598 and placebo.

Data that will be used for the estimand:

- **Endpoint #1:** data necessary for this estimand is complete closure status at Week 12 for single dose expansion and Week 16 for multiple dose expansion. For the subjects discontinued during the study investigational period with ICEs, the complete closure status just before the ICE will be used as the status at Week 12 for single dose expansion and Week 16 for multiple dose expansion.
- **Endpoints #2 and #3:** data necessary for these estimands are TMP size per total area of tympanic membrane and TMP size at each visit, respectively. For the subjects discontinued during the study investigational period with ICEs, only data obtained before the ICE will be used in the Mixed model repeated measures (MMRM) to handle discontinued subjects in some sense if the subjects remained in the study under missing at random (MAR) assumption.

5.4 Exploratory Endpoints

Single dose phase:

- Complete closure of TMP for post baseline visits at Day 2, Day 3 (evaluations at this visit will only be performed for cohorts 1, 2 and 3), Week 1, Week 2 and Week 4 for SAD and Week 2, Week 4 and Week 8 for single dose expansion

  For the observed response definition, refer to Section 5.3.

- Change from baseline in the ratio of TMP size per total area of tympanic membrane for post baseline visits at Day 2, Day 3 (evaluations at this visit will only be performed for cohorts 1, 2 and 3), Week 1, Week 2 and Week 4 for SAD and Week 2, Week 4 and Week 8 for single dose expansion
- Change from baseline in TMP size for post baseline visits at Day 2, Day 3 (evaluations at this visit will only be performed for cohorts 1, 2 and 3), Week 1, Week 2 and Week 4 for SAD and Week 2, Week 4 and Week 8 for single dose expansion

Multiple dose phase:

- Complete closure of TMP for post baseline visits at Week 1, Week 2, Week 3, Week 4, Week 5 and Week 8 for MAD and Week 2, Week 4, Week 6, Week 8 and Week 12 for multiple dose expansion

For the observed response definition, refer to Section 5.3.

- Change from baseline in the ratio of TMP size per total area of tympanic membrane for post baseline visits at Week 1, Week 2, Week 3, Week 4, Week 5 and Week 8 for MAD and Week 2, Week 4, Week 6, Week 8 and Week 12 for multiple dose expansion

- Change from baseline in TMP size for post baseline visits at Week 1, Week 2, Week 3, Week 4, Week 5 and Week 8 for MAD and Week 2, Week 4, Week 6, Week 8 and Week 12 for multiple dose expansion

Single and multiple dose phases:

- Time to closure of TMP

  Time to closure of TMP (days):

  TMP closure date - first dose date + 1. If the TMP is not closed, then time to closure will be set to missing.

- Size of TMP ≤ 5% of pars tensa surface area after treatment

  Observed response:

  Using the non-missing size of TMP per pars tensa surface area, an observed response is

  Yes: if the post baseline size of the TMP per pars tensa surface area ≤ 5% and the size of the TMP per pars tensa surface area is smaller than baseline,

  No: if the above condition is false.

  Ratio of TMP size per total area of tympanic membrane will be used in the above calculation as an approximation for TMP per pars tensa surface area.

- Size of TMP ≤ 1% of pars tensa surface area (pin point perforation) after treatment

  Observed response:

  Using the non-missing size of TMP per pars tensa surface area, an observed response is

  Yes: if the post baseline size of the TMP per pars tensa surface area ≤ 1% and the size of the TMP per pars tensa surface area is smaller than baseline,

  No: if the above condition is false.

  Ratio of TMP size per total area of tympanic membrane will be used in the above calculation as an approximation for TMP per pars tensa surface area.

- Change from baseline in Air bone (A-B) gap by PTA

  For the change from baseline in A-B gap, a negative value (decrease from baseline) indicates an improvement.

  PTA is a behavioral test and generally the 1st quantitative hearing test done to assess the nature and degree of hearing loss in adults and in children.

  PTA air conduction and bone conduction tests analyzed together determines any hearing loss: type and magnitude of hearing loss; frequencies that are affected; and whether hearing loss is unilateral or bilateral. For both air and bone conduction, a lower value indicates better hearing threshold.

Type of hearing loss can be as follows:

- Conductive hearing loss: when air conduction thresholds are elevated relative to bone conduction thresholds, an "A-B gap" exists, indicates a conductive hearing loss.
- Sensorineural hearing loss: when air conduction and bone conduction thresholds showing the same amount of hearing loss, indicates sensorineural hearing loss.
- Mixed hearing loss: when both air and bone conduction thresholds are elevated, but air conduction thresholds are more elevated than bone conduction thresholds, indicates mixed hearing loss.

Definitions for mean bone conduction hearing, mean air conduction hearing, mean A-B gap and A-B gap (dB) for each frequency are provided below:

Mean bone conduction hearing (dB):

Arithmetic mean of the bone conduction hearing values at 500 Hz, 1, 2 and 4 kHz. If the value is missing for any of the 4 frequencies, then the mean value will be set to missing.

Mean air conduction hearing (dB):

Arithmetic mean of the air conduction hearing values at 500 Hz, 1, 2 and 4 kHz. If the value is missing for any of the 4 frequencies, then the mean value will be set to missing.

Mean A-B gap (dB):

Mean air conduction hearing - mean bone conduction hearing. If either one is missing or both are missing then the mean A-B gap is set to missing.

A-B gap (dB) for each frequency:

Air conduction hearing at the frequency level - bone conduction hearing at the frequency level. If either one is missing or both are missing then the A-B gap is set to missing. This will be derived for frequencies 500 Hz, 1 kHz, 2 kHz, and 4 kHz.

- Change from baseline in air conduction hearing by PTA

  Mean air conduction hearing will be derived as explained in the endpoint Percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing)

- Percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing)

  Observed response:

  Using the non-missing percentage change from baseline in mean A-B gap, an observed response is

  Yes: if the percentage change from baseline in mean A-B gap (derived using mean of the A-B gap results at 500 Hz, 1, 2 and 4 kHz) ≤ -50%,

  No: if the above condition is false.

- Improvement from baseline in air conduction hearing by PTA ≥ 15 dB

  For the change from baseline in air conduction hearing, a negative value (decrease from baseline) indicates an improvement.

  Observed response:

  Using the non-missing change from baseline mean air conduction hearing, an observed response is

  Yes: if the change from baseline in mean air conduction hearing by PTA ≤ -15 dB,

  No: if the above condition is false.

- A-B gap after treatment was 15 dB or less

  Observed response:

  Using the non-missing mean A-B gap, an observed response is

  Yes: if the post baseline mean A-B gap ≤ 15 dB,

  No: if the above condition is false.

- Air conduction hearing threshold after treatment was 30 dB or less

  For the air conduction hearing, a lower value indicates better hearing threshold.

  Observed response:

  Using the non-missing mean air conduction hearing, an observed response is

  Yes: if the post baseline mean air conduction hearing ≤ 30 dB,

  No: if the above condition is false.

- Type of tympanogram (A, B or C)

  For Type A, there are 2 additional subcategories: Ad and As.

- Equivalent ear canal volume (ECV) by tympanometry

Safety endpoint:

- Vital signs (body temperature, blood pressure and pulse rate)
- Microscopic ear examination

  At screening, both ears will be examined microscopically to identify the TMP and evaluate the eligibility of the subject for the study. Only treated ear will be examined at Day 1 and the following visits.

  On Day 1, Week 2 (multiple dose only), and Week 4 (multiple dose only), 1 h after the treatment, the following ear canal reactions of treated ear will be collected.

  Did the ear experience an abnormal ear sensation

  - No

- Yes
  - Burning
  - Stinging
  - Itching
  - Pain
  - Other

After Day 1, data for the following parameters will be collected:

- Gel visible in the treated ear (Yes, No)
- Gel visible in the middle ear (Yes, No)
- TMP of treated ear completely closed (Yes, No, Unknown)
- Ear pain (otalgia) in the treated ear (Yes, No)
- Status of the treated tympanic membrane (Normal, Abnormal, Abnormal: Myringitis, Abnormal: Hematoma, Abnormal: Other, Not visible)
- Status of the middle ear in the treated ear (Normal, Abnormal, Abnormal: Mucosal inflammation with effusion, Abnormal: Mucosal inflammation without effusion, Abnormal: Other, Not visible)

Local skin reaction around the application site will be examined at 60 minutes after the treatment at Day 1 and throughout the observation period. Skin toxicity will be evaluated by Modified Brighton Grading Scheme as shown below.

Grade	Description
0	no abnormalities
1	canal erythema
1b	canal erythema with otorrhea (ear canal fluid discharge)
2	canal erythema + swelling, TM still visible
2b	canal erythema + swelling, TM still visible with otorrhea
3	canal erythema + swelling, TM not visible
3b	canal erythema + swelling, TM not visible with otorrhea
4	canal erythema + swelling, TM not visible + erythema and swelling of the pinna
4b	canal erythema + swelling, TM not visible + erythema and swelling of the pinna with otorrhea

Pharmacokinetics:

- Serum concentration of ASP0598 (dose escalation part only)

6 STATISTICAL METHODOLOGY

6.1 General Considerations

Continuous data will be summarized descriptively including the number of subjects (n), mean, standard deviation (SD) and/or standard error, median, minimum and maximum.

Categorical data will be summarized by frequencies and percentages. Percentages by categories will be based on the number of subjects with no missing data, i.e. the percentages for the non-missing categories will add up to 100%.

For SAD and MAD, results will be presented by ASP0598 treatment group in each cohort and placebo group where placebo subjects will be pooled across cohorts.

For single dose expansion and multiple dose expansion, results will be presented by each ASP0598 treatment group and placebo group.

All statistical comparisons between ASP0598 and placebo will be conducted using 2-sided tests at $\alpha = 0.05$ significance level, unless stated otherwise. For dose expansion phase, a hierarchical testing method will be used to control familywise error rate where the highest dose group vs placebo will be tested first. Refer to Section 6.4.2 for details.

All data summarization and analyses will be performed using SAS® Version 9.4 or higher on Redhat Enterprise Linux. Specifications for tables, listings, and figures (TLF) formats can be found in the TLF specifications.

6.2 Study Population

6.2.1 Disposition of Subjects

The following subject data will be presented. Unique subjects are defined as rescreened subjects for whom the data from the last enrollment are used.

- Number of subjects with informed consent, unique subjects with informed consent, subjects with rescreening, subjects discontinued before randomization, unique subjects who discontinued before randomization and randomized for all subjects with informed consent (overall only),
- Screening disposition for all unique subjects with informed consent (overall only),
- Number and percentage of subjects who were randomized, were administered study drug, were not administered study drug, in the analysis sets by treatment group, ASP0598 total and 'total' over all treatment groups for all randomized subjects,
- Treatment disposition for all randomized subjects, SAF and FAS by each treatment group, ASP0598 total and 'total' over all treatment groups,
- Investigational period disposition for all randomized subjects, SAF and FAS by each treatment group, ASP0598 total and 'total' over all treatment groups, and
- Protocol version disposition for all unique subjects with informed consent by each treatment group and 'total' over all treatment groups for all randomized subjects, screen failures only and overall including screen failures,
- Number and percentage of subjects by site and country by treatment group and 'total' over all treatment groups for all randomized subjects.
- Number and percentage of subjects discontinued (cumulative by analysis visit) the study investigational period by analysis visit (Weeks 2, 4 and 8 for SAD, Weeks 2, 4, 8 and 12 for single dose expansion, Weeks 1, 2, 3, 4, 5, 8 and 12 for MAD, Weeks 2, 4, 6, 8, 12 and 16 for multiple dose expansion) and completed the study investigational period by

each treatment group for the FAS. For analysis visit windows, refer to Table 6. For Week 2 SAD visit window will be same as Week 2 single dose expansion window.

6.2.2 Protocol Deviations

The number and percentage of subjects with the following protocol deviation criteria will be summarized for each criterion and overall, by treatment group, ASP0598 total and 'total' over all treatment groups as well as by investigative site. Subjects deviating from a criterion more than once will be counted once for the corresponding criterion.

The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

6.2.3 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics will be summarized using descriptive statistics for all randomized subjects, SAF and FAS by treatment group, ASP0598 total (if applicable) and 'total' over all treatment groups.

Demographics variables:

Variable	Variable Type	Categories
Sex	Categorical	• Male • Female • Unknown
Race	Categorical	• White • Black or African American • Asian • American Indian or Alaska Native • Native Hawaiian or Other Pacific Islander • Other
Ethnicity	Categorical	• Not Hispanic or Latino • Hispanic or Latino
Table continued on next page		

Variable	Variable Type	Categories
Age	Continuous	NA
Age Group	Categorical	• ≤ 60 years • > 60 years
EudraCT Age Category	Categorical	• ≥ 18 to ≤ 64 years • ≥ 65 years to ≤ 84 years
Baseline weight	Continuous	NA
Height	Continuous	NA
Baseline body mass index (BMI)	Continuous	NA
Baseline BMI group	Categorical	• ≤ 30 kg/m^2 • > 30 kg/m^2
Primary Diagnosis:		
Onset of chronic tympanic membrane perforation (CTMP) (years) derived as (Informed consent date - onset date of CTMP)/365.25. Imputed date for onset date of CTMP may be used to calculate the onset of CTMP (Section 6.9.3.4).	Continuous	NA

CTMP duration group	Categorical	• ≤ 1 year
		• > 1 year
		  ○ > 1 to ≤ 10 years
		  ○ > 10 years

TMP present	Categorical	• Left ear • Right ear • Both ears
Treated ear	Categorical	• Left ear • Right ear
Primary Cause of TMP	Categorical	• Infection (CSOM) • Trauma • Placement of tube • Iatrogenic complication • Other

Table continued on next page

Variable	Variable Type	Categories
Areas of tympanic membrane involved in TMP	Categorical	• Anterior superior • Anterior inferior • Posterior superior • Posterior inferior • Anterior Superior/ Anterior Inferior • Anterior Inferior/ Posterior Inferior • Posterior Superior/ Posterior Inferior
Areas of tympanic membrane involved in TMP group	Categorical	• 1 • 2 • ≥ 3 Derived as number of areas of tympanic membrane involved in TMP.
Paper patch to treat the treated ear	Categorical	• Yes • No
Stratification factor TMP size group (expansion only)	Categorical	• ≤ 15% • > 15%
Targeted Medical History		

Tobacco history	Categorical	• Yes (Current)
		• No (Former or Never)
		  ○ Former
		  ○ Never

Diabetes history	Categorical	• Yes • No
Latex allergy history	Categorical	• Yes • No
Latex sensitivity history	Categorical	• Yes • No
Cannabis History		
History of Cannabis Use	Categorical	• Current • Former • Never

Baseline variables:

Variable	Variable Type	Categories
Secondary Variables:		
Baseline ratio of TMP size group per total area of tympanic membrane from central imaging	Categorical	• ≤ 15% • > 15%
Baseline TMP size from central imaging	Continuous	NA
Baseline ratio of TMP size per total area of tympanic membrane from central imaging	Continuous	NA
Other Efficacy Variables:		
Baseline mean air conduction hearing loss by PTA	Continuous	NA
Baseline mean air conduction hearing loss group by PTA	Categorical	• Normal (≤ 25 dB) • Mild hearing loss (> 25 to ≤ 40 dB) • Moderate hearing loss (> 40 to ≤ 55 dB) • Moderately severe hearing loss (> 55 to ≤ 70 dB) • Severe hearing loss (> 70 to ≤ 90 dB) • Profound loss (> 90 dB)

Medical history is coded in Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by System Organ Class (SOC) and Preferred Term (PT) for the SAF by treatment group, ASP0598 total (if applicable) and 'Total' over all treatment groups.

6.2.4 Previous and Concomitant Medications

A previous medication is defined as any medication taken before the dose of study drug.

A concomitant medication is defined as any medication taken on or after the dose of study drug through end of study visit. Refer to Section 6.9.1 for end day of end of study visit definition.

Medications that started prior to the dose of study drug and continued on or after the dose of study drug will be counted in both previous and concomitant medications.

Imputed dates for medication start and stop dates may be used to determine the medication is a previous or concomitant medication or both (Section 6.9.3.2).

Previous and concomitant medications will be listed only.

6.3 Study Drugs Exposure and Compliance

Actual dose of study drug will be summarized using descriptive statistics, entire planned volume of study drug administered (Yes, No), was there needle stick of injury during study

drug administration (Yes, No), area of injury (Ear Canal Skin, Tympanic membrane, Other) and multiple dose phase only: total number of doses administered (1, 2 and 3) will be summarized using number and percentage of subjects for the SAF by treatment group.

Actual dose of study drug (µg) = Administration concentration (µg/mL) x Actual volume of study drug (mL).

Administration concentration values for each dose are provided in Table 1 of the protocol.

Imputed date for study drug dose date may be used (Section 6.9.3.3).

6.4 Analysis of Efficacy

Efficacy analyses will be presented by treatment group for FAS, unless specified otherwise.

For the endpoint complete closure of TMP at Week 12/Week 16 in dose expansion, based on the interim analysis results, if 3 treatment groups are selected, hierarchical testing method will be used to control familywise error rate where the highest dose group vs placebo will be tested first. Refer to Section 6.4.2 for details. If 2 treatment groups are selected, no multiplicity adjustment is needed.

For continuous endpoints, descriptive statistics will be used to summarize baseline value, post baseline value and change from baseline at each specified post baseline analysis visit by treatment group.

For categorical endpoints, number and percentage of subjects will be used to summarize baseline value and post baseline value at each specified post baseline analysis visit by treatment group. Difference from placebo will be presented, where applicable.

Table 1 and Table 2 provides list of variables that will be summarized for categorical endpoints using number and percentage of subjects and for continuous endpoints using descriptive statistics respectively.

Table 3 provides an overview of efficacy analyses of secondary and exploratory variables for dose expansion phase.

Table 1 Categorical Efficacy Variables for Number and Percentage of Subjects

Variable	Analysis Visits
Observed response for complete closure of TMP as assessed by site investigator	**SAD:** Baseline, Day 2, Day 3*, Week 1, Week 2, Week 4, Week 8 and End of Study (EOS) **Single dose expansion:** Baseline, Week 2, Week 4, Week 8, Week 12 and EOS **MAD:** Baseline, Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12 and EOS **Multiple dose expansion:** Baseline, Week 2, Week 4, Week 6, Week 8, Week 12, Week 16 and EOS
Table continued on next page	

Variable	Analysis Visits
Observed response for complete closure of TMP as assessed by central reader	**SAD:** Baseline, Day 2, Day 3*, Week 1, Week 2, Week 4, Week 8 and End of Study (EOS) **Single dose expansion:** Baseline, Week 2, Week 4, Week 8, Week 12 and EOS **MAD:** Baseline, Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12 and EOS **Multiple dose expansion:** Baseline, Week 2, Week 4, Week 6, Week 8, Week 12, Week 16 and EOS
Imputed response for complete closure of TMP as assessed by site investigator	**SAD:** Week 8 **Single dose expansion:** Week 12 primary analysis and Week 12 sensitivity analysis **MAD:** Week 12 **Multiple dose expansion:** Week 12 and Week 16 primary analysis and Week 16 sensitivity analysis
Imputed response for complete closure of TMP as assessed by central reader	**SAD:** Week 8 **Single dose expansion:** Week 12 primary analysis and Week 12 sensitivity analysis **MAD:** Week 12 **Multiple dose expansion:** Week 12 and Week 16 primary analysis and Week 16 sensitivity analysis
Reduction from baseline in size of TMP to less than or equal to 5% of pars tensa surface area ● Observed response ● Observed response where subjects with ≤ 5% at baseline are excluded	**SAD:** Week 8 and EOS **Single dose expansion:** Week 8, Week 12 and EOS **MAD:** Week 12 and EOS **Multiple dose expansion:** Week 12, Week 16 and EOS
Reduction from baseline in size of TMP to less than or equal to 1% of pars tensa surface area ● Observed response ● Observed response where subjects with ≤ 1% at baseline are excluded	
Observed response for percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing)	**SAD:** Week 8 and EOS **Single dose expansion and MAD:** Week 12 and EOS **Multiple dose expansion:** Week 16 and EOS
Observed response for improvement from baseline in air conduction hearing by PTA ≥ 15 dB	
Observed response for A-B gap after treatment was 15 dB or less	
Observed response for air conduction hearing threshold after treatment was 30 dB or less	
Type of tympanogram	**SAD:** Baseline, Week 8 and EOS **Single dose expansion and MAD:** Baseline, Week 12 and EOS **Multiple dose expansion:** Baseline, Week 12, Week 16 and EOS

*Day 3 evaluations will only be performed for cohorts 1, 2 and 3

Table 2 Continuous Efficacy Variables for Descriptive Statistics

Variable	Plots	Analysis Visits
Ratio of TMP size per total area of tympanic membrane	**SAD and MAD:** Mean +/- SE for the change from baseline vs Analysis Visit by treatment group (overlay) **Dose expansion:** Least squares mean +/- SE for the change from baseline vs Analysis Visit by treatment group (overlay)[a]	**SAD:** Baseline, Day 2, Day 3[b], Week 1, Week 2, Week 4, Week 8 and EOS **Single dose expansion:** Baseline, Week 2, Week 4, Week 8, Week 12 and EOS **MAD:** Baseline, Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12 and EOS **Multiple dose expansion:** Baseline, Week 2, Week 4, Week 6, Week 8, Week 12, Week 16 and EOS EOS visit will not be included in the plot.
TMP size		
Time to closure of TMP[c]	Not Applicable	Not Applicable
Air conduction hearing at 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz and 8 kHz	**SAD, MAD and Dose expansion:** Individual subject plot of air conduction hearing and bone conduction hearing for each frequency and mean by Analysis Visit (Overlay)	SAD: Baseline, Week 8 and EOS **Single dose expansion and MAD:** Baseline, Week 12 and EOS **Multiple dose expansion:** Baseline, Week 12, Week 16 and EOS
Mean air conduction hearing		
Bone conduction hearing at 500 Hz, 1 kHz, 2 kHz and 4 kHz		
Mean bone conduction hearing		
A-B gap at 500 Hz, 1 kHz, 2 kHz, and 4 kHz		
Mean A-B gap		
ECV by tympanometry	Not Applicable	

[a] from the MMRM model as described in Section 6.4.2 and Section 6.4.3.

[b] Day 3 evaluations will only be performed for cohorts 1, 2, and 3

[c] change from baseline is not applicable.

For the secondary endpoint complete closure of TMP at Week 12 (single dose expansion) and Week 16 (multiple dose expansion), number and percentage of subjects will be provided by treatment group for the following subgroups:

- Sex,
- Race,
- Age group,
- BMI group,
- CTMP duration group (≤ 1 year, > 1 to ≤ 10 years, > 10 years),
- Tobacco history (Yes, No),
- Causation of TMP,
- Diabetes history
- Stratification factor TMP size group,
- Baseline TMP size group per total area of tympanic membrane from central imaging.

For the dose expansion secondary endpoints (change from baseline in ratio of TMP size per total area of tympanic membrane and change from baseline in TMP size), descriptive statistics will be used to summarize the Week 12 (single dose expansion) and Week 16 (multiple dose expansion) analysis visit results by treatment group for the subgroup baseline TMP size group per total area of tympanic membrane from central imaging.

For the categories of the subgroup variables, refer to Section 6.2.3.

Table 3 Overview of Efficacy Analysis for Dose Expansion

Endpoint Type	Analysis Variable	Analysis Method
Secondary Efficacy	Imputed response for complete closure of TMP at Week 12 for single dose expansion and Week 16 for multiple dose expansion ● Primary analysis imputation ● Sensitivity analysis imputation	Mantel–Haenszel (MH) test: adjusted for baseline perforation size group by each ASP0598 treatment group Fisher-exact test and Clopper-Perason method (no stratification factor) if Mantel and Fleiss criterion not satisfied
	Change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 12 for single dose expansion and Week 16 for multiple dose expansion ● Primary analysis: No imputation ● Sensitivity analysis: discontinuation reason based multiple imputation (MI)[a]	MMRM model: Treatment group and week as factors, baseline TMP size as a covariate, as well as an interaction of treatment by week and an interaction of baseline TMP size by week
	Change from baseline in TMP size at Week 12 for single dose expansion and Week 16 for multiple dose expansion ● Primary analysis: No imputation ● Sensitivity analysis: discontinuation reason based MI[a]	MMRM model: Treatment group and week as factors, baseline TMP size as a covariate, as well as an interaction of treatment by week and an interaction of baseline TMP size by week
Exploratory Efficacy	Relationship between change from baseline in the ratio of TMP size per total area of tympanic membrane and mean A-B gap at Week 12 for single dose expansion and Week 16 for multiple dose expansion	Linear regression: Change from baseline in the ratio of TMP size per total area of tympanic membrane as predictor variable and change from baseline in mean A-B gap as response

[a] Discontinuation-reason based MI will use a "Jump to Reference" algorithm (where placebo is the reference group) for subjects who discontinue the study investigational period due to lack of efficacy or AEs and standard regression-based MI for subjects with missing data for other reasons.

6.4.1 Analysis of Primary Efficacy Endpoint(s)

Not applicable.

6.4.2 Analysis of Secondary Efficacy Endpoints

SAD and MAD

No statistical hypothesis testing will be performed.

Single dose expansion

- Subjects with complete closure of TMP at Week 12

  Primary Analysis:

  At week 12, to understand the relationship between complete closure of TMP and treatment group, MH test adjusting for baseline perforation size group (≤ 15%, > 15%) will be used. Analysis will be performed using the data for the estimand (refer to

Section 5.3 for details) and done separately for each ASP0598 treatment group vs placebo. Here baseline perforation size group is the same as baseline TMP size group per total area of tympanic membrane from central imaging.

From this analysis, the following results will be presented:

- Proportion of subjects with complete closure of TMP in each treatment group (each of ASP0598 treatment group and placebo),
- For the comparisons between each of the ASP0598 treatment group vs placebo, risk difference (ASP0598 – placebo), 2-sided p-value for overall general association between treatment group and response and 2-sided 95% stratified Newcombe Confidence Interval (CI) computed using MH weights for the risk difference. If the CI cannot be obtained using Newcombe method then they will be presented using Sato method.

  If the Mantel and Fleiss criterion (across-strata sum of expected vales for a particular cell has a difference of at least 5 from both the minimum possible sum and the maximum possible sum of the observed values) is not satisfied, the above analysis will be performed with no stratification factor and exact p-value using Fisher-exact method and CI using Clopper-Pearson method will be presented.

  Based on the interim analysis results, if 3 treatment groups are selected for the dose expansion phase, hierarchical testing method will be used to control familywise error rate where the highest dose group vs placebo will be tested first at a 2-sided significance level of 0.05 and if the null hypothesis is rejected for this comparison, low dose group vs placebo will be tested at a 2-sided significance level of 0.05.

Sensitivity analysis:

The above analysis will be repeated using sensitivity analysis imputation definition.

- Change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 12
- Change from baseline in TMP size at Week 12

Primary analysis:

For the change from baseline in TMP size at Week 12, a MMRM will be used with treatment group and week (Week 2, 4, 8 and 12) as factors, baseline TMP size as a covariate, as well as an interaction of treatment by week and an interaction of baseline TMP size by week.

For the change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 12, a MMRM will be used with treatment group and week (Week 2, 4, 8 and 12) as factors, baseline ratio of TMP size per total area of tympanic membrane as a covariate, as well as an interaction of treatment by week and an interaction of baseline ratio of TMP size per total area of tympanic membrane by week.

The repeated measures are not equally spaced. The model will assume unstructured

covariance among the within subject repeated measurements. Fitting a model can be computationally intensive and if the algorithm does not converge, spatial power covariance structure and then compound symmetry as a covariance structure will be tried to achieve convergence. Denominator degrees of freedom will be estimated using the Kenward-Roger approximation. Analysis will be performed using the data for the estimand (refer to Section 5.3 for details). From this analysis, the following results will be presented:

- Least square (LS) mean estimate, standard error (SE), and 2-sided 95% CI for change from baseline to each treatment week within a treatment group (placebo and each ASP0598 treatment group),
- For comparisons between each of the ASP0598 treatment group vs placebo:
  - Difference in LS mean estimates (ASP0598 – placebo), SE and corresponding 2-sided 95% CI for the change from baseline at each week.
  - The differences in the LS mean estimates will be used to obtain 2-sided p-value for the difference.

Sensitivity analysis:

The above analysis will be repeated under the assumption that data is MNAR using discontinuation-reason based MI. For the discontinuation-reason based MI, refer to Section 6.9.3.5 for details. From this analysis, the following results will be presented using the pooled results per step 3 of Section 6.9.3.5.

- LS mean estimate, SE, and 2-sided 95% CI for change from baseline to each treatment week within a treatment group (placebo and each ASP0598 treatment group),
- For comparisons between each of the ASP0598 treatment group vs placebo:
  - Difference in LS mean estimates (ASP0598 – placebo), SE and corresponding 2-sided 95% CI for the change from baseline at each week.
  - The differences in the LS mean estimates will be used to obtain 2-sided p-value for the difference.
- Descriptive statistics at each visit will be derived using the mean of the M=100 imputed datasets.

Checks of model assumptions will be carried using residual plots of scaled residuals against the predicted values and scaled residuals normal plot.

If the lack of model assumptions are detected, additional model using generalized linear mixed model may be fitted as appropriate.

Multiple dose expansion

- Subjects with complete closure of TMP at Week 16

Primary Analysis:

At week 16, to understand the relationship between complete closure of TMP and treatment group, MH test adjusting for baseline perforation size group will be used. Refer to single dose expansion endpoint subjects with complete closure of TMP at Week 12 primary analysis for details.

Sensitivity analysis:

The above analysis will be repeated using sensitivity analysis imputation definition.

- Change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 16
- Change from baseline in TMP size at Week 16

Primary Analysis:

For the change from baseline in TMP size at Week 16, a MMRM will be used with treatment group and week (Week 2, 4, 6, 8, 12 and 16) as factors, baseline TMP measurement as a covariate, as well as an interaction of treatment by week and an interaction of baseline TMP measurement by week. For the change from baseline in the ratio of TMP size per total area of tympanic membrane at Week 16, the MMRM model will contain treatment group and week as factors, baseline ratio of TMP size per total area of tympanic membrane as a covariate, as well as an interaction of treatment by week and an interaction of baseline ratio of TMP size per total area of tympanic membrane by week. Refer to single dose expansion endpoint change from baseline in TMP size at Week 12 primary analysis for details.

Sensitivity analysis:

The above analysis will be repeated under the assumption that data is MNAR using discontinuation-reason based MI. Refer to single dose expansion endpoint change from baseline in TMP size at Week 12 sensitivity analysis for details.

6.4.3 Analysis of Exploratory Endpoints

Continuous endpoints:

SAD and MAD

No statistical analysis will be performed.

For the mean air conduction hearing loss group, a shift table of category changes from baseline value to post baseline value at each analysis visit (Week 8 and EOS for SAD, Week 12 and EOS for MAD) will be presented.

Dose expansion

- Change from baseline in the ratio of TMP size per total area of tympanic membrane for post baseline visits at Week 2, Week 4, and Week 8 for single dose expansion

- Change from baseline in TMP size for post baseline visits at Week 2, Week 4, and Week 8 for single dose expansion
- Change from baseline in the ratio of TMP size per total area of tympanic membrane for post baseline visits at Week 2, Week 4, Week 6, Week 8, and Week 12 for multiple dose expansion
- Change from baseline in TMP size for post baseline visits at Week 2, Week 4, Week 6, Week 8, and Week 12 for multiple dose expansion

  The above change from baseline dose expansion phase endpoints will be analyzed using the MMRM model as described in Section 6.4.2 Single Dose Expansion.

To understand the relationship between change from baseline in the ratio of TMP size per total area of tympanic membrane and mean A-B gap at Week 12 for single dose expansion and Week 16 for multiple dose expansion, linear regression will be used with change from baseline in mean A-B gap as response and change from baseline in the ratio of TMP size per total area of tympanic membrane as predictor variable. A scatter plot with linear regression line, regression equation and 95% CI for the slope will be provided.

For the mean air conduction hearing loss group, a shift table of category changes from baseline value to post baseline value at each analysis visit (Week 12 and EOS for single dose expansion, Week 12, Week 16 and EOS for multiple dose expansion) will be presented.

Categorical endpoints:

Refer to Section 5.4 for the list of categorical endpoints. No statistical analysis will be performed.

6.5 Analysis of Safety

Safety analyses will be presented by treatment group and ASP0598 total for SAF, unless specified otherwise.

6.5.1 Adverse Events

AEs will be coded using MedDRA.

For the AEs, the following summaries will be presented:

- An overview of TEAEs and deaths
  - Number of TEAEs
  - Number and percentage of subjects with TEAEs
  - Number of drug-related TEAEs
  - Number and percentage of subjects with drug-related TEAEs
  - Number of serious TEAEs
  - Number and percentage of subjects with serious TEAEs
  - Number of serious drug-related TEAEs
  - Number and percentage of subjects with serious drug-related TEAEs
  - Number of TEAEs leading to treatment discontinuation (MAD and MDE only)

- Number of deaths

- Number and percentage of subjects with TEAEs by SOC and PT for the following:
  1) TEAEs
  2) Drug-related TEAEs
  3) TEAEs Leading to Treatment Discontinuation (MAD and MDE)
  4) Serious TEAEs and including number of events
  5) Drug-related serious TEAEs and including number of events
  6) Common (≥15% in any treatment group) TEAEs for expansion only
  7) TEAEs excluding serious AEs and including number of events and number of threshold events. Here threshold equals 5%.This summary will be created only if at least one serious TEAE is present.

- An overview of TEAEs of interest
  - Number of TEAEs of interest
  - Number and percentage of subjects with TEAEs of interest
  - Number of drug-related TEAEs of interest
  - Number and percentage of subjects with drug-related TEAEs of interest
  - Number of serious TEAEs of interest
  - Number and percentage of subjects with serious TEAEs of interest
  - Number of serious drug-related TEAEs of interest
  - Number and percentage of subjects with serious drug-related TEAEs of interest

  The above summary will present results for any TEAEs of interest and each of the TEAEs of interest: Cholesteatoma or neoplasm, Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness) and Otitis media or otitis externa.

- Number and percentage of subjects with TEAEs of special interest by SOC and PT for the following:
  1) TEAEs of special interest for Cholesteatoma or neoplasm
  2) TEAEs of special interest for Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness)
  3) TEAEs of special interest for Otitis media or otitis externa
  4) Serious TEAEs of special interest for Cholesteatoma or neoplasm and including number of events
  5) Serious TEAEs of special interest for Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness) and including number of events
  6) Serious TEAEs of special interest for Otitis media or otitis externa and including number of events

6.5.2 Bone Conduction Hearing at 1, 2, 4 kHz by PTA

Descriptive statistics will be used to summarize the bone conduction hearing at each frequency (1, 2 and 4 kHz) and the mean baseline value, post baseline value and change from

baseline at each specified post baseline analysis visit by treatment group.

A summary will also present the number and percentage of subjects with an increase of ≥ 20 dB from baseline for each frequency and the mean bone conduction hearing at each post baseline analysis visit. This summary will also present results for an increase of ≥ 10 dB from baseline at 2 consecutive frequencies (≥ 10 dB at 1 and 2 kHz or ≥ 10 dB at 2 and 4 kHz) for each post baseline analysis visit.

Analysis visits:

SAD	Baseline, Day 2, Day 3[a], Week 1, Week 2, Week 4, Week 8, EOS and Maximum[b]
Single dose expansion	Baseline, Week 2, Week 4, Week 8, Week 12, EOS and Maximum[b]
MAD	Baseline, Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12, EOS and Maximum[b]
Multiple dose expansion	Baseline, Week 2, Week 4, Week 6, Week 8, Week 12, Week 16, EOS and Maximum[b]

[a] Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

[b] Maximum during study period.

6.5.3 Tinnitus Visual Analog Scale (TVAS)

Descriptive statistics will be used to summarize the treated ear TVAS baseline value, post baseline value and change from baseline at each specified post baseline analysis visit by treatment group. Refer to Section 6.5.2 for the list of analysis visits. Number and percentage of subjects will be used to summarize an increase from baseline for the TVAS at each specified post baseline analysis visit by treatment group.

- An increase in tinnitus in any ear.
- An increase in tinnitus in treated ear.

Analysis visits:

SAD	Day 2 to Week 2, Day 2, Day 3[a], Week 1, Week 2, Week 4, Week 8 and Maximum*
Single dose expansion	Day 2 to Week 2, Week 2, Week 4, Week 8, Week 12 and Maximum[b]
MAD	Day 2 to Week 2, Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12, EOS and Maximum[b]
Multiple dose expansion	Day 2 to Week 2, Week 2, Week 4, Week 6, Week 8, Week 12, Week 16, EOS and Maximum[b]

For Day 2 to Week 2, maximum post baseline value during the analysis window will be used.

[a] Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

[b] Maximum post baseline value during study period.

6.5.4 Microscopic Ear Examination

On Day 1, Week 2 (multiple dose only) and Week 4 (multiple dose only), 1 hour after the treatment, number and percentage of subjects will be used to summarize the following canal reactions of treated ear by treatment group:

- Did the Ear experience an abnormal ear sensation
  - No
  - Yes
    - Burning
    - Stinging
    - Itching
    - Pain
    - Other

  Percentages for the subcategories of 'Yes' will be based on number of subjects with abnormal ear sensation=Yes.

- After Day 1, for categorical endpoints status of the middle ear and status of the treated tympanic membrane, number and percentage of subjects will be used to summarize baseline value and post-baseline value at each specified post-baseline analysis visit by treatment group. Other parameters will be listed only.

  Status of the treated tympanic membrane (Normal, Abnormal, Abnormal: Myringitis, Abnormal: Hematoma, Abnormal: Other, Not visible)

  Status of the middle ear in the treated ear (Normal, Abnormal, Abnormal: Mucosal inflammation with effusion, Abnormal: Mucosal inflammation without effusion, Abnormal: Other, Not visible)

  Percentages for the subcategories of 'Abnormal' will be based on number of subjects with abnormal.

Analysis visits:

SAD	Day 2, Day 3*, Week 1, Week 2, Week 4, Week 8 and EOS
Single dose expansion	Week 2, Week 4, Week 8, Week 12 and EOS
MAD	Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12 and EOS
Multiple dose expansion	Week 2, Week 4, Week 6, Week 8, Week 12, Week 16 and EOS

*Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

Number and percentage of subjects will be used to summarize the local skin reaction at the application site by grade as assessed by Modified Brighton Grading Scheme.

- Day 1 post dose through end of study visit by grade, grade 1 or above, grade 3 or above
- Day 1 post dose by grade, grade 1 or above, grade 3 or above
- Day 2 by grade, grade 1 or above, grade 3 or above (SAD only)
- Day 3 by grade, grade 1 or above, grade 3 or above (SAD cohorts 1, 2 and 3 only)
- 2nd dose: post dose through end of study by grade, grade 1 or above, grade 3 or above (MAD and multiple dose expansion)
- 2nd dose: post dose on the dosing day by grade, grade 1 or above, grade 3 or above (MAD and multiple dose expansion)

- 3rd dose: post dose through end of study by grade, grade 1 or above, grade 3 or above (MAD and multiple dose expansion)
- 3rd dose: post dose on the dosing day by grade, grade 1 or above, grade 3 or above (MAD and multiple dose expansion)
- Days 2 to 18 by grade, grade 1 or above, grade 3 or above
- ≥ Days 19 by grade, grade 1 or above, grade 3 or above

When assessing severity, if the event changes in severity (0, 1, 1b, 2, 2b, 3, 3b, 4 and 4b where Grade 4b is the worst grade) during a time interval for a subject, the event with the worst grade will be chosen. If the event is reported multiple times with missing grade(s) and with non-missing grade(s) during an analysis period for a subject, then the subject will be counted for the non-missing worst grade. If the event is reported multiple times with all missing grade(s) for a subject, then the subject will be counted as a missing grade.

Refer to Section 6.9.1 for end day of end of study visit definition.

6.5.5 Clinical Laboratory Evaluation

Not applicable.

6.5.5.1 Liver Safety Assessment

Not applicable.

6.5.6 Vital Signs

For vital signs parameters: systolic blood pressure, diastolic blood pressure, pulse rate and body temperature, descriptive statistics will be used to summarize baseline value, post-baseline value and change from baseline at each specified post-baseline analysis visit by treatment group. Refer to Section 6.5.2 for the list of analysis visits excluding maximum.

6.5.7 Electrocardiograms

Not applicable.

6.6 Pharmacokinetic

Descriptive statistics (n, mean, SD, minimum, median, maximum, coefficient of variation (CV), geometric mean, and geometric CV) will be used to summarize baseline value, post-baseline value, and change from baseline at each post-baseline time point for serum concentrations of ASP0598 by treatment group including placebo using the SAF.

Analysis time points:

SAD: Baseline, Day 1 0.5 hour post dose, Day 1 1 hour post dose and Day 3 (Cohorts 1, 2, and 3.) or Day 2 (Cohorts 4, 5, and 6).

MAD: Baseline, Day 1 0.5 hour post dose, Day 1 1 hour post dose, Week 4 predose, Week 4 0.5 hour post dose, Week 4 1 hour post dose.

Dose expansion: Not Applicable.

6.7 Other Analyses

No other analyses are planned.

6.8 Interim Analysis (and Early Discontinuation of the Clinical Study)

Two interim analyses are planned and will be conducted after completion of the last cohort of the SAD and MAD, respectively. The decision to open the single dose expansion and multiple dose expansion will be made based on the safety and efficacy results of the above interim analyses. During each interim analysis, the Data Monitoring Committee (DMC) team will review the data and make the final decision to open the dose expansion. Three different treatment groups including placebo or two different treatment groups including placebo will be selected to open the expansion phase. Additional details and analyses will be described in a DMC charter/an IAP.

6.9 Additional Conventions

6.9.1 Analysis Windows for Safety Variables

For the assessments where the summaries and/or analyses are presented by analysis visit during study period, post baseline values will not contribute to the safety analyses:

- SAD: if the study day of the value is > minimum(study day of end of study visit, 57) + 7 days.
- Single dose expansion and MAD: if the study day of the value is > minimum(study day of end of study visit, 85) + 7 days.
- Multiple dose expansion: if the study day of the value is > minimum (study day of end of study visit, 113) + 7 days.

For the assessments where the summaries and/or analyses are not presented by analysis visit during study period (AEs, previous and concomitant medications and modified brighton grading scheme), end day of end of study visit is defined as follows:

- SAD: minimum(study day of end of study visit, 57) + 7 days.
- Single dose expansion and MAD: minimum(study day of end of study visit, 85) + 7 days.
- Multiple dose expansion: minimum (study day of end of study visit, 113) + 7 days.

6.9.1.1 Duplicate Values on the Same Day

If more than one value is reported on the same day, then the value based on the following convention will be used for analysis visit window programming. This needs to be done before creating the analysis visits or time points.

Variable Type	Assessment	Convention
Continuous variable	Bone Conduction by PTA	Last value on that day based on non-missing time. If time is missing for all values on the same day, then the value corresponding to the scheduled visit will be used.
	TVAS	
	Vital Signs	
Categorical variable	Microscopic ear examination	Last value on that day based on non-missing time. If time is missing for all values on the same day, then the value corresponding to the scheduled visit will be used.

6.9.1.2 Analysis Visit Windows

Table 4 Analysis Visit Windows: Bone Conduction by PTA, TVAS, Microscopic Ear Examination and Vital Signs

		Analysis Visit Window (Study Day)			
Analysis Visit	**Target Study Day**	**SAD**	**Single dose expansion**	**MAD**	**Multiple dose expansion**
Baseline [a]	1	≤ 1	≤ 1	≤ 1	≤ 1
Day 2[b]	2	2	NA	NA	NA
Day 2[c]	2	2 to 3	NA	NA	NA
Day 3[d]	3	3 to 4	NA	NA	NA
Week 1	8	5 to 11	NA	2 to 11	NA
Week 2	15	12 to 18	2 to 18	12 to 18	2 to 22
Week 3	22	NA	NA	19 to 25	NA
Week 4	29	19 to 39	19 to 39	26 to 32	23 to 36
Week 5	36	NA	NA	33 to 39	NA
Week 6	43	NA	NA	NA	37 to 50
Week 8	57	≥ 40	40 to 74	40 to 74	51 to 71
Week 12	85	NA	≥ 75	≥ 75	72 to 99
Week 16	113	NA	NA	NA	≥ 100
Minimum [e]		≥ 2	≥ 2	≥ 2	≥ 2
Maximum [f]		≥ 2	≥ 2	≥ 2	≥ 2
EOS [g]		≥ 2	≥ 2	≥ 2	≥ 2

[a] Baseline value for the above data is based on the last available non-missing value on or prior to the first dose day (Day 1) of study drug within the visit window.

[b] For cohorts 1, 2 and 3.

[c] For cohorts 4, 5 and 6.

[d] Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

[e] Minimum value for the above data is based on the minimum of non-missing post-baseline value within the visit window.

[f] Maximum value for the above data is based on the maximum of non-missing post-baseline value within the visit window.

[g] EOS value for the above data is based on the last available non-missing post-baseline value within the visit window.

For microscopic ear examination, 1 hour post dose analysis timepoint on dosing days will be assigned same as the nominal timepoint.

To identify the value for the analysis, the following rules will apply excluding baseline, minimum, maximum and EOS:

1. For an analysis visit, if a subject has more than one result with a value included within a window, the value closest to the target study day will be used. In case of ties between values located on different sides of the target study day, the later assessment will be used.

2. For the categorical parameter, if a subject has more than one result included within a window, the assessment closest to the target study day will be used. In case of ties between results located on different sides of the target study day, the later assessment will be used.

Planned time points will be used as analysis time points. Concentrations will be excluded from analysis if the actual time from dosing (actual sample date/time – dose date/time) is outside the time window as specified below.

Table 5 Analysis Visit Windows: Serum ASP0598 or Placebo Concentrations

Analysis Time Point	Analysis Time Window
Baseline (Pre-dose)	Actual time from dosing ≤ 0
0.5 hour post dose	Actual time from dosing = 30 ± 5 minutes
1 hour post dose	Actual time from dosing = 60 ± 5 minutes

6.9.1.3 Value Below Limit of Quantification

1) Serum concentration values (ASP0598 or Placebo) below limit of quantification (BLQ) will be set to 0 for the calculation of summary statistics. Geometric mean and Geometric CV will not be calculated if at least 1 concentration value is equal to 0.

2) If more than 50% of the concentrations are BLQ at a given time point, SD and CV will not be calculated in the summary statistics.

6.9.2 Analysis Windows for Efficacy Variables

For the assessments where the summaries and/or analyses are presented by analysis visit during study period, post baseline values will not contribute to the efficacy analyses:

- SAD: if the study day of the value is > minimum(study day of end of study visit, 57) + 7 days.
- Single dose expansion and MAD: if the study day of the value is > minimum(study day of end of study visit, 85) + 7 days.
- Multiple dose expansion: if the study day of the value is > minimum(study day of end of study visit, 113) + 7 days.

6.9.2.1 Duplicate Values on the Same Day

If more than one value is reported on the same day, then the result based on the following convention will be used for analysis visit window programming. This needs to be done before creating any analysis visits or time points including baseline or screening.

Variable Type	Assessment	Convention
Continuous variable	Imaging	Last value on that day based on non-missing time. If time is missing for all values on the same day, then the value corresponding to the scheduled visit will be used.
	PTA Assessments	
	Tympanometry	
Categorical variable	Imaging	For complete closure of TMP, Last value on that day based on non-missing time. If time is missing for all values on the same day, then the value corresponding to the scheduled visit will be used.

6.9.2.2 Analysis Visit Windows

Table 6 Analysis Visit Windows: Imaging

		Analysis Visit Window (Study Day)			
Analysis Visit	**Target Study Day**	**SAD**	**Single dose expansion**	**MAD**	**Multiple dose expansion**
Baseline [a]	1	≤ 1	≤ 1	≤ 1	≤ 1
Day 2[b]	2	2	NA	NA	NA
Day 2[c]	2	2 to 3	NA	NA	NA
Day 3[d]	3	3 to 4	NA	NA	NA
Week 1	8	5 to 11	NA	2 to 11	NA
Week 2	15	12 to 18	2 to 18	12 to 18	2 to 22
Week 3	22	NA	NA	19 to 25	NA
Week 4	29	19 to 39	19 to 39	26 to 32	23 to 36
Week 5	36	NA	NA	33 to 39	NA
Week 6	43	NA	NA	NA	37 to 50
Week 8	57	≥ 40	40 to 74	40 to 74	51 to 71
Week 12	85	NA	≥ 75	≥ 75	72 to 99
Week 16	113	NA	NA	NA	≥ 100
EOS [e]		≥ 2	≥ 2	≥ 2	≥ 2

For the results corresponding to post-dose timepoints immediately after, 0.5 hour and 1 hour post-dose on the dosing days, analysis timepoint will be assigned same as the nominal timepoint and these results will not be used to identify the value for analysis.

[a] Baseline value for the above data is based on the last available non-missing value on or prior to the first dose day (Day 1) of study drug within the visit window.

[b] For cohorts 1, 2 and 3.

[c] For cohorts 4, 5 and 6.

[d] Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

[e] EOS value for the above data is based on the last available non-missing post-baseline value within the visit window.

Table 7 Analysis Visit Windows: PTA Assessments, Tympanometry and Tympanogram

		Analysis Visit Window (Study Day)			
Analysis Visit	**Target Study Day**	**SAD**	**Single dose expansion**	**MAD**	**Multiple dose expansion**
Baseline [a]	1	≤ 1	≤ 1	≤ 1	≤ 1
Week 8	57	≥ 40	NA	NA	NA
Week 12	85	NA	≥ 75	≥ 75	72 to 99
Week 16	113	NA	NA	NA	≥ 100
EOS [b]		≥ 2	≥ 2	≥ 2	≥ 2

[a] Baseline value for the above data is based on the last available non-missing value on or prior to the first dose day (Day 1) of study drug within the visit window.

[b] EOS value for the above data is based on the last available non-missing post-baseline value within the visit window.

To identify the value for the analysis, the following rules will apply excluding baseline and EOS:

1. For an analysis visit, if a subject has more than one result included within a window, the value closest to the target study day will be used. In case of ties between values located on different sides of the target study day, the later assessment will be used.

2. For the categorical parameter, if a subject has more than one result included within a window, the assessment closest to the target study day will be used. In case of ties between results located on different sides of the target study day, the later assessment will be used.

6.9.3 Imputation Rules

6.9.3.1 Imputation of AE Onset Date

For AEs, a missing or incomplete onset date will be imputed according to the following conventions:

AE Onset Date Scenario	**Convention**
Completely missing or only the year is known	Non-missing first dose date of study drug
Only the month and year is known	Set surrogate onset date = AE onset date where day is set to the first day of that month and then apply the following rules: 1. If the month and year of the onset date is prior to the month and year of the first dose of study drug, then the surrogate onset date will be the imputed onset date. 2. If the month and year of the onset date is on or after the month and year of the first dose of study drug, then the imputed onset date will be the latest of the following non-missing dates: • First dose date of study drug • Surrogate onset date

If the above imputed onset date is after a complete AE end date, the imputed onset date will be the same as the complete AE end date.

6.9.3.2 Imputation of Previous and Concomitant Medication Dates

For previous and concomitant medications, a missing or incomplete start and stop dates will be imputed according to the following conventions:

Start Date Scenario	Convention
Missing day	first day of the month under consideration
Missing month	January
Missing year	year of the informed consent date
Completely missing	Informed consent date

Stop Date Scenario	Convention
Missing day and not ongoing	last day of the month under consideration
Missing month and not ongoing	December
(Missing year or completely missing) and not ongoing	December 31st, 2099
Ongoing	Remains missing

If the above imputed start date is after the stop date, then the imputed start date will be one day prior to the stop date.

6.9.3.3 Imputation of Study Drug Start Date

For the randomized subjects that received study drug, a missing or partial first dose date of study drug will be imputed as the non-missing dispense date at randomization.

6.9.3.4 Onset Date of CTMP

Onset Date Scenario	Convention
Missing day, but month and year are present	the day will be imputed as the 15th day of the month
Missing day and month, but year is present	the day and month will be imputed as 30 June of the year
Missing year, but day and month are present	No imputations will occur, and the subject will be excluded from all summaries related to CTMP duration
Completely missing	Informed consent date

If the above imputed date falls after the informed consent date, then the onset date will be taken as equal to the informed consent date.

6.9.3.5 Discontinuation-Reason based MI

The MMRM analysis that will be used to perform the primary analysis of the secondary endpoints assumes that missingness is at random. That is, the model assumes that the trajectory of TMP size (or ratio of TMP size per total area of tympanic membrane) over time for subjects who withdrew the study is similar to the trajectory for those observed in their own treatment group which is valid so long as that assumption is reasonable.

Discontinuation-reason based MI will be used to examine the sensitivity of the primary analysis results to departures from that underlying assumption and will assess a situation where data for subjects who discontinue early follow a pattern which is missing not at random.

First Markov chain Monte Carlo (MCMC) method will be used to partially impute non-monotone missing data for completed and discontinued subjects from the study investigational period for each treatment group separately. After this is done, MI will be used to impute the monotone missing data for the subjects discontinue the study investigational period as follows:

MI will be used for imputation of any missing data, using “Jump to Reference” algorithm (where placebo is the reference group) [Carpenter et al. 2013] under the assumption of MNAR for subjects who discontinue due to lack of efficacy or AEs and standard regression based MI under the assumption of MAR for subjects with missing data for other reasons. The analysis will be implemented using the general three-step process (imputation phase, analysis phase and pooling phase) described in O’Kelly and Ratitch, 2014.

1. The imputation phase will implement MI via sequential modelling for both the “Jump to Reference” algorithm and standard regression-based MI. In particular, when implementing the “Jump to Reference” algorithm via sequential modelling, only baseline values are used in the imputation of missing values for subjects in the ASP0598 active groups who discontinue study investigational period due to lack of efficacy or AEs (this variation of the “Jump to Reference” algorithm is also referred to as the “Unconditional Reference” approach). The imputation phase will generate M imputed datasets, where M =100.
2. The analysis phase will perform the primary MMRM analysis model of the change from baseline secondary efficacy endpoints as described in Section 6.4.2 for each of the M = 100 imputed datasets (which now contain complete data, where missing data have been filled in).
3. Rubin’s rules will be used to generate an overall set of pooled results which combines the analysis results from the M = 100 imputed datasets.

7 REVISION AND RATIONALE

7.1 List of Changes in SAP Version 3.0 from Version 2.0

The changes from the approved SAP from Version 2.0 (Dated 22-Nov-2020) to Version 3.0 (Dated 06-Jul 2022) that impact analyses are listed with the rationale in the table below.

SAP Sections	Description	Rationale
4.1	Changed definition of FAS to match protocol	The protocol changed the definition of the FAS to account for
5.4, Table 1, Table 2, 6.5.2, 6.5.3, 6.5.4, 6.6, Table 4, Table 6	Changed references to Day 3 to note that Day 3 assessments were completed for cohorts 1, 2, and 3 only.	The protocol was revised to remove Day 3 for SAD to consolidate visits and procedures for the subjects. The revision to add a + 1 day visit window to Day 2 is made so that assessments (including the pharmacokinetic [PK] sample) can take place on Day 1 or Day 3 depending on the site or subject's schedule.
Table 1	Added line Observed response for complete closure of TMP as assessed by site investigator and Imputed Response for complete closure of TMP as assessed by site investigator	The tables for complete TMP closure and imputation of TMP closure were changed to include both the site investigator assessment and central reader assessment.
6.5.1	Added TEAEs causing treatment discontinuation (only for MAD and MDE)	These TEAEs are collected for MAD and MDE.

7.2 List of Changes in SAP Version 2.0 from Version 1.0

The changes from the approved SAP Version 1.0 (Dated 23-Mar-2020) to Version 2.0 (Dated 22-Nov-2020) that impact analyses are listed with the rationale in the table below.

SAP Sections	Description	Rationale
6.4, 6.4.3	For the regression analysis of change from baseline in TMP size and mean air conduction hearing at Week 12 treatment group as a classification variable is removed. Also predictor variable is updated as change from baseline in ratio of TMP size per total area of tympanic membrane and response is updated as change from baseline in mean A-B gap.	An improvement in TMP size should improve the air conduction hearing hence the response and predictor variables are reversed. The analysis is to understand the relationship between the response variable and predictor hence the classification variable is removed. Change from baseline in ratio of TMP size per total area of tympanic membrane is used because it is normalized per the total area of tympanic membrane. Change from baseline in mean A-B gap is used because air conduction and bone conduction tests analyzed together determines any hearing loss.
6.5.4	For Modified Brighton Grading Scheme, time intervals Days 2 to 11 is updated as Days 2 to 18, Day 19 to 39 is updated as ≥ Days 19. ≥ Days 40 time interval is removed.	Provides comparisions across SAD, MAD, single dose expansion and multiple dose expansion in a consistent manner.
6.9.1.1	Details to address duplicate values on the same day for Microscopic ear examination is added.	Missing hence added.
9.1, 9.2, 9.3	For the categorical variable coming from imaging data, updated about addressing duplicates values on the same day as the continuous variables.	For consistency across variables coming from imaging data.
6.9.1.2, 6.9.2.2	Visit Window for Week 2 single dose expansion is updated.	Due to Protocol Version 2.0 (Week 1 is removed).
	Adverse Events of Interest list is revised using MedDRA version 23.0.	Due to MedDRA version update which is updated to capture COVID-19 related terms. The terms in Sections 9.2 and 9.3 remained the same compared to MedDRA version 22.0.

8 REFERENCES

ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

ICH Harmonized Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

Carpenter JR, Roger JH & Kenward MG. Analysis of longitudinal trials with protocol deviation: a framework for relevant, accessible, assumptions, and inference via multiple imputation. Journal of Biopharmaceutical Statistics. 2013; 23(6): 1352-1371.

O'Kelly M. & Ratitch B. Clinical Trials with Missing Data A Guide for Practitioners, Statistics in Practice (2014): 189 - 190.

9 APPENDICES

9.1 Appendix 1: Pre-Specified Criteria for Adverse Events of Interest: Cholesteatoma or Neoplasm

All selected terms are based on MedDRA Version 23.0

Neoplasm

1. Aural neoplasms benign (PTs)

Preferred Term Code	Preferred Term
10052241	Aural cystadenoma
10003794	Aural polyp
10069051	Benign ear neoplasm
10004292	Benign middle ear neoplasm
10008649	Cholesterin granuloma of middle ear
10008660	Cholesterol granuloma
10061332	Paraganglion neoplasm

2.Aural neoplasms malignant (PTs)

Preferred Term Code	Preferred Term
10055016	Ear neoplasm
10055017	Ear neoplasm malignant
10082277	External ear neoplasm malignant
10061267	Malignant middle ear neoplasm

Cholesteatoma (PTs)

Preferred Term Code	Preferred Term
10008642	Cholesteatoma

9.2 Appendix 2: Pre-Specified Criteria for Adverse Events of Interest: Ototoxic symptoms (Tinnitus, Sensorineural hearing loss, Dizziness)

All selected terms are based on MedDRA Version 23.0

Ototoxicity symptoms (PTs)

Preferred Term Code	Preferred Term
10043882	Tinnitus
10047340	Vertigo
10013573	Dizziness
10011878	Deafness
10061373	Sudden hearing loss

Ototoxicity (PTs)

Preferred Term Code	Preferred Term
10033109	Ototoxicity

9.3 Appendix 3: Pre-Specified Criteria for Adverse Events of Interest: Otitis media or Otitis externa

All selected terms are based on MedDRA Version 23.0

Middle ear infections (PTs)

Preferred Term Code	Preferred Term
10061557	Allergic otitis media
10078160	Bezold abscess
10081430	Eosinophilic otitis media
10063095	Gradenigo's syndrome
10065838	Middle ear inflammation
10061302	Myringitis
10078830	Noninfective myringitis
10033078	Otitis media
10033079	Otitis media acute
10065176	Otitis media bacterial
10033081	Otitis media chronic
10065175	Otitis media fungal
10067322	Otitis media haemophilus
10033083	Otitis media moraxella
10080507	Otitis media pneumococcal
10033085	Otitis media post measles
10080508	Otitis media staphylococcal
10065177	Otitis media viral
10033102	Otosalpingitis
10034762	Petrositis

External ear infections (PTs)

Preferred Term Code	Preferred Term
10000295	Abscess of external auditory meatus
10075072	Allergic otitis externa
10014014	Ear lobe infection
10015729	External ear cellulitis
10065837	External ear inflammation
10019959	Herpes simplex otitis externa
10063491	Herpes zoster oticus
10033072	Otitis externa
10065179	Otitis externa bacterial
10033076	Otitis externa candida
10052557	Otitis externa fungal
10065178	Otitis externa viral
10062067	Perichondritis

9.4 Appendix 4: Author and Approver Signatures

(E-signatures are attached at the end of document.)