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Clinical Protocol SPI-1005-151 v2.0

**A PHASE 1b, RANDOMIZED, DOUBLE-BLIND, PLACEBO-
CONTROLLED STUDY TO EVALUATE THE SAFETY,
PHARMACOKINETICS AND PHARMACODYNAMICS OF SPI-1005
IN ADULTS WITH MENIERE'S DISEASE**

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Statistical Analysis Plan

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Abbreviations

AE	adverse event
ANOVA	analysis of variance
AP	action potential
AUC	area under the curve
CBC	complete blood count
Chem-20	Chemistry-20 blood panel
C _{max}	maximum concentration
CRF	case report form
CI	confidence interval
dB HL	hearing level (in decibels)
ECochG	electrocochleography
ICP-MS	inductively coupled plasma-mass spectrometry
ITT	intention-to-treat
kHz	kilohertz
LCMS	liquid chromatography - mass spectrometry
MMRM	mixed-effects model repeated measures
PD	pharmacodynamics
PK	pharmacokinetics
PT	preferred term
PTA	pure tone audiometry
SAP	statistical analysis plan
StdDev	standard deviation
SNR	signal-to-noise ratio
SOC	system organ class
SP	summation potential
SPI	Sound Pharmaceuticals, Inc.
t _{1/2}	half life
TEAE	treatment emergent adverse events
TFI	Tinnitus Functional Index
T _{max}	time to maximum concentration
UA	Urinalysis
VS	vital signs
VSS	Vertigo Symptoms Scale
WINT	Words-in-Noise Test
WR	Word recognition

1. Introduction

This Statistical Analysis Plan (SAP) contains the list of summary tables, listings and figures for protocol SPI-1005-151.

This SAP should be read in conjunction with protocol SPI-1005-151 and the case report forms. Any changes to these documents may necessitate updates to the SAP.

The display shells associated with the tables, listings and figures described herein are included in a separate specifications document.

2. Study Design and Objectives

2.1 Study Objectives

The objectives of this study are to evaluate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of three dose levels (200, 400 and 600 mg) of oral SPI-1005 given twice daily for 21 days compared to placebo in patients with Meniere's disease.

The primary objective of this study is to determine the:

- Safety and tolerability of SPI-1005 treatment in adult patients with Meniere's disease using histories, physical exams, vital signs (VS), adverse events (AEs), and blood tests (complete blood count [CBC], Chemistry-20 [Chem-20]).

The secondary objectives of this study are to determine the:

- Pharmacokinetics: Peak/trough assessments will be determined for ebselen, its major metabolite, and selenium.
- Severity of sensorineural hearing loss in decibels (dB) using repeated pure-tone audiometry (PTA) at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kilohertz (kHz) and word recognition (WR) using the Words-in-Noise Test (WINT) at 24, 20, 16, 12, 8, 4 and 0 Signal-to-Noise Ratio (SNR).
- Pharmacodynamic response using PTA and electrocochleography (ECochG).
- Severity of vertigo using the Vertigo Symptoms Scale (VSS; short form).
- Severity of tinnitus using the Tinnitus Functional Index (TFI).

2.2 Study Design

The study is a randomized, double-blind, placebo-controlled, dose-escalating, safety, PK, and PD study of oral SPI-1005 in adults with Meniere's disease. All patients will undergo baseline audiometric testing and have their severity of sensorineural hearing loss, tinnitus and vertigo determined before the start of SPI-1005 treatment. At the end of the 21-day course of SPI-1005, and 28 days following the cessation of SPI-1005, patients will have their hearing loss and vertigo assessed. Tinnitus will be assessed weekly, and 7 and 28 days following treatment. ECochG will be performed at baseline, at the end of the SPI-1005 treatment, and 28 days after the cessation of SPI-1005 treatment. Six outpatient visits will be performed over a 7-9 week period.

Enrollment of 40 adult patients, with approximately equal number of males and females, will be sufficient to provide safety, PK and PD results to guide future dose selection. Enrollment

into the study will be performed using 3 successive cohorts:

- Cohort 1: 200 mg po BID (n=10) or placebo (n=3) x 21 days;
- Cohort 2: 400 mg po BID (n=10) or placebo (n=3) x 21 days;
- Cohort 3: 600 mg po BID (n=10) or placebo (n=4) x 21 days.

Before, during, and 28 days after administration of SPI-1005, patients will be evaluated for safety by history, physical examination, vital signs and blood tests (CBC and Chem-20). Peak and trough plasma levels of ebselen and its major metabolite will be determined using liquid chromatography-mass spectrometry (LCMS) at certain time points. Additionally, plasma will be analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for plasma selenium levels at the corresponding time points. Patients will be monitored for adverse events at specific clinic visits.

The timing of all safety and efficacy assessments is provided in [Table 1](#).

Table 1 Schedule of Assessments for Protocol SPI-1005-151

Clinic Visit	Study Day	Briefing/ Consent	Blood/ Urine Testing	History/ Physical /Vital Signs	Hearing Evaluation (PTA, WR) ⁵	ECochG	Vertigo Evaluation (VSS) ⁶	Tinnitus Functional Index
CV1 (screening/baseline)	-14 to -1	X	X ^{1,2}	X	X	X	X	X
CV2	7		X ^{1,3}	X				X
CV3	14		X ³	X				X
CV4	21		X ^{1,4}	X	X	X	X	X
CV5 (7 days post treatment)	28		X ²	X				X
CV6 (28 days post treatment)	49		X ^{1,2}	X	X	X	X	X

[1] Chem-20 and CBC. Urine pregnancy test on CV1 screening visit only.

[2] Blood collection for total plasma selenium levels and plasma ebselen and its metabolite levels (random sample at baseline and Clinic Visits 5 and 6). Only plasma selenium will be tested on the baseline sample.

[3] Blood collection for total plasma selenium levels and plasma ebselen and its metabolite levels (trough prior to morning dose of SPI-1005 on Clinic Visit 2 and 3).

[4] Blood collection for total plasma selenium levels and plasma ebselen and its metabolite levels (trough prior to morning dose of SPI-1005 on study day 21; followed by study drug administration; followed by peak measurement 2 hours following study drug administration).

[5] Hearing evaluation using pure-tone audiometry (PTA) at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz and WR using WINT at 24, 20, 16, 12, 8, 4 and 0 SNR. Tympanometry will be performed at CV1.

[6] Vertigo assessment using the VSS.

3. Changes from the Planned Analyses in the Protocol

3.1 Protocol Amendments Impacting Analyses

Not applicable.

3.2 Other Changes to Analyses Prior to Study Unblinding

The primary focus of most analyses for this study is the differences among various treatments, and differences between the treatments can be obtained by writing contrasts that include all terms in the model. Hence, inferences for treatment differences can be performed without first performing a model selection step. Backwards elimination of insignificant fixed effect terms and interactions will not be used. It is a technique better suited for multiple regression settings.

3.3 Changes to Planned Analyses Post Study Unblinding

Any changes or additions to the planned analyses that are determined after the study has been unblinded will be documented in the clinical study report.

4. Conventions and General Methodology

EMB Statistical Solutions, LLC will generate the statistical analyses detailed in this SAP. All computations for statistical analyses will be performed using SAS® software, Version 9.3 or later. All SAS programs used in the production of statistical summary tables, data listings, and figures will be validated with independent programming prior to finalization. In addition, all program outputs will be independently reviewed. The validation process will be used to confirm that statistically valid methods were implemented and that all data manipulations and calculations were accurately done. Once validation is complete, the quality reviewer and senior statistical reviewer will perform a final review of the documents to ensure accuracy and consistency with this plan and consistency across tables and figures. Upon completion of validation and quality review procedures, all documentation will be collected and filed by the project statistician or designee.

Note that tables and figures will include an analyses where all SPI-1005 dose groups are pooled together in the display (ALL SPI-1005).

Before implementation of parametric methods of analysis, the distribution of primary analysis variables will be examined to determine if model assumptions are satisfied; accordingly, transformations or nonparametric methods of analysis may be used if warranted. However, in some cases, nonparametric analysis may be the initially proposed method due to the expected distribution of response. Whenever alternative methods of analysis are required, the description of the new method along with the rationale for its use will be documented in the clinical study report.

Data listings will be sorted by patient identification number and clinic visit or time.

4.1 Unblinding Plan

This is a double-blinded study. In the event of a serious or unexpected adverse event that requires knowledge of an individual patient's treatment assignment, the treatment assigned for that individual patient may be released. In such cases, an investigator will first contact the Sponsor to discuss the event and rationale for unblinding. Then, the Sponsor will direct the appropriate unblinded personnel to communicate the patient's treatment assignment to the investigator. All cases where an individual patient's treatment assignment is unblinded prior to the data lock will be documented by the Sponsor.

After the last patient randomized into the study completes Clinic Visit 6 (28 days post-treatment) and all data management data activities have been completed – data entered, coding completed, and all queries resolved – the Study Unblinding Authorization will be signed off, thereby approving release of the randomization schedule by the Randomization Statistician.

4.2 Analysis Population Sets

The primary analysis population for efficacy is the intention-to-treat (ITT) population. The **ITT population** is defined as all randomized patients. All analyses using the ITT population will be performed using the treatments assigned at randomization

Safety will be analyzed using the **Safety Population**. The Safety population is comprised of all randomized patients for whom at least one dose of study treatment was received. All safety analyses will be performed using the treatments actually received.

Patients who violated some portion of the protocol will be identified using the following criteria:

- Patient withdrawn from study due to noncompliance with the protocol, as determined by the investigator.
- Patient failed to meet inclusion or exclusion criteria
- Patient significantly non-compliant with study protocol and/or treatment, as determined by the investigator.
- Patient use of an ototoxic medication (e.g. aminoglycoside antibiotics [gentamicin, tobramycin, amikacin, streptomycin]; platinum-containing chemotherapies [cisplatin, carboplatin, oxaliplatin]; or loop diuretic [furosemide]) prohibited by the protocol during the study period.

Separate analyses that include only those patients who are compliant with the protocol are not planned. A listing of all protocol violations will be provided.

4.3 Handling of Dropouts and Missing Data

No data imputations are planned for this study.

Analyses of the efficacy parameters are based on models that allow random missingness of observations among the patients and will include all randomized patients. Analyses of the safety endpoints, many of which are incidence based, will include all patients in the Safety Population, unless specifically stated otherwise. Baseline characteristics will be based on the data collected at Clinic Visit 1. If a patient is missing a baseline observation, then that patient will not be included in the baseline analysis of that parameter or the analysis of the change from baseline. Similarly, time point specific analyses of clinical laboratories and vital signs will include only those patients with data from the scheduled data collections.

4.4 Interim Analysis and Adjustment for Multiplicity

There were no planned interim analyses for this study.

5. Patient Accounting and Disposition

The number of ITT patients entered, randomized, treated, and the reasons for withdrawal will be summarized with counts and percentages overall and by treatment group. Plus, the dates of the first and last visit for the study will also be provided.

6. Baseline Characteristics

Demographic data and the baseline characteristics height, weight, and air and potentially bone conduction thresholds will be collected during the Clinic Visit 1. Summaries of baseline characteristics for the randomized treatment groups will be generated using the ITT population. Patients who are missing measurements will not be included in the summary for that variable.

Screening data for laboratory variables and vital signs, which are also collected throughout the study, will not be analyzed as baseline variables. However, the screening assessments will be considered as the baseline records and incorporated in the safety analyses of those variables. Descriptions of these analyses are detailed in later sections of the SAP.

Data collected for the inclusion/exclusion criteria as well as examinations or tests for determining eligibility for enrollment, i.e. medical history, physical exams, and pregnancy tests, will be summarized (i.e. listing). Such data will be retained in the database in case a patient-level review becomes necessary. Omitting this information from the summaries does not hinder the evaluation of the treatments in this study since any abnormalities encountered as a result of these tests either prevented a patient's enrollment in the study or was recorded as part of the patient's medical history and was thus taken into account when recording adverse events.

6.1 Demographics

Demographic variables collected prior to randomization include date of birth, sex, race, and ethnicity. Age at study entry will be based on the age of the patient on the date the informed consent is signed. Age at study entry will be summarized as a continuous variable. In addition, Age Group will be defined as a dichotomous variable using the median age for the entire ITT population as the cut-off ($\leq$ median ITT age versus $>$ median ITT age).

Six categorical variables (i.e., White, Black or African American, Native Hawaiian or other Pacific Islander, Asian, American Indian or Alaskan Native, and Race Unknown) will be used to identify a patient's race, and more than one race can be affirmatively identified for a patient. Ethnicity, which is defined as Hispanic or Latino versus Not Hispanic or Latino, will also be summarized by treatment.

6.2 Baseline Anthropometrics

Patient height and weight will also be collected prior to randomization. Both variables will be reported in metric units (height in cm and weight in kg) and will be summarized for the treatment groups as continuous variables along with body mass index (BMI; in kg/m^2). Body mass index will also be summarized as a dichotomous variable using the categories: ($< 30 \text{ kg/m}^2$ versus $\geq 30 \text{ kg/m}^2$).

6.3 Baseline Audiological Characteristics

6.3.1 Pure Tone Audiometry

During screening, Pure Tone Audiometry (PTA) using air conduction will be performed. Air conduction testing will be collected at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz. Bone conduction PTA at 0.25, 0.5, 1, 2, and 4 kHz will be performed only where air conduction thresholds exceed 15 dB HL.

The following variables, which identify normal hearing thresholds, will be derived for each patient and summarized by randomized treatment assignment:

- the maximum air conduction threshold across ears and the frequencies 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz;
- the maximum absolute difference in air conduction threshold between the ears across the frequencies 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz;
- the maximum air-bone gap across ears and the frequencies 0.25, 0.5, 1, 2, and 4 kHz.

Descriptive statistics for the baseline air conduction thresholds will be provided for each combination of treatment, frequency and ear. In addition, descriptive statistics will also be produced for the mean across the frequencies 0.25, 0.5, and 1 kHz in each ear.

6.3.2 Word Recognition

Word recognition using the WINT at 24, 20, 16, 12, 8, 4 and 0 Signal-to-Noise Ratio (SNR) will be completed at Clinic Visit 1. Five words from a standardized list of words will be read into each ear for each of the 7 SNRs for a total of 35 words per ear. Each list contains 35 different words. The sum of correct scores across all SNRs (24 to 0) will be totaled. The percent of correct scores will be derived by dividing the sum of correct scores by 35 and multiplying by 100.

Descriptive statistics for the baseline percentage of words correctly identified will be provided for each combination of treatment and ear.

6.3.3 Electrocochleography

Electrocochleography will be completed at Clinic Visit 1. The ratios of the area under the curve (AUC) and amplitude involving the summing potential (SP) and the action potential (AP), the variables are SP/AP AUC ratio and SP/AP Amplitude ratio, respectively, will be reported in each ear.

Descriptive statistics for the electrocochleography parameters will be provided for each combination of treatment and ear.

6.3.4 Vertigo Symptoms Scale

The VSS – Short form will be completed by the patient and scored by study personnel at Clinic Visit 1. The variables Vertigo/Balance Sub-score (range 0-32), Autonomic/Anxiety Sub-score (range 0-28), and the Total Score (range 0-60) will be recorded in the CRF.

Descriptive statistics for each parameter will be provided by treatment.

6.3.5 Tinnitus Function Index

The TFI will be completed by the patient and scored by study personnel at Clinic Visit 1. Each of the variables Intrusive Sub-scale (range 0-100), Sense of Control Sub-scale (range 0-100), Cognitive Sub-scale (range 0-100), Sleep Sub-scale (range 0-100), Auditory Sub-scale (range 0-100), Relaxation Sub-scale (range 0-100), Quality-of-Life Sub-scale (range 0-100), Emotional Sub-scale (range 0-100), the Overall Score (range 0-100), and the response to Question 2 'How Strong or Loud was your tinnitus over the past week?' (range 0-10) will be recorded in the CRF.

Descriptive statistics for each parameter will be provided by treatment.

7. Study Exposure, Compliance, and Concomitant Treatment

The Safety Population will be used to summarize these variables.

7.1 Study Drug Exposure

Patients are expected to swallow 6 capsules per day, 3 capsules taken twice daily (AM and PM dose), on each of the 21 days of the treatment period for an expected total of 126 ($6 \times 21 = 126$). The total number of capsules received during the study will be summarized using the categories: 1 to 42 capsules, 43 to 84 capsules, 85 to 126 capsules, and ≥ 127 capsules.

The date of last dose of study drug was not captured in the CRF. However, the date and time of dose at Clinic Visits 2 (Day 7), 3 (Day 14), and 4 (Day 21) was captured in the CRF. The number (percent) of patients who received drug on Days 7, 14, and 21 will be reported for each treatment group.

7.2 Compliance

The CRF does include the question 'Was patient compliant with study drug protocol?', so compliance for each treatment group will be reported at Clinic Visits 2, 3 and 4 using the number (percent) of patients for whom the investigators answer 'Yes' and 'No'.

7.3 Concomitant Treatments

Concomitant medications taken during this study will be recorded in the CRF, but they will not be coded. Only a by-patient listing of all concomitant medications will be provided. This listing will be reviewed by Clinical personnel prior to unblinding the study to identify protocol violations.

8. Safety Analyses

As noted in [Section 2.2](#), all safety endpoints will be analyzed using the Safety Population.

8.1 Adverse Events

At each clinic visit patients are to be asked about any negative experiences that they are having. Those adverse events that are reported or observed after the first dose of study medication will be classified as Treatment Emergent Adverse Events (TEAE) and reported in

the CRF along with any action taken.

Adverse events will be coded using the MedDRA® dictionary, version 18.1 or later. Incidence of TEAE will be reported using number (percent) of patients for each treatment arm by system organ class (SOC) and preferred term (PT). The total number of events reported for each TEAE will also be reported. No statistical testing will be performed. The following categories of TEAE will be reported:

- all TEAE
- TEAE either possibly or definitely related (or missing relatedness) to study drug
- serious TEAE
- TEAE resulting in study drug withdrawal
- TEAE by maximal severity.

8.2 Clinical laboratory parameters

8.2.1 Chem-20, Total Selenium & CBC with Differentials

Certified medical laboratory technicians will collect blood samples for CBC, Chem-20 and plasma selenium at Clinic Visits 1, 2, 4, and 6. Plasma selenium will also be collected on Clinic Visits 3 and 5. The samples collected at Clinic Visit 1 will be considered the baseline values, and change from baseline will be derived for each post-baseline sample.

Analyses will be conducted using the baseline value, the post-baseline values collected at the scheduled clinic visits, plus the last measurement collected. Actual values and the change from baseline will be descriptively summarized by visit and treatment group using n, mean, median, standard deviation, minimum, and maximum. In addition, shifts from baseline to the last measurement will be presented for each parameter. Unscheduled assessments will be included when determining the last measurement. No statistical testing will be performed for the clinical laboratory parameters.

8.2.2 Plasma Ebselen and Metabolite Concentrations

At all clinic visits, except Clinic Visit 1, plasma samples will be collected for analysis of ebselen and its major metabolite 2- glucuronyl selenobenzanilide as well as selenium. For Clinic Visits 2, 3, and 4 the plasma sample will be collected just prior to the first dose of the day and at Clinic Visit 4 another sample will be collected 2 hours after dosing. A previous single-dose, Phase 1 PK study with the SPI-1005 formulation of ebselen ([Lynch and Kil \[2009\]](#)) demonstrated that T_{max} for ebselen ranged from 1.5 to 2.3 hours and $t_{1/2}$ ranged from 6.4 to 16.7 hours for doses ranging from 200 mg to 1600 mg. Hence, the samples collected 2 hours post dose should reflect peak concentration levels while the pre-dose samples should reflect trough levels.

Peak (C_{max}) and trough plasma ebselen levels increased proportionately with dose in the Phase 2 study (SPI-1005-202), while C_{max} was not dose proportional in the Phase 1 study (SPI-1005-101). The current study (SPI-1005-151) will utilize the same dose levels as the Phase 2 study (SPI-1005-202), which formed a much narrower range than the Phase 1 study (SPI-1005-101), and the dose proportionality of the peak and trough concentrations will be assessed. In addition, the Phase 1 (SPI-1005-101) study showed a strong correlation between plasma selenium concentration and the combined concentrations of plasma ebselen and its major and minor metabolites, while a weaker correlation was observed in the

Phase 2 study (SPI-1005-202). A plot of the plasma selenium concentrations versus the combined concentrations of plasma ebselen and its major metabolite will be produced for this study.

The descriptive statistics geometric mean, geometric standard deviation, median, minimum and maximum for the peak and trough values plus the peak-to-trough ratio will be provided for each treatment group. Dose proportionality will be assessed by regressing the difference between ln-transformed (natural log-transformed) peak and trough values on ln-transformed dose. Proportionality will be suggested if a strong linear relationship is established and the 95% confidence interval (CI) for the slope includes the value of 1.

To assess the potential of using plasma selenium as a reliable surrogate of plasma ebselen, combined plasma ebselen and metabolite concentrations will be plotted against selenium plasma concentrations using a simple linear regression. Separate slope parameters by SPI-1005 dose level will not be included when assessing surrogacy.

8.3 Vital signs and weight

The vital signs systolic and diastolic blood pressure (mmHg), respiratory rate (breaths per minute), pulse rate (beats per minute), oral temperature (°F), and weight (kg) will be collected at every clinic visit. The measurements taken at Clinic Visit 1 will be considered the baseline values, and change from baseline will be derived for each post-baseline collection.

Analyses will be conducted using the baseline value, the post-baseline values collected at the scheduled clinic visits, and last post-baseline measurement of a parameter regardless if the collection was scheduled. Actual values and the change from baseline will be descriptively summarized by visit and treatment group using n, mean, median, standard deviation, minimum, and maximum. No statistical testing will be performed for the vital sign parameters.

9. Efficacy Analyses

9.1 Pure Tone Audiometry

Pure Tone Audiometry using air conduction will be performed in both ears at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz at Clinic Visits 1 (Baseline), 4 (21 days of treatment) and 6 (28 days post-treatment). Analysis of the air conduction threshold measurements will be performed to identify potential differences in the severity of hearing loss among the randomized treatment arms.

Analysis of the change from baseline in air conduction thresholds will be performed using MMRM methods using the triply repeated measurements (ears, frequencies and visits) for each patient. The model will have fixed effects for treatment, ear, frequency and visit, all 2-way interactions, and the 3-way interaction treatment-by-frequency-by-visit plus a random effect for patient, which will be nested within treatment, and baseline as a covariate. The covariance structure among the repeated measurements for a patient will be assumed to be common across patients and modeled as the Kronecker product of a completely symmetric covariance (2x2) for ears, an unstructured 8x8 covariance matrix among the frequency levels, and an unstructured (2x2) covariance matrix for time (visit). This analysis will be performed using the SAS procedure PROC MIXED. Note that if the variance parameter for the patient random effect is sufficiently small that the model fails to adequately converge

then it may be dropped from the model. Similarly, an unstructured covariance matrix for ears may be used if incorporating the completely symmetric structure is problematic.

The efficacy analysis that examines the pairwise comparisons of the SPI-1005 dose levels with placebo, and for ALL SPI-1005 dose levels combined with placebo, will be obtained using contrast statements from PROC MIXED. Contrasts between each of the SPI-1005 dose levels with placebo will be presented for Day 21 of treatment and 28 days post-treatment for each frequency level. In addition, contrasts among the treatments for each of the clinic visits will be provided for the mean across 0.25, 0.5, and 1 kHz, averaged across ears and for each ear separately.

The tabular output for these MMRM analyses will be presented in two tables. The first table will present at each time and frequency the least squares means, standard errors, 95% confidence intervals and p-values for each marginal treatment mean plus the least squares means, standard errors, 95% confidence intervals and p-values for the treatment contrast versus placebo. The second table will present the model terms p-values along with the least squares means, standard errors and 95% confidence intervals and p-values for each main effect marginal. In addition, a line graph will be used to show the means and standard errors by frequency, for each visit and treatment group.

An analysis of PTA will identify patients that show a significant improvement from baseline on the PTA at any low frequency (i.e. 0.25, 0.5, or 1.0 kHz). The analysis will be performed at Clinic Visits 4 and 6. A patient will be classified as showing a significant improvement at the post-baseline visit if the improvement from baseline for at least one of the thresholds, at 0.25, 0.5, or 1.0 kHz, is 10 dB or more. This classification will be done for both ears. The number of patients with improvement in both ears, one ear, and neither ear will be summarized, and the treatment groups will be compared with placebo, and ALL SPI-1005 dose levels combined will be compared with placebo, using a Mann-Whitney U test. The number of patients with improvement in either ear will be summarized, and the treatment groups will be compared with placebo, and ALL SPI-1005 treatment groups combined will be compared with placebo, using Fisher's Exact test.

Bar graphs of the percentage of subjects in each treatment group with a 10 dB or more improvement in either ear at any low frequencies will be created for each post-baseline time point. A Cochran-Armitage trend test will compare these percentages across treatments.

9.2 Word Recognition

Word recognition using the WINT at 24, 20, 16, 12, 8, 4 and 0 Signal-to-Noise Ratio (SNR) will be completed at Clinic Visits 1, 4 and 6. For each ear the total WINT score and the WINT percent score will be derived and results of the analysis will be presented both in terms of the total WINT score and WINT % score.

Analysis of the change from baseline in total WINT score will be performed using MMRM methods using the doubly repeated measurements (ears and visits) for each patient. The model will have fixed effects for treatment, ear, and visit, and all 2-way interactions plus a random effect for patient, which will be nested within treatment, and the baseline total WINT score as a covariate. The covariance structure among the repeated measurements for a patient will be assumed to be common across patients and modeled as the kronecker product of a completely symmetric covariance (2x2) for ears and an unstructured (2x2) covariance matrix for time (visit). This analysis will be performed using the SAS procedure PROC MIXED. Note that if the variance parameter for the patient random effect is

sufficiently small that the model fails to adequately converge then it may be dropped from the model. Similarly, an unstructured covariance matrix for ears may be used if incorporating the completely symmetric structure is problematic.

The efficacy analysis that examines the pairwise comparisons of the SPI-1005 dose levels with placebo, and for ALL SPI-1005 dose levels combined with placebo, will be obtained using contrast statements from PROC MIXED. Contrasts of the least square means for the change from baseline in the Total WINT score between each of the SPI-1005 dose levels with placebo will be presented for Day 21 of treatment and 28 days post-treatment. Estimates of the contrasts for the WINT percent score will be obtained by dividing estimates of the contrasts for the Total WINT score by 35 and multiplying by 100.

The tabular output for these MMRM analyses will be presented in two tables. The first table will present at each visit the least squares means, standard errors, 95% confidence intervals and p-values for each marginal treatment mean plus the least squares means, standard errors, 95% confidence intervals and p-values for the treatment contrast versus placebo. The second table will present the model terms p-values along with the least squares means, standard errors and 95% confidence intervals and p-values for each main effect marginal.

An analysis of the Total WINT score will identify patients that show a significant improvement in their Total WINT score from baseline. The analysis will be conducted at Clinic Visits 4 and 6. A patient will be classified as showing improvement for the analysis if the Total WINT score improves from the baseline value by 10% or more at the post-baseline visit. This will be done for both ears. The number of patients with improvement in both ears, one ear, and neither ear will be summarized, and the treatment groups will be compared with placebo, and ALL SPI-1005 treatment groups combined will be compared with placebo, using a Mann-Whitney U test. The number of patients with improvement in either ear will be summarized, and the treatment groups will be compared with placebo, and ALL SPI-1005 treatment groups combined will be compared with placebo, using Fisher's Exact test.

Bar graphs of the percentage of subjects in each treatment group with a 10% or more improvement in either ear will be created for each post-baseline time point. A Cochran-Armitage trend test will compare these percentages across treatments.

9.3 Electrocochleography

Electrocochleography will be completed at Clinic Visits 1, 4, and 6. The ratios of the area under the curve (AUC) and amplitude between for the summing potential (SP) and the action potential (AP), the variables are SP/AP AUC ratio and SP/AP Amplitude ratio, respectively, will be reported in each ear.

Analyses of the change from baseline in the ratios will be performed using MMRM analyses. The ratios are repeated for each patient since measurements are collected in each ear at specific clinic visits. The model for each ratio will have fixed effects for treatment, ear, and visit, and all 2-way interactions plus a random effect for patient, which will be nested within treatment, and baseline as a covariate. The covariance structure among the repeated measurements for a patient will be assumed to be common across patients and modeled as the kronecker product of a completely symmetric covariance (2x2) for ears, an unstructured (2x2) covariance matrix for time (visit). This analysis will be performed using the SAS procedure PROC MIXED.

The efficacy analysis that examines the pairwise comparisons of the SPI-1005 dose levels

with placebo, and for ALL SPI-1005 dose levels combined with placebo, will be obtained using contrast statements from PROC MIXED. Contrasts between each of the SPI-1005 dose levels with placebo will be presented for Day 21 of treatment and 28 days post-treatment.

The tabular output for these MMRM analyses will be presented in two tables. The first table will present at each visit the least squares means, standard errors, 95% confidence intervals and p-values for each marginal treatment mean plus the least squares means, standard errors, 95% confidence intervals and p-values for the treatment contrast versus placebo. The second table will present the model terms p-values along with the least squares means, standard errors and 95% confidence intervals and p-values for each main effect marginal.

9.4 Vertigo Symptoms Scale

The VSS – Short form will be completed by the patient and scored by study personnel at Clinic Visits 1, 4 and 6, and the variables Vertigo/Balance Sub-score (range 0-32), Autonomic/Anxiety Sub-score (range 0-28), and the Total Score (range 0-60) will be recorded in the CRF.

Analyses of the change from baseline in the scores will be performed using MMRM analyses. The scores are repeated for each patient since measurements are collected at specific clinic visits. The model for each ratio will have fixed effects for treatment, visit, and their 2-way interaction plus a random effect for patient, which will be nested within treatment, and baseline as a covariate. The covariance structure among the repeated measurements for a patient will be assumed to be common across patients and modeled as an unstructured (2x2) covariance matrix for time (visit). This analysis will be performed using the SAS procedure PROC MIXED.

The efficacy analysis that examines the pairwise comparisons of the SPI-1005 dose levels with placebo, and for ALL SPI-1005 dose levels combined with placebo, will be obtained using contrast statements from PROC MIXED. Contrasts between each of the SPI-1005 dose levels with placebo will be presented for Day 21 of treatment and 28 days post-treatment.

The tabular output for these MMRM analyses will be presented in two tables. The first table will present at each visit the least squares means, standard errors, 95% confidence intervals and p-values for each marginal treatment mean plus the least squares means, standard errors, 95% confidence intervals and p-values for the treatment contrast versus placebo. The second table will present the model terms p-values along with the least squares means, standard errors and 95% confidence intervals and p-values for each main effect marginal.

An analysis will examine the number (percent) of patients with a significant improvement in the VSS Total Score. A significant improvement is defined as a ≥ 6 point reduction from baseline, and this analysis will be performed at Clinic Visit 4 and Clinic Visit 6. Separate pairwise comparisons between the SPI-1005 dose levels and placebo, and between ALL SPI-1005 dose levels combined and placebo, will be performed using Fisher's Exact Tests.

Bar graphs of the percentage of subjects in each treatment group with a 6 point or more improvement in either ear will be created for each post-baseline time point. A Cochran-Armitage trend test will compare these percentages across treatments.

9.5 Tinnitus Function Index

The TFI will be completed by the patient and scored by study personnel at every clinic visit. Each of the sub-scale scores plus the response to ‘How Strong or Loud was your tinnitus over the past week?’ will be performed using MMRM analyses.

Each change from baseline for an outcome score is repeated for each patient since measurements are collected at specific clinic visits. The model for each sub-scale will have fixed effects for treatment, visit, and their 2-way interaction plus a random effect for patient, which will be nested within treatment, and baseline as a covariate. The covariance structure among the repeated measurements for a patient will be assumed to be common across patients and modeled as an unstructured 2x2 covariance matrix for time (visit). This analysis will be performed using the SAS procedure PROC MIXED.

The efficacy analysis that examines the pairwise comparisons of the SPI-1005 dose levels with placebo, and for ALL SPI-1005 dose groups combined with placebo, will be obtained using contrast statements from PROC MIXED. Contrasts between each of the SPI-1005 dose levels with placebo will be presented for Day 21 of treatment and 28 days post-treatment.

The tabular output for these MMRM analyses will be presented in two tables. The first table will present at each visit the least squares means, standard errors, 95% confidence intervals and p-values for each marginal treatment mean plus the least squares means, standard errors, 95% confidence intervals and p-values for the treatment contrast versus placebo. The second table will present the model terms p-values along with the least squares means, standard errors and 95% confidence intervals and p-values for each main effect marginal.

An analysis of TFI will identify patients that show a significant improvement on the TFI between baseline and Clinic Visit 4 and baseline and Clinic Visit 6. A patient will be classified as having a significant improvement for this analysis if the total TFI score decreases from baseline by 10 points or more. In addition, an analysis will identify patients that show a 2 point or more decrease on Question 2 (Tinnitus Loudness). Separate pairwise comparisons between the SPI-1005 dose levels and placebo, and between ALL SPI-1005 dose levels combined and placebo, will be performed using Fisher’s Exact Tests.

Bar graphs of the percentage of subjects in each treatment group that improved with a 10 point or more reduction in either ear will be created for each post-baseline time point for the total TFI score. Bar graphs of the percentage of subjects in each treatment group that improved with a 2 point or more reduction in either ear will be created for each post-baseline time point for Question 2. A Cochran-Armitage trend test will compare these percentages across treatments.

References

- Lynch E, Kil J: **Development of Ebselen, a Glutathione Peroxidase Mimic, for the Prevention and Treatment of Noise-Induced Hearing Loss** *Seminar in Hearing* 2009, 30(1):47-55.
- Kil, J., Lobarinas, E., Spankovich, C., Griffiths, S. K., Antonelli, P. J., Lynch, E. D., & Le Prell, C. G. (2017). **Safety and efficacy of ebselen for the prevention of noise-induced hearing loss: a randomised, double-blind, placebo-controlled, phase 2 trial.** *Lancet*. doi:10.1016/s0140-6736(17)31791-9