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)

ISN/Protocol 0598-CL-0101

Version 3.3

Incorporating Nonsubstantial Amendment 4 (see Section 13)

29 Aug 2022

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Sponsor:

Astellas Pharma Global Development Inc.
1 Astellas Way
Northbrook, IL 60062, US

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SIGNATURES

1. SPONSOR'S SIGNATURES

Required signatures (e.g., protocol authors and contributors, etc.) are located in [Section 14 Sponsor's Signatures].

3. INVESTIGATOR'S SIGNATURE

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)

ISN/Protocol 0598-CL-0101

Version 3.3

Incorporating Nonsubstantial Amendment 4

29 Aug 2022

I have read all pages of this protocol for which Astellas is the sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations. I will also ensure that sub-investigator(s) and other relevant members of my personnel have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: _____

Date (DD Mmm YYYY)

Printed Name: _____ _____

Address: _____

_____

CONTACT DETAILS OF SPONSOR'S KEY PERSONNEL

24-hour Contact for Serious Adverse Events See [Section 12.4.5 Reporting Procedures for Serious Adverse Events]	**Please fax or email the serious adverse events/special situations worksheet to:** **Astellas Pharma Global Development Inc.** **US Pharmacovigilance** **North America fax number: +1-888-396-3750** **North America alternate fax number: +1-847-317-1241** **Email: safety-us@astellas.com**
Medical Monitor/Study Physician	PPD
Clinical Research Contact(s)	*Parexel* PPD

1 PROTOCOL SUMMARY

1.1 Synopsis

Date and Version of Protocol Synopsis:	29 Aug 2022, Version 3.3
Sponsor: Astellas Pharma Global Development Inc. (APGD)	**Protocol Number:** 0598-CL-0101
Compound Name: ASP0598	**Phase of Development:** Phase 1/2

Title of Study:
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)

Planned Study Period:
From approximately 3Q2020 to 3Q2024

Study Objective(s) and Endpoint(s):

Objective	Endpoints
Primary	
• To evaluate the safety and tolerability of ASP0598 Otic Solution	• Treatment-emergent adverse events (TEAEs) • Incidence of adverse events of special interest as defined below: 1. Cholesteatoma or ear neoplasm 2. Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness) 3. Otitis media or otitis externa • Change from baseline in bone conduction hearing at 1, 2, 4 kHz by Pure Tone Audiometry (PTA) • Change from baseline in Tinnitus Visual Analog Scale (TVAS)

Table continued on next page

Objective	**Endpoints**
Secondary	
• To evaluate the efficacy of ASP0598 Otic Solution For secondary efficacy endpoints the hypothetical strategy will be applied.	*Efficacy* *Single Dose* ● Complete closure of Tympanic Membrane Perforation (TMP) at Week 8 for Single Ascending Dose (SAD) and at Week 12 for dose expansion ● 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 dose expansion ● Change from baseline in TMP size at Week 8 for SAD and at Week 12 for dose expansion *Multiple Dose* ● Complete closure of TMP at Week 12 for Multiple Ascending Dose (MAD) and at Week 16 for dose expansion ● 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 dose expansion ● Change from baseline in TMP size at Week 12 for MAD and at Week 16 for dose expansion
Exploratory	
• To evaluate otological parameters that may correlate with efficacy outcome and pharmacokinetics of ASP0598 Otic Solution	*Single Dose* ● Complete closure of TMP for post baseline visits at Day 2, Day 3*, Week 1, Week 2, and Week 4, for SAD and Week 2, Week 4, Week 8 for dose expansion ● Change from baseline in the ratio of TMP size per total area of tympanic membrane for post baseline visits at Day 2, Day 3*, Week 1, Week 2, and Week 4 for SAD and Week 2, Week 4, and Week 8 for dose expansion ● Change from baseline in TMP size for post baseline visits at Day 2, Day 3*, Week 1, Week 2, and Week 4 for SAD and Week 2, Week 4, and Week 8 for dose expansion * Day 3 evaluations will only be performed for cohorts 1, 2 and 3. *Multiple Dose* ● 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 dose expansion ● 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 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 dose expansion

Table continued on next page

	Single and Multiple Dose ● Time to closure of TMP ● Size of TMP ≤ 5% of pars tensa surface area after treatment ● Size of TMP ≤ 1% of pars tensa surface area (pin point perforation) after treatment ● Change from baseline in air bone (A-B) gap by PTA ● Change from baseline in air conduction hearing by PTA ● Percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing) ● Improvement from baseline in air conduction hearing by PTA ≥ 15 dB ● A-B gap after treatment was 15dB or less ● Air conduction hearing threshold after treatment was 30 dB or less ● Type of tympanogram (A, B or C) ● Equivalent ear canal volume (ECV) by tympanometry *Pharmacokinetics* ● Serum concentration of ASP0598 (dose escalation part only)
To evaluate the safety of ASP0598 Otic Solution	● Vital signs (body temperature, blood pressure and pulse rate)

Planned Total Number of Study Sites and Location(s): Approximately 20 study sites in the United States
Study Population: Subjects between 18 and 75 years of age at the time of informed consent with a clinical diagnosis of CTMP.
Number of Subjects to be Enrolled/Randomized: *Single Dose* Approximately 20 subjects in SAD (5 subjects per cohort) and up to 39 subjects in single dose expansion. *Multiple Dose* Approximately 16 subjects in MAD (8 subjects per cohort) and up to 39 subjects in multiple dose expansion.
Study Design Overview: This is a randomized, placebo-controlled and investigator and subject blinded study to evaluate the safety, tolerability, efficacy and pharmacokinetics in subjects with CTMP. The main part of this study is dose escalation. Dose escalation will start with single ascending dose (SAD) followed by multiple ascending dose (MAD, Q2W×3). Dose escalation will primarily assess safety and tolerability of single dose and multiple doses of ASP0598 Otic Solution. Dose escalation will consist of up to 4 cohorts for SAD and up to 2 cohorts for MAD, with different dose levels (Table 1). Each cohort will consist of 5 subjects (ASP0598 Otic Solution n = 4 and placebo n = 1) for SAD and 8 subjects (ASP0598 Otic Solution n = 6 and placebo n = 2) for MAD. Single and/or multiple dose expansion will be opened based on the safety and efficacy results following an interim analysis. Single dose expansion will be opened based on the results of the first interim analysis, which will occur after the completion of SAD. Multiple dose expansion will be opened based on the results of the second interim analysis, which will occur after the completion of MAD.

Table 1 Planned Dose Levels for Dose Escalation

Cohort	Dose regimen	Dose (µg)	Drug concentration (µg/mL)	Administration volume (mL)	ASP0598 Otic Solution (n)	Placebo (n)
1	Single	0.03	0.2	0.15	4	1
2	Single	0.15[a]	1.0[a]	0.15	4	1
3	Single	0.75[a]	5.0[a]	0.15	4	1
4	Single	2.25[a]	15[a]	0.15	4	1
5	Q2W×3	0.75[a]	5.0[a]	0.15	6	2
6	Q2W×3	2.25[a]	15[a]	0.15	6	2

[a] Planned dose levels. Doses may be adapted depending on emergent safety and tolerability data.

The study will consist of the following:

Single Dose

- Screening (Day -28 to -1)
- Randomization (Day 1)
- Observation [through Day 57 (SAD) or through Day 85 (single dose expansion)]

Multiple Dose

- Screening (Day -28 to -1)
- Randomization (Day 1)
- Observation [through Day 85 (MAD) or through Day 113 (multiple dose expansion)]
- Additional Treatment on Days 15 and 29

Prior to any study-related assessments, the Informed Consent Authorization Form will be signed by the subject at Screening (Visit 1). All subjects will enter into a screening period (Days -28 to -1) and will be evaluated for randomization eligibility after completing all the screening procedures. Eligible subjects will be randomized on Day 1 (Visit 2).

For a subject with bilateral TMP, once both ears have been identified as meeting all inclusion/exclusion criteria at Randomization on Day 1 (Visit 2), the ear with the worse hearing will be selected as the treated ear, unless a rationale exists to treat the other ear. In the case of equivalent hearing level in both ears, the investigator will decide the treatment side with subject and will document the reason in the source document and the electronic case report form (eCRF). In the case that the investigator is unable to decide the appropriate ear to treat, consultation with the sponsor may be required.

To Dose Escalation

Approximately 20 subjects for SAD and 16 subjects for MAD will be enrolled in the dose escalation part of the study. The initiation and selection of dose concentrations for MAD will be based on at least 3 cohorts from SAD. For SAD, after randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear. Subjects will return to the investigative site for assessments on Days 2, 3, 8, 15, 29, and 57 [end of study (EOS)]. Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

For MAD, after randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear and will receive additional treatments into the same ear on Days 15 and 29. Subjects will return to the investigative site for assessments on Days 8, 15, 22, 29, 36, 57, and 85 (EOS).

For SAD and MAD, subjects who terminate early from the study will complete EOS assessments.

For MAD only, subjects who discontinue treatment will be encouraged to complete all protocol defined study visits through the Day 85 (EOS).

The decision to escalate or stop escalation during SAD will be made by the Dose Escalation Committee (DEC). The DEC will operate as per the approved DEC Charter.

The DEC will decide dose escalation and the initiation and selection of doses for MAD cohorts based on the emerging safety and tolerability data from SAD. The tentative starting dose concentration of MAD will be 5.0 μg/mL, and another dose concentration up to a maximum feasible dose concentration of 15 μg/mL will be considered.

The first interim analysis will be conducted after completion of the last SAD cohort. The second interim analysis will be conducted after completion of the last MAD cohort.

The Data Monitoring Committee (DMC) will review the data and make the final decision to open single and/or multiple dose expansion. DMC operation, roles, and responsibilities will be described in the DMC Charter.

Subjects may be replaced at the discretion of the sponsor. If replaced, the replacement subject will be given the same treatment group as the original subject's randomized treatment group.

Dose Expansion

The decision to open single and/or multiple dose expansion will be made based on the safety and efficacy results of the interim analyses after completion of SAD and after completion of MAD by the DMC charter. Once the decision to open single and/or multiple dose expansion is made, the number of treatment groups (2 or 3 treatment groups, including 1 or 2 active treatment groups and 1 placebo group) and the dose for ASP0598 Otic Solution will be selected based on the safety and efficacy results of the interim analyses. All analyses will be described in the Interim Analysis Plan (IAP).

Up to 39 subjects will be randomized to single dose and/or multiple dose expansion. 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, or 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 or placebo (13 subjects in each treatment group).

Stratification at the time of randomization will be based on the % size of the TMP at the screening visit and subjects will be balanced equally between the treatment groups.

Dose expansion will assess safety and efficacy of ASP0598 Otic Solution.

Single Dose Expansion

After randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear at the investigative site. Subjects will return to the investigative site for assessments on Days 15, 29, 57, and 85 (EOS).

Subjects who terminate early from the study will complete EOS assessments at the time of discontinuation.

Multiple Dose Expansion

After randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear. Subjects may receive additional treatment in the same ear on Days 15 and 29. Subjects will return to the investigative site for assessments on Days 15, 29, 43, 57, 85, and 113 (EOS).

In cases where complete closure is confirmed on Day 15 or Day 29, the subject will not receive the next scheduled dose(s) but will complete all visit assessments as defined in the Schedule of Assessments through Day 113.

Subjects who terminate early from the study will complete EOS assessments at the time of discontinuation

Subjects who discontinue treatment will be encouraged to complete all protocol defined study visits through Day 113.

In single and/or multiple dose expansion, 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.

Inclusion/Exclusion Criteria:

Inclusion Criteria:

A subject is eligible for the study if all the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written Informed Consent and privacy language as per national regulation (e.g., Health Insurance Portability and Accountability Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Subject must be 18 to 75 years of age at the time of informed consent.
3. Subject has chronic tympanic membrane perforation (CTMP) documented as persisting longer than 3 months.
4. A female subject is eligible to participate if she is not pregnant (see Appendix 12.3: Contraception Requirements) and at least one of the following conditions applies:
    a. Not a woman of childbearing potential (WOCBP) as defined in Appendix 12.3: Contraception Requirements OR
    b. WOCBP who agrees to follow the contraceptive guidance as defined in Appendix 12.3: Contraception Requirements starting at screening and for at least 28 days after investigational product (IP) application.
5. Female subject must agree not to breastfeed starting at drug application on Day 1 and for at least 28 days after IP application.
6. Female subject must not donate ova starting on Day 1 and for at least 28 days after IP application.
7. A male subject with female partner(s) of child-bearing potential must agree to use contraception starting on Day 1 and for at least 28 days after IP application as detailed in Appendix 12.3.

8. A male subject must not donate sperm starting on Day 1 and for at least 28 days after IP application.
9. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom from Day 1 and for at least 28 days after IP application.
10. Subject must be willing and able to comply with the study requirements including refraining from using prohibited concomitant medication.
11. Subject agrees not to participate in another interventional study during the study period.
12. Subject has the ability to understand and the willingness to sign a written informed consent document.
13. Waivers to the inclusion criteria will NOT be allowed.

Exclusion Criteria:

A subject will be excluded from participation if any of the following apply:

1. Subject has one of following conditions, that is confirmed by the investigator, that may affect the ipsilateral side of the ear with CTMP:
    a. Perforation involving 3 or more quadrants.
    b. Pin hole perforation (only for the expansion cohort)
    c. Presence of tympanosclerosis adjacent to the perforation.
    d. Perforation involves malleus erosion.
    e. Absent malleus.
    f. Marginal perforation (i.e., involving the annulus or exposing the handle of malleus).
    g. TMP caused by electric/slag/blast/burn injury.
    h. Post radiated TMP.
    i. History of tympanic membrane repair by any type of live tissue.
    j. Active otorrhea or active treatment for otorrhea within the last 3 months prior to Screening.
    k. Bellucci otorrhea grade 3 or above.
    l. Active external ear canal inflammation (otitis externa, dermatitis) or within the last 3 months prior to Screening.
    m. Active diagnosis of Eustachian Tube dysfunction or diagnosis within 6 months prior to Screening.
    n. Craniofacial abnormalities, history of head and neck surgery within the last 3 months prior to Screening, history of radiation to head and neck.
    o. Recent (within 2 weeks) diagnosis of upper respiratory tract infection.
    p. Presence or history of cholesteatoma.
    q. Presence of pars-flaccida or pars tensa retraction or adhesion.
    r. Presence or history of tumors of the middle or external ear.
    s. Contraindications to tympanic membrane closure
    t. An audiometric finding indicates a characteristic of Carhart's notch which is an increase in bone conduction threshold with a peak at 2,000 Hz.
    u. Only hearing ear or better hearing ear and the contralateral ear $\geq$ 40 dB by average four-frequency (500, 1000, 2000 and 4000 Hz).
    v. Whole circumference of the tympanic membrane perforation is not visible by endoscope.
    w. Presence/history of eosinophilic otitis media in either ear.
2. Subject has a presence of adhesive otitis media in the contralateral ear.
3. Subject has a presence of any wound healing systemic condition.

4. Subject has Obstructive Sleep Apnea where the subject is required to use Continuous Positive Airway Pressure (CPAP) during the study period.
5. Subject is exposed in their daily life to high volume of water into the ear canal (e.g., swimmer or surfer).
6. Subject has health conditions that would prevent him/her from fulfilling the study requirements as judged by the clinical investigator on the basis of medical history and laboratory test (Serum Chemistries, CBC with Differential, Urinalysis) results at the screening visit.
7. Subject is receiving any other investigational agents during study participation.
8. Subject has any form of substance abuse, or psychiatric illness/social situations that would limit compliance with study requirements, or a condition that in the opinion of the investigator could invalidate communication with the investigator.
9. Subject has a known or suspected hypersensitivity to ASP0598, or any components of the formulation used.
10. Subject has had previous exposure with ASP0598.
11. Subject is unlikely to comply with the visits scheduled in the protocol, in the opinion of the investigator.

Waivers to the exclusion criteria will NOT be allowed.

Investigational Product(s):

Name:

ASP0598 is a recombinant human heparin-binding EGF-like (epidermal growth factor-like) growth factor (rhHB-EGF). ASP0598 Otic Solution contains rhHB-EGF that is diluted with a buffered aqueous solution to adjust the dose strength and mixed with a polymer solution to enable forming hydrogel and retention after administration.

Use:

Test product

Dose(s):

0.2, 1.0, 5.0, and 15.0 μg/mL

Administration Volume:

Up to a maximum volume of 0.15 mL/administration

Mode(s) of Administration:

Single Dose: Topical application on Day 1 onto the tympanic membrane through the external auditory canal via syringe.

Multiple Dose: Topical application on Days 1, 15, and 29 onto the tympanic membrane through the external auditory canal via syringe.

Comparator:

Name:

The matching placebo is a sterile solution of buffered aqueous solvent combined with a copolymer prior to otic administration.

Use :

Placebo

Dose(s):

Matching placebo for ASP0598 Otic Solution

Administration Volume:

Up to a maximum volume of 0.15 mL/administration

Mode(s) of Administration:

Single Dose: Topical application on Day 1 onto the tympanic membrane through the external auditory canal via syringe.

Multiple Dose: Topical application on Days 1, 15, and 29 onto the tympanic membrane through the external auditory canal via syringe.

Dose Modifications:

SAD and single dose expansion

Not Applicable.

MAD

On Day 15 or Day 29, treatment can be skipped at the investigator's discretion based on safety reasons that interrupt IP administration (e.g., ear canal skin irritation, mild otorrhea). Subjects should be encouraged to return to the office for evaluation and additional IP administration within the Day 15 or Day 29 visit windows. In cases where the investigator is unable to conduct IP administration for anything other than safety reasons, consultation with the sponsor may be required.

Multiple dose expansion

The statement above for MAD is also applicable to the multiple dose expansion. Additionally, the treatment on Day 15 or Day 29 will be skipped in cases where complete closure is confirmed. If the perforation is detected on Day 29 after treatment was skipped on Day 15, IP administration can be conducted.

Concomitant Treatment (Medication and Nonmedication Therapy) Restrictions or Requirements:

These restrictions are required after ASP0598 Otic Solution or placebo administration on Randomization (Day 1), until completion of study procedures at Day 57 (SAD)/Day 85 (single dose expansion or MAD) / Day 113 (multiple dose expansion) or End of Study (EOS) visit.

- Ear drop agents will be used only for the treatment of otitis media with suppurative ear discharge. Only Ciprofloxacin or Ofloxacin ear drops (does not include steroids) are allowed to be used in the ASP0598 Otic Solution or placebo treated ear. Investigators should minimize treatment with ear drops up to a maximum of 10 days and consider alternative treatment (e.g., oral antibiotics).
- Any other topical agents in the ear canal are prohibited to use in the ASP0598 Otic Solution or placebo treated ear.
- Any otologic procedures (e.g. paper patch or surgeries) to the ASP0598 Otic Solution or placebo treated ear are prohibited.

All concomitant medication usage will be noted and recorded during each study visit.

Other restrictions:

These restrictions are required after ASP0598 Otic Solution or placebo administration on Day 1 until completion of the study procedures at Day 57 (SAD)/Day 85 (single dose expansion) or Day 85 (MAD) / Day 113 (multiple dose expansion) or End of Study (EOS) visit.

- Subjects must refrain from showering or bathing for at least 24 hours after IP administration.
    - Showering or bathing are allowed 24 hours after IP administration. Water precautions are to be taken as per the guidelines in Appendix 12.8.

- Any water activities that risk submersion of the ear (e.g., swimming or surfing) are prohibited throughout the entire study period.
- Subjects are not allowed to use any materials (e.g., Q-tips) placed into the ear canal of the treated ear (ear buds or headphones placed at the entrance to the ear canal are considered acceptable). If ear cleaning is desired, then subjects can use a soft tissue placed gently at the entrance to the ear canal.
- Investigators will not clean the ASP0598 Otic Solution/placebo treated ear by suctioning throughout Observation period, unless subjects experience uncomfortable ear discharge. General ear cleaning will be allowed to visualize the whole tympanic membrane at Day 57 (SAD), Day 85 (single dose expansion and MAD), Day 113 (multiple dose expansion), or EOS visit, before imaging picture.
- Investigators may gently remove an obstacle that is blocking the ear canal if it prevents IP application or causes subjects to experience an uncomfortable sensation. If the obstacle is adherent to the tympanic membrane, it should not be removed; it is recommended to wait until the obstacle comes off.
- Subjects should refrain from activities that can increase the middle ear pressure including plane travel (at least 28 days after IP application), positive pressure ventilation (e.g., CPAP or BiPAP) devices, Valsalva maneuvers, excessive high-pressure nose blowing, or diving underwater.
- Subject must refrain from activities that can excessively increase body temperature (e.g. Sauna) for at least 3 days after IP application.
- Subject must refrain from activities that stimulate sweating excessively for at least 3 days after IP application.

Duration of Treatment:

SAD and single dose expansion: Topical application into the affected ear on Day 1.

MAD: Topical application into the affected ear on Days 1, 15, and 29.

Multiple Dose Expansion: Topical application into the affected ear on Day 1 and if the subject has not achieved complete closure of the TMP at Day 15 and Day 29, additional treatment will occur. If the subject achieves complete closure on either Day 15 or Day 29, no additional treatment is required at those visit(s).

Treatment Discontinuation Criteria:

SAD and single dose expansion

Not applicable because there is a single treatment of ASP0598 Otic Solution or placebo.

MAD and multiple dose expansion

Treatment Discontinuation

During the course of IP administration, if the subject experiences a drug-related adverse event (AE), the IP administration may be discontinued at the investigator's discretion.

In addition, IP administration will be discontinued if severe intensity occurs in any of the following situations:

- Subject develops cholesteatoma or suspicion of neoplasm in the treated ear as determined by the investigator.
- Subject develops suspected ototoxicity symptoms (e.g., sudden hearing loss at high frequencies (1, 2, 4 kHz), new high frequency tinnitus or new balance disorder) which are considered by the investigator to be related to the study drug.
- Subjects with persistent otitis media or otitis externa unresponsive to oral and topical antibiotics (as described in Previous and Concomitant Treatment [Medication and Nonmedication Therapy of the synopsis and protocol Section 6.5])

Dose Escalation:

Decision Process for Dose Escalation and Initiation of MAD

The DEC will decide dose escalation and the initiation and selection of doses for MAD cohorts based on the emerging safety and tolerability data from SAD. After all subjects in a cohort have been dosed and completed study procedures through Day 15, the overall safety and tolerability of the dose will be determined after evaluation of the blinded safety data [treatment emergent adverse events (TEAEs), adverse events (AEs) of special interest, otology examinations (imaging pictures of tympanic membrane, bone conduction hearing at 1, 2, 4 kHz by PTA, TVAS, Modified Brighton Grading Scale), and vital signs] for the current cohort. Cumulative AE data from the preceding cohort(s) will also be included in the assessment for dose escalation. Details will be defined in the DEC Charter.

Dose Escalation Stopping Rules

Depending on the nature, frequency and severity of the safety profile observed in the study, the DEC will decide whether to:

- Proceed with dose escalation and determine the next higher dose or,
- Stop dose escalation (i.e., no further dosing with IP).

Dose escalation will be stopped if 1 (or more) of the following apply:

- If 1 or more subjects in 1 cohort develop cholesteatoma or suspicion of ear neoplasm which is considered to be related to ASP0598.
- If 2 or more subjects in 1 cohort develop Ototoxicity (clinically significant report of vestibular symptoms, tinnitus, or acute hearing loss) which are considered to be related to the IP.
- If 2 or more subjects in 1 cohort develop persistent otitis media or otitis externa unresponsive to oral and topical antibiotics which is described in concomitant treatment requirements section of the synopsis which are considered to be related to the IP.
- If 1 or more subjects in 1 cohort experience AEs of severe intensity or 2 or more subjects in 1 cohort experience AEs of the same character of moderate intensity, which are considered to be related to the IP, and of clinical concern.

In the event that it is deemed relevant to unblind a subject for the purposes of a dose escalation decision, the DEC chair or delegate can request the treatment assigned to one or more from the study statistician. The DEC charter will include information on unblinding of study team members and other details.

Definition of the Maximum Tolerated Dose:

If the DEC decides to stop dose escalation based on available safety data, the current dose level will be considered as the minimum intolerable dose (MID). The dose just below the MID will be regarded as the maximum tolerated dose (MTD). If the dose escalation is stopped due to reaching exposure limit without dosing limiting safety findings, MTD cannot be determined.

Statistical Methods:

Sample Size Justification:

SAD

Formal sample size calculations were not performed. SAD 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) 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.

MAD

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

A sample size of 8 per cohort (n = 6 ASP0598 Otic Solution, n = 2 placebo) 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, 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).

Efficacy:

Dose Escalation

Continuous data will be summarized by using descriptive statistics (mean, SD, median, min, max) by treatment group, including placebo pooled across cohort. For categorical data, the number and percentage of subjects in each category will be presented by treatment group, including placebo pooled across cohort.

The following parameters will be summarized:

- Complete closure of TMP
- Change from baseline in the ratio of TMP size per total area of tympanic membrane
- Change from baseline in TMP size
- Time to closure of TMP
- Size of TMP ≤ 5% of pars tensa surface area after treatment
- Size of TMP ≤ 1% of pars tensa surface area (pin point perforation) after treatment
- Change from baseline in air bone (A-B) gap by PTA
- Change from baseline in air conduction hearing by PTA
- Percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing)
- Improvement from baseline in air conduction hearing by PTA ≥ 15 dB
- A-B gap after treatment was 15 dB or less
- Air conduction hearing threshold after treatment was 30 dB or less
- Type of tympanogram (A, B or C)
- Equivalent Ear Canal Volume (ECV) by tympanometry
- Vital signs (body temperature, blood pressure and pulse rate)

No statistical hypothesis testing will be performed.

Dose Expansion

The efficacy parameters shown above will be summarized by using descriptive statistics by treatment group.

For the proportion of subjects with complete closure of TMP at week 12 for single dose expansion and at week 16 for multiple dose expansion, stratified MH test by categorized baseline perforation size will be applied. For the subjects with intercurrent events (ICEs), the complete closure status just before the ICEs will be used as complete closure status at week 12 for single dose expansion and week 16 for multiple dose expansion. Based on the interim analysis results, if 3 treatment groups are selected for dose expansion, hierarchical testing method will be used to control familywise error rate where the highest dose group vs placebo will be tested first at 2-sided significance level of 0.05.

For the change from baseline in TMP size and 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, a mixed models repeated measures (MMRM) will be used with 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. For subjects with ICEs, only data obtained before the ICE will be used in the MMRM. No multiplicity will be made from change from baseline secondary endpoints.

Safety:

Dose Escalation

To characterize the safety profile, descriptive statistics will be presented for TEAEs, vital signs (body temperature, blood pressure and pulse rate), bone conduction hearing at 1, 2, 4 kHz by PTA and TVAS by treatment group, including placebo pooled across cohorts.

Dose Expansion

To characterize the safety profile, descriptive statistics will be presented for TEAEs, vital signs (body temperature, blood pressure and pulse rate), bone conduction hearing at 1, 2, 4 kHz by PTA and TVAS by treatment group.

Pharmacokinetics (dose escalation only):

Descriptive statistics will be presented for serum concentrations of ASP0598 by scheduled sample time. These summaries will be provided by treatment group, including placebo, pooled across cohorts.

Immunogenicity:

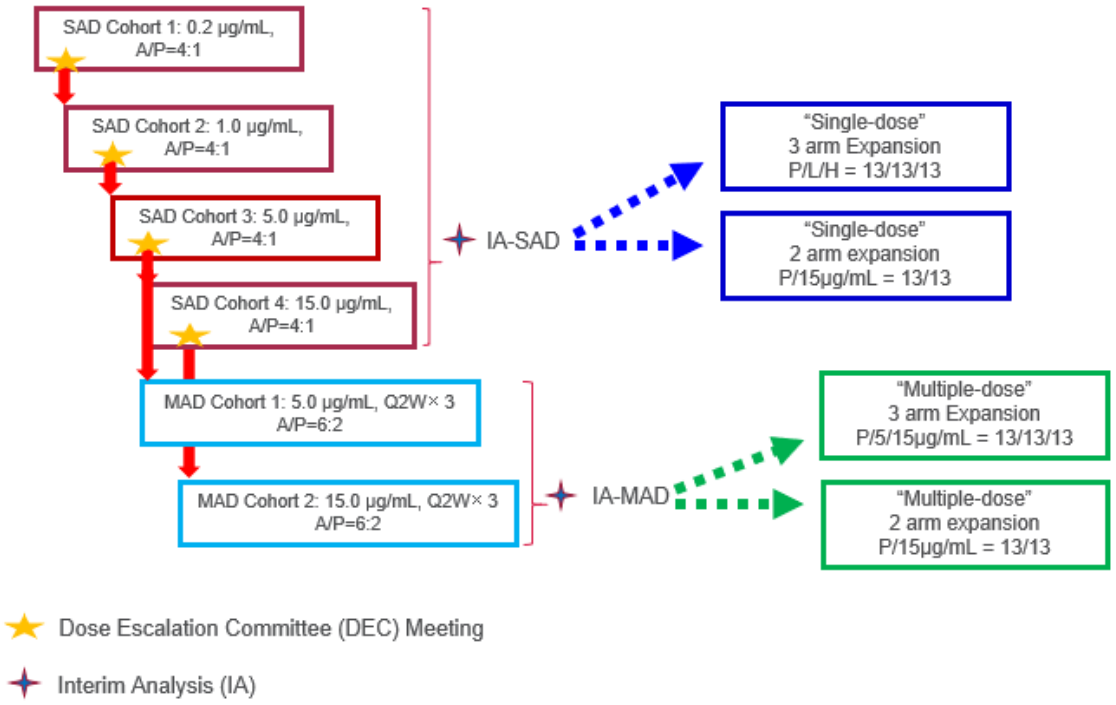
Not applicable

Interim Analyses:

The decision to open single and/or multiple dose expansion will be made based on the safety and efficacy results of the interim analysis after all SAD cohorts have completed and after all the MAD cohorts have completed, respectively. Two interim analyses are planned. The first interim analysis will be conducted after completion of the last cohort of SAD. The second interim analysis will be conducted after completion of MAD. The DMC will review the data and make the final decision to open single and/or multiple dose expansion. Two or three treatment groups including one placebo group will be selected to open single and/or multiple dose expansion. All analyses will be described in the Interim Analysis Plan (IAP).

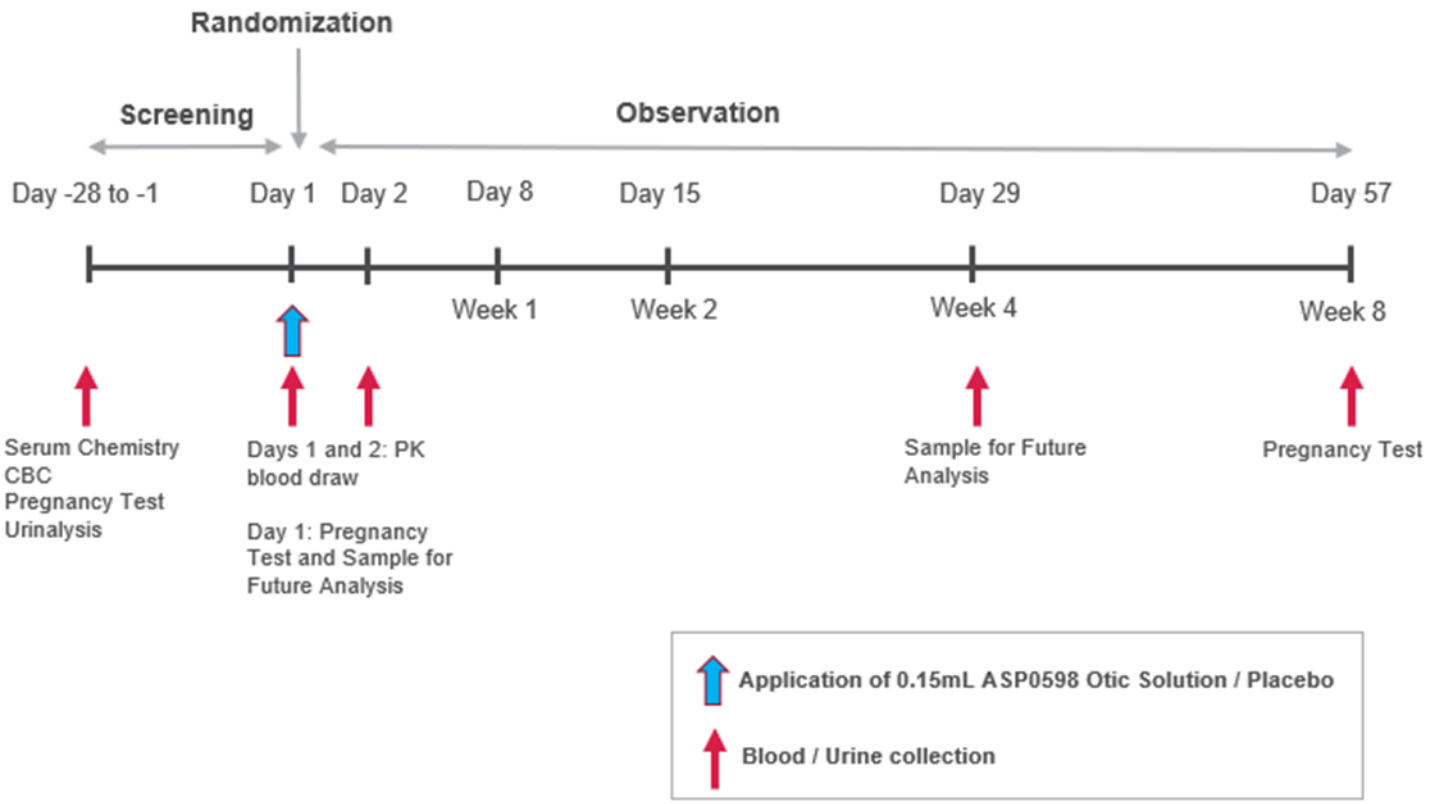
1.2 Study Schema

Figure 1 Study Design Schema



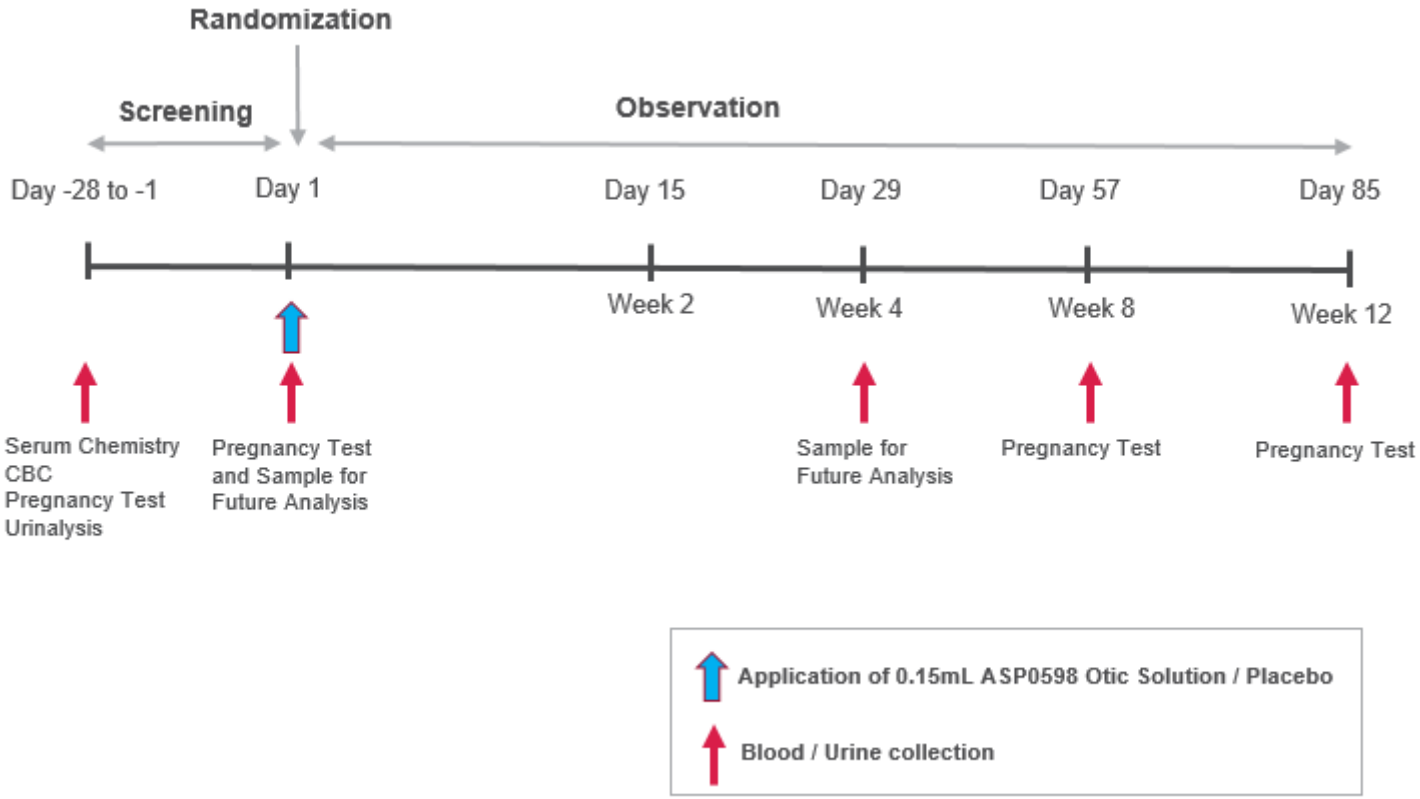
A: active; P: placebo; L: low-dose ASP0598; H: high-dose ASP0598

Figure 2 Study Flow Chart – SAD



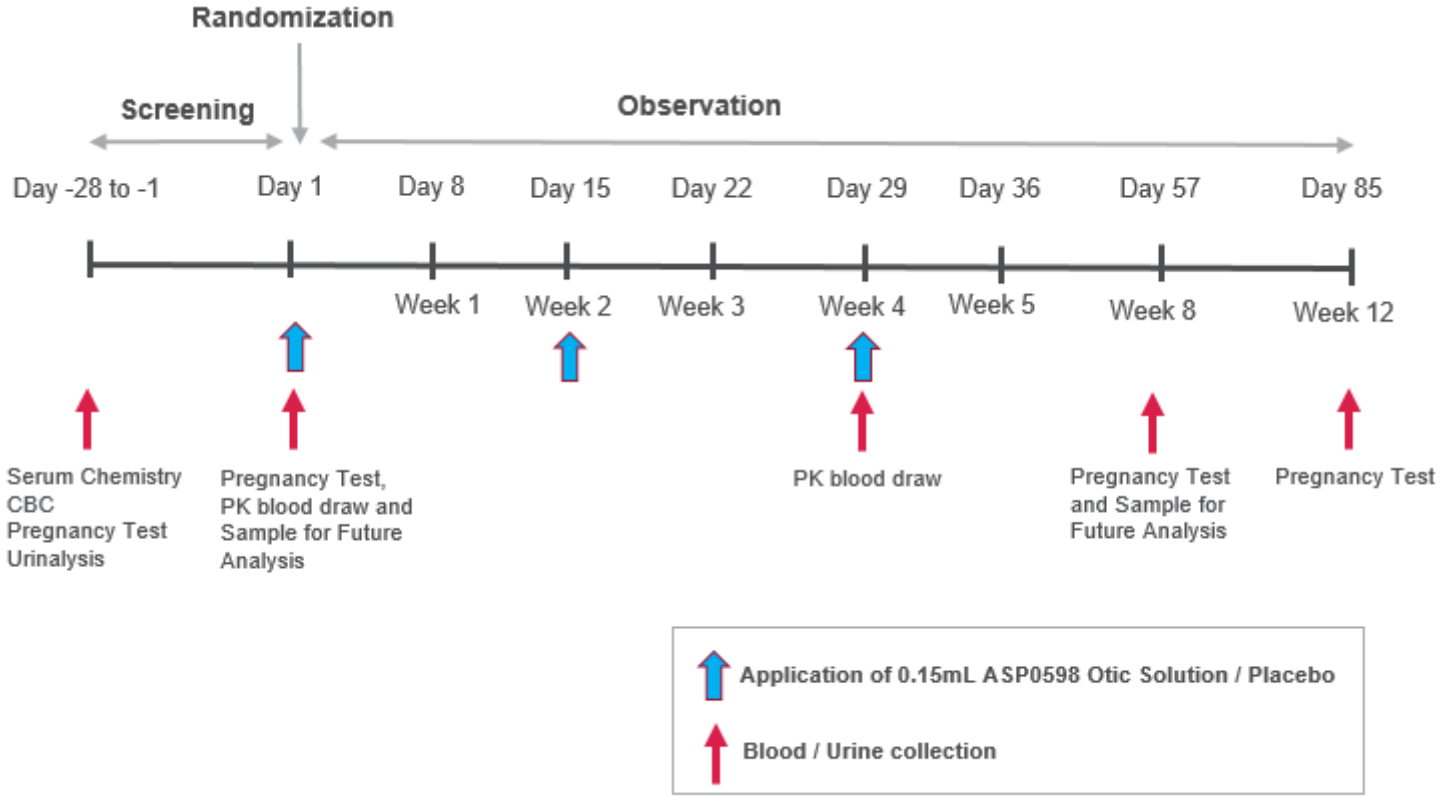
CBC: complete blood count; PK: pharmacokinetic; SAD: Single Ascending Dose

Figure 3 Study Flow Chart – Single Dose Expansion



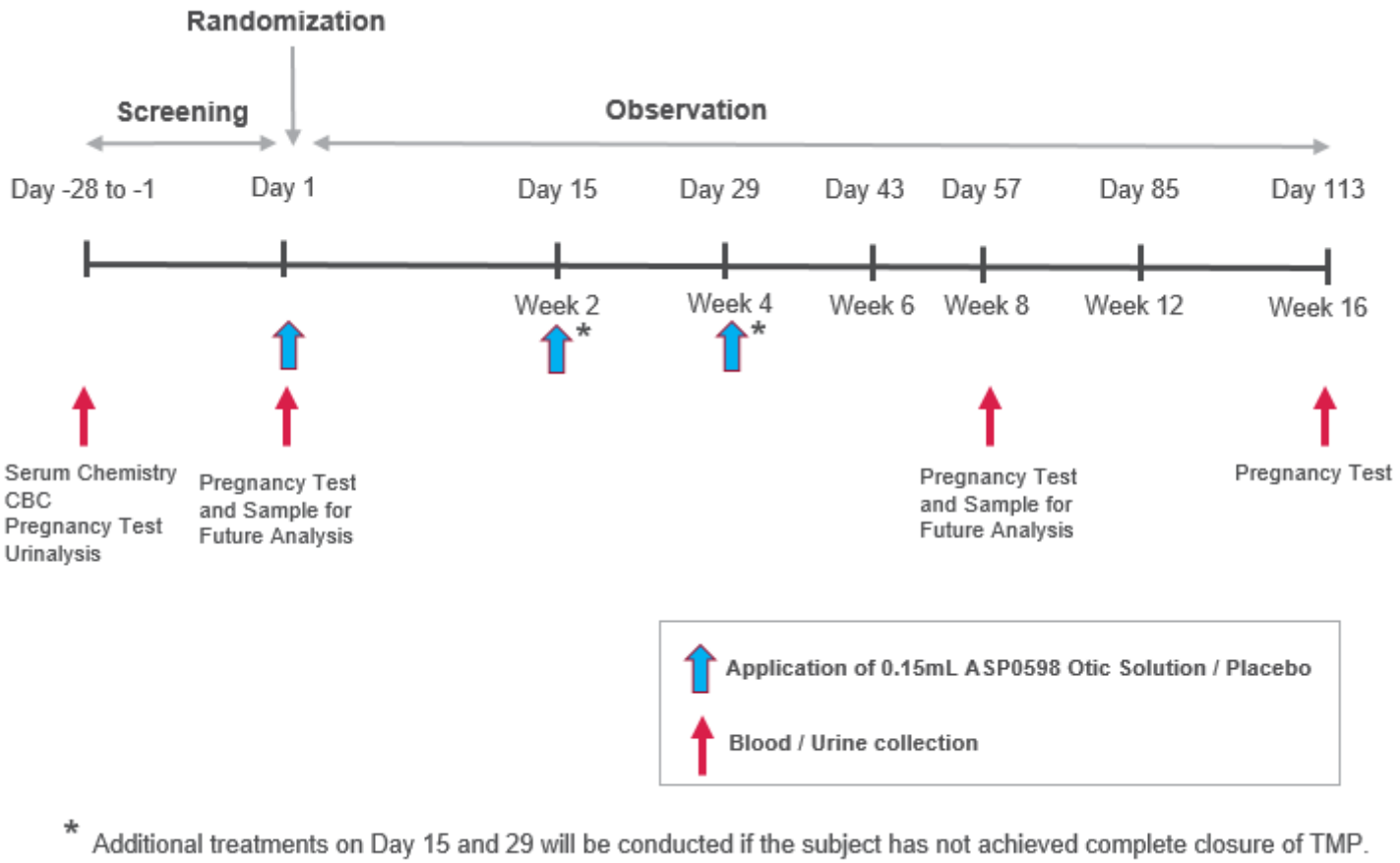
CBC: complete blood count

Figure 4 Study Flow Chart – MAD



CBC: complete blood count; MAD: Multiple Ascending Dose; PK: pharmacokinetic

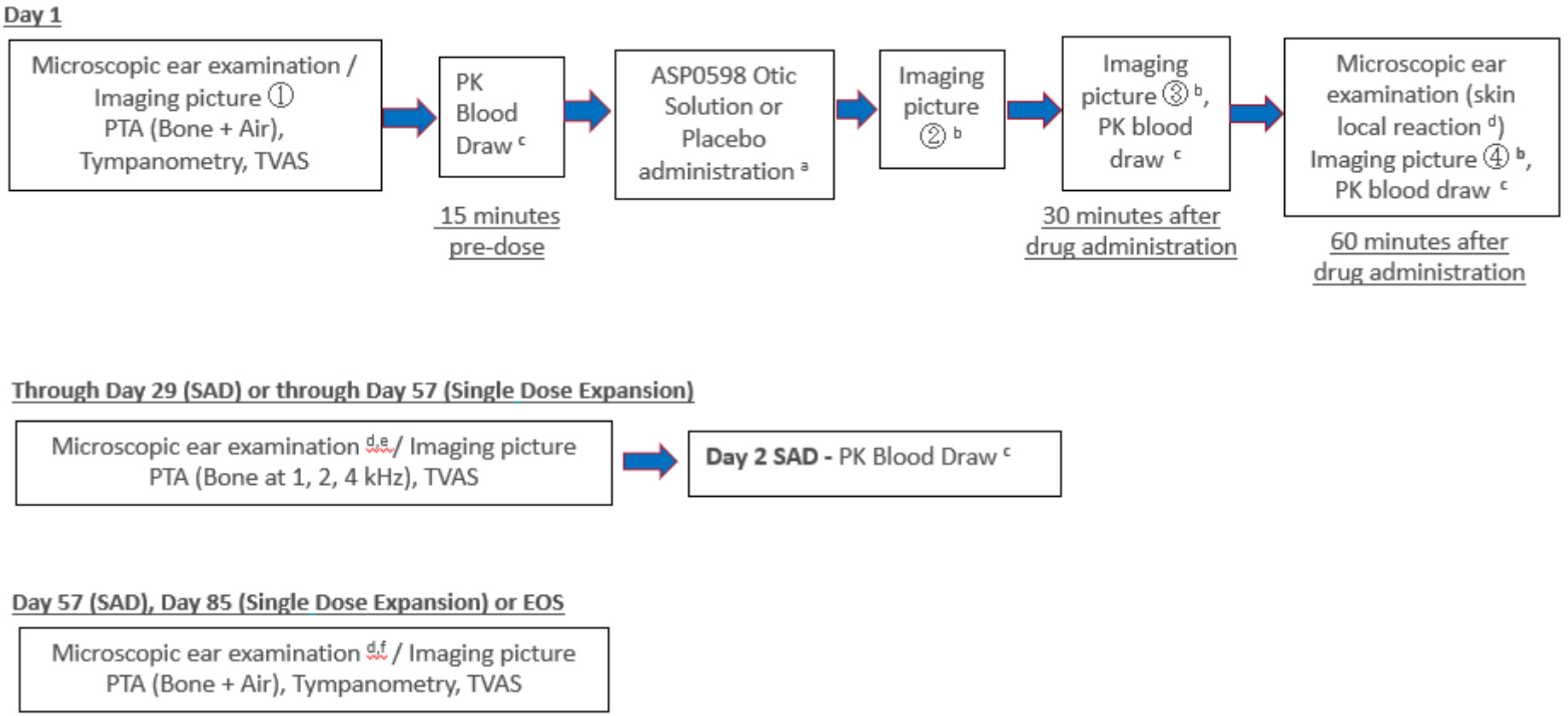
Figure 5 Study Flow Chart – Multiple Dose Expansion



* Additional treatments on Day 15 and 29 will be conducted if the subject has not achieved complete closure of TMP.

CBC: complete blood count

Figure 6 Otology Examination and PK Blood Draw Flow Chart – Single Dose

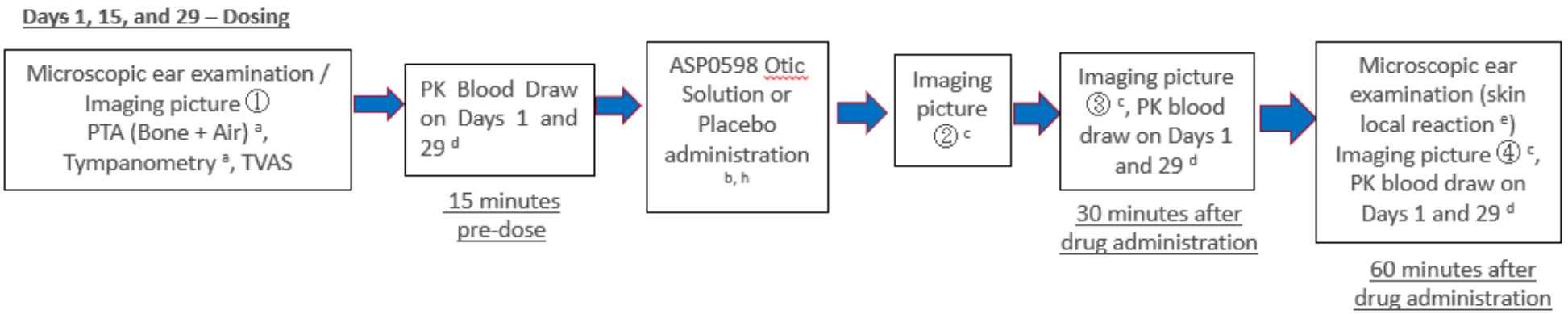


Footnotes appeared on next page

EOS: end of study; PK: pharmacokinetic; PTA: Pure Tone Audiometry; SAD: single ascending dose; TM: tympanic membrane; VAS: Tinnitus Visual Analog Scale

a. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
b. Day 1, imaging picture will be taken pre-dose, immediately after, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear.
c. PK blood samples will be collected on Day 1 at pre-dose (-15) minutes and at the following post-dose time points: 30 (±5) minutes and 60 (±5) minutes. PK blood sampling will be performed only in SAD after ASP0598 Otic Solution or placebo administration into the affected ear.
d. Skin local reaction at the ASP0598 Otic Solution/placebo application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at all visits.
e. Ear cleaning will not be conducted between Days 2 – 29 (SAD) or Days 2 – 57 for (single dose expansion), unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge).
f. At Day 57 (SAD) or Day 85 (single dose expansion) and/or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.

Figure 7 Otology Examination and PK Blood Draw Flow Chart – Multiple Dose



Footnotes appear on next page

EOS: end of study; MAD: multiple ascending dose; PK: pharmacokinetic; PTA: Pure Tone Audiometry; TM: tympanic membrane; TVAS: Tinnitus Visual Analog Scale.

a. Days 15 and 29, PTA will be conducted only for bone conduction hearing at 1, 2, 4 kHz and Tympanometry will not be conducted.
b. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
c. Days 1, 15, and 29, imaging picture will be taken pre-dose, immediately after, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear.
d. Day 1 and Day 29, PK blood draw will be performed pre-dose (-15) minutes, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear. PK sampling will be performed only during MAD.
e. Skin local reaction at the ASP0598 Otic Solution/placebo application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at all visits.
f. Ear cleaning will not be conducted between Days 2 – 57 (MAD) or Days 2 – 85 for (multiple dose expansion), unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge).
g. At Day 85 (MAD) or Day 113 (multiple dose expansion) and/or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
h. For multiple dose expansion, ASP0598 Otic Solution or placebo administration on Day 15 and Day 29 will be conducted in the case that the TMP closure is not confirmed. If the treatment is not conducted on these visits, imaging pictures and Microscopic ear examination for skin local reaction after the drug administration will be skipped.

1.3 Schedule of Assessments

Table 2 Schedule of Assessments – SAD

	Screening	Randomization	Observation				
Day	Day -28 to Day -1	Day 1	Day 2	Day 8	Day 15	Day 29	Day 57 Last Visit/ EOS
Week				Week 1	Week 2	Week 4	Week 8
Visit Windows			+ 1 day	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6	7
Study Procedures							
Informed Consent	X						
Verify Inclusion/Exclusion	X	X					
Medical History/Disease History/Demographics	X	X					
Physical Exam including Height and Weight	X						
Vital Signs [a]	X	X	X	X	X	X	X
Prior/Concomitant Medications [b]	X	X	X	X	X	X	X
Adverse Events Assessment [c]	X	X	X	X	X	X	X
Study Treatment Administration							
ASP0598 or placebo		X [d]					
Otology Examinations							
Microscopic Ear Examination	X [e]	X [f]	X [g]	X [g]	X [g]	X [g]	X [i]
Modified Brighton Grading Scheme Evaluation		X [f]	X	X	X	X	X
Imaging picture of tympanic membrane by endoscope	X [e]	X [h]	X	X	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)	X [e]	X	X [j]	X [j]	X [j]	X [j]	X
Air conduction by PTA	X [e]	X					X
Tympanometry	X [e]	X					X
Tinnitus Visual Analog Scale (TVAS)	X	X	X	X	X	X	X

Table continued on next page

	Screening	Randomization	Observation				
Day	Day -28 to Day -1	Day 1	Day 2	Day 8	Day 15	Day 29	Day 57 Last Visit/ EOS
Week				Week 1	Week 2	Week 4	Week 8
Visit Windows			+ 1 day	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6	7
Blood Collection							
Serum Chemistries	X						
CBC with Differential	X						
Pregnancy Test [k]	X	X					X
Pharmacokinetic Sample		X[l]	X				
Blood Sample for Future Analysis		X				X	
Urine Collection							
Urinalysis	X						

AE: adverse event; CBC: complete blood count; EOS: end of study; SAD: single ascending dose; TM: tympanic membrane

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 57 or EOS. Any AEs ongoing at Day 57 or EOS Visit must be followed until resolution or investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Microscopic Ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 29, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subject's experience uncomfortable ear discharge.
h. At Day 1, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 57 or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 2, 8, 15, 29 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.
l. Day 1, PK blood draw will be performed at the following time points: pre-dose (-15) minutes, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear.

Table 3 Schedule of Assessments – Single Dose Expansion

	Screening	Randomization	Observation			
Day	Day -28 to Day -1	Day 1	Day 15	Day 29	Day 57	Day 85 Last Visit/ EOS
Week			Week 2	Week 4	Week 8	Week 12
Visit Windows			+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6
Study Procedures						
Informed Consent	X					
Verify Inclusion/Exclusion	X	X				
Medical History/Disease History/Demographics	X	X				
Physical Exam including Height and Weight	X					
Vital Signs [a]	X	X	X	X	X	X
Prior/Concomitant Medications [b]	X	X	X	X	X	X
Adverse Events Assessment [c]	X	X	X	X	X	X
Study Treatment Administration						
ASP0598 or placebo		X [d]				
Otology Examinations						
Microscopic Ear Examination	X [e]	X [f]	X[g]	X[g]	X[g,]	X [i]
Modified Brighton Grading Scheme Evaluation		X [f]	X	X	X	X
Imaging picture of tympanic membrane by endoscope	X [e]	X[h]	X	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)	X [e]	X	X [j]	X [j]	X [j]	X
Air conduction by PTA	X [e]	X				X
Tympanometry	X [e]	X				X
Tinnitus Visual Analog Scale (TVAS)	X	X	X	X	X	X

Table continued on next page

	Screening	Randomization	Observation			
Day	Day -28 to Day -1	Day 1	Day 15	Day 29	Day 57	Day 85 Last Visit/ EOS
Week			Week 2	Week 4	Week 8	Week 12
Visit Windows			+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6
Blood Collection						
Serum Chemistries	X					
CBC with Differential	X					
Pregnancy Test [k]	X	X			X	X
Blood Sample for Future Analysis		X		X		
Urine Collection						
Urinalysis	X					

AE: adverse event; CBC: complete blood count; EOS: end of study; TM: tympanic membrane

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 57 or EOS. Any AEs ongoing at Day 85 or EOS Visit must be followed until resolution or investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Microscopic Ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 57, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge.
h. At Day 1, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 85 or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 15, 29, and 57 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.

Table 4 Schedule of Assessments – MAD

	Screening	Randomization	Observation						
Day	Day – 28 to Day -1	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 57	Day 85 Last Visit/ EOS
Week			Week 1	Week 2	Week 3	Week 4	Week 5	Week 8	Week 12
Visit Windows			+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6	7	8	9
Study Procedures									
Informed Consent	X								
Verify Inclusion/Exclusion	X	X							
Medical History/Disease History/Demographics	X	X							
Physical Exam including Height and Weight	X								
Vital Signs [a]	X	X	X	X	X	X	X	X	X
Prior/Concomitant Medications [b]	X	X	X	X	X	X	X	X	X
Adverse Events Assessment [c]	X	X	X	X	X	X	X	X	X
Study Treatment Administration									
ASP0598 or placebo [d]		X		X		X			
Otology Examinations									
Microscopic Ear Examination	X [e]	X [f]	X [g]	X [f, g]	X [g]	X [f, g]	X [g]	X [g]	X [i]
Modified Brighton Grading Scheme Evaluation		X [f]	X	X [f]	X	X [f]	X	X	X
Imaging picture of tympanic membrane by endoscope	X [e]	X [h]	X	X [h]	X	X [h]	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)	X [e]	X	X [j]	X [j]	X [j]	X [j]	X [j]	X [j]	X
Air conduction by PTA	X [e]	X							X
Tympanometry	X [e]	X							X
Tinnitus Visual Analog Scale (TVAS)	X	X	X	X	X	X	X	X	X

Table continued on next page

	Screening	**Randomization**	**Observation**						
Day	Day – 28 to Day -1	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 57	Day 85 Last Visit/ EOS
Week			Week 1	Week 2	Week 3	Week 4	Week 5	Week 8	Week 12
Visit Windows			+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6	7	8	9
Blood Collection									
Serum Chemistries	X								
CBC with Differential	X								
Pregnancy Test [k]	X	X						X	X
Pharmacokinetic Sample		X [l]				X			
Sample for Future Analysis		X						X	
Urine Collection									
Urinalysis	X								

AE: adverse event; CBC: complete blood count; EOS: end of study; TM: tympanic membrane

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 85 or EOS. Any AEs ongoing at Day 85 or EOS Visit must be followed until resolution or investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Days 1, 15 and 29, microscopic ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 57, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge).
h. At Days 1, 15, and 29, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 85 and/or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 8, 15, 22, 29, 36 and 57 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.
l. Day 1 and Day 29, PK blood draw will be performed at the following time points: pre-dose (-15) minutes, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear.

Table 5 Schedule of Assessments – Multiple Dose Expansion

	Screening	Randomization	Observation					
Day	Day – 28 to Day -1	Day 1	Day 15	Day 29	Day 43	Day 57	Day 85	Day 113 Last Visit/ EOS
Week			Week 2	Week 4	Week 6	Week 8	Week 12	Week 16
Visit Windows			+/- 5 days	+/- 5 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number	1	2	3	4	5	6	7	8
Study Procedures								
Informed Consent	X							
Verify Inclusion/Exclusion	X	X						
Medical History/Disease History/Demographics	X	X						
Physical Exam including Height and Weight	X							
Vital Signs [a]	X	X	X	X	X	X	X	X
Prior/Concomitant Medications [b]	X	X	X	X	X	X	X	X
Adverse Events Assessment [c]	X	X	X	X	X	X	X	X
Study Treatment Administration								
ASP0598 or placebo [d]		X	X [l]	X [l]				
Otology Examination								
Microscopic Ear Examination	X [e]	X [f]	X [f, g]	X [f, g]	X [g]	X [g]	X [g]	X [i]
Modified Brighton Grading Scheme Evaluation		X [f]	X [f]	X [f]	X	X	X	X
Imaging picture of tympanic membrane by endoscope	X [e]	X [h]	X [h]	X [h]	X	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)	X [e]	X	X [j]	X [j]	X [j]	X [j]	X [j]	X
Air conduction by PTA	X [e]	X					X	X
Tympanometry	X [e]	X					X	X
Tinnitus Visual Analog Scale (TVAS)	X	X	X	X	X	X	X	X
Blood Collection								
Serum Chemistries	X							
CBC with Differential	X							
Pregnancy Test [k]	X	X				X		X
Sample for Future Analysis		X				X		
Urine Collection								
Urinalysis	X							

Footnotes appear on next page

AE: adverse event; CBC: complete blood count; EOS: end of study; TM: tympanic membrane; TMP: tympanic membrane perforation

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 113 or EOS. Any AEs ongoing at Day 113 or EOS Visit must be followed until resolution or investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Microscopic Ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 85, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge).
h. At Days 1, 15, and 29, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 85 (SAD) or Day 113 (single dose expansion) and/or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 15, 29, 43, 57, and 85 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.
l. ASP0598 Otic Solution or placebo administration on Day 15 and Day 29 will be conducted in the case that the TMP closure is not confirmed.

2 INTRODUCTION

2.1 Background

Target Disease

Chronic Tympanic Membrane Perforation (CTMP) is defined as stable maintenance of tympanic membrane perforation over 3 months, in contrast to acute tympanic membrane perforations, which heal within 7 to 10 days [Seonwoo et al, 2013]. CTMP affects both pediatric and adult patients and can result from disease, trauma, or medical procedures. Due to the continuous opening in the tympanic membrane, chronic perforation is usually attributed to chronic inflammation in the middle ear, resulting in suppurative otitis media [Seonwoo et al, 2013; Aggarwal et al, 2006]. Surgical repairs such as myringoplasty or tympanoplasty are employed and are currently the main treatments for patients with CTMP. Successful tympanic membrane closure rates vary between studies, ranging from 35% to 99% [Aggarwal et al, 2006]. Disadvantages associated with these surgical procedures include anesthesia-associated risks and the inconvenience of surgery. Therefore, a non-invasive treatment is desired in order to address the unmet need.

ASP0598 and Its Pharmacological Concept

Preclinical studies revealed that proliferation and migration of keratinocytes across layers of the tympanic membrane facilitate closure of the perforation site during the healing process [Santa Maria et al, 2010a]. Keratinocyte proliferation and migration are essential to cutaneous wound healing, and wound stimuli induce keratinocyte shedding of epidermal growth factor (EGF) receptor ligands in vitro, particularly the ligand HB-EGF [Tokumaru et al, 2000]. HB-EGF (an 87 amino acid glycoprotein) is a member of the EGF family of proteins that is encoded by the HB-EGF gene. Preclinical studies showed that HB-EGF was upregulated by over 8-fold by 12 hours following perforation of the tympanic membrane and remained upregulated for a prolonged time compared to constantly expressed EGF in rats [Santa Maria et al, 2010b]. Thus, HB-EGF appears to play a crucial role in tympanic membrane wound healing after acute tympanic membrane perforation. In a study [Santa Maria et al, 2015a], KB-R7785, which inhibits HB-EGF expression, was administered for 1 week to mice with tympanic membrane perforation (CTMP animal model); persistent tympanic membrane perforation longer than 3 months was observed in these mice. In addition, single topical administration of recombinant mouse pro-heparin-binding EGF-like growth factor with a sustained-release dose via a bioabsorbable chitosan-based polymer to the CTMP animal model showed complete closure of tympanic membrane perforation in 83.3% of animals, while application of other growth factors, such as EGF and fibroblast growth factor 2, healed only 15.8% and 31.6%, respectively, of the tympanic membrane perforations in the animal model [Santa Maria et al, 2015a]. These data from the literature suggest that topical, non-invasive administration of HB-EGF is a potentially effective treatment for patients with CTMP.

ASP0598 Otic Solution contains recombinant human heparin-binding EGF-like growth factor that is diluted with a buffered aqueous solution to adjust the dose strength and mixed with a

polymeric solution to enable the formation and retention of hydrogel after administration into the ear.

2.1.1 Nonclinical and Clinical Data

Detailed information can be found in the Investigational Brochure (IB).

Pharmacology

ASP0598 enhanced the proliferation of primary human and mouse epidermal keratinocytes with half-maximal effective concentrations (EC_{50}) of 11.72 and 52.92 ng/mL, respectively.

A single dose of topical application of ASP0598 Otic Solution (0.04 μg/mL to 5 μg/mL as final concentrations of ASP0598) onto a perforated tympanic membrane increased the healing rate, meaning that ASP0598 Otic Solution increased the number of animal ears with complete perforation closure in a mouse model of CTMP. The minimum efficacious concentration in this model was 0.2 μg/mL.

Pharmacokinetics

The toxicokinetics of ASP0598 were assessed in mice and cynomolgus monkeys. After a single administration into the external auditory canal or transtympanic administration of ASP0598 Otic Solution at concentrations of 15 μg/mL and 150 μg/mL, there was no discernible absorption of ASP0598. Administration of [^{89}Zr]ASP0598 Otic Solution toward tympanic membranes in cynomolgus monkeys followed by positron emission tomography (PET) scans showed the presence of residual [^{89}Zr]ASP0598 Otic Solution around the tympanic membranes from 0 to 14 days after administration. Radioactivity in all plasma samples collected from these same animals was at background levels.

Toxicology

The safety of ASP0598 Otic Solution has been evaluated in 8 good laboratory practice (GLP) toxicity studies in CBA/CaJ mice and cynomolgus monkeys: 2 pivotal, extended single-dose toxicity studies following administration into the external auditory canal and 2 pivotal, single-dose ototoxicity studies following transtympanic administration, 2 pivotal, repeat-dose toxicity studies following administration into the external auditory canal and 2 pivotal, repeat-dose ototoxicity studies following transtympanic administration.

Single dose administration of ASP0598 Otic Solution into the external auditory canal of either mice or cynomolgus monkeys resulted in no adverse findings or observations at concentrations up to 150 μg/mL. These results were confirmed following transtympanic administration of ASP0598 Otic Solution in both species. However, non-adverse, minimal to moderate procedure and/or vehicle related, histopathological changes were observed in cynomolgus monkeys following a single dose administration of ASP0598 Otic Solution into the external auditory canal or transtympanically.

Administration of ASP0598 Otic Solution to mice by the external auditory canal or transtympanically resulted in serum concentrations of HB-EGF near or below the limit of quantification, while in cynomolgus monkeys, ASP0598 Otic Solution administration

resulted in serum concentrations of HB-EGF that remained at baseline values. These data indicate that the external auditory canal or transtympanic route of administration of ASP0598 Otic Solution was not associated with discernible systemic exposure to HB-EGF.

Based on these results, no observed adverse effect level (NOAEL) was set at the highest concentration of 150 μg/mL in both the mouse and cynomolgus monkey studies following single external auditory canal or transtympanic administration.

Finally, results from 4 GLP repeat dose toxicity studies in mice and cynomolgus monkeys were comparable with the results from the single dose administration assessments. In these studies, ASP0598 Otic Solution was administered either into the external auditory canal or transtympanically 3 times at 2-week intervals. No test article-related adverse effects were noted in either species when ASP0598 Otic Solution was applied either into the external auditory canal or transtympanically to the middle ear. For both mice and cynomolgus monkeys the repeat dose results indicated that the NOAEL was also 150 μg/mL.

Clinical Studies

As of the date of the preparation of this protocol, no clinical studies of ASP0598 have been conducted. Therefore, the safety profile of ASP0598 Otic Solution has not been established.

2.1.2 Summary of Key Safety Information for Investigational Product(s)

Given the limited safety information with ASP0598, there are currently no expected serious adverse reactions for ASP0598. Detailed reference safety information (RSI) can be found in the IB.

2.2 Study Rationale

This is the first clinical trial for ASP0598 Otic Solution. Surgical procedure is the only available treatment for CTMP and includes anesthesia-associated risks and the inconvenience of surgery. ASP0598 Otic Solution will be developed as a non-invasive treatment in order to address the unmet need. This study will be a randomized, placebo-controlled and investigator and subject blinded study to evaluate the safety, tolerability, efficacy and pharmacokinetics in subjects with CTMP.

2.3 Risk Benefit Assessment

The non-clinical profile of ASP0598 Otic Solution demonstrates its potential efficacy on tympanic membrane perforation, localized distribution at the administration site with no systemic exposure to HB-EGF, and a favorable safety profile, supporting its clinical investigation. In this study, skin local reaction in the external ear canal will be assessed after topical application of ASP0598 Otic Solution. An appropriate administration to the tympanic membrane and observation of administration site are required to minimize exposure to the middle ear. In addition, monitoring of auditory functions (otology examinations) is recommended.

Overall, the risk associated with the participation of subjects with CTMP in this study is considered acceptable.

3 STUDY OBJECTIVE AND ENDPOINTS

3.1 Primary Objective and Endpoints

Objective	Endpoints
• To evaluate the safety and tolerability of ASP0598 Otic Solution	• Treatment-emergent adverse events (TEAEs) • Incidence of adverse events of special interest as defined below: 1. Cholesteatoma or ear neoplasm 2. Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness) 3. Otitis media or otitis externa • Change from baseline in bone conduction hearing at 1, 2, 4 kHz by Pure Tone Audiometry (PTA) • Change from baseline in Tinnitus Visual Analog Scale (TVAS)

3.2 Secondary Objective and Endpoints

Objective	Endpoints
• To evaluate the efficacy of ASP0598 Otic Solution For secondary efficacy endpoints the hypothetical strategy will be applied.	*Efficacy* *Single Dose* • Complete closure of Tympanic Membrane Perforation (TMP) at Week 8 for Single Ascending Dose (SAD) and at Week 12 for dose expansion • 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 dose expansion • Change from baseline in TMP size at Week 8 for SAD and at Week 12 for dose expansion *Multiple Dose:* • Complete closure of TMP at Week 12 for Multiple Ascending Dose (MAD) and at Week 16 for dose expansion • 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 dose expansion • Change from baseline in TMP size at Week 12 for MAD and at Week 16 for dose expansion

For the dose expansion, hypothetical strategy will be applied to analyze the secondary endpoints. Details of intercurrent events (ICEs) and estimand are shown below.

3.2.1 Intercurrent Events

The following ICEs may happen:

- Treatment discontinuation due to any reason
- Study discontinuation due to any reason

3.2.2 Treatment regimen under evaluation

Randomized treatment administered as directed per Schedule of Assessments.

3.2.3 Estimand

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

Efficacy endpoints:

1. Complete closure of TMP at Week 12 for single dose expansion and Week 16 for multiple dose expansion.
2. 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.
3. 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:** 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 Otic Solution and placebo.
- **Endpoint #2:** 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 Otic Solution and placebo.
- **Endpoint #3:** 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 Otic Solution and placebo.

Data that will be used for the analysis:

- **Endpoint #1:** data necessary for this analysis is complete closure status at Week 12 for single dose expansion and Week 16 for multiple dose expansion. For the subjects who have discontinued treatment or the study before these data are collected due to 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 analyses are TMP size per total area of tympanic membrane and TMP size at each visit, respectively. For the subjects who have discontinued treatment or the study before these data are collected due to 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.

3.3 Exploratory Objectives and Endpoints

Objective	Endpoints
To evaluate otological parameters that may correlate with efficacy outcome and pharmacokinetics of ASP0598 Otic Solution	*Single Dose:* ● Complete closure of TMP for post baseline visits at Day 2, Day 3* Week 1, Week 2, Week 4 for SAD and Week 2, Week 4, and Week 8 for dose expansion ● Change from baseline in the ratio of TMP size per total area of tympanic membrane for post baseline visits at Day 2, Day 3*, Week 1, Week 2, and Week 4 for SAD and Week 2, Week 4, and Week 8 for dose expansion ● Change from baseline in TMP size for post baseline visits at Day 2, Day 3*, Week 1, Week 2, and Week 4 for SAD and Week 2, Week 4, and Week 8 for dose expansion * Day 3 evaluations will only be performed for cohorts 1, 2 and 3. *Multiple Dose:* ● 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 dose expansion ● 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 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 dose expansion *Single and Multiple Dose:* ● Time to closure of TMP ● Size of TMP ≤ 5% of pars tensa surface area after treatment ● Size of TMP ≤ 1% of pars tensa surface area (pin point perforation) after treatment ● Change from baseline in air bone (A-B) gap by PTA ● Change from baseline in air conduction hearing by PTA ● Percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing) ● Improvement from baseline in air conduction hearing by PTA ≥ 15 dB ● A-B gap after treatment was 15 dB or less ● Air conduction hearing threshold after treatment was 30 dB or less ● Type of tympanogram (A, B or C) ● Equivalent ear canal volume (ECV) by tympanometry *Pharmacokinetics* ● Serum concentration of ASP0598 (dose escalation part only)
Table continued on next page	

Objective	Endpoints
To evaluate the safety of ASP0598 Otic Solution	• Vital signs (body temperature, blood pressure and pulse rate)

4 STUDY DESIGN AND DOSE RATIONALE

4.1 Study Design

This is a randomized, placebo-controlled and investigator and subject blinded study to evaluate the safety, tolerability, efficacy and pharmacokinetics in subjects with CTMP.

The main part of this study is dose escalation. Dose escalation will start with single ascending dose (SAD) followed by multiple ascending dose (MAD, Q2W x3).

Dose escalation will primarily assess safety and tolerability of single dose and multiple doses of ASP0598 Otic Solution. Dose escalation will consist of up to 4 cohorts for SAD and up to 2 cohorts for MAD, with different dose levels (Table 6). Each cohort will consist of 5 subjects (ASP0598 Otic Solution n = 4 and placebo n = 1) for SAD and 8 subjects (ASP0598 Otic Solution n = 6 and placebo n = 2) for MAD.

Single and/or multiple dose expansion will be opened based on the safety and efficacy results following an interim analysis. Single dose expansion will be opened based on the results of the first interim analysis, which will occur after the completion of SAD. Data will be summarized for all time points available. Multiple dose expansion will be opened based on the results of the second interim analysis, which will occur after the completion of MAD.

Table 6 Planned Dose Levels for Dose Escalation

Cohort	Dose regimen	Dose (µg)	Drug concentration (µg/mL)	Administration volume (mL)	ASP0598 Otic Solution (n)	Placebo (n)
1	Single	0.03	0.2	0.15	4	1
2	Single	0.15[a]	1.0[a]	0.15	4	1
3	Single	0.75[a]	5.0[a]	0.15	4	1
4	Single	2.25[a]	15[a]	0.15	4	1
5	Q2W×3	0.75[a]	5.0[a]	0.15	6	2
6	Q2W×3	2.25[a]	15[a]	0.15	6	2

[a] Planned dose levels, doses may be adapted depending on emergent safety and tolerability data.

This study will consist of the following:

Single Dose:

- Screening (Days -28 to -1)
- Randomization (Day 1)
- Observation [through Day 57 (SAD) or through Day 85 (single dose expansion)]

Multiple Dose:

- Screening (Day -28 to -1)
- Randomization (Day 1)
- Observation [through Day 85 (for MAD) or through Day 113 (for multiple dose expansion)]
- Additional Treatment on Days 15 and 29

Prior to any study-related assessments, the Informed Consent Authorization Form will be signed by the subject at Screening (Visit 1). All subjects will enter into a screening period (Days -28 to -1) and will be evaluated for randomization eligibility after completing screening procedures. Eligible subjects will be randomized on Day 1 (Visit 2).

For a subject with bilateral TMP, once both ears have been identified as meeting all inclusion/exclusion criteria at Randomization on Day 1 (Visit 2), the ear with the worse hearing will be selected as the treated ear, unless a rationale exists to treat the other ear. In the case of equivalent hearing level in both ears, the investigator will decide the treatment side with subject and will document the reason in the source document and the eCRF. In the case that the investigator is unable to decide the appropriate ear to treat, consultation with the sponsor may be required.

Dose Escalation

Approximately 20 subjects for SAD and 16 subjects for MAD will be enrolled in dose escalation part of the study. The initiation and selection of dose concentrations for MAD will be based on at least 3 cohorts of SAD.

For SAD, after randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear. Subjects will return to the investigative site for assessments on Days 2, 3, 8, 15, 29, and 57 [End of Study (EOS)]. Day 3 evaluations will only be performed for cohorts 1, 2 and 3.

For MAD, after randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear and will receive additional treatments into the same ear on Days 15 and 29. Subjects will return to the investigative site for assessments on Days 8, 15, 22, 29, 36, 57, and 85 (EOS).

For SAD and MAD, subjects who terminate early from the study will complete EOS assessments.

For MAD only, Subjects who discontinue treatment will be encouraged to complete all protocol defined study visits through the Day 85 (EOS).

The decision to escalate or stop escalation during SAD will be made by the Dose Escalation Committee (DEC). The DEC will operate as per the approved DEC Charter.

The DEC will decide dose escalation and the initiation and selection of doses for MAD cohorts based on the emerging safety and tolerability data from SAD. The tentative starting

dose concentration of MAD will be 5.0 μg/mL, and another dose concentration up to a maximum feasible dose concentration of 15 μg/mL will be considered.

The first interim analysis will be conducted after completion of the last SAD cohort. Data will be summarized for all time points available. The second interim analysis will be conducted after completion of the last MAD cohort.

The Data Monitoring Committee (DMC) will review the data and make the final decision to open single and/or multiple dose expansion. The DMC operation, roles and responsibilities will be described in the DMC Charter.

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.

Dose Expansion

The decision to open single and/or multiple dose expansion will be made based on the safety and efficacy results of the interim analyses after completion of SAD and after completion of MAD by the DMC per the charter. Once the decision to open single and/or multiple dose expansion is made, the number of treatment groups (2 or 3 treatment groups including 1 or 2 active treatment groups and 1 placebo group) and the dose for ASP0598 Otic Solution will be selected based on the safety and efficacy result of the interim analyses. All analyses will be described in the Interim Analysis Plan (IAP).

Up to 39 subjects will be randomized to single dose and/or multiple dose expansion. 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, or 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 or placebo (13 subjects in each treatment group).

Stratification at the time of randomization will be based on the % size of the TMP at the screening visit and subjects will be balanced equally between the treatment groups. Dose expansion will assess safety and efficacy of ASP0598 Otic Solution.

Single Dose Expansion

After randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear at the investigative site. Subjects will return to the investigative site for assessments on Days 15, 29, 57, and 85 (EOS).

Subjects who terminate early from the study will complete EOS assessments at the time of discontinuation.

Multiple Dose Expansion

After randomization on Day 1, subjects will receive ASP0598 Otic Solution or placebo administration into the affected ear. Subjects may receive additional treatment in the same

ear on Days 15 and 29. Subjects will return to the investigative site for assessments on Days 15, 29, 43, 57, 85 and 113 (EOS).

In cases where complete closure is confirmed on Day 15 or Day 29, the subject will not receive the next scheduled dose(s), but will complete all visit assessments as defined in the Schedule of Assessments through Day 113.

Subjects who terminate early from the study will complete EOS assessments at the time of discontinuation.

Subjects who discontinue treatment will be encouraged to complete all protocol defined study visits through the Day 113.

In single dose expansion and/or multiple dose expansion, 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.

4.1.1 Decision Process for Dose Escalation, Dose Escalation Stopping Rules and Definition of the Maximum Tolerated Dose

4.1.1.1 Composition of Dose Escalation Committee

Proceeding to the next higher dose requires an agreement of the DEC, which is comprised of Sponsor representatives. The DEC Charter will provide detailed additional information.

4.1.1.2 Decision Process for Dose Escalation and MAD Initiation

The DEC will decide dose escalation and the initiation and selection of doses for MAD cohorts based on emerging safety and tolerability data from SAD. After all subjects in a cohort have been dosed and completed study procedures through Day 15, the overall safety and tolerability of the dose will be determined after evaluation of the blinded safety data [treatment emergent adverse events (TEAEs), adverse events (AEs) of special interest, otology examinations (imaging pictures of tympanic membrane, bone conduction hearing at 1, 2, 4 kHz by PTA, TVAS, Modified Brighton Grading Scale, and vital signs] for the current cohort. Cumulative AE data from the preceding cohort(s) will also be included in the assessment for dose escalation. Details will be defined in the DEC Charter.

4.1.1.3 Dose Escalation Stopping Rules

Depending on the nature, frequency and severity of the safety profile observed in the study, the DEC will decide whether to:

- Proceed with dose escalation and determine the next higher dose or,
- Stop dose escalation (i.e., no further dosing with IP).

Dose escalation will be stopped if 1 (or more) of the following apply:

- If 1 or more subjects in 1 cohort develop cholesteatoma or suspicion of ear neoplasm which is considered to be related to the ASP0598.
- If 2 or more subjects in 1 cohort develop Ototoxicity (clinically significant report of vestibular symptoms, tinnitus, or acute hearing loss) which are considered to be related to the IP.

- If 2 or more subjects in 1 cohort develop persistent otitis media or otitis externa unresponsive to oral and topical antibiotics which is described in concomitant treatment requirements section of the synopsis which are considered to be related to the IP.
- If 1 or more subjects in 1 cohort experience AEs of severe intensity or 2 or more subjects in 1 cohort experience AEs of the same character of moderate intensity, which are considered to be related to the IP, and of clinical concern.

In the event that it is deemed relevant to unblind a subject for the purposes of a dose escalation decision, the DEC chair or designee can request the treatment assigned to one or more subjects from the study statistician. The DEC charter will include information on unblinding of study team members and other details.

4.1.1.4 Definition of the Maximum Tolerated Dose

If the DEC decides to stop dose escalation based on available safety data, the current dose level will be considered as the minimum intolerable dose (MID). The dose just below the MID will be regarded as the maximum tolerated dose (MTD). If the dose escalation is stopped due to reaching exposure limit without dosing limiting safety findings, MTD cannot be determined.

4.2 Decision Process for Dose Expansion

A Data Monitoring Committee (DMC) will be implemented and is responsible for the interim evaluation of safety and efficacy data and decision to open single and/or multiple dose expansion. The DMC membership will be described in a separate DMC Charter.

Two interim analyses will be conducted. The analyses will be performed after completion of last cohorts from of SAD and MAD, respectively.

The analysis will be conducted by the Astellas study statistician, with results reviewed by the DMC as per IAP. Details of the interim analysis procedure and the criteria to open single and/or multiple dose expansion will be described in the DMC charter.

4.3 Dose Rationale

SAD

The starting dose for SAD is 0.2 µg/mL administered in a single 0.15 mL dose volume. This starting concentration is equivalent to the minimum efficacious concentration in a nonclinical pharmacology study in a mouse CTMP model (minimum efficacious dose = 0.2 µg/mL, 5-µL single-dose volume). The dose volume has been scaled for clinical application based on tympanic membrane surface area between mice (3 mm^2) and humans (68.3 – 90 mm^2).

The nonclinical pharmacology study showed concentration-dependent efficacy of ASP0598 [Section 2.1.1]. In addition, given the nature of ASP0598 Otic Solution as a topical agent with local exposure and toxicity, the concentration (µg/mL) of ASP0598 Otic Solution was used as the basis for selecting the doses for the first-in-human study.

GLP studies were conducted to evaluate the toxicity after single-dose administration of ASP0598 Otic Solution in 2 different species: mice and cynomolgus monkeys [Section 2.1.1]. The no adverse effect levels (NOAEL) were determined in the GLP toxicity studies as follows:

- NOAEL after a single administration into external auditory canal:
  150 µg/mL administered in a 10-µL volume in mice.
  150 µg/mL administered in a 100-µL volume in cynomolgus monkeys.
  In both species, the NOAEL was the highest concentration tested in the study.
- NOAEL after a single transtympanic dose administration:
  150 µg/mL administered in a 2-µL volume in mice.
  150 µg/mL administered in a 100-µL volume in cynomolgus monkeys.
  In both species, the NOAEL was the highest concentration tested in the study.

The first-in-human starting concentration of 0.2 µg/mL has a 750-fold safety margin over the NOAEL concentration from the GLP toxicity studies.

The first-in-human maximum concentration is tentatively set at a concentration 15 µg/mL in a 0.15 mL volume dose, which is the maximum feasible concentration in the clinic. This maximum concentration has a 10-fold safety margin over the NOAEL concentration from the GLP toxicity studies. Dose adjustments will be based on altering the concentration of ASP0598 in the solution to 1.0, 5.0, and 15 µg/mL while the administration volume will be held constant at 0.15 mL. More than a 3-fold dose-increment, especially at lower concentration levels, is considered acceptable, considering the large safety margin as well as the non-steep concentration-response relationship observed in the CTMP mouse model.

MAD

The initiation and selection of dose concentrations for MAD will be based on emerging safety and tolerability data, and at least 3 dose cohorts of SAD should be available for the decision to initiate MAD. The starting dose of MAD will be 5.0 µg/mL or lower, and another dose concentration up to the maximum feasible dose (15 µg/mL) or the NOAEL, whichever is lower, will be selected. The toxicology profile associated with repeat doses of ASP0598 given 3 times at 2-week intervals has been tested in CBA/CaJ mice and cynomolgus monkeys and did not produce any significant toxicological findings. A bi-weekly dose schedule is selected that is considered convenient for patients and otolaryngology clinics, and it is predicted to resemble single treatments with minimal chance of drug accumulation. Therefore, Q2W × 3 is selected for the dose frequency of the multiple dose.

4.4 End of Study Definition

End of the study is defined as the last visit or scheduled procedure shown in schedule of assessments for the last subject in the study.

5 STUDY POPULATION

All screening assessments must be completed and reviewed to confirm the potential subject meets all eligibility criteria. Prospective approval of protocol deviations (PDs) to eligibility criteria (also known as protocol waivers or exemptions) is not permitted.

5.1 Inclusion Criteria

A subject is eligible for the study if all the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written Informed Consent and privacy language as per national regulation (e.g., Health Insurance Portability and Accountability Authorization for US sites) must be obtained from the subject or legally authorized representative prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Subject must be 18 to 75 years of age at the time of informed consent.
3. Subject has chronic tympanic membrane perforation (CTMP) documented as persisting longer than 3 months.
4. A female subject is eligible to participate if she is not pregnant see Appendix 12.3: Contraception Requirements and at least one of the following conditions applies:
    a. Not a woman of childbearing potential (WOCBP) as defined in Appendix 12.3: Contraception Requirements OR
    b. WOCBP who agrees to follow the contraceptive guidance as defined in Appendix 12.3: Contraception Requirements starting at screening and for at least 28 days after investigational product (IP) application.
5. Female subject must agree not to breastfeed starting at drug application on Day 1 and for at least 28 days after IP application.
6. Female subject must not donate ova starting on Day 1 and for at least 28 days after IP application.
7. A male subject with female partner(s) of child-bearing potential must agree to use contraception starting on Day 1 and for at least 28 days after IP application as detailed in Appendix 12.3.
8. A male subject must not donate sperm starting on Day 1 and for at least 28 days after IP application.
9. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom from Day 1 and for at least 28 days after IP application.
10. Subject must be willing and able to comply with the study requirements including refraining from using prohibited concomitant medications.
11. Subject agrees not to participate in another interventional study during the study period.

12. Subject has the ability to understand and the willingness to sign a written informed consent document.

Waivers to the inclusion criteria will NOT be allowed.

5.2 Exclusion Criteria

A subject will be excluded from participation if any of the following apply:

1. Subject has one of following conditions, that is confirmed by the investigator, that may affect the ipsilateral side of the ear with CTMP:
    a. Perforation involving 3 or more quadrants.
    b. Pin hole perforation (only for the expansion cohort).
    c. Presence of tympanosclerosis adjacent to the perforation.
    d. Perforation involves malleus erosion.
    e. Absent malleus.
    f. Marginal perforation (i.e., involving the annulus or exposing the handle of malleus).
    g. TMP caused by electric/slag/blast/burn injury.
    h. Post radiated TMP.
    i. History of tympanic membrane repair by any type of live tissue.
    j. Active otorrhea or active treatment for otorrhea within the last 3 months prior to Screening.
    k. Bellucci otorrhea grade 3 or above.
    l. Active external ear canal inflammation (otitis externa, dermatitis) or within the last 3 months prior to Screening.
    m. Active diagnosis of Eustachian Tube dysfunction or diagnosis within 6 months prior to Screening.
    n. Craniofacial abnormalities, history of head and neck surgery within the last 3 months prior to Screening, history of radiation to head and neck.
    o. Recent (within 2 weeks) diagnosis of upper respiratory tract infection.
    p. Presence or history of cholesteatoma.
    q. Presence of pars-flaccida or pars tensa retraction or adhesion.
    r. Presence or history of tumors of the middle or external ear.
    s. Contraindications to tympanic membrane closure.
    t. An audiometric finding indicates a characteristic of Carhart's notch which is an increase in bone conduction threshold with a peak at 2,000 Hz.
    u. Only hearing ear or better hearing ear and the contralateral ear ≥ 40 dB by average four-frequency (500, 1000, 2000 and 4000 Hz).
    v. Whole circumference of the tympanic membrane perforation is not visible by endoscope.
    w. Presence/history of eosinophilic otitis media in either ear.
2. Subject has a presence of adhesive otitis media in the contralateral ear.
3. Subject has a presence of any wound healing systemic condition.

4. Subject has Obstructive Sleep Apnea where the subject is required to use Continuous Positive Airway Pressure (CPAP) during the study period.
5. Subject is exposed in their daily life to high volume of water into the ear canal (e.g., swimmer or surfer).
6. Subject has health conditions that would prevent him/her from fulfilling the study requirements as judged by the clinical investigator on the basis of medical history and laboratory test (Serum Chemistries, CBC with Differential, Urinalysis) results at the screening visit.
7. Subject is receiving any other investigational agents during study participation.
8. Subject has any form of substance abuse, or psychiatric illness/social situations that would limit compliance with study requirements, or a condition that in the opinion of the investigator could invalidate communication with the investigator.
9. Subject has a known or suspected hypersensitivity to ASP0598, or any components of the formulation used.
10. Subject has had previous exposure with ASP0598.
11. Subject is unlikely to comply with the visits scheduled in the protocol, in the opinion of the Investigator.

Waivers to the exclusion criteria will NOT be allowed.

5.3 Restrictions During the Study

These restrictions are required after ASP0598 Otic Solution/placebo administration at Day 1 until completion of the study procedures at Day 57 (SAD), Day 85 (single dose expansion), or Day 85 (MAD), Day 113 (multiple dose expansion), or EOS visit.

- Subjects must refrain from showering or bathing for at least 24 hours after IP administration.
    - Showering or bathing are allowed 24 hours after IP administration. Water precautions are to be taken as per the guidelines in Appendix 12.8.
- Any water activities that risk submersion of the ear (e.g., swimming or surfing) are prohibited throughout the entire study period.
- Subjects are not allowed to use any materials (e.g., Q-tips) placed into the ear canal of the treated ear (ear buds or headphones placed at the entrance to the ear canal are considered acceptable). If ear cleaning is desired, then subjects can use a soft tissue placed gently at the entrance to the ear canal.
- Investigators will not clean the ASP0598 Otic Solution/placebo treated ear by suctioning throughout Observation period, unless subjects experience uncomfortable ear discharge. General ear cleaning will be allowed to visualize the whole tympanic membrane at Day 57 (SAD), Day 85 (single dose expansion or MAD), Day 113 (multiple dose expansion) or End of Study (EOS) visit, before imaging picture.

- Investigators may gently remove an obstacle that is blocking the ear canal if it prevents IP application or causes subjects to experience an uncomfortable sensation. If the obstacle is adherent to the tympanic membrane, it should not be removed; it is recommended to wait until the obstacle comes off.
- Subjects should refrain from activities that can increase the middle ear pressure including plane travel (at least 28 days after IP application), positive pressure ventilation (e.g., CPAP or BiPAP) devices, Valsalva maneuvers, excessive high-pressure nose blowing or diving underwater.
- Subject must refrain from activities that can excessively increase body temperature (e.g., Sauna) for 3 days after IP application.
- Subject must refrain from activities that stimulate sweating excessively for 3 days after IP application.

5.4 Screen Failures

A screen failure is defined as a potential subject who signed the Informed Consent Form (ICF), but did not meet 1 or more criteria required for participation in the study and was not randomized.

For screen failures, the demographic data, date of signing the ICF, inclusion and exclusion criteria, AEs up to the time of screen failure and reason for screen failure will be collected in the eCRF.

5.4.1 Rescreening

Results of screening assessments that do not meet the parameters required by eligibility criteria (e.g., clinical laboratory tests, vital signs, physical examination, ear examination, etc.) may be repeated once within the 28-day screening period without the need to register the subject as a screen failure. If more than 28 days elapses from the date of signing the ICF, the subject must be documented as a screen failure. In order to re-screen, a new ICF must be signed and the subject entered into screening with a new subject identification number. Rescreening is only allowed once for an individual subject.

6 INVESTIGATIONAL PRODUCT(S)

6.1 Investigational Product(s) Administered

Table 7 Investigational Product(s)

Name	***ASP0598 Otic Solution***	***Buffered Aqueous Solvent with Copolymer***
Use	Test Product	Placebo
Dosage Formulation	Liquid for reconstitution and administration	Solution for administration
Physical Description	Colorless to pale yellow, clear to slightly opalescent liquid solution	Colorless to pale yellow, clear to slightly opalescent liquid solution
Unit Dose Strength	0.2, 1.0, 5.0, and 15.0 µg/mL	N/A
Table continued on next page		

Packaging and Labeling	Clear glass, single-use vial with a rubber stopper and an aluminum crimping cap.	Clear glass, single-use vial with a robber stopper with an aluminum crimping cap.
Route of Administration	Single administration onto the tympanic membrane through the external auditory canal via syringe.	Single administration onto the tympanic membrane through the external auditory canal via syringe.
Administration Instruction	See Section 6.4 and Pharmacy Manual and other supporting materials	See Section 6.4 and Pharmacy Manual and other supporting materials
IMP or Non-IMP	IMP	IMP
Sourcing	Provided centrally by the sponsor	Provided centrally by the sponsor

Refer to the pharmacy manual and product label for detailed information regarding preparation, handling and storage of the IP and other study treatment(s).

6.1.1 Investigational Product Application

IP (ASP0598 Otic Solution or placebo) will be prepared by the pharmacist or designee according the IRT treatment allocation using the pharmacy manual. The final IP Otic Solution will be brought to the Study Physician or designee in the 1 mL Luer Lock™ syringe. Ensure the final IP Otic Solution is administered within the appropriate window of time, depending on the assigned treatment. Refer to the Pharmacy Manual, Section 9.3, for detailed information. The final IP Otic Solution should be stored at room temperature (20-25°C) and protected from light prior to administration. The following information should be provided by the pharmacist / designee with the final IP Otic Solution as per institutional standards: subject's initials, randomization number, end time of IP preparation, and administer by (time).

The Study Physician or designee will put the 22G sheathed blunt-end needle onto the 1 mL Luer Lock™ syringe prior to administration. The Study Physician or designee should prime the syringe prior to otic administration so that up to a maximum volume of 0.15 mL is applied onto the tympanic membrane.

After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Packaging and Labeling

All IP used in this study will be prepared, packaged and labeled under the responsibility of qualified personnel at APGD or sponsor's designee in accordance with APGD or sponsor's designee standard operating procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local laws/regulations.

Each carton and vial will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as investigational drug.

Refer to the pharmacy manual and product label for detailed information regarding packaging, labeling, and preparation of the IP.

6.2.2 Handling, Storage and Accountability

1. The pharmacist or designee must confirm appropriate temperature conditions have been maintained during transit for all IP received and any discrepancies are reported and resolved before use of the IP.
2. Only subjects enrolled in the study may receive IP and only authorized study site personnel may supply or administer IP. Only IP with appropriate expiry/retest dating may be dispensed.
3. All IP must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions and access must be limited to the investigator and authorized study site personnel.
4. The pharmacist, investigator, institution or the head of the medical institution (where applicable) is responsible for accountability, reconciliation and record maintenance (i.e., receipt, reconciliation and final disposition records).
5. Further guidance and instruction on final disposition of used and unused IP is provided in the pharmacy manual.

Refer to the pharmacy manual for detailed information regarding handling, storage and accountability of the IP.

6.3 Randomization and Blinding

This is an investigator and subject blinded study. Subjects will be randomized by IRT to receive ASP0598 Otic Solution or placebo in a blinded manner such that neither the investigator nor the subject will know which IP is being administered.

Only the pharmacist and designated staff will be unblinded to treatment. All other study site staff will remain blinded to subjects' treatment assignment.

6.3.1 Blinding Method

In order to maintain the blind, the ASP0598 Otic Solution and placebo will be received in the same volume and route from the pharmacist or designee. The pharmacist or designee will receive the unblinded treatment assignment from IRT in order to prepare the correct concentration of ASP0598 Otic Solution or placebo. The subject and investigator will be blinded to the treatment assignment.

Dose Escalation

Only one cohort will be enrolled at a time. The randomization number will be assigned based on information obtained from the IRT system.

Dose Expansion

Subjects will be randomized through the IRT system in a 1:1:1 ratio, if 3 treatment groups are selected or 1:1 ratio, if 2 treatment groups are selected based on the interim analysis.

6.3.2 Confirmation of the Indistinguishability of the Investigational Product

The appearance of both the dosage form and packaging of ASP0598 Otic Solution are identical to those of their matching placebo.

6.3.3 Retention of the Assignment Schedule and Procedures for Treatment Code Breaking

The randomization list and treatment assignment blind will be maintained by the IRT system.

6.3.4 Breaking the Treatment Code for Emergency

The treatment code for each randomized subject will be obtained from the IRT system in the event of a medical emergency requiring knowledge of the treatment assigned to the subject. The IRT system will be programmed with blind-breaking instructions that can only be requested by the investigator or sub-investigators designated to have access to perform blind-breaking. In case of a medical emergency, the investigator has the sole responsibility for determining if unblinding of the subject's treatment assignment is warranted. Subject safety must always be the first consideration in making such determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's treatment assignment unless this could delay emergency treatment for the subject.

Prior to the initial IP shipment, the investigator must have confirmed ability to access code-break through the IRT system and must have a designated backup (e.g., redundant processes) to support emergency unblinding requirements.

Prior to randomization, subjects should be provided with information that includes the study site emergency contact number and back-up contact number in case of a medical emergency. Any unblinding by the investigational personnel must be reported immediately to the sponsor and include an explanation of why the IP was unblinded. If unblinding is associated with a serious adverse event (SAE), the investigator is to follow the instructions in [Section 12.4.5 Reporting Procedures for Serious Adverse Events].

Care still be taken to limit knowledge of the treatment assignment, in case this can affect the blinding of other subjects or future study assessment for the subject.

6.3.5 Breaking the Treatment Code by the Sponsor

The sponsor may break the treatment code for subjects who experience a suspected unexpected serious adverse reaction (SUSAR), in order to determine if the individual case or a group of cases requires expedited regulatory reporting. Individual emergency codes will be provided to the limited personnel who are responsible to break the codes for all SUSAR cases for reporting purposes.

6.3.6 Assignment and Allocation

SAD

In each cohort, a total of 5 subjects will be randomized in a 4:1 ratio of ASP0598 Otic Solution (4 subjects) and placebo (1 subject).

MAD

In each cohort, a total of 8 subjects will be randomized in a 6:2 ratio of ASP0598 Otic Solution (6 subjects) and placebo (2 subjects).

Dose Expansion

- 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, or 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 or placebo (13 subjects in each treatment group).

Subjects will be stratified at the time of randomization by % size of the TMP (≤ 15%, > 15%) at the Screening Visit. Subjects will be balanced equally between the treatment groups. TMP size group will be determined by the Screening images sent from the investigative site to the central imaging vendor.

For SAD, MAD, and dose expansion, randomization will be performed via 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. The randomization schedule that determines subject treatment will be computer-generated by IRT before the beginning of the study. The unblinded study site personnel will dispense the treatment according to the IRT system's assignment. Specific procedures for randomization through the IRT are contained in the study-specific IRT manual.

6.4 Investigational Product Compliance

Single Dose

IP will be administered by the PI or designee on Day 1. Ensure the final IP Otic Solution is administered within the appropriate window of time, depending on the assigned treatment. Refer to the Pharmacy Manual, Section 9.3, for detailed information. Subjects will be provided Ear Care Recommendations (Appendix 12.8) that must be reviewed and acknowledged on Day 1 in the source document. The subject should follow this guidance for the duration of their participation. The investigator will examine the treated ear at every visit.

Multiple Dose

IP will be administered by the PI or designee on Days 1, 15, and 29. Ensure the final IP Otic Solution is administered within the appropriate window of time, depending on the assigned treatment. Refer to the Pharmacy Manual, Section 9.3, for detailed information. Subjects

will be provided Ear Care Recommendations (Appendix 12.8) that must be reviewed and acknowledged at each IP administration visit in the source document. The subject should follow this guidance for the duration of their participation. The investigator will examine the treated ear at every visit.

6.5 Previous and Concomitant Treatment (Medication and Nonmedication Therapy)

These restrictions are required after ASP0598 Otic Solution or placebo administration on Randomization (Day 1), until completion of study procedures at Day 57 (SAD) / Day 85 (single dose expansion or MAD) / Day 113 (multiple dose expansion) or EOS visit.

- Ear drop agents will be used only for the treatment of otitis media with suppurative ear discharge. Only Ciprofloxacin or Ofloxacin ear drops (does not include steroids) are allowed to be used in the ASP0598 Otic Solution or placebo treated ear. Investigators should minimize treatment with ear drops up to a maximum of 10 days and consider alternative treatment (e.g., oral antibiotics).
- Any other topical agents in the ear canal are prohibited to use in the ASP0598 Otic Solution or placebo treated ear.
- Any otologic procedures (e.g., paper patch or surgeries) to the ASP0598 Otic Solution or placebo treated ear are prohibited.

All concomitant medication usage will be noted and recorded during each study visit.

6.6 Dose Modification

SAD and single dose expansion

Not Applicable.

MAD

On Day 15 or Day 29, treatment can be skipped at the Investigator's discretion based on safety reasons that interrupt IP administration (e.g., ear canal skin irritation, mild otorrhea). Subjects should be encouraged to return to the office for evaluation and additional IP administration within the Day 15 or Day 29 visit windows. In the case that the investigator is unable to conduct IP administration for anything other than safety reasons, consultation with the sponsor may be required.

Multiple dose expansion

The statement above for MAD is also applicable to the multiple dose expansion. Additionally, the treatment on Day 15 or Day 29, treatment will be skipped in cases where complete closure is confirmed. If the perforation is detected on Day 29 after treatment was skipped on Day 15, IP administration can be conducted.

6.7 Criteria for Continuation of Treatment

SAD and single dose expansion

Not applicable because there is a single treatment of ASP0598 Otic Solution or placebo.

MAD and multiple dose expansion

Treatment Discontinuation

During the course of IP administration, if the subject experiences a drug-related AE, the IP administration may be discontinued at the investigator's discretion.

In addition, IP administration will be discontinued if severe intensity occurs in any of the following:

- Subject develops cholesteatoma or suspicion of neoplasm in the treated ear as determined by investigator.
- Subjects develops suspected ototoxicity symptoms (e.g., sudden hearing loss at high frequencies (1, 2, 4 kHz), new high frequency tinnitus or new balance disorder) which are considered by the investigator to be related to the study drug.
- Subjects with persistent otitis media or otitis externa unresponsive to oral and topical antibiotics (as described in Previous Concomitant Treatment [Medication and Nonmedication Therapy of the synopsis and protocol Section 6.5])

7 STUDY PROCEDURES AND ASSESSMENTS

Refer to the Alternate Schedule of Assessments in [Section 12.11 Clinical Study Continuity] for acceptable alternate methods to assess safety and efficacy parameters in the event the study is interrupted due to a crisis (e.g., natural disaster, pandemic).

7.1 Efficacy Assessments

7.1.1 Imaging picture of tympanic membrane by endoscope

At Screening, an imaging picture of the tympanic membrane for both ears will be captured by endoscope. After the treated ear is determined at Day 1, an imaging picture will be taken only of the treated ear throughout the study.

An imaging picture of the tympanic membrane will be recorded to support evaluation of efficacy endpoints at all visits. TMP closure is defined as TMP closure without presence of pin hole. All images from each visit will be sent to a central imaging vendor for measurement and interpretation. TMP size calculation and evaluation of complete closure will be performed by central imaging vendor and detailed instruction will be described in Independent Review Charter. See the SAD and Single Dose Expansion Schedule of Assessments (Table 2 and Table 3) and MAD and Multiple Dose Expansion Schedule of Assessments (Table 4 and Table 5) for timing of procedures.

7.1.2 Microscopic Ear Examination

The site investigator will examine the tympanic membrane by microscope. TMP closure will be evaluated at all visits except for Screening and Day 1, and will be recorded in the eCRF.

7.1.3 Pure Tone Audiometry

Pure Tone Audiometry (PTA) is a behavioral test and generally the first quantitative hearing test done to assess the nature and degree of hearing loss in adults and in children. This

measure involves the peripheral and central auditory systems. Pure tone air conduction and bone conduction tests determine whether or not there is any hearing loss; what type of hearing loss it is (conductive, sensorineural or mixed hearing loss); the frequencies that are affected (configuration); magnitude of hearing loss (intensity) and whether hearing loss is unilateral or bilateral. The result is plotted on an audiogram, which is a graph displaying intensity as a function of frequency.

PTA will be performed on the bilateral ears at Screening and will be tested only for the treated ear after the treatment side is determined by investigator at Day 1. This will be performed on a calibrated audiometer by a licensed audiologist.

For the efficacy evaluation, PTA will be measured following different frequencies:

Air: 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, and 8 kHz

Bone: 500 Hz, 1 kHz, 2 kHz, and 4 kHz

Follow the Schedule of Assessments (Table 2, Table 3, Table 4 and Table 5) for timing of procedures.

PTA result(s) (decibel at each frequency) will be recorded in the eCRF at each visit.

7.1.4 Tympanometry

Tympanometry (an objective test of middle-ear function) is an examination used to test the condition of the middle ear and mobility of the tympanic membrane (TM) by creating variations of air pressure in the ear canal. Tympanometry will be performed on the bilateral ear at Screening and only for the treated ear for the remaining study visits.

Tympanogram type (A, Ad, As, B or C) and ear canal volume (ECV) will be recorded in the eCRF. Tympanometry will be performed at Screening, Day 1 and Day 57 (SAD) or Day 85 (single dose expansion and MAD) or Day 113 (multiple dose expansion) or EOS Visits. The assessment will be performed by a licensed audiologist on a calibrated tympanometer. A print-out containing the tympanogram will be collected from the machine or, if no print out is available, the tympanogram should be drawn and stored in the source documents.

7.2 Safety Assessments

Single Dose study procedures and their timing are summarized in the Schedule of Assessments (Table 2 and Table 3). Multiple Dose study procedures and their timing are summarized in the Schedule of Assessments (Table 4 and Table 5). Protocol waivers or exemptions are not allowed.

Procedures conducted as part of a subject's routine clinical management (i.e., standard of care) obtained before signing the ICF may be utilized for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame, as defined in the Single Dose Schedule of Assessments (Table 2 and Table 3) and the Multiple Dose Schedule of Assessments (Table 4 and Table 5).

7.2.1 Adverse Events

See [Section 7.3 Adverse Events and Other Safety Aspects] for information regarding AE collection and data handling.

7.2.2 Laboratory Assessments

- Will be performed at Screening for eligibility purposes.
- The investigator or sub-investigator must review the laboratory report and document this review.
- Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or sub-investigator who is a qualified physician.
- Unscheduled laboratory assessments may be performed for Adverse Events.

7.2.3 Vital Signs

Vital signs will include systolic and diastolic blood pressure (mmHg), heart rate (bpm), and body temperature (°F / °C). Vital signs will be performed according to the Schedule of Assessments (Table 2, Table 3, Table 4, Table 5). Additional vital sign assessments may be taken as indicated or according to institutional standard procedure.

7.2.4 Physical Examination

A physical examination of the subject will be conducted by a medically qualified person at the Screening Visit. This will consist of an examination of general appearance, nose, throat, neck, and lymph nodes, as well as measurements of height and weight. A symptom-directed physical may be performed at any of the other visits, if necessary.

The screening physical examination also includes any significant, ongoing medical conditions.

7.2.5 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 the ASP0598 Otic Solution or placebo treated ear will be examined at Day 1 and at subsequent visits.

Local skin reaction around the application site will be examined at 60 minutes after the treatment at Day 1 for single dose and Days 1, 15, and 29 for multiple dose and throughout the observation period. Skin toxicity will be evaluated by the Modified Brighton Grading Scheme (Appendix 12.9) and recorded in the eCRF.

7.2.6 Pure Tone Audiometry for Ototoxicity Assessment

For the ototoxicity evaluation, bone conduction hearing level at 1, 2 and 4 kHz will be measured by PTA. PTA for ototoxicity assessment will be performed for the treated ear on a calibrated audiometer by a licensed audiologist.

Bone Conduction hearing level measured by PTA will be performed according to the Schedule of Assessments (Table 2, Table 3, Table 4, Table 5).

PTA result (decibel at each frequency) will be recorded in the eCRF at each visit.

7.2.7 Tinnitus Visual Analog Scale

Subjects will be given the Tinnitus Visual Analog Scale (Appendix 12.10) to rate their tinnitus at all visits. The Scale is a numeric scale and ranges from 0 (not at all strong or loud) to 10 (extremely strong or loud). The subject's response will be recorded in the eCRF at each visit. The scale is designed to assess the loudness of the subject's tinnitus, its annoyance, the degree of stress it was causing to the subject, and how well the subject was coping with the tinnitus.

7.2.8 Order of Assessments

Refer to and Figures 4 and 5 (Single Dose) and Figures 6 and 7 (Multiple Dose): Otology Examination Flow Chart for the order of assessments.

7.3 Adverse Events and Other Safety Aspects

The definitions of an AE or SAE can be found in [Appendix 12.4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up or Reporting].

The investigator and medically qualified designee(s) are responsible for detecting, documenting and recording events that met the definition of an AE or SAE.

7.3.1 Time Period for Collecting Adverse Event and Serious Adverse Event Information

In order to identify any events that may be associated with study procedures and could lead to a change in the conduct of the study, Astellas collects AEs even if the subject has not received IP. AE collection begins after the signing of the ICF and will be collected until 14 days after the last study visit or when the subject is determined to be a screen failure.

7.3.2 Method of Detecting Adverse Events and Serious Adverse Events

The methods of recording, evaluating and assessing seriousness, causality and severity of AEs and SAEs are described in [Appendix 12.4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up or Reporting]. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

An AE with a change in severity is recorded as a new AE.

7.3.3 Follow-up of Adverse Events

All AEs occurring during or after the subject has discontinued the study are to be followed up on until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized by the investigator.

If after the protocol-defined AE collection period (see [Section 7.3.1 Time Period for Collecting Adverse Event and Serious Adverse Event Information]), an AE progresses to an SAE, or the investigator learns of any (S)AE (serious adverse event or adverse event) including death, where he/she considers there is reasonable possibility it is related to the IP or study participation, the investigator must promptly notify the sponsor.

7.3.4 Reporting of Serious Adverse Events

Prompt notification by the investigator to the sponsor of an SAE is essential, so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study intervention under clinical investigation are met.

In the case of an SAE, the investigator must contact the sponsor by fax or email immediately (within 24 hours of awareness).

Procedures for reporting SAEs to the sponsor are described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events].

7.3.5 Disease-related Events and/or Disease-related Outcomes Not Qualifying as Adverse Events or Serious Adverse Events

Under this protocol, the following event(s) will not be considered as an(S)AE:

- Disease progression: events including defined study endpoints that are clearly consistent with the expected pattern of progression of the underlying disease are not to be recorded as AEs. These data will be captured as efficacy assessment data as outlined in [Section 7.1 Efficacy Assessments]. If there is any uncertainty as to whether an event is due to anticipated disease progression and/or if there is evidence suggesting a causal relationship between the IP and the event, it should be reported as an (S)AE. All deaths up to 30 days after the final administration of IP must be reported as an SAE, even if attributed to disease progression.
- Pre-planned and elective hospital/clinical procedures/interventions or procedures for diagnostic, therapeutic, or surgical procedures for a pre-existing condition that did not worsen during the course of the study. These procedures are collected per the eCRF's completion guidelines.

7.3.6 Adverse Events of Special Interest

Incidence of adverse events of special interest as defined below:

1. Cholesteatoma or ear neoplasm
2. Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness)
3. Otitis media or otitis externa

Additional data may be collected for AEs of special interest (i.e., unscheduled microscopic pictures, histopathology, surgical procedures, or other treatments).

7.3.7 Special Situations

Certain special situations observed in association with the IP, such as incorrect administration (e.g., wrong dose of IP or background therapy) are collected in the eCRF, as PDs per [Section 10.3 Major Protocol Deviations] or may require special reporting, as described below. These special situations are not considered AEs, but do require to be communicated to Astellas as per the timelines defined below.

If a special situation is associated with, or results in, an AE, the AE is to be assessed separately from the special situation and captured as an AE in the eCRF. If the AE meets the

definition of an SAE, the SAE is to be reported as described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events] and the details of the associated special situation are to be included in the clinical description on the SAE worksheet.

The special situations are:

- Pregnancy
- Medication error, overdose and use outside protocol
- Misuse/abuse
- Occupational exposure
- Suspected drug-drug interaction

Instructions and procedures for reporting special situations are provided in [Appendix 12.4.6 Reporting Procedures for Special Situations].

7.3.8 Supply of New Information Affecting the Conduct of the Study

When new information becomes available that is necessary for conducting the study properly, the sponsor will inform all investigators involved in the study as well as the appropriate regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

The investigator will also inform the subjects, who will be required to sign an updated ICF in order to continue in the study.

7.3.9 Urgent Safety Measures

An urgent safety measure (USM) is an intervention that is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the sponsor, relevant competent authorities (CA), IRB/IEC, where applicable, in order to protect subjects from any immediate hazard to their health and/or safety. Either the investigator or the sponsor can initiate a USM. The cause of a USM can be safety-, product- or procedure-related.

7.3.10 Reporting Urgent Safety Measures

In the event of a potential USM, the investigator must contact the study physician (within 24 hours of awareness). Full details of the potential USM are to be recorded in the subject's medical records. The sponsor may request additional information related to the event to support their evaluation.

If the event is confirmed to be a USM, the sponsor will take appropriate action to ensure the safety and welfare of the subjects. These actions may include but are not limited to a change in study procedures or study treatment, halting further enrollment in the study, or stopping the study in its entirety. The sponsor or sponsor's designee will notify the relevant competent authorities and concerned ethics committee within the timelines required per current local regulations, and will inform the investigators, as required. When required, investigators must notify their IRB/IEC within timelines set by regional regulations.

7.4 Pharmacokinetics

Serum will be collected to assess the pharmacokinetic profile of ASP0598 Otic Solution as indicated in the Schedule of Assessments (Table 2 and Table 4).

The actual date and time of each blood sample collection will be collected in the source documents. Serum will be prepared according to procedures further specified in the laboratory manual. Samples will be shipped on dry ice to designated contract research organization (CRO) and analyzed using a validated method.

7.5 Immunogenicity

Banking blood samples for future analysis will be collected for all subjects at timepoints as detailed in the Schedules of Assessments (Table 2, Table 3, Table 4, and Table 5). The actual date and time of each blood sample collection will be recorded in the source documents. Serum will be prepared according to procedures further specified in the laboratory manual. Samples will be shipped to designated CRO. When deemed appropriate at a later date, banked serum sample may be used for potential future immune testing. The results of these tests will be described in a separate report and will not be incorporated in the integrated clinical study report.

7.6 Total Amount of Blood

The total amount of whole blood including clinical laboratory, pharmacokinetic sampling, and blood banking sample that will be collected during the entire study is approximately 20.0 mL per subject. For MAD cohorts only, the total amount of whole blood collected will be approximately 30.0 mL per subject.

8 DISCONTINUATION

8.1 Discontinuation of Individual Subject(s) From Study Treatment

SAD and single dose expansion

Not applicable because there is a single treatment of ASP0598 Otic Solution or placebo.

MAD and multiple dose expansion

Treatment Discontinuation

During the course of IP administration, if the subject experiences a drug-related adverse event (AE) the IP administration may be discontinued at the investigator's discretion.

In addition, study drug administration will be discontinued if any of the following occur:

- Subject develops cholesteatoma or suspicion of neoplasm in the treated ear as determined by investigator.
- Subjects develops suspected ototoxicity symptoms (e.g., sudden hearing loss at high frequencies (1, 2, 4 kHz), new high frequency tinnitus or new balance disorder) which are considered by the investigator to be related to the study drug.

- Subjects with persistent otitis media or otitis externa unresponsive to oral and topical antibiotics (as described in other area of synopsis)

A discontinuation from treatment is defined as a subject who enrolled in the study and for whom study treatment is permanently discontinued for any reason.

The subject is free to withdraw from the study treatment and/or study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to discontinue the subject from study treatment or to terminate a subject's involvement in the study at any time if the subject's clinical condition warrants it.

The reason for discontinuation from study treatment must be documented in the subject's medical records.

A subject must discontinue study treatment for any of the following reasons:

- Subject requests to stop treatment.
- Any clinical AE, laboratory abnormality or intercurrent illness, in the opinion of the investigator, indicates continued treatment is not in the best interest of the subject.

8.2 Discontinuation of Individual Subject(s) From Study

All subjects who discontinue study treatment will remain in the study and must continue to be followed for protocol-specific follow-up procedures as outlined in the Schedule of Assessments (Table 2, Table 3, Table 4, and Table 5). The only exception to this is when the subject specifically withdraws consent for any further contact with him/her or persons previously authorized by the subject to provide this information.

8.2.1 Lost to Follow-up

Every reasonable effort is to be made to contact any subject lost to follow-up during the course of the study to complete study-related assessments and record outstanding data.

8.3 Discontinuation of the Study Site

If an investigator intends to discontinue participation in the study, the investigator must immediately inform the sponsor.

8.4 Discontinuation of the Study

The sponsor may terminate this study prematurely, either in its entirety or at any study site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns. If the sponsor terminates the study for safety reasons, the sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

9 STATISTICAL METHODOLOGY

A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database lock. Any changes to the dose escalation of the SAP will be approved prior to

unblinding the dose escalation data for the prescribed interim analysis. Changes from the planned analyses in the final SAP that impact the statistical analyses will be justified in the clinical study report (CSR).

In general, continuous data will be summarized using descriptive statistics (number of subjects, mean, standard deviation and/or standard error, minimum, median and maximum), and categorical data will be summarized using number and percentage of subjects. Baseline will be defined as the last non-missing observation on or prior to the administration of study drug, unless otherwise specified.

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 ASP0598 treatment group and placebo group.

All statistical comparisons between ASP0598 and placebo will be made using a 2-sided test at $\alpha = 0.05$ significance level and 2-sided 95% confidence intervals (CIs) will be reported for each treatment group and difference from placebo.

9.1 Sample Size

SAD

Formal sample size calculations were not performed. SAD 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) 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.

MAD

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

A sample size of 8 per cohort (n = 6 ASP0598 Otic Solution, n = 2 placebo) 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, 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).

9.2 Analysis Sets

9.2.1 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.

9.2.2 Safety Analysis Set

SAD and single dose expansion

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 and pharmacokinetic data, subjects will be presented by actual treatment received.

MAD and multiple dose expansion

The 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. 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.

9.3 Demographics and Baseline Characteristics

9.3.1 Demographics

Demographics and baseline characteristics will be summarized for all randomized subjects, SAF and FAS by treatment group and ‘total’ over all treatment groups.

9.3.2 Subject Disposition

The following subject data will be presented:

- Number of subjects with informed consent, discontinued during the screening period and randomized for all subjects with informed consent (overall only),
- Screening disposition for all subjects with informed consent (overall only),
- Number and percentage of subjects who were randomized, were administered study drug, did not receive study drug administration, in the analysis sets by treatment group and ‘total’ over all treatment groups for all randomized subjects,
- Treatment disposition for all randomized subjects, SAF and FAS by each treatment group and ‘total’ over all treatment groups,
- Investigational period disposition for all randomized subjects, SAF and FAS by each treatment group and ‘total’ over all treatment groups, and

- Protocol version disposition for all 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.

9.3.3 Previous and Concomitant Treatment (Medication and Nonmedication Therapy)

All previous and concomitant treatment will be listed.

9.3.4 Medical History

Medical history is coded in Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized for the SAF by system organ class (SOC) and preferred term (PT), by treatment group and 'total' over all treatment groups.

9.3.5 Investigational Product Exposure

SAD and single dose expansion

Actual dose of study drug will be summarized using descriptive statistics and entire planned volume of study drug administered (Yes, No), needle stick injury during study drug administration (Yes, No) and area of needle stick injury will be summarized using number and percentage of subjects for the SAF by treatment group.

MAD and multiple dose expansion

Actual total dose of study drug will be summarized using descriptive statistics, entire planned volume of study drug administered (Yes, No) and total number of doses administered (1, 2 and 3), needle stick injury during study drug administration (Yes, No) and area of needle stick injury will be summarized using number and percentage of subjects for the SAF by treatment group.

9.4 Analysis of Efficacy

Efficacy analysis will be conducted on the FAS.

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.

9.4.1 Analysis of Primary Endpoint

Not Applicable.

9.4.2 Analysis of Secondary Endpoints

SAD and single dose expansion

Categorical endpoint:

- Complete closure of TMP at Week 8 for SAD and at Week 12 for dose expansion.

Observed response is defined as follows using the non-missing result:
Yes: if a subject with complete closure of TMP,
No: if the above condition is false.

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

MAD and multiple dose expansion

Categorical endpoint:

- Complete closure of TMP at Week 12 for MAD and at Week 16 for dose expansion.
  Observed response is defined as follows using the non-missing result:
  Yes: if a subject with complete closure of TMP,
  No: if the above condition is false.

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 dose expansion.
- Change from baseline in TMP size at Week 12 for MAD and at Week 16 for dose expansion.

9.4.2.1 Analysis

SAD

No statistical hypothesis testing will be performed.

Single dose expansion

- Subjects with complete closure of TMP at Week 12

  At week 12, to understand the relationship between complete closure of TMP and treatment group, Mantel–Haenszel (MH) test adjusting for baseline perforation size group will be used. Analysis will be performed using the data for the estimand (refer to Section 3.2 for details) and done separately for each ASP0598 treatment group vs placebo.

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 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 values 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, 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, low dose group vs placebo will be tested at a 2-sided significance level of 0.05.

- 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.

For the change from baseline in TMP size at Week 12, a mixed models repeated measures (MMRM) will be used with treatment group and week (Week 2, 4, 8 and 12) 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 12, 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. The model will assume unstructured covariance among the within subject repeated measurements. If this is not feasible, an additional covariance structure will be considered. Analysis will be performed using the data for the estimand (refer to Section 3.2 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.

No multiplicity adjustment will be applied for the above change from baseline secondary endpoints.

MAD

No statistical hypothesis testing will be performed.

Multiple dose expansion

- Subjects with complete closure of TMP at Week 16

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 Patients with complete closure of TMP at Week 12 for details.

- 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.

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 for details.

No multiplicity adjustment will be applied for the above change from baseline secondary endpoints.

9.4.2.2 Sensitivity Analysis

Sensitivity Analysis will be described in the SAP.

9.4.2.3 Subgroup Analysis

SAD

No subgroup analysis is planned.

MAD

No subgroup analysis is planned.

Dose expansion

Subgroup summary of proportion of subjects with complete closure of TMP at week 12 for single dose expansion and week 16 for multiple dose expansion will be performed on the following subgroups: sex, race, age group (≤ 60 years and > 60 years), BMI group (≤ 30 kg/m^2 and >30 kg/m^2), baseline disease duration (≤ 1 year, > 1 year to ≤ 10 years and >10 years), smoking status, causation of TMP and stratification factor TMP size group. The SAP will provide more details on subgroup analyses.

9.4.3 Analysis of Exploratory Endpoints

SAD and single dose expansion

Categorical endpoints:

- 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 dose expansion.

  For observed response definition, refer to Section 9.4.2.
- Size of TMP ≤ 5% of pars tensa surface area after treatment.

  Observed response is defined as follows using the non-missing result:

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

  Observed response is defined as follows using the non-missing result:

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

  For A-B gap, decrease (reduction) from baseline indicates an improvement.

  Observed response is defined as follows using the non-missing result:

  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 air conduction hearing, decrease (reduction) from baseline indicates an improvement.

  Observed response is defined as follows using the non-missing result:

  Yes: if the change from baseline in mean air conduction hearing by PTA (derived using mean of the results at 500 Hz, 1, 2 and 4 kHz) ≤ -15 dB

  No: if the above condition is false
- A-B gap after treatment was 15 dB or less.

  Observed response is defined as follows using the non-missing result:

  Yes: if the post baseline result in the mean A-B gap (derived using mean of A-B gap results at 500 Hz, 1, 2 and 4 kHz) ≤ 15 dB

  No: if the above condition is false
- Air conduction hearing threshold after treatment was 30 dB or less.

  Observed response is defined as follows using the non-missing result:

  Yes: if the post baseline result in mean air conduction hearing threshold by PTA (derived using mean of results at 500 Hz, 1, 2 and 4 kHz) ≤ 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.

Continuous endpoints:

- 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 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 dose expansion

  The above change from baseline in dose expansion endpoints will be analyzed using the MMRM model as described in Section 9.4.2.
- Time to closure of TMP
- Change from baseline in A-B gap by PTA
- Change from baseline in air conduction hearing by PTA
- ECV by tympanometry

For the exploratory endpoints excluding the above change from baseline in dose expansion endpoints, no statistical analysis will be performed.

MAD and multiple dose expansion

Categorical endpoints:

- 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 dose expansion

  For observed response definition, refer to Section 9.4.2
- Reduction from baseline in size of TMP to less than or equal to 5% of pars tensa surface area
- Reduction from baseline in size of TMP to less than or equal to 1% of pars tensa surface area (pin point perforation)
- Percentage improvement from baseline in A-B gap by PTA ≥ 50% (improvement of conductive hearing)
- Improvement from baseline in air conduction hearing by PTA ≥ 15 dB
- A-B gap after treatment was 15 dB or less
- Air conduction hearing threshold after treatment was 30 dB or less
- Type of tympanogram (A, B or C)

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

Continuous endpoints:

- 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 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 dose expansion

  The above change from baseline dose expansion endpoints will be analyzed using the MMRM model as described in Section 9.4.2.
- Time to closure of TMP
- Change from baseline in A-B gap by PTA
- Change from baseline in air conduction hearing by PTA
- ECV by tympanometry

For the exploratory endpoints excluding the above change from baseline dose expansion endpoints, no statistical analysis will be performed.

9.5 Analysis of Safety

Safety analysis will be conducted using the SAF, unless otherwise specified.

The primary endpoints to evaluate the safety and tolerability of ASP0598 Otic Solution are shown below:

- TEAEs
- Incidence of AEs of special interest:
  1. Cholesteatoma or ear neoplasm
  2. Ototoxic symptoms (tinnitus, sensorineural hearing loss, dizziness)
  3. Otitis media or otitis externa
- Change from baseline in bone conduction hearing at 1, 2, 4 kHz by PTA
- Change from baseline in TVAS

No statistical hypothesis testing will be performed.

Continuous data will be summarized using descriptive statistics and categorical data will be summarized using number and percentage of subjects.

9.5.1 Adverse Events

AEs will be coded using MedDRA.

A TEAE is defined as an AE observed after starting administration of the study drug through EOS visit. A drug-related TEAE is defined as any TEAE with a causal relationship assessed as “yes” by the investigator.

An overview of TEAEs and Death by treatment group will be presented. In addition, the following summaries will be presented by SOC and PT:

- TEAEs by treatment group
- Drug-related TEAEs by treatment group
- Serious TEAEs by treatment group
- Drug-related serious TEAEs by treatment group

An overview of TEAEs of special interest by treatment group will be presented. The following summaries will be presented by SOC and PT:

- TEAEs of special interest by treatment group
- Serious TEAEs of special interest by treatment group

Details about selection of AEs of special interest will be provided in the SAP.

9.5.2 Bone conduction hearing at 1, 2, 4 kHz by PTA and TVAS

For the mean bone conduction hearing and TVAS, 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 the mean bone conduction hearing by PTA, the summaries will also present the number and percentage of subjects with an increase of ≥ 10 to 20, ≥ 20 to 30, ≥ 30 to 40, and ≥ 40 dB from baseline at each post baseline analysis visit.

9.5.3 Concentration-response Relationship Analysis

Not Applicable

9.5.4 Microscopic Ear Examination

Parameters and categories will be described in the SAP.

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.

9.6 Analysis of Pharmacokinetics

Descriptive statistics will include n, mean, SD, minimum, median, maximum, coefficient of variation (CV), geometric mean, and geometric CV.

Descriptive statistics will be used to summarize baseline value, post baseline value, and change from baseline at each post-baseline timepoint for serum concentrations of ASP0598 by treatment group including placebo using the SAF.

9.7 Analysis of Immunogenicity

Not Applicable.

9.8 Other Analyses

Not Applicable.

9.8.1 Analysis of Exploratory Biomarker(s)

Not Applicable.

9.9 Major Protocol Deviations

Major PDs as defined in [Section 10.3 Major Protocol Deviations] will be summarized for all randomized subjects by treatment group and overall, as well as by study site.

Major PD data will be listed by study site and subject.

The major PD criteria will be uniquely identified in the summary table and listing.

9.10 Interim Analysis (and Early Discontinuation of the Study)

SAD Interim Analysis

The decision to open single dose expansion will be made based on the safety and efficacy results of the interim analysis. One interim analysis is planned during SAD and will be conducted after completion of last cohort of the SAD. The DMC will review the data and make the final decision to open single dose expansion. Two different ASP0598 treatment groups and placebo or one ASP0598 treatment group and placebo will be selected to open single dose expansion. All analyses will be described in the IAP.

MAD Interim Analysis

The decision to open multiple dose expansion will be made based on the safety and efficacy results of the interim analysis. One interim analysis is planned during MAD and will be conducted after completion of last cohort of MAD. The DMC will review the data and make the final decision to open multiple dose expansion. Two different ASP0598 treatment groups and placebo or one ASP0598 treatment group and placebo will be selected to open multiple dose expansion. All analyses will be described in an IAP.

9.11 Additional Conventions

If the start and stop dates of AEs and concomitant medications are incomplete or onset date of TMP is incomplete, imputed dates will be used to determine whether an AE is/is not treatment emergent or to allocate a concomitant medication to the study period it was taken or to calculate time since TMP onset.

See the SAP for details of the definition for analysis windows to be used for analyses by visit.

Relevant listings will present the actual partial dates; imputed dates will not be shown.

10 OPERATIONAL CONSIDERATIONS

10.1 Data Collection

The investigator or site designee will enter data collected using an electronic data capture system. In the interest of collecting data in the most efficient manner, the investigator or designee should record data (including clinical laboratory values, if applicable) in the eCRF within 5 days after the subject's visit. For SAD and MAD, the investigator or site designee must enter data collected into the RAVE data management system within 24 hours after the completion of the Screening, Day 1, Day 8 and Day 15 visits.

The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with the source. These documents should be appropriately maintained by the study site.

The monitor should verify the data in the eCRFs with the source and confirm that there are no inconsistencies among them.

Clinical laboratory tests are performed at the local laboratory. Clinical laboratory results will be documented in the subject's source records. All PK samples and additional samples collected for future analysis will be shipped to the central laboratory for storage and shipping to the laboratory responsible for analyzing the samples. PK data will be transferred electronically to the sponsor or designee at predefined intervals during the study.

All procedures conducted under the protocol must be documented.

The investigator or designee is responsible for eCRF and source data completion and will ensure that all data and queries are accurate, complete and are verifiable with the source. The source should be appropriately maintained by the study site.

Electronic data sources and any supporting documents should be available for review/retrieval by the sponsor/designee at any time.

10.2 Demographics and Baseline Characteristics

10.2.1 Demographics

Demographic information will be collected for all subjects at Screening and will include age or date of birth, sex, race, and ethnicity (as allowed by local regulation).

10.2.2 Medical History

Medical history will include all significant medical conditions that have occurred or are ongoing at the time of consent. Details that will be collected include the onset date and recovery date for condition that are ongoing at the time of consent.

10.2.3 Diagnosis of the Target Disease, Severity and Duration of Disease

A complete medical history of the target disease will be recorded during the screening period and entered into the eCRF.

10.3 Major Protocol Deviations

A PD is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change. All deviations from the protocol are to be recorded. A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited.

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and must protect the rights, safety and well-being of subjects. The investigator should not implement any deviation from, or changes of, the protocol, unless it is necessary to eliminate an immediate hazard to subjects.

A major PD is 1 that may potentially impact the completeness, accuracy or reliability of data contributing to the primary endpoint or affect the rights, safety or well-being of a subject. Major PDs will have additional reporting requirements.

When a major deviation from the protocol is identified for an individual subject, the investigator or designee must ensure the sponsor is notified. The sponsor will follow up with the investigator, as applicable, to assess the deviation and the possible impact to the safety and/or efficacy of the subject to determine subject continuation in the study.

The major PD criteria that will be summarized at the end of the study are as follows:

PD1 - Entered into the study even though the subject 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

The investigator will also assure that deviations meeting IRB/IEC and appropriate regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and appropriate regulatory authorities will be provided to the sponsor and maintained within the Trial Master File (TMF).

10.4 STUDY ORGANIZATION

10.4.1 Dose Escalation Committee

For SAD and MAD, the Dose Escalation Committee (DEC) is responsible for dose escalation decisions. The DEC will consist of Astellas team members and an advisor. For detailed information on the dose escalation decision process, refer to Section 4.1.1 Decision Process for Dose Escalation, Dose Escalation Stopping Rules and Definition of the Maximum Tolerated Dose. A separate DEC Charter will be maintained detailing the membership, roles, and responsibilities of the DEC.

10.4.2 Data Monitoring Committee

The Data Monitoring Committee is an Astellas internal committee consisting of Astellas team members and an advisor. It is not independent and is responsible for the interim evaluation of the safety and efficacy data to open the single and multiple dose expansion cohorts. For detailed information on the dose expansion process, refer to Section 4.2 Decision Process for Dose Expansion. A separate DMC Charter will be maintained detailing the operation, roles, and responsibilities of the DMC.

11 REFERENCES

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12 APPENDICES

12.1 Ethical, Regulatory and Study Oversight Considerations

12.1.1 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

12.1.2 Institutional Review Board/Independent Ethics Committee/Competent Authorities

GCP requires that the protocol, any protocol amendments, investigator's brochure, ICF and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an IRB/IEC. The IRB/IEC will review the ethical, scientific and medical appropriateness of the study before it is conducted. IRB/IEC approval of the protocol, ICF and subject information and/or advertising, as relevant, will be obtained prior to initiation of any study-specific procedures.

Any substantial amendments to the protocol will require competent authority and IRB/IEC approval before implementation, except for changes necessary to eliminate an immediate hazard to subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies and procedures established by the IRB/IEC.
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.
- Providing oversight of the conduct of the study at the study site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, EU Regulation No. 536/2014 for studies (if applicable), and all other applicable local regulations.

12.1.3 Protocol Amendment and/or Revision

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments: substantial amendments and/or nonsubstantial amendments. Depending on the nature of the amendment, either IRB/IEC or competent authority approval or notification may be required. The changes will become effective only after the approval of the sponsor, investigator, IRB/IEC and appropriate regulatory authorities.

Amendments to this protocol must be signed by the sponsor and investigator. Written verification of IRB/IEC approval will be obtained before any amendment is implemented. Modifications to the protocol that are administrative in nature do not require IRB/IEC approval, but will be submitted to the IRB/IEC for their information, if required by local regulations.

If there are changes to the ICF, written verification of IRB/IEC approval must be forwarded to the sponsor. An approved copy of the new ICF must also be forwarded to the sponsor.

12.1.4 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.1.5 Informed Consent of Subjects

12.1.5.1 Subject Information and Consent

The investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the ICF will be reviewed, signed and dated by the subject, the person who administered the ICF and any other signatories according to local requirements. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that the ICF was signed prior to any study-related procedures and that the subject received a signed copy of the ICF.

The signed ICFs will be retained by the investigator and made available (for review only) to the study monitor, auditor and appropriate regulatory authorities and other applicable individuals upon request.

12.1.5.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information

1. The investigator or his/her representative will immediately inform the subject verbally whenever new information becomes available that may be relevant to the subject's consent or may influence the subject's willingness to continue participating in the study (e.g., report of serious adverse drug reaction). The communication must be documented in the subject's medical records and whether the subject is willing to remain in the study or not must be confirmed and documented.
2. The investigator must update the subject's ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the subject on all updated ICFs throughout their participation in the study. The investigator or his/her designee must reconsent subjects with the updated ICF even if relevant information was provided verbally. The investigator or his/her representative who obtained the written informed consent and the subject should sign and date the ICF. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must be made in the subject's records documenting the reconsent process.

12.1.6 Source Documents

Source data must be available at the study site to document the existence of the subjects and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are handwritten on paper or entered electronically. If source data are created (first entered), modified, maintained, achieved, retrieved or transmitted electronically via computerized systems (and/or other kind of electronic devices) as part of regulated study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol-related assessments, AE tracking, electronic clinical outcome assessments and/or drug accountability.

Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments, and audit trail information, if applicable). All printed records must be kept in the subject file and be available for archiving.

12.1.7 Record Retention

The investigator will archive all study data (e.g., subject identification code list, source data eCRFs and investigator's file) and relevant correspondence. These documents are to be kept on file for the appropriate term determined by local regulation (for US study sites, 2 years after approval of the NDA or discontinuation of the IND). The investigator agrees to obtain the sponsor's agreement prior to disposal, moving or transferring of any study-related records. The sponsor will archive and retain all documents pertaining to the study according to local regulations.

Data generated by the methods described in the protocol will be recorded in the subject’s medical records and/or study progress notes.

12.1.8 Subject Confidentiality and Privacy

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited unless the subject provides written consent or approval. Additional medical information may be given only after approval of the subject to the investigator or to other appropriate medical personnel responsible for the subject's well-being.

The sponsor shall not disclose any confidential information on subjects obtained during the performance of their duties in the study without justifiable reasons.

Even though any individuals involved in the study, including the study monitors and auditors, may get to know matters related to a subject's privacy due to direct access to source documents, or from other sources, they may not disclose the content to third parties.

The sponsor affirms the subject's right to protection against invasion of privacy. Only a subject identification number will identify subject data retrieved by the sponsor. However, the sponsor requires the investigator to permit the sponsor, sponsor's representative(s), the IRB/IEC and when necessary, representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

The sponsor agrees to comply and process personal data in accordance with all applicable privacy laws and regulations, including, without limitation, the Personal Information Protection Law in Japan and privacy laws in the US. If the services will involve the collection or processing of personal data (as defined by applicable data protection legislation) within the European Economic Area (EEA), then the sponsor shall serve as the controller of such data, as defined by the EU Data Protection Directive (DPD), and investigator and/or third party shall act only under the instructions of the sponsor in regard to personal data. If the sponsor is not based in the EEA, the sponsor must appoint a third party to act as its local data protection representative or arrange for a co-controller established in the EU for data protection purposes in order to comply with the DPD.

12.1.9 Arrangement for Use of Information and Publication of the Study

Information concerning the test product, patent applications, processes, unpublished scientific data, the investigator's brochure and other pertinent information is confidential and remains the property of the sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the sponsor will use the information obtained during the study in connection with the development of the drug and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this study, the investigator understands that he/she has an obligation to provide the sponsor with all data obtained during the study.

Publication of the study results is discussed in the study agreement.

12.1.10 Signatory Investigator for Clinical Study Report

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final CSR that forms part of a marketing authorization application, be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator(s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. The representative for the coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the sponsor prior to database hard-lock.

12.2 Procedure for Study Quality Control

12.2.1 Study Monitoring

The sponsor or delegated CRO is responsible for monitoring the study to ensure that the rights, safety and well-being of subjects are protected, the study is properly conducted in adherence to the current protocol and GCP and the study data reported by the investigator/sub-investigator are accurate, complete and verifiable with the source. The sponsor is responsible for assigning the study monitor(s) to this study for proper monitoring. They will monitor the study in accordance with planned monitoring procedures.

12.2.2 Direct Access to Source Data/Documents

The investigator and the study site must accept monitoring and auditing by the sponsor or delegated CRO, as well as inspections from the IRB/IEC and appropriate regulatory authorities. In these instances, they must provide all study-related records including source documents when they are requested by the sponsor monitors and auditors, the CRO, the IRB/IEC or appropriate regulatory authorities. The confidentiality of the subject's identity shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

12.2.3 Data Management

Data management will be coordinated by the Data Science department or designee of the sponsor in accordance with the SOPs for data management. All study-specific processes and definitions will be documented by data management. eCRF completion will be described in the eCRF instructions. Coding of medical terms and medications will be performed using MedDRA and the WHO Drug Dictionary, respectively.

12.2.4 Quality Assurance

The sponsor is implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP and applicable regulatory requirement(s). Where applicable, the QA and QC systems and written SOPs of the CRO will be applied.

The sponsor or sponsor's designee may arrange to audit the study at any or all study sites and facilities. The audit may include on-site review of regulatory documents, CRFs and source documents. Direct access to these documents will be required by the auditors.

To support quality around subject safety and reliability of study results, quality tolerance limits (QTLs) are defined and monitored. QTLs represent the acceptable variation of study data, taking into consideration the current state of medical and statistical knowledge about the variables to be analyzed as well as the statistical design of the study. It is a level, point, or value associated with a parameter that should trigger an evaluation if a deviation is detected to determine if there is a possible systematic issue (i.e., a trend has occurred). The QTLs defined for this study are provided below.

Table 8 Quality Tolerance Limit

QTL #: Name and Parameter	Definition	Parameter Justification
TMP Imaging Compliance	Number of randomized subjects that have received study drug who do not have an acceptable TMP image at specific study visits.	Missing images can have a negative impact on key secondary endpoints

QLT: quality tolerance limit; TMP: tympanic membrane perforation

QTL Management Activities:

Not Applicable.

12.3 Contraception Requirements

WOCBP who are eligible for participation in the study, including those who choose complete abstinence, must have pregnancy tests as specified in the schedule of assessments. Pregnancy test results must confirm that the subject is not pregnant.

WOMEN OF CHILDBEARING POTENTIAL DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS

A female is considered fertile (i.e., WOCBP) following menarche and until becoming postmenopausal unless permanently sterile.

Females in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal with 1 of the following (i.e., permanently sterile):
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy
- Postmenopausal

A postmenopausal state is defined as at least 12 months after last menstrual bleeding without an alternative medical cause.

In case the last menstrual bleeding cannot be clearly determined, confirmation with more than 1 follicle-stimulating hormone (FSH) measurement of at least > 40 IU/L (or higher per local institutional guidelines) is required.

Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use 1 of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status by repeated FSH measurements before study enrollment.

Documentation of any of these categories can come from the study site personnel's review of the female subject's medical records, medical examination or medical history interview.

CONTRACEPTION GUIDANCE FOR FEMALE SUBJECTS OF CHILDBEARING POTENTIAL

Female subjects of childbearing potential are eligible for participation in the study if they agree to use 1 of the highly effective methods of contraception listed below from the time of signing the ICF and until the end of relevant systemic exposure, defined as 28 days after the IP application.[a]

Highly effective methods of contraception (failure rate of < 1% per year when used consistently and correctly)[b]:

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation

  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
  - Oral
  - Injectable
  - Implantable
- Other combined (estrogen- and progesterone-containing) methods
  - Vaginal ring
  - Injectable
  - Implantable
  - Intrauterine hormone-releasing system or intrauterine device
- Other combined methods
- Vasectomized partner
  A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
- Sexual abstinence
  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the test product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject. It is not necessary to use any other method of contraception when complete abstinence is elected.

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

[b] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE SUBJECTS WITH PARTNER(S) OF CHILDBEARING POTENTIAL.

Male subjects with female partners of childbearing potential are eligible for participation in the study if they agree to the following during treatment and until the end of relevant systemic exposure defined as *28 days* after IP application.[a]

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator
- Use a condom
- Female partners of male subjects who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

12.4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

12.4.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a subject administered an IP, and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of IP whether or not considered related to the IP.

12.4.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, biochemistry or urinalysis) or other safety assessment (e.g., vital signs, physical examination, ECGs or radiographic scans), including those that worsen from baseline, that is considered to be clinically significant in the medical and scientific judgment of the investigator and not related to underlying disease, is to be reported as an (S)AE.

Any clinically significant abnormal laboratory finding or other abnormal safety assessment, which is associated with the underlying disease, does not require reporting as an (S)AE, unless judged by the investigator to be more severe than expected for the subject's condition.

Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

12.4.1.2 Potential Cases of Drug-induced Liver Injury

This is a single and multiple dose administration and liver safety monitoring will be applied if any AEs occur that prompt safety consideration.

Refer to [Appendix 12.5 Liver Safety Monitoring and Assessment] for detailed instructions on drug induced liver injury. Abnormal values in AST and/or ALT concurrent or with abnormal elevations in TBL that meet the criteria outlined in [Appendix 12.5 Liver Safety Monitoring and Assessment], in the absence of other causes of liver injury, are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and are always to be considered important medical events and reported per [Section 12.4.5 Reporting Procedures for Serious Adverse Events].

12.4.2 Definition of Serious Adverse Events

For this phase 1/2 study, there are no anticipated SAEs for which a single occurrence will be excluded from IND safety reporting.

An AE is considered "serious" if, in the view of either the investigator or sponsor, the event:

- Results in death
- Is life-threatening (An AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death; it

does not include an AE that, had it occurred in a more severe form, might have caused death.)
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly, or birth defect
- Requires inpatient hospitalization (except for planned procedures as allowed per study) or leads to prolongation of hospitalization (except if prolongation of planned hospitalization is not caused by an AE)
- Other medically important events (defined in paragraph below)

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These events, including those that may result in disability/incapacity, usually are considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

12.4.3 Criteria for Causal Relationship to Investigational Product

A medically qualified investigator is obligated to assess the relationship between IP and each occurrence of each (S)AE. This investigator will use medical judgment as well as the reference safety information Section 2.1.2 Summary of Key Safety Information to determine the relationship. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

The investigator is requested to provide an explanation for the causality assessment for each (S)AE and must document in the medical notes that he/she has reviewed the (S)AE and has provided an assessment of causality.

Following a review of the relevant data, the causal relationship between the IP and each (S)AE will be assessed by answering “yes” or “no” to the question “Do you consider that there is a reasonable possibility that the event may have been caused by the IP?”

When making an assessment of causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a “reasonable possibility” that an (S)AE may have been caused by the IP (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Has the subject been administered IP?
- Plausibility (i.e., could the event been caused by the suspect drug? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and study data, etc.)
- Dechallenge/dose reduction/rechallenge:
    - Dechallenge: Did the (S)AE resolve or improve after only stopping the dose of the suspect drug without any treatment?

  - Dose reduction: Did the (S)AE resolve or improve after reducing the dose of the suspect drug?
  - Rechallenge: Did the (S)AE reoccur if the suspected drug was reintroduced after having been stopped?
- Laboratory or other test results: a specific lab investigation supports the assessment of the relationship between the (S)AE and the IP (e.g., based on values pre-, during and post-treatment)
- Available alternative explanations independent of IP exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation
- Finally, judging which are more likely based on all the above contents, factors of reasonable possibility or confounding factors, comprehensive judgment of plausible temporal relationship between exposure to the IP and (S)AE onset and/or resolution will be provided. Did the (S)AE occur in a reasonable temporal relationship to the administration of the IP?

There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always assesses causality for every event before the initial transmission of the SAE data to the sponsor. With limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of "no" is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise his/her assessment of causality in light of new information regarding the SAE and shall send an SAE follow-up report and update the eCRF with the new information and updated causality assessment.

12.4.4 Criteria for Defining the Severity of an Adverse Event

AEs will be classified for severity using the Clinical Data Interchange Standards Consortium Study Data Tabulation Model Controlled (CDISC) Terminology as described in the table below.

Table 9 Criteria for the Severity of an Adverse Event

Classification	CDISC Definition
Mild	A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
Moderate	A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
Severe	A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

AE: adverse event; CDISC: Clinical Data Interchange Standards Consortium.

Modified Brighton Grading Scheme will assess local skin reaction at the application site.

12.4.5 Reporting Procedures for Serious Adverse Events

The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the sponsor by fax or email immediately (within 24 hours of awareness).

The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data as well as the investigator causality assessment including the explanation for the causality assessment.

If the SAE is associated with emergency unblinding by the investigator as outlined in [Section 6.3.4 Breaking the Treatment Code for Emergency], this is to be recorded on the SAE worksheet. On the SAE worksheet, the investigator is to include when unblinding took place in association with the SAE.

For contact details, see Contact Details of Sponsor's Key Personnel. Fax or email the SAE/special situations worksheet to:

Astellas Pharma Global Development Inc.
Pharmacovigilance
North America fax number: +1-888-396-3750
North America alternate fax number: +1-847-317-1241
Email: safety-us@astellas.com

If there are any questions, or if clarification is needed regarding the SAE, please contact the sponsor's medical monitor/study physician or their designee [CONTACT DETAILS OF SPONSOR'S KEY PERSONNEL].

Follow-up information for the event should be sent promptly (as soon as available but no longer than within 7 days of the initial notification.

Full details of the SAE should be recorded on the medical records, SAE/special situation worksheet and on the eCRF.

The following minimum information is **required**:

- International study number/study number
- Subject number, sex and age
- Date of report
- Description of the SAE (event and seriousness criteria)
- Causal relationship to the IP (including reason)
- IP provided (if any)

The sponsor or sponsor's designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality, and expectedness of the events (e.g., SUSAR reporting) according to current local/regional regulatory requirements. The sponsor or sponsor's designee will submit expedited safety

reports to competent authorities and concerned ethics committee per current local regulations, and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports as required by their IRB/IEC within timelines set by regional regulations (e.g., EMA, FDA) where required. Documentation of the submission to and receipt by the IRB/ IEC of expedited safety reports should be retained by the study site. In the US, FDA expedited IND reporting guidelines will be followed.

The sponsor will notify all investigators responsible for ongoing clinical studies with the test product of all SUSARs, which require submission per local requirements IRB/IEC.

The investigators should provide written documentation of IRB/IEC notification for each report to the sponsor.

The investigator may contact the sponsor's medical monitor/study physician for any other problem related to the rights, safety or well-being of the subject.

12.4.6 Reporting Procedures for Special Situations

12.4.6.1 Pregnancy

If a female subject becomes pregnant during the study dosing period or within 28 days after IP application on Day 1 , the investigator is to report the information to the sponsor according to the timelines in [Section 12.4.5 Reporting Procedures for Serious Adverse Events] using the SAE worksheet or a pregnancy form and in the eCRF.

The investigator will attempt to collect pregnancy information on any female partner of a male subject who becomes pregnant during the study dosing period or within 28 days after IP application on Day 1 from the discontinuation of dosing and report the information to sponsor according to the timelines in [Section 12.4.5 Reporting Procedures for Serious Adverse Event**s**] using the SAE worksheet or pregnancy form.

The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data, etc., should be included in this information.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female subject as an AE in the eCRF or SAE per [Section 12.4.5 Reporting Procedures for Serious Adverse Events]. For (S)AEs experienced by a female partner of a male subject, (S)AEs are to be reported via the SAE worksheet.

Additional information regarding the outcome of a pregnancy when also categorized as an SAE is mentioned below:

- ″Spontaneous abortion″ includes miscarriage, abortion and missed abortion.
- Death of a newborn or infant within 1 month after birth is to be reported as an SAE regardless of its relationship with the IP.
- If an infant dies more than 1 month after the birth, it is to be reported if a relationship between the death and intrauterine exposure to the IP is judged as *"*possible*"* by the investigator.
- Congenital anomaly (including anomaly in miscarried fetus)

Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination or other means as appropriate. (S)AEs experienced by the newborn/infant should be reported via the pregnancy reporting form. Generally, follow up will be no longer than 6 to 8 weeks following the estimated delivery date.

12.4.6.2 Medication Error, Overdose and "Off-label Use"

If a medication error (defined as an unintended failure in the treatment process that leads to, or has the potential to lead to, harm to the subject), overdose or "off-label use" (i.e., use outside of what is stated in the protocol) is suspected, refer to [Section 10.3 Major Protocol Deviations]. Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events] together with the details of the medication error, overdose and/or "off-label use."

12.4.6.3 Misuse/Abuse

Definition of misuse: Situations where the IP is/are intentionally and inappropriately used not in accordance with the intended use as defined in the protocol.

Definition of abuse: Persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects.

If misuse or abuse of the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events] together with details of the misuse or abuse of the IP.

12.4.6.4 Occupational Exposure

If occupational exposure (e.g., inadvertent exposure to the IP of study site personnel while preparing it for administration to the subject) to the IP occurs, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs occurring to the individual associated with or resulting from the special situation are to be reported on the special situations worksheet.

12.4.6.5 (Suspicion of) Transmission of Infectious Agent

If transmission of an infectious agent associated with the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness) and any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events] together with the details of the suspected transmission of infectious agent.

12.4.6.6 Suspected Drug-drug Interaction

If a drug-drug interaction associated with the IP is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events] together with details of the suspected drug-drug interaction.

12.5 Liver Safety Monitoring and Assessment

This is a single dose administration and liver safety monitoring will be applied if any AEs occur that prompt safety consideration.

The purpose of this appendix is to provide guidance for the monitoring of drug-induced liver injury during the course of the study. It should be noted that this section does not specify the EOS analyses of liver enzymes. The EOS liver enzymes analyses will be described in the SAP. Any subject enrolled in a study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × ULN or bilirubin > 2 × ULN should undergo detailed testing for liver enzymes (including at least ALP, ALT, AST and TBL). Testing should be repeated within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator and study team. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN is as shown below.

Table 10 Moderate and Severe Liver Abnormalities

	ALT or AST		TBL
Moderate	> 3 × ULN	or	> 2 × ULN
Severe	> 3 × ULN	and†	> 2 × ULN

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBL: total bilirubin; ULN: upper limit of normal

†Samples taken simultaneously or within maximum 24 hours.

In addition, the subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks.
- ALT or AST > 3 × ULN and† and* TBL > 2 × ULN or international normalized ratio (INR) > 1.5 (if INR testing is applicable/evaluated)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

† Samples taken simultaneously or within a maximum of 24 hours.

The investigator may determine that abnormal liver function results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history,

physical examination and clinical laboratory tests. The study site personnel are to complete the liver abnormality case report form (LA-CRF). Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated 2 to 3 times weekly, and then weekly or less if abnormalities stabilize or the IP has been discontinued and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a SAE. The sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to IP are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases are to be recorded as "AEs" within the eCRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic subjects, and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications are to be entered in the eCRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
    - Acute viral hepatitis (A, B, C, D, E or other infectious agents)
    - Ultrasound or other imaging to assess biliary tract disease
    - Other clinical laboratory tests, including INR and direct bilirubin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Study Treatment Discontinuation

In the absence of an explanation for increased liver function tests, such as viral hepatitis, preexisting or acute liver disease, or exposure to other agents associated with liver injury, the subject may be discontinued from study treatment. The investigator may determine that it is not in the subject's best interest to continue study treatment. Discontinuation of study treatment should be considered if:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks

- ALT or AST > 3 × ULN and† TBL > 2 × ULN or INR > 1.5) (if INR testing is applicable/evaluated)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

† Samples taken simultaneously or within a maximum of 24 hours.

In addition, if close monitoring for a subject with moderate or severe hepatic laboratory tests is not possible, study treatment should be discontinued.

Hy's Law definition: Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10% to 50% mortality (or transplant).

The 2 "requirements" for Hy's Law are:

1. Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in AT elevations > 3 × ULN ("2 × ULN elevations are too common in treated and untreated subjects to be discriminating").
2. Cases of increased total bilirubin (at least 2 × ULN) with concurrent AT elevations at least 3 × ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert's syndrome [Temple, 2006].

FDA Guidance for Industry titled, "Drug-induced Liver Injury: Premarketing Clinical Evaluation" issued by the FDA on July 2009:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (nonhepatotoxic) control drug or placebo.
2. Among subjects showing such AT elevations, often with ATs much greater than 3 × ULN, 1 or more also show elevation of serum TBL to > 2 × ULN, without initial findings of cholestasis (elevated serum ALP).
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.

References

Temple R. Hy's Law: Predicting Serious Hepatotoxicity. Pharmacoepidemiol Drug Saf. 2006;15(4):241-3.

The purpose of this appendix is to provide guidance for the monitoring of drug-induced liver injury during the course of the study. It should be noted that this section does not specify the EOS analyses of liver enzymes. The EOS liver enzymes analyses will be described in the SAP. Any subject enrolled in a study with active drug therapy and who reveals an increase of serum aminotransferases (AT) to > 3 × ULN (to > 5 × ULN in subjects with liver metastases) or total bilirubin > 2 × ULN should undergo detailed testing for liver enzymes (including at least ALP,

ALT, AST and TBL). Testing should be repeated within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator and study team. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN is as shown below.

Table 11 Moderate and Severe Liver Abnormalities

	ALT or AST		TBL
Moderate	> 3 × ULN (in subjects without liver metastases), > 5 × ULN (in subjects with liver metastases)	or	> 2 × ULN
Severe	> 3 × ULN	and†	> 2 × ULN

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBL: total bilirubin; ULN: upper limit of normal

†Samples taken simultaneously or within maximum 24 hours.

In addition, the subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks (in the absence of liver metastases)
- ALT or AST > 3 × ULN and† TBL > 2 × ULN or international normalized ratio (INR) > 1.5 (If INR testing is applicable/evaluated)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

† Samples taken simultaneously or within a maximum of 24 hours.

The investigator may determine that abnormal liver function results, other than those described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and clinical laboratory tests. The study site personnel are to complete the liver abnormality case report form (LA-CRF). Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated 2 to 3 times weekly then weekly or less if abnormalities stabilize or the IP has been discontinued and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology may be considered an important medical event and may be reported as an SAE. The sponsor

should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to IP are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases are to be recorded as AEs in the eCRF. Illnesses and conditions such as hypotensive events and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic subjects and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications, including dose, are to be entered in the eCRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
  - Acute viral hepatitis (A, B, C, D, E or other infectious agents)
  - Ultrasound or other imaging to assess biliary tract disease
  - Other clinical laboratory tests including INR, direct bilirubin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Study Treatment Discontinuation

In the absence of an explanation for increased liver function tests, such as viral hepatitis, preexisting or acute liver disease, presence of liver metastases, or exposure to other agents associated with liver injury, the subject may be discontinued from study treatment. The investigator may determine that it is not in the subject's best interest to continue study treatment. Discontinuation of study treatment should be considered if:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks (in subjects without liver metastases
- ALT or AST > 3 × ULN and† TBL > 2 × ULN or INR > 1.5 (If INR testing is applicable/evaluated)
- ALT or AST > 5 × ULN and† (TBL > 2 × ULN in subjects with liver metastases)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

† Samples taken simultaneously or within a maximum of 24 hours.

In addition, if close monitoring for a subject with moderate or severe hepatic laboratory tests is not possible, study treatment should be discontinued.

Hy's Law Definition:

1. Evidence that a drug can cause hepatocellular-type injury, generally shown by a higher rate than control of people with 3 × AT elevations over the ULN (2 × elevations are too common in treated and untreated subjects to be discriminating).
2. Cases of increased bilirubin (to at least 2 × ULN) in people with concomitant AT elevation to at least 3 × ULN (but it is almost invariably higher) and no evidence of intra-or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert's syndrome [Temple, 2006].

FDA Guidance for Industry titled "Drug-induced Liver Injury: Premarketing Clinical Evaluation" issued by the FDA on July 2009:

FDA Guidance for Industry:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (nonhepatotoxic) control drug or placebo.
2. Among subjects showing such AT elevations, often with AT levels much greater than 3 × ULN, 1 or more also show elevation of serum TBL to > 2 × ULN, without initial findings of cholestasis (elevated serum ALP).
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.

References

Temple R. Hy's Law: Predicting Serious Hepatotoxicity. Pharmacoepidemiol Drug Saf. 2006;15(4):241-3.

12.6 List of Excluded Concomitant Medications

These restrictions are required after ASP0598 Otic Solution or placebo administration on Randomization (Day 1), until completion of study procedures at Day 57 (SAD) / Day 85 (single dose expansion or MAD) / Day 113 (multiple dose expansion) or EOS visit.

- Ear drop agents will be used only for the treatment of otitis media with suppurative ear discharge. Only Ciprofloxacin or Ofloxacin ear drops (does not includes steroids) are allowed to be used in the ASP0598 Otic Solution or placebo treated ear. Investigators should minimize treatment with ear drops up to a maximum of 10 days and consider alternative treatment (e.g., oral antibiotics).
- Any other topical agents in the ear canal are prohibited to use in the ASP0598 Otic Solution or placebo treated ear.
- Any otologic procedures (e.g., paper patch or surgeries) to the ASP0598 Otic Solution or placebo treated ear are prohibited.

12.7 Laboratory Assessments

Laboratory tests will be performed at screening for eligibility assessment and sent to the local laboratory for analysis.

Table 12 Clinical Laboratory Tests

Panel/Assessments	Parameters to be Analyzed
Hematology	Hematocrit Hemoglobin Platelets Red blood cell count White blood cell count White blood cell count differential
Biochemistry	Albumin Alanine aminotransferase Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Chloride Creatinine Creatinine kinase Glucose Lactate dehydrogenase Phosphate Potassium Serum HCG for female subjects Sodium Total bilirubin (total and direct) Total protein
Urinalysis	Bilirubin Glucose Ketones pH Protein Red blood cells

12.8 Ear Care and Water Precautions

These restrictions are required after IP administration at Day 1 until completion of the study procedures at Day 57 (SAD), Day 85 (single dose expansion or MAD), Day 113 (multiple dose expansion) or EOS visit.

- Subjects must refrain from showering or bathing for at least 24 hours after IP administration.
    - Showering or bathing are allowed 24 hours after IP administration. When showering or bathing, follow "How to Keep Your Ear Dry" guidelines.
- Any water activities that risk submersion of the ear (e.g., swimming or surfing) are prohibited throughout the entire study period.
- Are not allowed to use any materials (e.g., Q-tips) placed into the ear canal of the treated ear (ear buds or headphones placed at the entrance to the ear canal are considered acceptable). If ear cleaning is desired, then a soft tissue can be used and placed gently at the entrance to the ear canal.
- Should refrain from activities that can increase the middle ear pressure including plane travel (at least 28 days after IP application), positive pressure ventilation (e.g., CPAP or BiPAP) devices, Valsalva maneuvers, excessive high-pressure nose blowing, diving underwater.
- Must refrain from activities that can excessively increase body temperature (e.g., Sauna) for 3 days after IP application.
- Must refrain from activities that stimulate sweating excessively for 3 days after IP application.

How to Keep Your Ear Dry

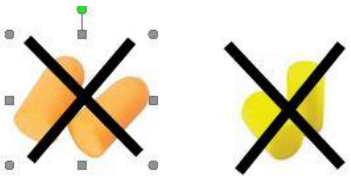
To keep water out of your ear when showering and bathing, place half of a cotton ball rolled in **1 tablespoon of petroleum jelly** to the ear.

1. The cotton ball should be completely saturated with petroleum jelly.
2. Place this in the bowl part of the outer ear, covering your ear canal. **Do not shove the cotton ball into the ear canal.** Smooth the edges of the cotton ball down to ensure a good seal.
3. Please use a new cotton ball each time and do not submerge your head underwater with this technique.

Other options for keeping your ear dry:

Over the counter options include plastic or soft, moldable silicone earplugs.

It is ***not*** recommended to use foam earplugs to keep water out of the ear.



12.9 Modified Brighton Grading Scheme

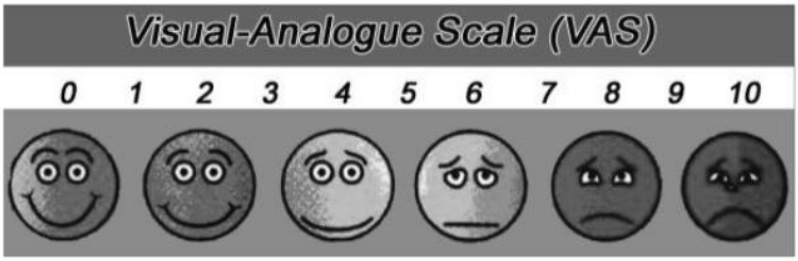
Grade	
0	no abnormalities
1	canal erythema
1b	canal erythema with otorrhea (ear canal fluid discharge)
2	canal erythema + swelling, TM still visible
2b	canal erythema + swelling, TM still visible with otorrhea
3	canal erythema + swelling, TM not visible
3b	canal erythema + swelling, TM not visible with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

Lindisfarne ME, Berwick J, Das P. Clinical Review: Acute Otitis Externa. Available from: https://www.gponline.com.

12.10 Tinnitus Visual Analog Scale (TVAS)

Tinnitus Visual Analog Scale

Mark how loud your tinnitus is today



12.11 Clinical Study Continuity

INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).

BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study subjects and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated Schedule of Assessments/ Table 2, Table 3, Table 4, and Table 5 unless the site PI discusses the need with the Astellas Medical Monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed as described in Section 7: Study Procedures and Assessments due to a crisis.

INFORMED CONSENT

Subjects who need to follow any or all of the alternate measures outlined in this Appendix will be required to provide informed consent which explicitly informs them of the nature of, and rationale for these changes, and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the PI or designee will obtain oral agreement from the subject followed by written documentation as soon as is feasible. A separate addendum to the study informed consent will be provided to document the participant's consent of the changes.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with subjects who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if one or more of the following conditions are met due to the crisis:

- Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible
- Site facilities have been closed for clinical study conduct
- Site has been restricted to treating patients with conditions outside of the scope of the study
- Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel
- Participant(s) have temporarily relocated from the current study site to an alternate study site to avoid placing a burden on the participant with respect to travel

- Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel
- Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety

Adherence to the protocol as reflected in the Schedule of Assessment (Table 2, Table 3, Table 4, and Table 5) is expected, where plausible. The alternate measures as noted in Table 13, Table 14, Table 15 and Table 16 below are only permissible in the event of a crisis, and after discussing the need to implement the alternate measure with the Astellas Medical Monitor. Patients with CTMP are generally healthy and not at higher risk of COVID-19 infection. IP must be administered in a medical facility by a trained and qualified physician. All otology examinations and procedures, including imaging of the tympanic membrane, must be conducted at the study site by a trained and qualified physician or audiologist. This is to allow for continuity of receiving IP (applicable to MAD). The following assessments can occur outside the medical facility for subjects participating in the study in a time of crisis: blood draws, vital signs, concomitant medications, TVAS, and AE assessments.

If alternate measures are implemented for a participant, the site should record the justification for implementing the alternate measure(s) and which alternate measure(s) that was implemented at a particular visit in the participant's source document.

Table 13 Schedule of Assessments – SAD

	Alternative Approach	Screening	Randomization	Observation				
Day		Day -28 to Day -1	Day 1	Day 2	Day 8	Day 15	Day 29	Day 57 Last Visit/ EOS
Week					Week 1	Week 2	Week 4	Week 8
Visit Windows				+1 day	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6	7
Informed Consent		X						
Verify Inclusion/Exclusion		X	X					
Medical History/Disease History/Demographics		X	X					
Physical Exam including Height and Weight		X						
Vital Signs [a]	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X	X
Prior/Concomitant Medications [b]		X	X	X	X	X	X	X
Adverse Events Assessment [c]		X	X	X	X	X	X	X
ASP0598 or placebo			X [d]					
Microscopic Ear Examination	If delayed, should be completed as soon as possible	X [e]	X [f]	X [g]	X [g]	X [g]	X [g]	X [i]
Modified Brighton Grading Scheme Evaluation			X [f]	X	X	X	X	X
Imaging picture of tympanic membrane by endoscope		X [e]	X [h]	X	X	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)		X [e]	X	X [j]	X [j]	X [j]	X [j]	X
Air conduction by PTA		X [e]	X					X
Tympanometry		X [e]	X					X
Tinnitus Visual Analog Scale (TVAS)	Remote/Virtual/Telemedicine Visits allowed for non-dosing visits.	X	X	X	X	X	X	X

Table continued on next page

Day	Alternative Approach	Screening	Randomization	Observation				
		Day -28 to Day -1	Day 1	Day 2	Day 8	Day 15	Day 29	Day 57 Last Visit/ EOS
Week					Week 1	Week 2	Week 4	Week 8
Visit Windows				+1 day	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6	7
Serum Chemistries	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X						
CBC with Differential		X						
Pregnancy Test [k]		X	X					X
Pharmacokinetic Sample			X [l]	X				
Blood Sample for Future Analysis			X				X	
Urinalysis	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X						

AE: adverse event; CBC: complete blood count; EOS: end of study; PK: pharmacokinetic; SAD: Single Ascending Dose; TM: tympanic membrane

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 57 or EOS. Any AEs ongoing at Day 57 or EOS Visit must be followed until resolution or Investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Microscopic Ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 29, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subject's experience uncomfortable ear discharge.
h. At Day 1, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 57 or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 2, 3, 8, 15, 29 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.
l. Day 1, PK blood draw will be performed at the following time points: pre-dose (-15) minutes, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear.

Table 14 Schedule of Assessments – Single Dose Expansion

	Alternative Approach	Screening	Randomization	Observation			
Day		Day -28 to Day -1	Day 1	Day 15	Day 29	Day 57	Day 85 Last Visit/ EOS
Week				Week 2	Week 4	Week 8	Week 12
Visit Windows				+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6
Study Procedures							
Informed Consent		X					
Verify Inclusion/Exclusion		X	X				
Medical History/Disease History/Demographics		X	X				
Physical Exam including Height and Weight		X					
Vital Signs [a]	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X
Prior/Concomitant Medications [b]		X	X	X	X	X	X
Adverse Events Assessment [c]		X	X	X	X	X	X
Study Treatment Administration							
ASP0598 or placebo			X [d]				
Otology Examinations							
Microscopic Ear Examination	If delayed, should be completed as soon as possible	X [e]	X [f]	X[g]	X[g]	X[g,]	X [i]
Modified Brighton Grading Scheme Evaluation			X [f]	X	X	X	X
Imaging picture of tympanic membrane by endoscope		X [e]	X[h]	X	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)		X [e]	X	X [j]	X [j]	X [j]	X
Air conduction by PTA		X [e]	X				X
Tympanometry		X [e]	X				X
Tinnitus Visual Analog Scale (TVAS)	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X

Table continued on next page

Day	Alternative Approach	Screening	Randomization	Observation			
		Day -28 to Day -1	Day 1	Day 15	Day 29	Day 57	Day 85 Last Visit/ EOS
Week				Week 2	Week 4	Week 8	Week 12
Visit Windows				+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6
Blood Collection							
Serum Chemistries		X					
CBC with Differential		X					
Pregnancy Test [k]	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X	X			X	X
Blood Sample for Future Analysis			X		X		
Urine Collection							
Urinalysis	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X					

AE: adverse event; CBC: complete blood count; EOS: end of study; TM: tympanic membrane

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 57 or EOS. Any AEs ongoing at Day 85 or EOS Visit must be followed until resolution or Investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Microscopic Ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 57, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge.
h. At Day 1, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 85 or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 15, 29, and 57 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.

Table 15 Schedule of Assessments – MAD

	Alternative Approach	Screening	Randomization	Observation						
Day		Day – 28 to Day -1	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 57	Day 85 Last Visit/ EOS
Week				Week 1	Week 2	Week 3	Week 4	Week 5	Week 8	Week 12
Visit Windows				+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6	7	8	9
Study Procedures										
Informed Consent		X								
Verify Inclusion/Exclusion		X	X							
Medical History/Disease History/Demographics		X	X							
Physical Exam including Height and Weight		X								
Vital Signs [a]	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X	X	X	X
Prior/Concomitant Medications [b]		X	X	X	X	X	X	X	X	X
Adverse Events Assessment [c]		X	X	X	X	X	X	X	X	X
Study Treatment Administration										
ASP0598 or placebo [d]			X		X		X			
Otology Examinations										
Microscopic Ear Examination	Remote/Virtual/Telemedicine Visits allowed.	X [e]	X [f]	X [g]	X [f, g]	X [g]	X [f, g]	X [g]	X [g]	X [i]
Modified Brighton Grading Scheme Evaluation			X [f]	X	X [f]	X	X [f]	X	X	X
Imaging picture of tympanic membrane by endoscope		X [e]	X [h]	X	X [h]	X	X [h]	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)		X [e]	X	X [j]	X [j]	X [j]	X [j]	X [j]	X [j]	X
Air conduction by PTA		X [e]	X							X
Tympanometry		X [e]	X							X
Tinnitus Visual Analog Scale (TVAS)	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X	X	X	X

Table continued on next page

	Alternative Approach	Screening	Randomization	Observation						
Day		Day – 28 to Day -1	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 57	Day 85 Last Visit/ EOS
Week				Week 1	Week 2	Week 3	Week 4	Week 5	Week 8	Week 12
Visit Windows				+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6	7	8	9
Blood Collection										
Serum Chemistries	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X								
CBC with Differential		X								
Pregnancy Test [k]		X	X						X	X
Pharmacokinetic Sample			X [l]				X			
Sample for Future Analysis			X						X	
Urine Collection										
Urinalysis	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X								

AE: adverse event; CBC: complete blood count; EOS: end of study; MAD: Multiple Ascending Dose; PK: pharmacokinetic; TM: tympanic membrane

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.
c. AEs will be collected from the time of informed consent through Day 85 or EOS. Any AEs ongoing at Day 85 or EOS Visit must be followed until resolution or Investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Days 1, 15 and 29, microscopic ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 57, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge).
h. At Days 1, 15, and 29, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.

Footnotes continued on next page

i. At Day 85 and/or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 8, 15, 22, 29, 36 and 57 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.
l. Day 1 and Day 29, PK blood draw will be performed at the following time points: pre-dose (-15) minutes, 30 (±5) minutes and 60 (±5) minutes after ASP0598 Otic Solution or placebo administration into the affected ear.

Table 16 Schedule of Assessments – Multiple Dose Expansion

	Alternative Approach	Screening	Randomization	Observation					
Day		Day – 28 to Day -1	Day 1	Day 15	Day 29	Day 43	Day 57	Day 85	Day 113 Last Visit/ EOS
Week				Week 2	Week 4	Week 6	Week 8	Week 12	Week 16
Visit Windows				+/- 5 days	+/- 5 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6	7	8
Study Procedures									
Informed Consent		X							
Verify Inclusion/Exclusion		X	X						
Medical History/Disease History/Demographics		X	X						
Physical Exam including Height and Weight		X							
Vital Signs [a]	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X	X	X
Prior/Concomitant Medications [b]		X	X	X	X	X	X	X	X
Adverse Events Assessment [c]		X	X	X	X	X	X	X	X
Study Treatment Administration									
ASP0598 or placebo [d]			X	X [1]	X [1]				

Table continued on next page

	Alternative Approach	Screening	Randomization	Observation					
Day		Day – 28 to Day -1	Day 1	Day 15	Day 29	Day 43	Day 57	Day 85	Day 113 Last Visit/ EOS
Week				Week 2	Week 4	Week 6	Week 8	Week 12	Week 16
Visit Windows				+/- 5 days	+/- 5 days	+/- 3 days	+/- 3 days	+/- 3 days	+/- 3 days
Visit number		1	2	3	4	5	6	7	8
Otology Examination									
Microscopic Ear Examination	If delayed, should be completed as soon as possible	X [e]	X [f]	X [f, g]	X [f, g]	X [g]	X [g]	X [g]	X [i]
Modified Brighton Grading Scheme Evaluation			X [f]	X [f]	X [f]	X	X	X	X
Imaging picture of tympanic membrane by endoscope		X [e]	X [h]	X [h]	X [h]	X	X	X	X
Bone conduction by Pure Tone Audiometry (PTA)		X [e]	X	X [j]	X [j]	X [j]	X [j]	X [j]	X
Air conduction by PTA		X [e]	X					X	X
Tympanometry		X [e]	X					X	X
Tinnitus Visual Analog Scale (TVAS)	Remote/Virtual/Telemedicine Visits allowed.	X	X	X	X	X	X	X	X
Blood Collection									
Serum Chemistries	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X							
CBC with Differential		X							
Pregnancy Test [k]		X	X				X		X
Sample for Future Analysis			X				X		
Urine Collection									
Urinalysis	Visit collection of samples at local facility is acceptable if results can be made available to investigative site	X							

AE: adverse event; CBC: complete blood count; EOS: end of study; SAD: Single Ascending Dose; TM: tympanic membrane; TMP: tympanic membrane perforation

a. Vital Signs include blood pressure, body temperature, and heart rate.
b. Includes all medications taken within 28 days prior to ASP0598 Otic Solution or placebo administration at Day 1.

Footnotes continued on next page

c. AEs will be collected from the time of informed consent through Day 113 or EOS. Any AEs ongoing at Day 113 or EOS Visit must be followed until resolution or Investigator determines the event to be stable. AEs should be assessed at least monthly.
d. ASP0598 Otic Solution or placebo will be applied onto the remaining tympanic membrane under a microscope with a 22-gauge blunt needle. After ASP0598 Otic Solution or placebo administration, subjects will be required to remain on their side with the treated ear facing up for at least 15 minutes. Please see the Pharmacy Manual, Section 9.4: Dose Administration for detailed instructions.
e. Otology examinations will be performed for both ears at the screening visit.
f. Microscopic Ear examination will be performed prior to and 60 (±5) minutes after ASP0598 Otic Solution/placebo administration. Skin local reaction at the application site will be evaluated by Modified Brighton Grading Scheme (Appendix 12.9) at 60 (±5) minutes after ASP0598 Otic Solution/placebo administration.
g. Ear cleaning will not be conducted between Days 2 – 85, unless it is absolutely necessary. Investigators will not clean the treated ear by suctioning unless subjects experience uncomfortable ear discharge).
h. At Days 1, 15, and 29, imaging pictures will be taken at the following time points: pre-dose, immediately after, 30 (±5) minutes, and 60 (±5) minutes after the ASP0598 Otic Solution or placebo administration.
i. At Day 85 (SAD) or Day 113 (single dose expansion) and/or EOS, the whole TM should be visible before taking imaging picture. If the whole TM is not visible, the investigator should clean the ear canal.
j. Bone conduction testing at Day 15, 29, 43, 57, and 85 will be tested only at 1, 2 and 4 kHz (ototoxicity assessment).
k. Urine or serum pregnancy test will be performed in women of childbearing potential.
l. ASP0598 Otic Solution or placebo administration on Day 15 and Day 29 will be conducted in the case that the TMP closure is not confirmed.

INVESTIGATIONAL MEDICINAL PRODUCT SUPPLY

If any of the conditions outlined above in the Subjects Procedures Assessment are met, one or all of the following mitigating strategies will be employed, as needed, to ensure continuity of IMP supply to the subjects:

- Increase stock of IMP on site to reduce number of shipments required, if site space will allow.

DATA COLLECTION REQUIREMENTS

Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.

Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window, or other modifications) due to the crisis.

12.12 List of Abbreviations and Definition of Key Study Terms

List of Abbreviations

Abbreviations	Description of abbreviations
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
bpm	Beats per minute
BUN	blood urea nitrogen
CA	Competent authority
CBC	complete blood count
CCSI	company core safety information
CI	Confidence interval
C_{max}	maximum concentration
CPAP	Continuous Positive Airway Pressure
CRF	case report form
CRO	contract research organization
CSR	Clinical study report
CTCAE	common terminology criteria for adverse events
CTMP	Chronic Tympanic Membrane Perforation
CV	Coefficient of variation
dB	Decibels
DEC	Dose Escalation Committee
DILI	drug-induced liver injury
DMC	Data Monitoring Committee
DPD	Data Protection Directive
eCRF	electronic case report form
EC_{50}	Effective Concentration
ECV	Equivalent Ear Canal Volume
EEA	European Economic Area
EGF	Epidermal growth-like factor
EMA	European Medicines Agency
EOS	End of Study
FAS	full analysis set
GCP	Good Clinical Practice
GCPL	Global Pharmacology Lead
GML	Global Medical Lead

Abbreviations	Description of abbreviations
GMP	Good Manufacturing Practices
GGT	gamma-glutamyltransferase
Hz	hertz
IAP	Interim Analysis Plan
IB	Investigational Brochure
ICE	Intercurrent event
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IP	investigational product
IMP	investigational medicinal product
IRB	Institutional Review Board
IRT	interactive response technology
ISN	international study number
kHz	kilohertz
LA-CRF	liver abnormality case report form
LS	Least square
MAD	Multiple Ascending Dose
MAR	Missing at random
MEDRA	Medical Dictionary for Regulatory Activities
MH	Mantel-Haenszel
MID	Minimum Intolerable Dose
mL	milliliter
mm	Millimeters
mmHg	Millimeters of mercury
MMRM	mixed models repeated measures
MTD	Maximum tolerated dose
NOAEL	No observed adverse event level
PD	protocol deviation
PET	Positron emission tomography
PK	Pharmacokinetic
PTA	Pure Tone Audiometry
PT	Preferred Term
QA	quality assurance
QC	quality control
RBC	red blood cell

Abbreviations	Description of abbreviations
RNA	Ribonucleic acid
RSI	reference safety information
(S)AE	serious adverse event or adverse event
SAD	Single Ascending Dose
SAE	serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SDV	source document verification
SE	Standard error
SFL	screen failure log
SOC	System organ class
SOP	standard operating procedure
SPC	summary of product characteristics
SUSAR	suspected unexpected serious adverse reactions
TEAE	treatment-emergent adverse event
TBL	total bilirubin
TM	Tympanic membrane
TMF	Trial Master File
TMP	Tympanic membrane perforation
TVAS	Tinnitus visual analog scale
ULN	upper limit of normal
USM	Urgent safety matter
WBC	white blood cell
WOCBP	Women of child bearing potential
µg	microgram

Definition of Key Study Terms

Terms	Definition of Terms
Baseline	Assessments of subjects as they enter a study before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study. Note: Not all endpoints are themselves assessments since certain endpoints might apply to populations or emerge from analysis of results. That is, endpoints might be facts about assessments (e.g., prolongation of survival).
Enroll	To register or enter a subject into a study. Note: Once a subject has received the IP or placebo, the protocol applies to the subject.
Intervention	The drug, device, therapy or process under investigation in a study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety and pharmacoeconomics).
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test product or comparative drug (sometimes without randomization) is given to a subject and continues until the last assessment after completing administration of the test product or comparative drug.
Randomization	The process of assigning subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias. NOTE: Unequal randomization is used to allocate subjects into groups at a differential rate; for example, three subjects may be assigned to a treatment group for every one assigned to the control group.
Screening	A process of active consideration of potential subjects for randomization in a study.
Screen failure	Potential subject who signed the ICF, but did not meet 1 or more criteria required for participation in the study and was not randomized.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent form until just before the test product or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first study site initiation date to the last study site completing the study.
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

13 PROTOCOL AMENDMENT SUMMARY OF CHANGES

ISN/Protocol 0598-CL-0101 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)

Amendment 4 [Nonsubstantial] 29 Aug 2022

This amendment is considered to be nonsubstantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union because it neither significantly impacts the safety or physical/mental integrity of participants nor the scientific value of the study.

Overall Rationale for the Amendment:

Language was revised in the protocol to offer the elderly population more opportunity to participate in this First In Human study.

Summary of Changes

Table 1 Nonsubstantial Changes

Section Number	Description of Change	Brief Rationale
Synopsis	The planned study period is updated to end 3Q2024.	To update the study period according to the latest timeline.
Synopsis, 5.1	Inclusion criterion #2 is updated to allow for enrollment of subjects 18 to 75 years of age.	To allow a more elderly population to participate in the study after the study team identified a significant unmet need based on study site feedback. Considering this is the First In Human study, an upper age limit was included based on the predicted mechanisms of action. There is no safety concern for increasing the upper age limit given the nature of the CTMP patient population and the observed safety data to date.
7.6	Add that for MAD cohorts only, the total amount of whole blood collected will be approximately 30.0 mL per subject.	To reflect the correct amount of blood sample taken for MAD cohorts.
Throughout	Minor administrative-type changes, e.g., typos, format, numbering, consistency throughout the protocol.	To provide clarifications to the protocol and to ensure complete understanding of study procedures.

14 SPONSOR SIGNATURES