Frequency Therapeutics/[redacted] STATISTICAL ANALYSIS PLAN

A Phase 2a, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single and Repeat-Dose, Multicenter, Exploratory Efficacy Study of FX-322 Administered by Intratympanic Injection in Adults with Stable Sensorineural Hearing Loss

FX-322-202 Protocol (Version 5.0, 08 February 2021)

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Frequency Therapeutics/[redacted]
STATISTICAL ANALYSIS PLAN
ACKNOWLEDGMENT AND SIGNATURE SHEET

A Phase 2a, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single and Repeat-Dose, Multicenter, Exploratory Efficacy Study of FX-322 Administered by Intratympanic Injection in Adults with Stable Sensorineural Hearing Loss

Approved: [redacted]

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Version History

SAP Version	Approval Date	Change(s)	Rationale
1.0	26NOV2019	Not Applicable	Original Version
2.0	08OCT2020	Include change from baseline analyses for TFI, HSI, and HHIA. Make updates for Protocol Version 3.0, for interim analysis, and handling of treated/untreated ears for Full Analysis Set and Safety Analysis Set analyses.	Version 2.0
3.0	15JAN2021	Make updates for Protocol Version 4.0 and for interim analysis.	Version 3.0
4.0	10FEB2021	Make updates to unblind at the subject level after the interim analysis, as specified in Protocol Version 5.0. Clarify covariance structure for HSI and HHIA analyses.	Version 4.0

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LIST OF ABBREVIATIONS

AC	Air Conduction
AE	Adverse Event
AESI	Adverse Event of Special Interest
AR(1)	Autoregressive (1)
ATC	Anatomical Therapeutic Chemical
BC	Bone Conduction
BCA	Bone Conduction Average
BIC	Bayesian Information Criteria
C-SSRS	Columbia-Suicide Severity Rating Scale
CI	Confidence Interval
CNC	Consonant-Nucleus-Consonant
CRF	Case Report Form
CSR	Clinical Study Report
CTMS	Clinical Trial Management System
dB	Decibel
EHFA	Extended High Frequency Audiometry
FAS	Full Analysis Set
GEE	Generalized Estimating Equation
HHIA	Hearing Handicap Inventory for Adults
HSI	Hearing Screening Inventory
IA	Interim Analysis
ICH	International Conference on Harmonisation
LS	Least Squares
MAR	Missing At Random
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed-effect Model for Repeated Measures
NIHL	Noise Induced Hearing Loss
NR	No Response
PPAS	Per Protocol Analysis Set
PTA	Pure Tone Average
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SfAS	Safety Analysis Set
SL	Sensation Level
SPL	Sound Pressure Level
SSNHL	Sudden Sensorineural Hearing Loss

TEAE	Treatment-Emergent Adverse Event
TFI	Tinnitus Functional Index
TM	Tympanic Membrane
TR	Thornton-Raffin
UN	Unstructured
WR	Word Recognition in quiet
WIN	Words-In-Noise testing

1. PURPOSE OF THE ANALYSES

The purpose of this statistical analysis plan (SAP) is to provide detailed information to aid in the implementation of the statistical analysis and reporting of the study data for use in the interim analysis (IA) and clinical study report (CSR). It briefly summarizes the protocol, describes the analysis sets that will be analyzed, and describes the analyses to be performed. The details of the specific statistical methods that will be used are provided. If additional analyses are required to supplement the planned analyses described in this SAP, they may be completed and will be identified in the CSR as post hoc. Table, figure, and listing specifications are in separate documents.

Plans for the IA are specified in Section 10.

This SAP was written with due consideration of the recommendations outlined in the most recent International Conference on Harmonisation (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials and the most recent ICH E3 Guideline, entitled Guidance for Industry: Structure and Content of Clinical Study Reports and was finalized prior to unblinding for the interim analysis.

2. PROTOCOL SUMMARY

2.1. Primary Study Objectives

1. To assess the exploratory efficacy by audiologic response following single and repeat doses of FX-322 in subjects with stable sensorineural hearing loss.

2. To assess the local and systemic safety of single and repeat doses at of FX-322 in subjects with stable sensorineural hearing loss.

2.2. Study Design

This is a Phase 2a, prospective, randomized, double-blind, placebo-controlled, single and repeat-dose, multicenter, exploratory efficacy study of FX-322, administered by intratympanic injection, in adults with stable sensorineural hearing loss.

The study will have 3 phases: Screening, Treatment, and Follow-up. The Screening phase can occur up to 30 days prior to study drug administration (Baseline/Treatment Day 1). The Treatment phase will be 21 days (4 treatment visits 7 days apart) and the Follow-up phase will be 7 months from Baseline (Day 1).

2.2.1. Screening

During the Screening phase, all subjects will be screened to determine study eligibility. All subjects will return for the Baseline visit and will be evaluated to ensure they continue to meet appropriate inclusion/exclusion criteria applicable at that visit.

2.2.2. Treatment

Approximately 96 subjects are planned to be enrolled in this study. The subjects will be randomized to receive FX-322 or placebo according to the different treatment group schedules listed below:

Group Dosing Schedule					
Treatment Group	**# of Subjects**	**Day 1**	**Day 8**	**Day 15**	**Day 21**
1	24	FX-322	Placebo	Placebo	Placebo
2	24	FX-322	FX-322	Placebo	Placebo
3	24	FX-322	FX-322	FX-322	FX-322
4	24	Placebo	Placebo	Placebo	Placebo

Once a subject has been identified as meeting all inclusion/exclusion criteria, the qualifying ear with the worse hearing will be selected, unless a rationale exists to treat the other ear (e.g., the worse hearing ear does not meet the study criteria). The study treatment or placebo will be administered on Day 1, Day 8, Day 15, and Day 21. The placebo will be matched to FX-322 in consistency and color.

Each subject will be placed in the supine position. Topical anesthesia will be administered to the tympanic membrane. Under a microscope, a 25-gauge needle will be used to inject FX-322 or placebo into the middle ear at the junction of the posterior inferior and posterior superior quadrant with the needle tip directed towards the round window. The posterior superior quadrant should be avoided to prevent injury to the ossicles. After injection, the subject will continue to lie with the injected ear facing up for 10-15 minutes. The injections will be performed at an appropriate location designated by a Board-certified Otolaryngologist trained and experienced in performing intratympanic injections. Safety monitoring will include recording of adverse events (AEs), safety laboratory assessments, and monitoring of tympanometry and otoscopic exams.

2.2.3. Follow-up

Subjects will be required to return for safety, otologic, and audiologic assessments at Days 15, 60, 90, 150, and 210 after the initial injection. Subjects will be contacted by phone for safety assessments at Days 30, 120, and 180.

2.3. Study population

The study population will include male and female adults (18 to 65 years inclusive), otherwise healthy with stable sensorineural hearing loss. Protocol Sections 8.4.2 and 8.4.3 define the inclusion and exclusion criteria for the study.

2.4. Sample size

The sample size of approximately 24 subjects per group was considered adequate to explore the potential efficacy of FX-322 associated with various dosing schedules and was not based on formal statistical evaluation.

3. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following is a list of general analysis and reporting conventions to be applied for this study.

- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form n (%). If a count is 0, no percentage will be shown. To ensure completeness, summaries for categorical and discrete variables will include all categories, even if no subjects had a response in a particular category. Missing data for categorical variables will have a 'Missing' category added at the end and the count will be presented without a percentage. Percentages for categorical variables will exclude the 'Missing' category.

- Continuous variables will be summarized using mean, standard deviation (SD), minimum, maximum, median, and number of subjects. The mean, median, and confidence intervals (CI) will be rounded and reported to 1 more level of precision than the original observations, and the SD will be rounded and reported to 2 more levels of precision than the original observations. The minimum and maximum will be the same precision as the original data.

- Following SAS default rules, the median will be reported as the rounded average of the two middle numbers if the dataset contains even numbers.

- P-values will be rounded and reported to 3 decimal places if greater than 0.001. If the rounded p-value is less than 0.001, '<0.001' will be reported. If the rounded p-value is >0.999, '>0.999' will be reported. P-values and significant levels will be reported as 0.05 rather than .05.

- No preliminary rounding will be performed; rounding will only occur after analysis. To round, consider digit to right of last significant digit: if < 5 then round down, if >=5 then round up.

- All listings will be sorted in order of treatment group, subject, and time of assessment (e.g., visit, time, and/or event).

- Dates in listings will be displayed as yyyy-mm-dd (e.g., 2019-08-22).

- Age (in years) will be calculated using the date of birth and the Visit 1 (Screening) date in the following SAS algorithm: floor((intck('month', date of birth, Visit 1 date)

– (day(Visit 1 date) < day(date of birth))))/12. In the analysis datasets, tables, and listings, age will be reported as the integer part of the derived age, with no rounding.

- All analysis will be performed using the SAS System version 9.4.
- All tables that summarize results by treatment group for subjects in the Full Analysis Set (FAS) or Per Protocol Analysis Set (PPAS), defined in Section 4, will include each of the FX-322 treatment groups, a pooled FX-322 treatment group, and the placebo treatment group. Subjects in the FAS or PPAS will be classified into randomized treatment groups based on their randomized treatment.

Randomized Treatment Group	**# of Subjects**	**Day 1**	**Day 8**	**Day 15**	**Day 21**
FX-322 1x	24	FX-322	Placebo	Placebo	Placebo
FX-322 2x	24	FX-322	FX-322	Placebo	Placebo
FX-322 4x	24	FX-322	FX-322	FX-322	FX-322
FX-322 Pooled	72	FX-322	Placebo or FX-322	Placebo or FX-322	Placebo or FX-322
Placebo	24	Placebo	Placebo	Placebo	Placebo

For assessments completed by ear for each subject, the unit of analysis will be the ear and treated and untreated ears will be summarized separately. Treated ears will be defined as the qualified ears for treatment that received at least one dose of study treatment per the randomized treatment schedule, and untreated ears will be the ears not qualified for treatment. For analyses by ear, the treatment group for each ear will be the subject's randomized treatment group, regardless of actual administration in the given ear. All treated (qualified) ear data will be analyzed, including those with dosing administration errors. All untreated (not qualified) ear data will be analyzed up until any dosing administration error (wrong ear injection). For analyses by ear, data for untreated (not qualified) ears after a dosing administration error will be excluded from FAS and PPAS analyses.

- All tables that summarize results by treatment group for subjects in the Safety Analysis Set (SfAS) will include 1 FX-322 dose received, 2 FX-322 doses received, 4 FX-322 doses received, and placebo (0 FX-322 doses received). For SfAS analyses by subject, all subjects in the SfAS will be classified into actual treatment groups based on the actual number of FX-322 doses received in any ear (qualified or not qualified). In the event that any subject(s) receive 3 FX-322 doses, separate listings for subject(s) receiving 3 FX-322 doses will be created. Subjects receiving 3 FX-322 doses will be included in the total column.

Actual Treatment Group	# of Subjects Planned	Days 1-21
FX-322 1x	24	1 dose of FX-322
FX-322 2x	24	2 doses of FX-322
FX-322 4x	24	4 doses of FX-322
Placebo	24	0 doses of FX-322

For assessments completed by ear for each subject, the unit of analysis will be the ear and treated and untreated ears will be summarized separately. An ear will be considered treated from the time it receives any dose, whether the qualifying ear or not. A subject's treated ear will be classified into an actual treatment group based on the actual number of FX-322 doses received in that ear. An ear will be considered not treated up until the time it receives any dose, whether the qualifying ear or not. Untreated ears will be classified in the Placebo group up until the time it receives any dose, after which the ear will be classified into an actual treatment group based on the actual number of FX-322 doses received in that ear. Pre-dosing measurements will be mapped to treated or untreated based on dosing at Day 1. Subjects with treatment received in the non-qualified ear may contribute more than 1 treated ear to SfAS analyses. In the event that any subject/ear combination(s) receive 3 FX-322 doses, separate reports for subject/ear combination(s) receiving 3 FX-322 doses will be created, as applicable. Subject/ear combination(s) receiving 3 FX-322 doses will be included in the FX-322 total ears column.

For example, a subject treated with FX-322 2x in the qualified ear at Days 1 and 8 and treated with FX-322 2x in the not qualified ear at Days 15 and 21 will be summarized as follows:

a. For summaries by subject: This subject will be included in the FX-322 4x section of the table.
b. For summaries by ear:
    i. The qualified ear data will be reported at all visits in the FX-322 2x section of the table in the Treated column.
    ii. The not qualified ear data up to and including Day 15 will be reported in the Placebo section of the table in the Untreated column.
    iii. The not qualified ear data after Day 15 will be reported at visits after Day 15 in the FX-322 2x section of the table in the Treated column.

4. ANALYSIS SAMPLES

The following definitions will be used to derive the analysis sets for this study.

- Full Analysis Set

The Full Analysis Set is defined as all randomized subjects who receive at least one dose of study drug (regardless of whether a full dose was administered) in the qualified ear per the

randomized treatment schedule. Subjects will be analyzed according to their randomized treatment group, as described in Section 3. For assessments completed by ear for each subject, treated (qualified) and untreated (not qualified) ears will be summarized separately, as described in Section 3. The FAS will be used to explore efficacy.

- Per Protocol Analysis Set

The Per Protocol Analysis Set is defined as subjects receiving all four doses of study drug or placebo in the qualified ear to be treated per the randomization treatment schedule and without major protocol deviations that could interfere with interpretation of the results. These major protocol deviations will be identified prior to breaking the study blind for the IA. Protocol deviations that occur after unblinding for the IA will be evaluated for major/minor classification by the blinded medical and study team prior to breaking the study blind for the CSR. If different from the FAS, the PPAS may be used in additional sensitivity analyses for efficacy. PPAS subjects will be analyzed according to their randomized treatment group, as described in Section 3. For assessments completed by ear for each subject, treated (qualified) and untreated (not qualified) ears will be summarized separately, as described in Section 3.

- Safety Analysis Set

The Safety Analysis Set will include all subjects exposed to study drug or placebo in any ear and will be analyzed according to the actual treatment group regardless of their randomized treatment assignment, as described in Section 3. For safety assessments by subject, the total number of FX-322 received in any ear will be used to assign actual treatment group. For safety assessments completed by ear for each subject, treated and untreated ears will be summarized separately, as described in Section 3. The SfAS will be used for the analysis of safety.

5. STUDY SUBJECTS

5.1. Disposition of Subjects

The disposition of all consented subjects will be summarized in tables by treatment group and overall per Section 3. Subjects who failed screening or were not randomized will only be included in the overall column. The following disposition information will be summarized:

- The number of subjects consented (signed Informed Consent).
- The number and percentage of subjects who failed screening and the reasons for failure (Did not meet criteria, Withdrew consent, Lost to follow-up, Other).
- The number of subjects not randomized versus randomized and the number and percentage of subjects for each randomization stratification factor (Etiology of

hearing loss: Sudden sensorineural hearing loss (SSNHL), Noise-induced hearing loss (NIHL); Word Recognition Score at Screening: >=50% correct, <50% correct).

- The number and percentage of subjects in each analysis set (FAS, PPAS, SfAS).
- The number and percentage of subjects receiving study treatment at each treatment visit (Day 1, 8, 15, 21).
- The number and percentage of subjects who completed the study through Visit 9 (Day 210).
- The number and percentage of subjects who discontinued the study early and the reasons for withdrawal (Adverse Event, Death, Lack of Efficacy, Lost to Follow-up, Non-Compliance With Study Drug, Physician Decision, Pregnancy, Progressive Disease, Protocol Violation, Recovery, Study Terminated by Sponsor, Technical Problem, Withdrawal by Subject, Other).
- The number and percentage of subjects who discontinued the study early for reasons related to COVID-19 (Subject has confirmed COVID-19, Subject decision, Site decision).

Percentages for the number of subjects who failed screening and the reasons for failure will be based on the number of subjects who signed informed consent. All other percentages will use the number of randomized subjects as the denominator. All rows will be summarized by randomized treatment group.

The number and percentage of subjects who attended each visit will be summarized by randomized treatment group for the FAS.

A Kaplan-Meier plot of time from the date of randomization to the date of study completion or early discontinuation will be provided by randomized treatment group for all subjects randomized. Subjects who completed the study will be censored at their study completion date. For the IA, ongoing subjects will be censored at their Day 90 date, if available, or the data snapshot date if the Day 90 date is not available.

Subject disposition data for all subjects randomized will also be listed. A separate listing of subjects who failed screening with the reason for screen failure will be provided.

A listing of all subjects affected by the COVID-19 pandemic (discontinued the study early, had remote visits performed, or missed visits for reasons related due to COVID-19) will be provided by site and subject to document how the subject's participation was impacted by COVID-19.

5.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for demographic and other baseline characteristics will be reported for the FAS and SfAS analysis sets by treatment group and overall. The summary for FAS subjects will be by randomized treatment group and the summary for SfAS subjects will be by actual treatment group. Characteristics to be summarized include:

- Demographic: age, race, ethnicity, and sex.
- Targeted hearing loss history: when hearing loss started, ear(s) with hearing loss, etiology of hearing loss, other symptoms of hearing loss, and hearing aid use.
- Vital Signs: height, body weight, and BMI at screening.
- Pure Tone Average (0.5, 1, 2, and 4 kHz) at screening for the qualified ear.
- Bone Conduction Average (0.5, 1, 2, and 4 kHz) at screening for the qualified ear.
- Word Recognition number of recognized words at screening for the qualified ear.

Demographic and other baseline characteristic data will also be listed for subjects in the FAS.

Medical history and urine drug screen data will be listed for subjects in the FAS.

6. STUDY OPERATIONS

6.1. Protocol Deviations

All protocol deviations will be recorded in [redacted] clinical trial management system (CTMS) and reported in listings for randomized subjects. The listings will include the subject ID, treatment group, date of the deviation, protocol deviation text, and protocol deviation type. The medical and study team will perform a blinded review of the protocol deviations prior to the IA unblinding and database snapshot to classify the protocol deviations as major or minor. Protocol deviations that occur after unblinding for the IA will be evaluated for major/minor classification by the blinded medical and study team prior to study unblinding and database lock. The indicator identifying the deviation as major or minor will be included in the listings.

If the number of major protocol deviations is 10 or more, major protocol deviations will be summarized in tabular format by treatment group, including overall, and type of deviation for randomized subjects.

6.2. Randomization

Subjects will be randomized 1:1:1:1 to four treatment groups to receive multiple doses of FX-322 (0.2 mL) and/or placebo (0.2 mL). A single injection of FX-322 or placebo will be administered on Day 1 by intratympanic injection followed by subsequent injections of either FX-322 or placebo on Day 8, Day 15 and Day 21 as per treatment group. Randomized subjects will be allocated to each of 4 treatment groups (24 in each group).

Treatment Group Number (Label)	# of Subjects	Day 1	Day 8	Day 15	Day 21
1 (FX-322 1x)	24	FX-322	Placebo	Placebo	Placebo
2 (FX-322 2x)	24	FX-322	FX-322	Placebo	Placebo
3 (FX-322 4x)	24	FX-322	FX-322	FX-322	FX-322
4 (Placebo)	24	Placebo	Placebo	Placebo	Placebo

7. ENDPOINT EVALUATION

7.1. Overview of Efficacy Analysis Methods

7.1.1. Multicenter Studies

Study subjects will be enrolled from multiple study sites. For the primary efficacy and safety analyses, study data will be analyzed and summarized as a whole, and no formal accommodation for site-to-site variation will be made.

Sensitivity analyses will be performed for select word recognition endpoints (percentage of recognized words, absolute percent change from baseline, relative percent change from baseline) to assess the possible presence and effect of site-to-site heterogeneity. Basic descriptive analyses of demographics, baseline characteristics or other assessments may be repeated for each site individually to support the word recognition sensitivity analyses.

7.1.2. Assessment Time Windows

The following study visits are scheduled.

Table 7-1 Study Visit Windows

Visit	Visit Number	Assessment/Day	Target Day	Target Window	Analysis Window[a]
Screening	1	-30 to 0	0	0	<0
Baseline/ Treatment	2a	1	1	1	1
Treatment	2b	8	8	6-10 (+/- 2 days)	2-11
	2c	15	15	13-17 (+/- 2 days)	12-17
	2d	21	21	19-23 (+/- 2 days)	18-24
Follow-up	3	30	30	25-45 (+/- 15 days)	25-45
	4	60	60	45-75 (+/- 15 days)	46-75
	5	90	90	75-105 (+/- 15 days)	76-105
	6	120	120	105-135 (+/- 15 days)	106-135
	7	150	150	135-165 (+/- 15 days)	136-165
	8	180	180	165-195 (+/- 15 days)	166-195
	9	210	210	195-225 (+/- 15 days)	196-225

[a]Analysis windows are defined using the mid-points between each scheduled visit, and will be used to map early termination or unscheduled visit data to scheduled visits.

All scheduled visit data per Protocol Version 4.0, including assessments performed outside the target window, will be included in summary tables, figures, and listings. Scheduled visit data will be presented according to the nominal case report form (CRF) recorded visit and not re-mapped according to the analysis windows in Table 7-1. Early termination visit and unscheduled visit efficacy data will be mapped to scheduled visits using the analysis windows in Table 7-1. Early termination visit and unscheduled visit efficacy data will be included in tables and figures if no scheduled visit data is available for the nominal CRF recorded visit. If more than one unscheduled assessment (early termination or unscheduled) is performed within an analysis window, the assessment performed closest to the target day will be used. For safety tables and figures, early termination visit and unscheduled safety data will not be mapped to scheduled visits using analysis windows. Data for assessments that were planned to be performed at on-site study visits in Protocol Version 2.0 and were removed in Protocol Version 3.0 due to the switch to telephone visits will be excluded from tables and figures. All data, scheduled and unscheduled, will be included in listings.

7.1.3. Timing of Analyses

The IA will be performed after all enrolled subjects complete Visit 5 (Day 90) and the database snapshot is completed.

All CSR analyses will be performed after the study is completed and the database is locked.

7.1.4. Multiple Comparisons/Multiplicity

No adjustments for multiple comparisons will be made. All p-values will be considered descriptive.

7.1.5. Model Fitting

The following types of models may be fit for the efficacy endpoints: Mixed-effect model for repeated measures (MMRM), generalized estimating equations (GEE), or logistic regression.

For the MMRM models, a single covariance structure will be applied to all MMRM analyses for an efficacy endpoint. Different covariance structures may be applied to different efficacy endpoints. For the Word Recognition in Quiet (WR) and Words-in-Noise (WIN) analyses, the covariance structure for each endpoint will be identified by comparing model fit for the relative percent change from baseline in percentage of recognized words using two different covariance structures (unstructured [UN] and autoregressive (1) [AR(1)]), as described in Section 7.3.4. For the audiometry analyses, the covariance structure will be identified by comparing model fit for the mean overall Pure Tone Average (PTA) using the two different covariance structures, as described for WR and WIN. For the Tinnitus Functional Index (TFI) analyses, the TFI total score will be used to identify the covariance structure for all TFI endpoint analyses. Covariance structures will also be identified for the Hearing Handicap Inventory for Adults (HHIA) and the Hearing Screening Inventory (HSI) analyses, separately, using the total scores. The covariance structures identified for the IA may be different than the covariance structures for the CSR analyses, based on the evaluation for model fit. Further details for each of the planned models and analyses are provided in subsequent sections.

Models and descriptive summaries for the IA will only include data through Visit 5 (Day 90). Data through Visit 9 (Day 210) will be included in the models and summarized descriptively for the CSR.

7.1.6. Missing Data

Due to the exploratory nature of the study, no formal imputation on missing data will be performed. Missing data due to the COVID-19 pandemic will remain missing in the planned statistical summaries and analyses. Unless otherwise specified, incomplete data for the efficacy endpoints will be assumed missing at random (MAR) and analyzed using the MMRM approach.

For the WR and WIN, the number and percentage of subjects completing each word list at each visit will be summarized for treated ears by randomized treatment group for subjects in the FAS. For the audiometry endpoints, the number and percentage of subjects with non-missing, no response, or not done at each frequency and visit will be summarized for treated ears by randomized treatment group for subjects in the FAS.

Where recorded, visits impacted by the COVID-19 pandemic will be identified in data listings.

Table 7-2 Efficacy Variables and Analysis Methods

Efficacy Endpoint	Analysis Method(s)	Section
Word Recognition in Quiet		7.3
Number of recognized words	• Descriptive summary for treated and untreated ears. • MMRM in treated ears. • Risk ratios of treated ears to untreated ears for all subjects, subjects with bilateral hearing loss, and subjects without bilateral hearing loss.	7.3.1
Percentage of recognized words	• Descriptive summary for treated and untreated ears. • MMRM in treated ears. • Sensitivity: Descriptive summary for treated ears by site.	7.3.2
Absolute percent change from baseline in percentage of recognized words	• Descriptive summary for treated and untreated ears. • Line graphs (with standard error bars) for observed means for treated ears. • Line graphs (with standard error bars) for adjusted (least squares) means for treated ears. • MMRM in treated ears. • Sensitivity: Descriptive summary for treated ears by site.	7.3.3
Relative percent change from baseline in percentage of recognized words	• Descriptive summary for treated and untreated ears. • Line graphs (with standard error bars) for observed means for treated ears. • Line graphs (with standard error bars) for adjusted (least squares) means for treated ears. • MMRM in treated ears. • Sensitivity: Descriptive summary for treated ears by site.	7.3.4
Improvement >=10% (5 words) in percentage of recognized words	• Descriptive summary for treated ears. • GEE in treated ears.	7.3.5
Arcsine-transformed WR	• Descriptive summary for treated ears. MMRM in treated ears.	7.3.6
WR categories	• Descriptive summary for treated ears. • Bar charts for treated ears.	7.3.7

Efficacy Endpoint	Analysis Method(s)	Section
Shift in WR categories	• Descriptive summary for treated ears.	7.3.8
Clinical Improvement in WR, using Thornton-Raffin Confidence Intervals (CI_{TR})	• Descriptive summary for treated ears. • Scatterplots for treated ears. • GEE in treated ears.	7.3.9
Clinical Improvement in WR, using Carney Schlauch Confidence Intervals (CI_{CS})	• Descriptive summary for treated ears. • Scatterplots for treated ears. • GEE in treated ears.	7.3.10
Incidence of incorrect phonemes	• Heat maps for treated ears.	7.3.11
Words-In-Noise		7.4
Number of recognized words	• Descriptive summary for treated and untreated ears. • MMRM in treated ears. • Risk ratio of treated ears to untreated ears for all subjects, subjects with bilateral hearing loss, and subjects without bilateral hearing loss.	7.4.1
Percentage of recognized words	• Descriptive summary for treated and untreated ears. • Line graphs (with standard error bars) for observed means for treated ears (at each signal-to-noise ratio). • MMRM in treated ears.	7.4.2
Absolute percent change from baseline in percentage of recognized words	• Descriptive summary for treated and untreated ears. • Line graphs (with standard error bars) for observed means for treated ears. • Line graphs (with standard error bars) for adjusted (least squares) means for treated ears. • MMRM in treated ears.	7.4.3
Relative percent change from baseline in percentage of recognized words	• Descriptive summary for treated and untreated ears. • Line graphs (with standard error bars) for observed means for treated ears. • Line graphs (with standard error bars) for adjusted (least squares) means for treated ears. • MMRM in treated ears.	7.4.4
Improvement >=10% (5 words) in percentage of recognized words	• Descriptive summary for treated ears. • GEE in treated ears.	7.4.5

Efficacy Endpoint	Analysis Method(s)	Section
Spearman-Kärber estimates of dB thresholds for word recognition (25th, 50th (median) and 75th percentiles)	• Descriptive summary for treated ears. • Scatter plots for treated ears. • MMRM in treated ears.	7.4.6
Improvement >=3 dB shifts in median Spearman-Kärber estimates	• Descriptive summary for treated ears. • GEE in treated ears.	7.4.7
Incidence of incorrect phonemes	• Heat maps for treated ears.	7.4.8
Pure Tone Audiometry		7.5
Air Conduction (AC) and Bone Conduction (BC) audiometry dB hearing thresholds by frequency	• Descriptive summary for treated and untreated ears. • MMRM in treated ears.	7.5.1
AC and BC Improvement >=10 dB from baseline for two contiguous frequencies	• Descriptive summary for treated ears. • GEE in treated ears.	7.5.2
AC and BC Improvement >=15 dB from baseline for any frequency	• Descriptive summary for treated ears. • GEE in treated ears.	7.5.3
AC and BC Composite Improvement	• Descriptive summary for treated ears. • GEE in treated ears.	7.5.4
Mean overall Pure Tone Average (PTA) (AC over 0.5, 1, 2, and 4 kHz) and Bone Conduction Average (BCA) (BC over 0.5, 1, 2 and 4 kHz)	• Descriptive summary for treated ears. • MMRM in treated ears. • Descriptive summary of shift from baseline in PTA categories for treated ears.	7.5.5
Mean low AC dB hearing threshold (AC only over 0.25, 0.5, 1, and 2 kHz frequencies)	• Descriptive summary for treated ears. • MMRM in treated ears.	7.5.6

Efficacy Endpoint	Analysis Method(s)	Section
Mean high AC dB hearing threshold (AC only over 3, 4, 6, and 8 kHz frequencies)	• Descriptive summary for treated ears. • MMRM in treated ears.	7.5.7
Air-Bone Imbalance at 0.5, 1, 2, 3, and 4 kHz	• Descriptive summaries for treated ears.	7.5.8
Composite Endpoint		
Composite WR, WIN and Pure Tone Audiometry Endpoint	• Descriptive summary for treated ears. • Logistic Regression in treated ears.	7.5.9
Extended High Frequency Audiometry (EHFA)		7.6
AC audiometry dB hearing thresholds by frequency	• Descriptive summary for treated and untreated ears. • MMRM in treated ears.	7.6.1
AC Improvement >=10 dB from baseline for two contiguous frequencies	• Descriptive summary for treated ears. • GEE in treated ears.	7.6.2
AC Improvement >=15 dB from baseline for any frequency	• Descriptive summary for treated ears. • GEE in treated ears.	7.6.3
AC Composite Improvement	• Descriptive summary for treated ears. • GEE in treated ears.	7.6.4
Mean dB hearing threshold (AC over 9-16 kHz)	• Descriptive summary for treated ears. • MMRM in treated ears.	7.6.5
Shift from no signal to any signal by frequency	• Descriptive summary for treated ears.	7.6.6
Shift from no signal to any signal in two contiguous frequencies	• Descriptive summary for treated ears.	7.6.7
Shift from no signal to any signal in three contiguous frequencies	• Descriptive summary for treated ears.	7.6.8

Efficacy Endpoint	Analysis Method(s)	Section
Tinnitus Functional Index		7.7
Mean total score, individual subscale scores, and loudness score	• Descriptive summary. • MMRM for total score and loudness score.	7.7.1
Change from baseline in mean total score, individual subscale scores, and loudness score	• Descriptive summary. • MMRM for total score and loudness score. • Correlation analyses.	7.7.2
Hearing Handicap Inventory for Adults		7.8
Mean total score and individual subscale scores	• Descriptive summary. • MMRM.	7.8.1
Change from baseline in mean total score and individual subscale scores	• Descriptive summary. • MMRM. • Correlation analyses.	7.8.2
Hearing Screening Inventory		7.9
Mean total score	• Descriptive summary. • MMRM.	7.9.1
Change from baseline in mean total score	• Descriptive summary. • MMRM. • Correlation analyses.	7.9.2

7.2. Primary Endpoint

There is no single primary endpoint for this study.

7.3. Word Recognition in Quiet

Word Recognition is measured using the incidence of correctly recognized words from recorded consonant-nucleus-consonant (CNC) word lists. The WR test consists of 50 words and the number of and assessed percentage of correctly recognized words out of 50 will be recorded. If all 50 words are not assessed in each ear, the WR test score will not be calculated.

WR will be performed at Screening, Day 15, 60, 90, 150, and 210 visits. Testing will be performed at 30 dB sensation level (SL) regarding the pure tone average of 0.5, 1, and 2 kHz

at Screening. Word lists will be rotated at each visit. Additional details can be found in the study Audiology Manual of Procedures.

Baseline scores for analysis will be computed from the Screening visit. There will be no imputation for missing scores.

7.3.1. Number of recognized words

- *Computation of the Endpoint*

At each visit and by ear (left, right), the number of words classified as correctly recognized out of 50 will be recorded. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3.

In subjects in the FAS with hearing loss in both ears per the Targeted Hearing Loss History CRF page, the ratio of the number of recognized words for treated ear versus untreated ear will be computed.

- *Analysis of the Endpoint*

The number of recognized words will be summarized descriptively for treated and untreated ears separately by visit and treatment group for subjects in the FAS.

Treatment group comparisons will be performed using the approach described in Section 7.3.4 for subjects in the FAS. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words, as described in Section 7.3.4, will be implemented with a repeated statement in SAS.

The ratio of the number of recognized words for treated ears versus untreated ears will be summarized descriptively by visit and treatment group for all subjects in the FAS, subjects in the FAS with bilateral hearing loss, and subjects in the FAS without bilateral hearing loss.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.3.2. Percentage of recognized words

- *Computation of the Endpoint*

Using the number of recognized words in Section 7.3.1, the percentage of recognized words will be computed by visit as the number of recognized words divided by 50 and multiplied by 100, separately for each ear.

- *Analysis of the Endpoint*

The percentage of recognized words will be summarized descriptively for treated and untreated ears using the approach described in Section 7.3.1 for subjects in the FAS.

Treatment group comparisons will be performed using the approach described in Section 7.3.4 for subjects in the FAS. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words, as described in Section 7.3.4, will be implemented with a repeated statement in SAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

The possible presence of site-to-site heterogeneity will be evaluated by descriptively summarizing the percentage of recognized words for treated ears by site, visit, and treatment group for subjects in the FAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.3.3. Absolute percent change from baseline in percentage of recognized words

- *Computation of the Endpoint*

The absolute percent change from baseline to each post-baseline visit in percentage of recognized words will be computed as 100*[(w/50) – (b/50)] where “w” is the frequency of words recognized at the post-baseline visit and “b” is the frequency of words recognized at baseline.

- *Analysis of the Endpoint*

The absolute percent change from baseline in percentage of recognized words will be summarized descriptively for treated and untreated ears using the approach described in Section 7.3.1 for subjects in the FAS.

Line graphs (with standard error bars) will be created for the adjusted (least squares) mean absolute percent change from baseline in percentage of recognized words for treated ears by post-baseline visit and treatment group for subjects in the FAS. Post-baseline visit will be plotted on the x-axis and the adjusted (least squares) mean absolute percent change from baseline in percentage of recognized words for treated ears will be plotted on the y-axis. Each treatment group will be presented as a separate line in the plot. The line graphs will be repeated for the observed mean absolute percent change from baseline in percentage of recognized words for treated ears.

Treatment group comparisons will be performed using the approach described in Section 7.3.4 for subjects in the FAS. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words, as described in Section 7.3.4, will be implemented with a repeated statement in SAS.

- *Sensitivity Analyses of the Endpoint*

With the exception of the line graphs, the analyses will be repeated for subjects in the PPAS.

The possible presence of site-to-site heterogeneity will be evaluated using the approach described in Section 7.3.2 for subjects in the FAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.3.4. Relative percent change from baseline in percentage of recognized words

- *Computation of the Endpoint*

The relative percent change from baseline to each post-baseline visit in percentage of recognized words will be computed as 100*[(w-b)/b] where "w" is the frequency of words recognized at the post-baseline visit and "b" is the frequency of words recognized at baseline.

- *Analysis of the Endpoint*

The relative percent change from baseline in percentage of recognized words will be summarized descriptively for treated and untreated ears using the approach described in Section 7.3.1 for subjects in the FAS.

Line graphs (with standard error bars) for the adjusted (least squares) means and observed means will be created using the approach described in Section 7.3.3 for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for the relative percent change from baseline in percentage of recognized words will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. Separate models will be fit specifying UN and AR(1) covariance structures (implemented with a repeated statement in SAS). The covariance structure providing the lowest corrected Akaike's Information Criteria (AICc) and Bayesian Information Criteria (BIC) values from the model will be implemented for the relative percent change from baseline and all other WR endpoints. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated Least squares (LS) Mean differences by visit) and overall (e.g., Type III Sums of Squares).

- *Sensitivity Analyses of the Endpoint*

With the exception of the line graphs, the analyses will be repeated for subjects in the PPAS.

The possible presence of site-to-site heterogeneity will be evaluated using the approach described in Section 7.3.2 for subjects in the FAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.3.5. Improvement >=10% (5 words) in percentage of recognized words

- *Computation of the Endpoint*

Improvement >=10% in percentage of recognized words at each post-baseline visit for treated ears will be computed as 1 if the absolute percent change from baseline in percentage of recognized words >=10% and as 0, otherwise where absolute percent change from baseline is non-missing.

- *Analysis of the Endpoint*

The incidence of improvement >=10% in percentage of recognized words at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for incidence of improvement >=10% in percentage of recognized words may be analyzed using a generalized estimating equation based on the binomial distribution with logit link. The fixed covariates may include treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. Covariates for WR score at baseline and etiology of hearing loss may be dropped if the model fails to converge. A random effect for within-subject, repeated measures variability may be included. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Odds ratios, 95% CIs, and p-values may be reported and comparisons will be made by visit and overall.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.3.6. Arcsine-transformed WR

- *Computation of the Endpoint*

The arcsine-transformed WR (Studebaker, 1985) will be computed by visit for treated ears as

$$RR = \text{aaaaaassssss}\sqrt{ww\ (ss + 1)} + \text{aaaaaassssss}\sqrt{(ww + | | + 1)}$$

where "R" is the transformed radians, "w" is the frequency of words recognized, and "n" is the number of words in the word test (n = 50 for WR assessments).

- *Analysis of the Endpoint*

The arcsine-transformed WR will be summarized descriptively for treated ears using the approach described in Section 7.3.1 for subjects in the FAS.

Treatment group comparisons will be performed using the approach described in Section 7.3.4 for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.3.7. WR Categories

- *Computation of the Endpoint*

Using the percentage of recognized words in Section 7.3.2 by visit and for each ear, percentages will be categorized as follows, using the Schoepflin (2012) justification:

- Excellent: >85%
- Good: 68 to 85%
- Fair: 51 to 67%
- Poor 33 to 50%
- Very Poor: 15 to 32%
- Extremely Poor: <15%

- *Analysis of the Endpoint*

The distribution of WR categories at each baseline and post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

Bar charts will be created for the distribution of WR categories at each baseline and post-baseline visit for treated ears by visit and treatment group for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

With the exception of the bar charts, the analyses will be repeated for subjects in the PPAS.

7.3.8. Shift in WR Categories

- *Computation of the Endpoint*

Using the WR Categories in Section 7.3.7 by visit for treated ears, shifts from baseline to each post baseline-visit will be computed (e.g., Fair to Good).

- *Analysis of the Endpoint*

The distribution in the shift in WR categories from baseline to each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.3.9. Clinical Improvement in WR, using Thornton-Raffin Confidence Intervals (CI_{TR})

- *Computation of the Endpoint*

Clinical improvement in WR at post-baseline visits for treated ears will be defined by comparing Thornton-Raffin (1978) 95% confidence intervals (CI_{TR}) (Section 14.1) at the baseline visit versus the percentage of recognized words at the post-baseline visits. It will be defined as 'Improved' if the subject's post-baseline score is higher than the upper limit of the subject's baseline 95% CI_{TR}, as 'No change' if the post-baseline score falls within or on the bounds of the baseline 95% CI_{TR}, and 'Reduction' if the post-baseline score is lower than the baseline lower limit of the 95% CI_{TR}.

The Thornton-Raffin 95% CI_{TR} reported in Section 14.1 will be used directly and not computed.

- *Analysis of the Endpoint*

The incidence of clinical improvement in WR (Improvement, No Change, Reduction) based on CI_{TR} at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

Scatter plots will be used to summarize clinical improvement in the percentage of recognized words (Section 7.3.2) in treated ears. The percentage of recognized words at baseline will be plotted on the x-axis and the percentage of recognized words post-baseline will be plotted in the y-axis, with a separate plot for each post-baseline visit. The baseline Thornton-Raffin 95% confidence intervals in Section 14.1 will be overlaid in the plot.

In treated ears only for subjects in the FAS, treatment group comparisons for incidence of clinical improvement in WR may be analyzed using a generalized estimating equation based on the multinomial distribution with generalized logit (nominal) link. The fixed covariates may include treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. Covariates for WR score at baseline and etiology of hearing loss may be dropped if the model fails to converge. A random effect for within-subject, repeated measures variability may be included. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Proportional odds ratios, 95% CIs, and p-values may be reported and comparisons will be made by visit and overall.

- *Sensitivity Analyses of the Endpoint*

With the exception of the scatter plots, the analyses will be repeated for subjects in the PPAS.

7.3.10. Clinical Improvement in WR, using Carney Schlauch Confidence Intervals (CI_{CS})

- *Computation of the Endpoint*

Clinical improvement in WR at post-baseline visits for treated ears will be defined by comparing Carney Schlauch (2007) 95% confidence intervals (CI_{CS}) (Section 14.2) at the baseline visit versus the percentage of recognized words at the post-baseline visits. It will be defined as 'Improved' if the subject's post-baseline score is higher than the upper limit of the subject's baseline 95% CI_{CS}, as 'No change' if the post-baseline score falls within or on the bounds of the baseline 95% CI_{cs}, and 'Reduction' if the post-baseline score is lower than the baseline lower limit of the 95% CI_{cs}.

The Carney Schlauch 95% CI_{cs} reported in Section 14.2 will be used directly and not computed.

- *Analysis of the Endpoint*

Descriptive summaries, scatter plots, and treatment group comparisons may be performed for clinical improvement in WR (Improvement, No Change, Reduction) based on CI_{CS} as described in Section 7.3.9. The Carney Schlauch 95% confidence intervals will be utilized instead of the Thornton-Raffin 95% confidence intervals.

- *Sensitivity Analyses of the Endpoint*

With the exception of the scatter plots, the analyses will be repeated for subjects in the PPAS.

7.3.11. Incidence of incorrect phonemes

- *Computation of the Endpoint*

In each word recognition list, there are 150 phonemes represented across the 50 words with exactly 3 phonemes per word. The 150 phonemes are not uniformly or uniquely represented, but distributed with frequencies empirically approximating real-world use (Causey et al., 1984). For each unique phoneme, the percentage not recognized correctly will be computed by subject, visit, and ear.

- *Analysis of the Endpoint*

Heat maps will be produced by visit and treatment group for treated ears to graphically display the incidence of incorrect phonemes for subjects in the FAS. The x-axis will include a column for each unique phoneme and the y-axis will include a row for each subject. The shading for each cell of the map will represent the percentage of phonemes not recognized correctly.

Shading Color	Percentage Incorrect
	>=90%
	80 to <90%
	70 to <80%
	60 to <70%
	50 to <60%
	40 to <50%
	30 to <40%
	20 to <30%
	10 to <32%
	<=10%

Post hoc analyses may be performed on select unique phonemes of interest, based on the final results of the heat map described above. The post hoc analyses may include heat maps by visit for treated ears also taking into account the location of the phoneme within the word (phoneme/location) for subjects in the FAS. Post hoc treatment group comparisons may also be performed.

- *Sensitivity Analyses of the Endpoint*

None.

7.4. Words-In-Noise

WIN is measured using the incidence of correctly recognized words in the presence of background noise using multitalker babble (Wilson et al., 2003). Materials are recorded at 7 signal-to-noise ratios (0, 4, 8, 12, 16, 20, 24 dB) that are presented in a descending manner. The test consists of 2 lists of 35 recorded words (70 total) for each ear and the number of and percentage of correctly recognized words at each signal-to-noise ratio will be recorded separately by ear. The overall WIN score will be recorded as the percentage of correctly recognized words across all signal-to-noise ratios by visit and ear. If all 70 words are not assessed in each ear, the WIN test score will not be calculated.

The WIN test will be performed at Screening, Day 15, 60, 90, 150, and 210 visits. Testing will be performed at 80 dB sound pressure level (SPL) and word lists will be rotated at each visit. Additional details can be found in the Audiology Manual of Procedures.

Baseline scores for analysis will be computed from the Screening visit. There will be no imputation for missing scores.

7.4.1. Number of recognized words

- *Computation of the Endpoint*

At each visit and by ear (left, right), the number of words classified as correctly recognized at each signal-to-noise ratio will be recorded. The overall WIN score will also be computed as the number of words correctly recognized across all signal-to-noise ratios. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3.

In subjects in the FAS with hearing loss in both ears per the Targeted Hearing Loss History CRF page, the ratio of the number of recognized words for treated ear versus untreated ear will be computed.

- *Analysis of the Endpoint*

The number of recognized words at each signal-to-noise ratio and overall will be summarized descriptively for treated and untreated ears separately by visit and treatment group for subjects in the FAS.

Treatment group comparisons for the number of recognized words overall will be performed using the approach described in Section 7.4.4 for subjects in the FAS. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words, as described in Section 7.4.4, will be implemented with a repeated statement in SAS. Treatment group comparisons will not be performed for each signal-to-noise ratio.

The ratio of the number of recognized words for treated ears versus untreated ears will be summarized descriptively by visit and treatment group for all subjects in the FAS, subjects in the FAS with bilateral hearing loss, and subjects in the FAS without bilateral hearing loss.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.4.2. Percentage of recognized words

- *Computation of the Endpoint*

Using the number of recognized words in Section 7.4.1, the percentage of recognized words will be computed for each signal-to-noise ratio by visit as the number of recognized words divided by 10 and multiplied by 100, separately for each ear. The percentage of recognized words overall by visit will be computed as the number of recognized words divided by 70 and multiplied by 100, separately for each ear.

- *Analysis of the Endpoint*

The percentage of recognized words at each signal-to-noise ratio and overall will be summarized descriptively for treated and untreated ears using the approach described in Section 7.4.1 for subjects in the FAS.

Line graphs (with standard error bars) will be created for the observed percentage of recognized words at each signal-to-noise ratio for treated ears by visit and treatment group for subjects in the FAS. The signal-to-noise ratio will be plotted on the x-axis and the

percentage of recognized words will be plotted on the y-axis. There will be separate lines for each treatment group and possibly separate plots for each visit. All treatment groups and visits may be included in a single plot, if the lines are not too crowded.

Treatment group comparisons for the percentage of recognized words overall will be performed using the approach described in Section 7.4.4 for subjects in the FAS. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words, as described in Section 7.4.4, will be implemented with a repeated statement in SAS. Treatment group comparisons will not be performed for each signal-to-noise ratio.

- *Sensitivity Analyses of the Endpoint*

With the exception of the line graphs, the analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.4.3. Absolute percent change from baseline in percentage of recognized words

- *Computation of the Endpoint*

The absolute percent change from baseline to each post-baseline visit in percentage of recognized words at each signal-to-noise ratio will be computed as 100*[(w/10) – (b/10)] where “w” is the frequency of words recognized at the post-baseline visit and “b” is the frequency of words recognized at baseline. The absolute percent change from baseline to each post-baseline visit in percentage of recognized words overall will be computed using the same algorithm, but with 70 words in the denominator instead of 10.

- *Analysis of the Endpoint*

The absolute percent change from baseline in percentage of recognized words at each signal-to-noise ratio and overall will be summarized descriptively for treated and untreated ears using the approach described in Section 7.4.1 for subjects in the FAS.

Line graphs (with standard error bars) will be created for the adjusted (least squares) mean absolute percent change from baseline in percentage of recognized words overall for treated ears by post-baseline visit and treatment group for subjects in the FAS. Post-baseline visit will be plotted on the x-axis and the adjusted (least squares) mean absolute percent change from baseline in percentage of recognized words overall for treated ears will be plotted on the y-axis. Each treatment group will be presented as a separate line in the plot. The line graphs will be repeated for the observed mean absolute percent change from baseline in percentage of recognized words overall for treated ears.

Treatment group comparisons for the absolute percent change from baseline in percentage of recognized words overall will be performed using the approach described in Section 7.4.4

for subjects in the FAS. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words overall, as described in Section 7.4.4, will be implemented with a repeated statement in SAS. Treatment group comparisons will not be performed for each signal-to-noise ratio.

- *Sensitivity Analyses of the Endpoint*

With the exception of the line graphs, the analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.4.4. Relative percent change from baseline in percentage of recognized words

- *Computation of the Endpoint*

The relative percent change from baseline to each post-baseline visit in percentage of recognized words at each signal-to-noise ratio will be computed as 100*[(w-b)/b] where "w" is the frequency of words recognized at the post-baseline visit and "b" is the frequency of words recognized at baseline. The relative percent change from baseline to each post-baseline visit in percentage of recognized words overall will be computed using the same algorithm, but with "w" and "b" scores for all 70 words at each visit.

- *Analysis of the Endpoint*

The relative percent change from baseline in percentage of recognized words at each signal-to-noise ratio and overall will be summarized descriptively for treated and untreated ears using the approach described in Section 7.4.1 for subjects in the FAS.

Line graphs (with standard error bars) for the adjusted (least squares) means and observed means will be created using the approach described in Section 7.4.3 for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for the relative percent change from baseline in percentage of recognized words overall will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. As described in Section 7.3.4, separate models will be fit specifying UN and AR(1) covariance structures (implemented with a repeated statement in SAS). The covariance structure providing the lowest corrected AICc and BIC values from the model will be implemented for the relative percent change from baseline and all other WIN endpoints. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares). Treatment group comparisons will not be performed for each signal-to-noise ratio.

- *Sensitivity Analyses of the Endpoint*

With the exception of the line graphs, the analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.4.5. Improvement >=10% (7 words) in percentage of recognized words overall

- *Computation of the Endpoint*

Improvement >=10% in percentage of recognized words overall at each post-baseline visit for treated ears will be computed as 1 if the absolute percent change from baseline in percentage of recognized words overall >=10% and as 0, otherwise where the absolute percent change is not missing. Improvement >=10% (7 words) in percentage of recognized words will not be computed for each signal-to-noise ratio.

- *Analysis of the Endpoint*

The incidence of improvement >=10% in percentage of recognized words overall at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for incidence of improvement >=10% in percentage of recognized words overall may be analyzed using a generalized estimating equation based on the binomial distribution with logit link. The fixed covariates may include treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. Covariates for WR score at baseline and etiology of hearing loss may be dropped if the model fails to converge. A random effect for within-subject, repeated measures variability may be included. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Odds ratios, 95% CIs, and p-values may be reported and comparisons will be made by visit and overall.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.4.6. Spearman-Kärber estimates of dB thresholds for word recognition (25^{th}, 50^{th} (median) and 75^{th} percentiles)

- *Computation of the Endpoint*

The Spearman-Kärber estimates (25^{th}, 50^{th} (median) and 75^{th} percentiles) of dB thresholds for word recognition (Wilson et al., 1973) at each visit for treated ears will be computed as

$$dddd = ss + (pp_{cc} * dd) - (dd * ww)/aa$$

where "p_c" is the percentile of interest, "I" is the initial dB presentation level (fixed at 24), "d" is the attenuation dB step size (fixed at 4), "c" is the number of words per decrement (fixed at 10), and "w" is the frequency of words recognized overall at each visit.

- *Analysis of the Endpoint*

Spearman-Kärber estimates (25th, 50th (median) and 75th percentiles) will be summarized descriptively for treated ears by visit and treatment group for subjects in the FAS.

For each post-baseline visit, scatter plots of the median Spearman-Kärber estimates at baseline versus the post-baseline visit will be produced by treatment group for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for the median Spearman-Kärber estimate will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. The covariance structure (UN or AR(1)) that provides the best model fit for the relative percent change from baseline in percentage of recognized words overall, as described in Section 7.4.4, will be implemented with a repeated statement in SAS. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares).

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.4.7. Improvement >=3 dB shifts in median Spearman-Kärber estimates

- *Computation of the Endpoint*

Improvement >= 3 dB shifts in median Spearman-Kärber estimates at each post-baseline visit for treated ears will be computed as 1 if the post-baseline Spearman-Kärber estimate – baseline Spearman-Kärber estimate is >=3 and as 0, otherwise where the baseline and post-baseline Spearman-Kärber estimates are not missing.

- *Analysis of the Endpoint*

The incidence of improvement >=3 dB shifts in median Spearman-Kärber estimates at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

Treatment group comparisons may be performed using the approach described in Section 7.4.5 for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.4.8. Incidence of incorrect phonemes

- *Computation of the Endpoint*

For each unique phoneme, the percentage not recognized correctly over all signal-to-noise ratios will be computed by subject, visit, and ear.

Post hoc endpoints may be derived for phonemes not recognized correctly at each signal-to-noise ratio.

- *Analysis of the Endpoint*

Heat maps will be produced for the percentage of phonemes not recognized correctly by visit and treatment group for treated ears using the same approach described in Section 7.3.11.

Heat maps at each signal-to-noise ratio may also be produced post-hoc. Additional post hoc analyses may be produced on select unique phonemes of interest, based on the final results in the heat map described above. The post hoc analyses may include heat maps by visit for treated ears also taking into account the location of the phoneme with in the word (phoneme/location) for subjects in the FAS. Post hoc treatment group comparisons may also be performed.

- *Sensitivity Analyses of the Endpoint*

None.

7.5. Pure Tone Audiometry

The pure tone audiometry test will be used to determine a subject's threshold for hearing at the following frequencies transmitted via air and bone: air tested at 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz; and bone tested at 0.5, 1, 2, 3 and 4 kHz. If both masked and unmasked results are available for the same visit, date, ear, and frequency, the masked results will be used for analysis. This test will be performed at Screening, Day 15, 60, 90, 150, and 210.

Baseline scores for analysis will be computed from the Screening visit. For measurements post-Screening, missing response values for a given frequency, with classification of "NR" (no response due to subject audiometric thresholds greater than the maximal calibrated dB of the equipment), will be imputed using the maximum dB for the audiometry equipment of a given site plus 5 dB and classified as no signal at that frequency. Missing response values at Screening will remain missing.

7.5.1. Air and Bone Conduction audiometry dB hearing thresholds by frequency

- *Computation of the Endpoint*

At each visit and by ear (left, right), the AC and BC audiometry dB hearing thresholds will be recorded for each frequency (Air: 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz; Bone: 0.5, 1, 2, 3, and 4 kHz). Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing values will be imputed per Section 7.5.

- *Analysis of the Endpoint*

Separately for AC and BC, the hearing threshold at each frequency will be summarized descriptively for treated and untreated ears separately by visit and treatment group for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for AC hearing threshold at each frequency will be analyzed using separate mixed-effect, repeated measures models using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. The covariance structure (UN or AR(1)) that provides the best model fit for the PTA, as described in Section 7.5.5, will be implemented with a repeated statement in SAS. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares) for each frequency. The analysis will be repeated for BC hearing thresholds.

A scatter plot of the mean AC audiometry thresholds by frequency will be prepared for treated and untreated ears for each treatment group with lines connecting each point.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.5.2. AC and BC Improvement >=10 dB from baseline for two contiguous frequencies

- *Computation of the Endpoint*

AC Improvement >=10 dB from baseline for two contiguous frequencies at each post-baseline visit for treated ears will be computed as 1 if the post-baseline threshold – baseline threshold is >=10 dB for two contiguous frequencies and as 0, otherwise. The endpoint will utilize imputed values per Section 7.5. The BC Improvement >=10 dB will be derived using the same approach.

- *Analysis of the Endpoint*

The incidence of AC improvement >=10 dB from baseline for two contiguous frequencies at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS. The summary will be repeated for BC improvement.

In treated ears only for subjects in the FAS, treatment group comparisons for incidence of AC improvement >=10 dB from baseline for two contiguous frequencies may be analyzed using a generalized estimating equation based on the binomial distribution with logit link. The fixed covariates may include treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. Covariates for WR score at baseline and etiology of hearing loss may be dropped if the model fails to converge. A random effect for within-subject, repeated measures variability may be included. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Odds ratios, 95% CIs, and p-values may be reported and comparisons will be made by visit and overall. The analysis will be repeated for BC improvement.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.5.3. AC and BC Improvement >=15 dB from baseline for any frequency

- *Computation of the Endpoint*

AC Improvement >=15 dB from baseline for any frequency at each post-baseline visit for treated ears will be computed as 1 if the post-baseline threshold – baseline threshold is >=15 dB for any frequency and as 0, otherwise. The endpoint will utilize imputed values per Section 7.5. The BC Improvement >=15 dB will be derived using the same approach.

- *Analysis of the Endpoint*

The incidence of AC improvement >=15 dB from baseline for any frequency at each post-baseline visit for treated ears will be summarized using the approach described in Section 7.5.2 for subjects in the FAS. The summary will be repeated for BC improvement.

Treatment group comparisons for AC improvement and BC improvement may be performed using the approach described in Section 7.5.2 for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.5.4. AC and BC Composite Improvement

- *Computation of the Endpoint*

The AC Composite Improvement endpoint is defined as the occurrence of either or both of the following conditions for a given subject's ear for a given visit: 1) AC Improvement >=10 dB from baseline for two contiguous frequencies as described in Section 7.5.2 or 2) AC improvement >=15 dB from baseline for any frequency as defined in Section 7.5.3. BC Composite Improvement is defined using the same approach.

- *Analysis of the Endpoint*

The incidence of AC Composite Improvement at each post-baseline visit for treated ears will be summarized using the approach described in Section 7.5.2 for subjects in the FAS. The summary will be repeated for BC Composite Improvement.

Treatment group comparisons for AC Composite Improvement and BC Composite Improvement may be performed using the approach described in Section 7.5.2 for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.5.5. Mean overall PTA (AC over 0.5, 1, 2, and 4 kHz) and BCA (BC over 0.5, 1, 2 and 4 kHz)

- *Computation of the Endpoint*

At each visit and by ear (left, right), the mean overall PTA will be derived by averaging the AC audiometry dB hearing thresholds over 0.5, 1, 2, and 4 kHz frequencies. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing values will be imputed per Section 7.5. The BCA will be derived using the same approach. If hearing thresholds are missing for any of the 0.5, 1, 2, and 4 kHz frequencies (after imputation of records with classification of "NR"), PTA or BCA will be missing, respectively.

Mean overall PTA for each ear will be categorized into <26, 26-40, 41-55, 56-70, and >70 for each visit and shifts from baseline to each post-baseline visit will be computed (e.g., 26-40 to 41-55).

- *Analysis of the Endpoint*

The mean overall PTA will be summarized descriptively for treated ears separately by visit and treatment group for subjects in the FAS. The summary will be repeated for BCA.

In treated ears only for subjects in the FAS, treatment group comparisons for mean overall PTA will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss

(SSNHL versus NIHL), visit, and interaction between visit and treatment group. As described in Section 7.3.4, separate models will be fit specifying UN and AR(1) covariance structures (implemented with a repeated statement in SAS). The covariance structure providing the lowest corrected AICc and BIC values from the model will be implemented for the PTA and all other audiometry endpoints. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares). The treatment group comparisons will also be performed for BCA.

The distribution in the shift in mean overall PTA categories for treated ears from baseline to each post-baseline visit will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.5.6. Mean low AC dB hearing threshold (AC over 0.25, 0.5, 1, and 2 kHz)

- *Computation of the Endpoint*

At each visit and by ear (left, right), the mean low AC dB hearing threshold will be derived by averaging the AC audiometry dB hearing thresholds over 0.25, 0.5, 1, and 2 kHz frequencies. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing values will be imputed per Section 7.5.

- *Analysis of the Endpoint*

The mean low AC dB hearing threshold will be summarized descriptively for subjects in the FAS as described in Section 7.5.5. Treatment group comparisons will also be performed as described in Section 7.5.5.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.5.7. Mean high AC dB hearing threshold (AC over 3, 4, 6 and 8 kHz)

- *Computation of the Endpoint*

At each visit and by ear (left, right), the mean high AC dB hearing threshold will be derived by averaging the AC audiometry dB hearing thresholds over 3, 4, 6 and 8 kHz frequencies. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing values will be imputed per Section 7.5.

- *Analysis of the Endpoint*

The mean high AC dB hearing threshold will be summarized descriptively for subjects in the FAS as described in Section 7.5.5. Treatment group comparisons will also be performed as described in Section 7.5.5.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.5.8. Air-Bone Imbalance at 0.5, 1, 2, 3, and 4 kHz

- *Computation of the Endpoint*

At each visit and by ear (left, right), Air-Bone imbalance is computed as AC-BC dB hearing thresholds at 0.5, 1, 2, 3, and 4 kHz. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing threshold values will be imputed per Section 7.5 prior to the derivation.

Air-bone imbalance at each frequency will be categorized into <=10 dB versus >10 dB for each visit and shifts from baseline to each post-baseline visit will be computed (e.g., <=10 dB to >10 dB).

- *Analysis of the Endpoint*

The Air-Bone imbalance at each frequency (0.5, 1, 2, 3, and 4 kHz) will be summarized descriptively for treated ears by visit and treatment group for subjects in the FAS.

The distribution in the shift in air-bone imbalance for treated ears at each frequency from baseline to each post-baseline visit will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.5.9. Composite WR, WIN and Pure Tone Audiometry Endpoint

- *Computation of the Endpoint*

The composite endpoint will be assessed in treated ears and defined as simultaneously meeting both of the following conditions at any visit (Day 90 or 210):

- A post-baseline >=10% WR improvement OR a >=10% WIN improvement, and
- A post-baseline 10 dB improvement in 2 contiguous frequencies or a post-baseline 15 dB improvement in any frequency for AC thresholds

Post-baseline >=10% WR improvement is defined in Section 7.3.5 and >=10% WIN improvement is defined in Section 7.4.5. Post-baseline >= 10 dB improvement in 2 contiguous frequencies for AC thresholds is defined in Section 7.5.2 and post-baseline >= 15 dB in any frequency for AC thresholds is defined in Section 7.5.3.

The composite endpoint will be computed as 1 if both conditions are met in the treated ear at the same visit for any of the post-baseline visits (Day 90 and 210) and as 0, otherwise.

- *Analysis of the Endpoint*

The incidence of the composite WR, WIN and pure tone audiometry endpoint for treated ears will be summarized using frequencies and percentages by treatment group for subjects in the FAS.

In treated ears only for subjects in the FAS, treatment group comparisons for the composite endpoint will be analyzed using a logistic regression model. The fixed covariates will include treatment group, WR score at baseline (<50% versus >=50%), and etiology of hearing loss (SSNHL versus NIHL). The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Odds ratios, their 95% CIs, and p-values will be reported.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.6. Extended High Frequency Audiometry

EHFA will be performed to determine a subject's threshold dB for hearing at frequencies beyond those in standard pure tone audiometry. Over-air frequencies used (no bone testing) are 9, 10, 11.2, 12.4, 14, and 16 kHz. If both masked and unmasked results are available for the same visit, date, ear, and frequency, the masked results will be used for analysis. This test will be performed at Screening, Day 15, 60, 90, 150, and 210.

Baseline scores for analysis will be computed from the Screening visit. For measurements post-Screening, missing response values for a given frequency with classification of "NR" (no

response due to subject audiometric thresholds greater than the maximal calibrated dB of the equipment), will be imputed using the maximum dB for the audiometry equipment of a given site plus 5 dB and classified as no signal at that frequency. Missing response values at Screening will remain missing.

7.6.1. Air audiometry dB hearing thresholds by frequency

- *Computation of the Endpoint*

At each visit and by ear (left, right), the AC audiometry dB hearing thresholds will be recorded for each frequency (9, 10, 11.2, 12.4, 14, and 16 kHz). Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing values will be imputed per Section 7.6.

- *Analysis of the Endpoint*

Descriptive summaries, treatment group comparisons, and the scatter plot will be performed for AC audiometry dB hearing thresholds as described in Section 7.5.1.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.6.2. AC Improvement >=10 dB from baseline for two contiguous frequencies

- *Computation of the Endpoint*

AC Improvement >=10 dB from baseline for two contiguous frequencies at each post-baseline visit for treated ears will be computed as 1 if the post-baseline threshold – baseline threshold is >=10 dB for two contiguous frequencies and as 0, otherwise. The endpoint will utilize imputed values per Section 7.6.

- *Analysis of the Endpoint*

Descriptive summaries and treatment group comparisons may be performed for AC improvement >=10 dB from baseline for two contiguous frequencies as described in Section 7.5.2.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.6.3. AC Improvement >=15 dB from baseline for any frequency

- *Computation of the Endpoint*

AC Improvement >=15 dB from baseline for any frequency at each post-baseline visit for treated ears will be computed as 1 if the post-baseline threshold – baseline threshold is >=15 dB for any frequency and as 0, otherwise. The endpoint will utilize imputed values per Section 7.6.

- *Analysis of the Endpoint*

Descriptive summaries and treatment group comparisons may be performed for AC improvement >=15 dB from baseline for any frequency as described in Section 7.5.3.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.6.4. AC Composite Improvement

- *Computation of the Endpoint*

The AC Composite Improvement endpoint is defined as the occurrence of either or both of the following conditions for a given subject's ear for a given visit: 1) AC Improvement >=10 dB from baseline for two contiguous frequencies as described in Section 7.6.2 or 2) AC improvement >=15 dB from baseline for any frequency as defined in Section 7.6.3.

- *Analysis of the Endpoint*

The incidence of AC Composite Improvement at each post-baseline visit for treated ears will be summarized using the approach described in Section 7.6.2 for subjects in the FAS.

Descriptive summaries and treatment group comparisons may be performed for AC Composite Improvement as described in Section 7.5.4.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.6.5. Mean dB hearing threshold (AC over 9-16 kHz)

- *Computation of the Endpoint*

At each visit and by ear (left, right), the mean dB hearing threshold will be derived by averaging the AC audiometry dB hearing thresholds over 9-16 kHz frequencies. Left and right ears will be mapped to treated and untreated for analysis, as described in Section 3. Missing values will be imputed per Section 7.6.

- *Analysis of the Endpoint*

Descriptive summaries and treatment group comparisons will be performed for mean dB hearing threshold as described in Section 7.5.5.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

If there are any dosing administrations errors such that a not qualified ear is treated, the descriptive summaries by treated and untreated ears will be repeated for subjects in the FAS including all treated and untreated ear data.

7.6.6. Shift from no signal to any signal by frequency

- *Computation of the Endpoint*

For each frequency, shifts from no signal to any signal will be computed in subjects in the FAS with no signal (missing/imputed hearing response) at baseline in the treated ear. At each post-baseline visit, subjects with any signal (any non-missing/not imputed hearing response) in the treated ear will be flagged as 1 and as 0, otherwise. The endpoint will utilize imputed values per Section 7.6.

- *Analysis of the Endpoint*

For each frequency, the incidence of shifts from no signal at baseline to any signal at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects with no signal at baseline in the FAS.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.6.7. Shift from no signal to any signal in two contiguous frequencies

- *Computation of the Endpoint*

Shifts from no signal in two contiguous frequencies at baseline to any signal in two contiguous frequencies at each post-baseline visit will be computed in subjects in the FAS with no signal (missing/imputed hearing response) at baseline in the treated ear for any two contiguous frequencies. At each post-baseline visit, subjects with any signal (any non-missing/not imputed hearing response) in the treated ear at the same two contiguous frequencies will be flagged as 1 and as 0, otherwise. The endpoint will utilize imputed values per Section 7.6.

- *Analysis of the Endpoint*

The incidence of shifts from no signal in two contiguous frequencies at baseline to any signal in two contiguous frequencies at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS with no signal in any two contiguous frequencies at baseline in the treated ear.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS

7.6.8. Shift from no signal to any signal in three contiguous frequencies

- *Computation of the Endpoint*

Shifts from no signal in three contiguous frequencies at baseline to any signal in three contiguous frequencies at each post-baseline visit will be computed in subjects in the FAS with no signal (missing/imputed hearing response) at baseline in the treated ear for any three contiguous frequencies. At each post-baseline visit, subjects with any signal (any non-missing/not imputed hearing response) in the treated ear in the same three contiguous frequencies will be flagged as 1 and as 0, otherwise. The endpoint will utilize imputed values per Section 7.6.

- *Analysis of the Endpoint*

The incidence of shifts from no signal in three contiguous frequencies at baseline to any signal in three contiguous frequencies at each post-baseline visit for treated ears will be summarized using frequencies and percentages by visit and treatment group for subjects in the FAS with no signal in three contiguous frequencies at baseline in the treated ear.

- *Sensitivity Analyses of the Endpoint*

The analyses will be repeated for subjects in the PPAS.

7.7. Tinnitus Functional Index

The TFI is a 25-item questionnaire that can be used to quantify the treatment-related change in tinnitus. The TFI includes individual item scores, a total score, and eight subscales addressing: the intrusiveness of tinnitus, the sense of control the patient has, cognitive interference, sleep disturbance, auditory issues, relaxation issues, quality of life, and emotional distress. All instrument items, except items 1 and 3, are on an inverted 0-10 scale with 0 being most favorable and 10 being least favorable. Items 1 and 3 are on an inverted 0% to 100% scale with 0% being most favorable and 100% being least favorable. The total score and individual subscale scores are the average item response (all subscales consist of 3 or 4 items) that is then scaled to 0-100. Detailed scoring instructions are in Section 14.3. The subject will complete the instrument (either directly onto the questionnaire in person, via web, or over the phone) at the Day 1, 60, 120, and 210 visits.

Baseline scores for analysis will be computed from the Day 1 visit. No imputation will be performed for missing data and Section 14.3 provides further detail regarding the impact of missing data on TFI scores. No sensitivity analyses will be performed for the TFI endpoints.

7.7.1. Mean total score, individual subscale scores, and loudness score

- *Computation of the Endpoint*

At each visit, the mean total TFI score and individual subscale scores will be computed per Section 14.3. The loudness score will be the response from item 2.

- *Analysis of the Endpoint*

The mean total TFI score, individual subscale scores, and loudness score will be summarized descriptively by visit and treatment group for subjects in the FAS.

For subjects in the FAS, treatment group comparisons for the mean total TFI score will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. As described in Section 7.3.4, separate models will be fit specifying UN and AR(1) covariance structures (implemented with a repeated statement in SAS). The covariance structure providing the lowest corrected AICc and BIC values from the model will be implemented for the TFI total score and all other TFI endpoints. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares). The analysis will be repeated for the loudness score.

7.7.2. Change from baseline in mean total score, individual subscale scores, and loudness score

- *Computation of the Endpoint*

The change from baseline to each post-baseline visit will be computed as w – b where "w" is the post-baseline score and "b" is the score at baseline.

- *Analysis of the Endpoint*

The change from baseline to each post-baseline visit in mean total TFI score, individual subscale scores, and loudness score will be summarized descriptively by visit and treatment group for subjects in the FAS.

For subjects in the FAS, treatment group comparisons for the change from baseline in mean total TFI score will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. The covariance structure (UN or AR(1)) that provides the best model fit for the TFI total score, as described in Section 7.7.1, will be implemented with a repeated statement in SAS. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares). The analysis will be repeated for the loudness score.

Correlation analyses between the mean change from baseline in total TFI score, individual subscale scores, and the loudness score versus the following WR, WIN and PTA continuous endpoints for treated ears will be performed for subjects in the FAS:

- WR:

- Percentage of recognized words (Section 7.3.2)
- Absolute percent change from baseline in word recognition (Section 7.3.3)
- Relative percent change from baseline in word recognition (Section 7.3.4)
- Arcsine-transformed word recognition (Section 7.3.6)

- WIN:
  - Percentage of recognized words (Section 7.4.2)
  - Absolute percent change from baseline in word recognition (Section 7.4.3)
  - Relative percent change from baseline in word recognition (Section 7.4.4)
  - Median Spearman-Kärber estimates of dB thresholds for word recognition (Section 7.4.6)
- PTA:
  - Mean overall PTA (AC over 0.5, 1, 2, and 4 kHz) (Section 7.5.5)

Pearson correlation coefficients for the specified correlations will be summarized by visit and treatment group for subjects in the FAS.

Scatter plots will also be used to display the correlations. The change from baseline in TFI score (total, subscale, or loudness) will be plotted on the x-axis and the efficacy endpoint (WR, WIN, PTA) for treated ears will be plotted on the y-axis, with a separate plot for each post-baseline visit. Treatment groups will be differentiated using different symbols.

Post hoc correlation analyses may be performed on select qualitative WR, WIN and PTA endpoints of interest.

7.8. Hearing Handicap Inventory for Adults

The HHIA is a 25-item self-assessment scales composed of two subscales (13 items for Emotional and 12 items for Social/Situational). All items are on a 3-level scale of 0 to 4 (No = 0, Sometimes = 2, Yes = 4). The total and subscale scores range from 0 (no handicap) to 100 (total handicap) and will be computed as the sum of item response scores for each scale divided by the maximum possible score for the scale. The total and subscale scores will be missing if any item response scores are missing. The subject will complete the assessment (either directly onto the questionnaire in person, via web, or over the phone) at the Day 1, 60, 120, and 210 visits.

Baseline scores for analysis will be computed from the Day 1 visit. No imputation will be performed for missing data and no sensitivity analyses will be performed for the HHIA endpoints.

7.8.1. Mean total score and individual subscale scores

- *Computation of the Endpoint*

At each visit, the mean total HHIA score will be computed as the sum of all item responses and divided by 100. The Emotional and Social/Situational scores will be the sum of all subscale items, divided by the maximum possible score (52 for Emotional and 48 for Social/Situational).

- *Analysis of the Endpoint*

The mean total HHIA score and individual subscale scores will be summarized descriptively by visit and treatment group for subjects in the FAS.

For subjects in the FAS, treatment group comparisons for the mean total HHIA score will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. As described in Section 7.3.4, separate models will be fit specifying UN and AR(1) covariance structures (implemented with a repeated statement in SAS). The covariance structure providing the lowest corrected AICc and BIC values from the model will be implemented for the HHIA total score and all other HHIA endpoints. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares). The analysis will be repeated for each HHIA subscale score.

7.8.2. Change from baseline in mean total score and individual subscale scores

- *Computation of the Endpoint*

The change from baseline to each post-baseline visit will be computed as w – b where "w" is the post-baseline score and "b" is the score at baseline.

- *Analysis of the Endpoint*

Descriptive summaries, treatment group comparisons, and correlation analyses will be performed for change from baseline in mean total HHIA score and change from baseline in mean HHIA subscale scores as described in Section 7.7.2. The covariance structure identified in Section 7.8.1 will be utilized.

7.9. Hearing Screening Inventory

The HSI is a 12-item questionnaire assessing hearing impairment. Items 1 to 8 are scored on a 5-point ordinal scale: Never = 1, Seldom, Occasionally, Frequently, and Always = 5. Items 9 to 12 are on different 5-point scale: Good = 1, Average, Slightly Below Average, Poor, Very Poor = 5. The total score is on a 0 to 60 scale and is the sum of all 12 response scores, with items 2, 3, 4, 7, and 8 being reverse-scored (inverted). The total score will be missing if any item response scores are missing. Lower scores are favorable and higher scores are unfavorable. The HSI is designed to assess subjective change during the study and will be given to the subject to complete at the Day 1 and 210 visits.

Baseline scores for analysis will be computed from the Day 1 visit. No imputation will be performed for missing data and no sensitivity analyses will be performed for the HSI endpoints.

7.9.1. Mean total score

- *Computation of the Endpoint*

At each visit, the mean total HSI score will be computed as the sum of all 12 responses scores. Items 1, 5, 6, 9, 10, 11, and 12 will maintain the original 5-point scoring and items 2, 3, 4, 7, and 8 will be reverse-scored (inverted). The total score is on a 0 to 60 scale.

- *Analysis of the Endpoint*

The mean total HSI score will be summarized descriptively by visit and treatment group for subjects in the FAS.

For subjects in the FAS, treatment group comparisons for the mean total HSI score will be analyzed using a mixed-effect, repeated measures model using fixed covariates for treatment group, WR score at baseline (<50% versus >=50%), etiology of hearing loss (SSNHL versus NIHL), visit, and interaction between visit and treatment group. As described in Section 7.3.4, separate models will be fit specifying UN and AR(1) covariance structures (implemented with a repeated statement in SAS). The covariance structure providing the lowest corrected AICc and BIC values from the model will be implemented for the HSI total score and all other HSI endpoints. The treatment group differences analyzed will be between each FX-322 group (including the pooled FX-322 group) versus placebo, and comparisons between the individual FX-322 groups (excluding the pooled FX-322 group). Comparisons will be made by visit (e.g., estimated LS Mean differences by visit) and overall (e.g., Type III Sums of Squares).

7.9.2. Change from baseline in mean total score

- *Computation of the Endpoint*

The change from baseline to each post-baseline visit will be computed as w – b where "w" is the post-baseline score and "b" is the score at baseline.

- *Analysis of the Endpoint*

Descriptive summaries, treatment group comparisons, and correlation analyses will be performed for change from baseline in mean total HSI score as described in Section 7.7.2. The covariance structure identified in Section 7.9.1 will be utilized.

7.10. Examination of Subgroups

Descriptive summaries will be prepared for the endpoints defined in Sections 7.3.2 (Percentage of WR recognized words) and 7.4.2 (Percentage of WIN recognized words) for the following subgroups using the FAS:

- Subject Demographics
  - Sex
  - Race

- Ethnicity
- Age (categories: 18 to <35, 35 to <50, 50 to 65, >65)

- Baseline Disease Characteristics
  - WR score at baseline (<50% versus >=50%)
  - Etiology of hearing loss (SSNHL versus NIHL)

8. SAFETY EVALUATION

8.1. Overview of Safety Analysis Methods

The assessment of local and systemic safety of single and repeated doses of FX-322 in subjects with stable sensorineural hearing loss is an objective of this study. The safety analyses will be performed for the safety analysis set and will include treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), clinical laboratory measurements, vital signs, physical examination, otoscopic examination, tympanometry, concomitant medications, and Columbia-Suicide Severity Rating Scale (C-SSRS) assessments. Tabular summaries of descriptive statistics will be presented for all subjects included in the Safety Analysis Set and subjects will be classified into actual treatment groups based on the actual number of FX-322 doses received in any ear (qualified or not qualified).

Where appropriate, treated will be distinguished from the untreated ear, as described in Section 3. For unqualified ears that receive study treatment in error, the date and time of the study drug administration, or date if time is missing, or visit if date is missing (except for ear-related AEs, as provided below), will be used to identify assessments that will be classified as treated for safety analyses by ear.

If an assessment occurs on the same date that an unqualified ear receives study treatment in error, and assessment time or treatment start time is missing, the assessment will be presumed to have occurred prior to study drug administration for purposes of defining the ear as treated (i.e., the ear would be considered untreated at that assessment), with the exception of ear-related AEs, in which case the ear would be considered treated. The same logic would be applied to assessments other than ear-related AEs if the assessment occurred on the same visit that an unqualified ear receives study treatment in error and one or both dates are missing. For ear-related AEs, partial dates will be imputed for purposes of identifying treated ears in the same manner they are for purposes of defining TEAEs, as provided below.

For all safety endpoints, baseline will be the last non-missing value before first administration of study drug.

For all shift from baseline analyses, percentages will be based on the number of subjects in each baseline category.

Safety data will not be imputed, except for partial and missing dates, which will be imputed only for defining TEAEs and concomitant medications. Imputed dates will not be presented in data listings.

Partial dates will be imputed for the purposes of defining TEAEs as follows:

- For a missing start day where the month and year are present, the start day will be set to the first day of the month, unless the following two conditions are met:

  1. the first day of the month is before the date of first administration of study drug and the month and year are the same as the month and year of the date of first administration of study drug, and

  2. the end date is on or after the date of first administration of study drug or the end date is completely missing.

  If the two above conditions are met, the start day will be set to the day of first administration of study drug.

- For a missing start day and month where the year is present, the start day and month will be set to January 1st, unless 1) January 1st is before the date of first administration of study drug and the year is the same as the year of the date of first administration of study drug, and 2) the end date is on or after the date of first administration of study drug or the end date is completely missing, in which case the start day and month will be set to that of the date of first administration of study drug.

- For a missing end day where the month and year are present, the end day will be set to the last day of the month, unless the month and year are the same as the month and year of the last contact date for the subject, in which case the end day will be set to that of the subject's last contact date.

- For a missing end day and month where the year is present, the end day and month will be set to the subject's last contact date, unless the year of the subject's last contact date is greater than the end year, in which case the end day and month will be set to December 31st.

Partial and completely missing dates will be imputed for the purposes of classifying concomitant medications as follows:

- Partial dates will be imputed following the same algorithm as above for TEAEs.

- For an entirely missing start date (i.e., day, month, and year are missing), the start date will be set to the date of first administration of study drug unless the stop date is prior to the date of first administration of study drug, in which case the start date will be set to the stop date.

- For an entirely missing stop date (i.e., day, month, and year are missing), the medication will be treated as ongoing.

8.2. Extent of Exposure

The distribution of actual FX-322 doses received by randomized treatment group will be summarized as the number and percentage of subjects receiving 0, 1, 2, 3, or 4 doses of FX-322 in any ear and separately, in the qualifying ear to be treated. The summary will be by randomized treatment group and overall for subjects in the FAS.

The number and percentage of subjects receiving study treatment at each treatment visit (Day 1, 8, 15, 21) per the randomized treatment schedule in the qualifying ear to be treated will be summarized by randomized treatment group and overall for subjects in the FAS. The qualifying ear to be treated (Left Ear or Right Ear) will be captured on the Targeted Hearing Loss History CRF page and is the ear to be treated at all four treatment visits.

The number and percentage of subjects receiving incorrect study treatment at each treatment visit in the qualifying ear to be treated will be summarized by randomized treatment group and overall for subjects in the FAS.

The number and percentage of subjects treated in the non-qualifying ear at each visit will be summarized by randomized treatment group and overall for subjects in the FAS.

Dosing interval patterns for the qualifying ear each treatment group will be evaluated using the duration of days between each study treatment administration at Days 8, 15 and 21. The number of days between the current treatment administration and the prior treatment administration will be computed. The duration of days between each study treatment administration will be summarized categorically (<2 days, 2-4 days, 5-7 days, 8-10 days, >10 days) by treatment group and overall for the qualifying ear for subjects in the FAS.

If there are any dosing administrations errors such that a not qualified ear is treated, a sensitivity analysis may be performed summarizing the dosing interval patterns for any ear. Study treatment administration at each treatment visit will also be listed for subjects in the FAS; this listing will include actual treatment administered in each ear actually treated.

8.3. Adverse Events

All AEs will be recorded from the time of the first treatment through the end of the follow up period. Adverse events occurring for the first time, or worsening in severity, on or after the first dose of study drug or placebo will be considered to be TEAEs. Only TEAEs will be presented in summary tables, but all AEs will be listed with a flag indicating if the AE was treatment-emergent. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) to identify the system organ class and preferred term. The MedDRA version used will be within 1 full version of the latest version at study end. Any ear-related AE, whether occurring in 1 or both ears, will only be counted once in subject incidence counts per system organ class and preferred term.

Treatment-emergent AEs will be summarized by treatment group and overall for all subjects in the SfAS. The summary will display the total number of TEAEs as well as the count and percentage of subjects experiencing any TEAE. The total number of TEAEs and the count and percentage of subjects experiencing at least 1 TEAE in each system organ class and preferred term will also be shown. All percentages will use the number of subjects in the SfAS by treatment group as the denominator. Therefore, if a subject has more than 1 AE within a system organ class, the subject will be counted only once in that system organ class. If a subject has more than 1 AE that codes to the same preferred term, the subject will be counted only once for that preferred term. The tabular summary will be sorted by descending frequency of system organ class and preferred term for overall incidence.

Treatment-emergent AEs will also be summarized by maximum relationship to study drug and maximum severity. Relationship to the drug will be scored as Related, Possible, Unlikely or Not Related. Related AEs will be classified as those scored as Related and Possible. Severity will be rated as Mild, Moderate, or Severe. All percentages will use the number of subjects in the SfAS by treatment group as the denominator. Summary tables will reflect a count and percentage of subjects experiencing at least 1 TEAE in each system organ class, preferred term and grouping, relationship, or severity. If a subject experiences more than 1 AE within system organ class or preferred term, that subject will be counted only once for that event under the maximum severity or most related category for the study drug. Similarly, in the event that relationship or severity data are missing, the study analysis will include a category for missing relationship or severity in the summary tables. The tabular summaries will be sorted by descending frequency of system organ class and preferred term based on the overall incidence.

For ear-related TEAE analyses where the ear is the unit of analysis, ears will be summarized according to whether it was the treated (time period after ear received any treatment), untreated ear (time period before ear received any treatment), or both ears (count of subjects in each treatment group). Ears will be presented by actual treatment group and overall as described in Section 3. Percentages for the treated ear summary will use the number of ears treated in the SfAS by treatment group as the denominator. Percentages for the untreated ear summary will use the number of ears not treated in the SfAS by treatment group as the denominator. Percentages for the both ear summary will use the number of subjects in the SfAS by treatment group as the denominator. In each classification of ear type (treated, untreated, both), if an ear has more than 1 AE within a system organ class, the ear will be counted only once in that system organ class. If an ear has more than 1 AE that codes to the same preferred term, the ear will be counted only once for that preferred term. For unqualified ears that receive study treatment in error, any ear-related AEs will be mapped to untreated or treated ear(s) based on when study drug administration occurred in the unqualified ear, as described in Section 8.1. Because an ear is deemed treated after receiving any study treatment as described in Section 3, it is possible for both ears to be deemed treated for ear-related TEAE analyses.

All AEs will be presented in data listings for subjects in the SfAS. A column will be included in the listings for whether the AE is treatment-emergent.

8.4. Deaths, Serious Adverse Events, and Other Significant Adverse Events

Treatment-emergent SAEs, TEAEs leading to study withdrawal, and TEAEs resulting in death will be summarized separately for all subjects in the SfAS by treatment group and overall. All percentages will use the number of subjects in the SfAS by treatment group as the denominator. Summary tables will reflect a count and percentage of subjects experiencing at least 1 TEAE in each system organ class and preferred term within each AE subset (serious, leading to study withdrawal, death). In addition, the number of subjects experiencing any TEAEs in the respective subset will be reported. The tabular summary will be sorted by descending frequency of system organ class and preferred term based on the overall incidence.

Adverse events leading to study withdrawal, AEs resulting in death, and SAEs will be presented in data listings for subjects in the SfAS.

8.4.1. Adverse Events of Special Interest (AESI)

Protocol Section 13 defines adverse events of special interest.

AESIs will be summarized by treatment group and overall for all subjects in the SfAS. The summary will display the total number of AESIs as well as the count and percentage of subjects experiencing any AESI. The total number of AESIs and the count and percentage of subjects experiencing at least 1 AESI in each system organ class and preferred term will also be shown. All percentages will use the number of subjects in the SfAS by treatment group as the denominator. Therefore, if a subject has more than 1 AE within a system organ class, the subject will be counted only once in that system organ class. If a subject has more than 1 AE that codes to the same preferred term, the subject will be counted only once for that preferred term. The tabular summary will be sorted by descending frequency of system organ class and preferred term for overall incidence.

AESIs will be presented in a data listing for subjects in the SfAS.

8.5. Clinical Laboratory Evaluation

Clinical laboratory evaluation data will be presented by laboratory parameter as descriptive statistics for the change from baseline to each visit and shifts from baseline to each visit by treatment group and overall for subjects in the SfAS. Unscheduled visit and early termination visit data will not be mapped to scheduled visits per Section 7.1.2. Unscheduled visit and early termination visit data will be excluded from summary tables and included in listings. The analyses for shifts from baseline to each visit will use laboratory-provided values of high (H), normal (N) or low (L) to classify laboratory results. Results that are reported as high panic (HP) or high normal (HN) will be classified as H, and results that are reported as low panic (LP) or low normal (LN) will be classified as L. Missing values will remain missing.

Pregnancy results will not be summarized, but will be included in listings with all other laboratory data for subjects in the SfAS.

8.6. Vital Signs, Physical Findings, and Other Observations Related to Safety

8.6.1. Vital Signs

Vital sign evaluation data will be presented by parameter as descriptive statistics for the change from baseline to each visit and shifts from baseline to each visit by treatment group and overall for subjects in the SfAS. Unscheduled visit and early termination visit data will not be mapped to scheduled visits per Section 7.1.2. Unscheduled visit and early termination visit data will be excluded from summary tables and included in listings. The analyses for shifts from baseline to each visit will use predefined clinically relevant categories to classify results into low, normal and high. Missing values will remain missing.

Table 8-1 Vital Sign Categories for Clinical Relevance

Vital Sign	Low	Normal	High
Systolic Blood Pressure (mmHg)	<90	90-120	>120
Diastolic Blood Pressure (mmHg)	<60	60-80	>80
Heart Rate (beats per minute)	<60	60-100	>100

Vital sign evaluation data will also be included in listings for subjects in the SfAS.

8.6.2. Physical Examinations

Baseline physical examination results of Normal, Abnormal and Not Done will be presented by body system as descriptive statistics by treatment group and overall for subjects in the SfAS. Missing values will remain missing.

Physical examination data will also be included in listings for subjects in the SfAS.

8.6.3. Otoscopic Examinations

Otoscopic examination data will be presented by visit for treated ears (time period after ear received any treatment) and untreated ears (time period before ear received any treatment), separately, as descriptive statistics for each classification of the examination by treatment group (actual treatment received) and overall for subjects in the SfAS, as described in Section 3. For unqualified ears that receive study treatment in error, otoscopic examination results will be mapped to untreated or treated ear(s) based on when study drug administration occurred in the unqualified ear, as described in Section 8.1. Because an ear is deemed treated after receiving any study treatment as described in Section 3, it is possible for both ears to be deemed treated at a visit. Unscheduled visit and early termination visit data will not be mapped to scheduled visits per Section 7.1.2.

Unscheduled visit and early termination visit data will be excluded from summary tables and included in listings. Missing values for each component will remain missing.

Otoscopic examinations will include the following classifications:

- Treated Ear: Right or Left. For subjects with a non-qualified ear that received study treatment, both ears will be classified as treated after the administration of study treatment in the non-qualified ear.
- External Inspection, Auricle and Meatus: Normal, Abnormal (Erythema, Cerumen, Otorrhea, Other).
- Internal Ear Examination:
    - Tympanic Membrane: Normal, Abnormal (Erythema, Bulging, Scarring, Granulation, Perforation, Other)
    - Tympanic Membrane – Perforation: Not Present, Present (Size of Perforation: Pinhole, <= 25% of the TM, > 25% and <= 50% of the TM, > 50% of the TM)
    - Middle Ear: Normal, Abnormal (Effusion, Otorrhea, Other)

The number and percentage of ears presenting with each otoscopic classification will be provided for treated ears (time period after ear received any treatment) and untreated ears (time period before ear received any treatment), separately, for each visit by treatment group (actual treatment received) and overall for subjects in the SfAS, as described in Section 3. The number and percentage of ears with shifts in their otoscopic classification from baseline to each visit will also be provided by ear (treated and untreated) for each treatment group (actual treatment received) and overall.

Otoscopic examination data, scheduled and unscheduled, will be presented in a data listing for subjects in the SfAS.

8.6.4. Tympanometry

Tympanometry data will be presented by visit for treated ears (time period after ear received any treatment) and untreated ears (time period before ear received any treatment), separately, as descriptive statistics for each component of the tympanogram by treatment group (actual treatment received) and overall for subjects in the SfAS, as described in Section 3. For unqualified ears that receive study treatment in error, otoscopic examination results will be mapped to untreated or treated ear(s) based on when study drug administration occurred in the unqualified ear, as described in Section 8.1. Because an ear is deemed treated after receiving any study treatment as described in Section 3, it is possible for both ears to be deemed treated at a visit. Unscheduled visit and early termination visit data will not be mapped to scheduled visits per Section 7.1.2.

Unscheduled visit and early termination visit data will be excluded from summary tables and included in listings. Missing values for each component will remain missing.

Tympanogram data will include the following classifications:

- Treated Ear: Right or Left. For subjects with a non-qualified ear that received study treatment, both ears will be classified as treated after the administration of study treatment in the non-qualified ear.
- Type of Tympanogram (A, B, C)
- Type B – Volume (<2.0 mL, >=2.0 mL)
- Ear Canal Volume (mL)
- Peak Admittance (mL)
- Peak Pressure (daPa)

Treated Ear, Type of Tympanogram and Type B – Volume data will be summarized as the number and percentage of ears with each category and Ear Canal Volume, Peak Admittance and Peak Pressure will be summarized as continuous variables per Section 3. Summaries will be for treated ears (time period after ear received any treatment) and untreated ears (time period before ear received any treatment), separately, for each visit by treatment group (actual treatment received) and overall for subjects in the SfAS, as described in Section 3.

The number and percentage of ears with shifts from baseline to each visit in type and volume of tympanogram (A, B-small volume and/or normal, B-large volume, or C) will also be provided by ear (treated and untreated) for each treatment group (actual treatment received) and overall.

All tympanogram data, scheduled and unscheduled, will be presented in a data listing for subjects in the SfAS.

8.6.5. Columbia-Suicide Severity Rating Scale Assessments

The Baseline version of the C-SSRS will be administered at Day 1 and the Since Last Visit version will be administered at Days 60, 120 and 210. Unscheduled visit and early termination visit data will not be mapped to scheduled visits per Section 7.1.2. Unscheduled visit and early termination visit data will be excluded from summary tables and included in listings. All suicidal ideation and behavioral variables collected will be summarized descriptively for each visit by treatment group (actual treatment received) and overall for subjects in the SfAS, as described in Section 3. Missing values will remain missing.

All C-SSRS data, scheduled and unscheduled, will be included in data listings for all subjects in the SfAS.

8.6.6. Concomitant Medications

All medications taken in the 14 days prior to the Screening visit through the end of the follow-up period will be recorded on the Concomitant Medications log. Prior and concomitant medications will be coded using the latest version of World Health Organization Drug Dictionary Enhanced when the study started to identify the drug class and preferred drug name.

Concomitant medications will include all medications that started, or were continuing, during or after administration of study drug. Prior medications will include all medications that started and stopped prior to the day of administration of the study drug. In the event that a subject begins a prior medication following first dose of study drug or placebo, the post-dose use will be considered concomitant while the prior use medication will still be reported.

The number and percentage of subjects using concomitant medications will be tabulated by Anatomical Therapeutic Chemical (ATC) and preferred term for all subjects in the SfAS by treatment group (actual treatment received) and overall. If a subject has more than 1 medication within an anatomic class, the subject will be counted only once in that anatomic class. Similarly, if a subject has more than 1 medication that codes to the same preferred term, the subject will be counted only once for that preferred term. All percentages will use the number of subjects in the SfAS by treatment group (actual treatment received) and overall, as described in Section 3, as the denominator. The tabular summary will be sorted by descending order based on the overall frequency.

Prior and concomitant medication data will also be presented in a data listing for subjects in the SfAS.

9. OTHER ANALYSES

The biomarker analyses specified in the study protocol are outside the scope of this SAP.

10. INTERIM ANALYSIS AND DATA MONITORING

The blinded data will be continuously monitored for safety. Interventional level aggregated data monitoring will be conducted once all enrolled subjects have completed the Day 90 visit (Visit 5) or were early discontinuations. An interim analysis of all Day 90 data may be performed to assist in data interpretation or to understand patterns of missingness in real-

time or to monitor data integrity. The study will continue as planned to final analysis when all patients have completed their day 210 visit.

The planned interim analysis will include select tables and figures planned for the CSR, with subjects grouped into randomized or actual treatment groups based on unblinded treatment assignments. Datasets supporting the select tables and figures will also be provided along with the raw randomization codes. In the tables and figures, model results and descriptive statistics through Visit 5 (Day 90) will be presented, as appropriate. With the exception of the subject disposition summary and Kaplan-Meier plot of time from the date of randomization to the date of study completion or early discontinuation, all data after Day 90 will be excluded.

The following tables and figures planned for the CSR analyses are planned for the IA: Subject Disposition for Consented subjects (Section 5.1), Demographic and Baseline Characteristics for the FAS (Section 5.2), Subject Visit Accountability for the FAS (Section 5.1), WR, WIN, and Audiometry accountability (Section 7.1.6), Major Protocol Deviations for Randomized Subjects (Section 6.1), Study Treatment Exposure for the FAS (Section 8.2), Word Recognition in Quiet for the FAS and PPS excluding analyses by site or subgroup (Section 7.3), Words-In-Noise for the FAS and PPS excluding analyses by site or subgroup (Section 7.4), Pure Tone Audiometry analyses for the FAS and PPS (Section 7.5), Composite Endpoint for the FAS and PPS (Section 7.5.9), TFI for the FAS (Section 7.7), HHI for the FAS (Section 7.8), HSI for the FAS (Section 7.9), Adverse Events for the SfAS (Section 8.3), Otoscopic Examination for the SfAS (Section 8.6.3), Tympanometry for the SfAS (Section 8.6.4), C-SSRS for the SfAS (Section 8.6.5), and concomitant medications for the SfAS (Section 8.6.6). Phoneme analyses, WR and WIN analyses by site or subgroup, as well as any sensitivity analyses for the FAS due to study treatment administration in the not qualified ear are excluded from the IA.

The study team will remain blinded to the data during the IA. An unblinded statistician and statistical programmer, separate from the blinded study team, will provide unblinded results of the IA to the Frequency Therapeutics Director of Biostatistics, Sam Wilson. After receiving the unblinded IA results, the Frequency Director of Biostatistics and any other Frequency team members who are unblinded at the time of the interim analysis will be removed from any blinded discussions or decision making regarding the FX-322-202 final analyses.

11. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

Protocol Section 14.1.5 specifies that adjusted p-values and confidence intervals may be computed for pairwise treatment comparisons for a given endpoint. Such adjusted analyses are no longer planned in the SAP and there will be no adjustments for multiplicity of group comparisons.

12. REFERENCES

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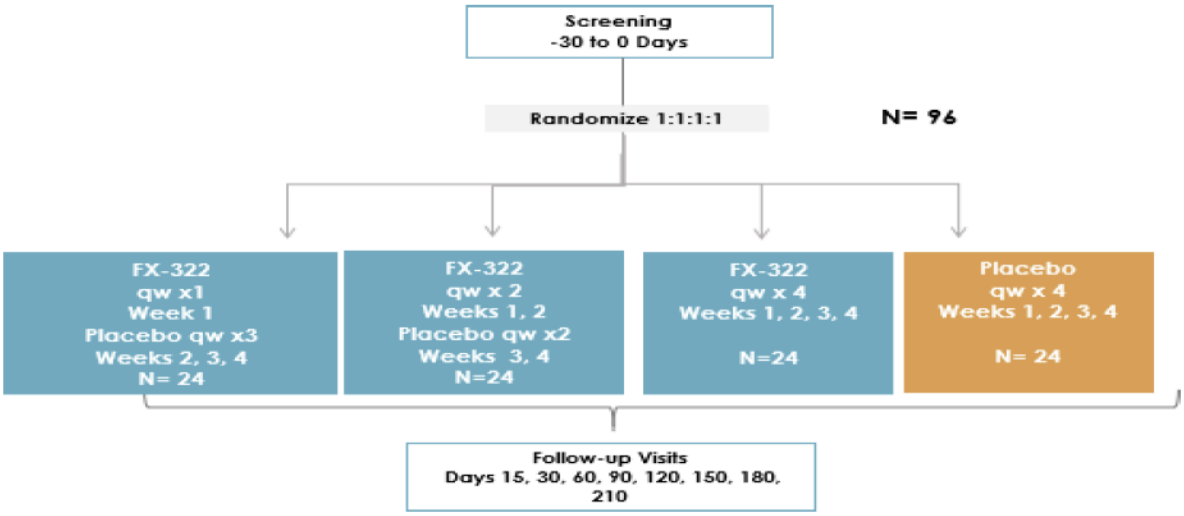
13. APPENDICES

13.1. Study Flow Chart



13.2. Schedule of Events

Visit	Screening	Baseline/ Treatment	Additional Treatments [e]	Phone Follow-up [e, f]	In-Clinic Follow-up [e]	Unscheduled Visit
Visit Number	**1**	**2a**	**2b, 2c, 2d**	**3, 6, 8**	**4, 5, 7, 9**	**UNS**
Assessment/Day	**-30 to 0**	**(Day 1)**	**Day 8, Day 15, Day 21**	**30, 120, 180**	**60, 90, 150, 210**	
Informed Consent	X					
Inclusion/ Exclusion Criteria	X	X[b]				
Demographics	X					
Medical History	X	X[b]				
Concomitant Medication	X	X[b, c]	X[b, c]	X	X	X
Physical Examination including weight and height	X					
Vital Signs (body temperature, pulse rate, bp)	X	X[b]	X[b]		X	X[i]
Tympanometry	X		X[b, d]		X	X[i]
Standard Pure Tone Audiometry	X		X[b, d]		X	X[i]
Extended High Frequency Audiometry	X		X[b, d]		X	X[i]
Word recognition, quiet	X		X[b, d]		X	X[i]
Words-In-Noise testing	X		X[b, d]		X	X[i]
Tinnitus Functional Index		X[b]		X[g]	X[g]	X[i]
Hearing Handicap Inventory for Adults		X[b]		X[g]	X[g]	X[i]
Hearing Screening Inventory		X[b]			X[h]	X[i]
Columbia Suicide and Severity Rating Scale		X[b]		X[g]	X[g]	X[i]
Otoscopy	X	X[b]	X[b]		X	X[i]
Urine Pregnancy Test (women of child bearing potential only)	X	X[b]	X[b]			X[i]
Urine Drug Screen	X[a]					
Safety Laboratory Assessments	X				X[h]	X[i]
Blood sample for biomarker analysis		X[b]			X[h]	
Study Medication (FX-322 or placebo)		X	X			
Adverse Events		X[c]	X[b, c]	X	X	X

a- See exclusion regarding positive drug tests
b- Assessment performed prior to injection.
c- Assessments performed after injection.
d- Perform only at Day 15 visit.
e- Visit windows: +/- 2 days for treatment visits, +/- 15 days for follow up visits.
f- Subjects will be administered questionnaires over the phone or the internet depending on site's preference.
g- Perform only at Day 60, 120, and 210 visits.
h- Perform only at Day 210 visit.
i- Perform at Investigator Discretion

14. ATTACHMENTS

14.1. Thornton and Raffin (1978) Word Recognition Scoring Chart

TABLE 4. Lower and upper limits of the 95% critical differences for percentage scores. Values within the range shown are not significantly different from the value shown in the percentage Score columns ($p > 0.05$).

% Score	n = 50	n = 25	n = 10	% Score	n = 100*
0	0-4	0-8	0-20	50	37-63
2	0-10			51	38-64
4	0-14	0-20		52	39-65
6	2-18			53	40-66
8	2-22	0-28		54	41-67
10	2-24		0-50	55	42-68
12	4-26	4-32		56	43-69
14	4-30			57	44-70
16	6-32	4-40		58	45-71
18	6-34			59	46-72
20	8-36	4-44	0-60	60	47-73
22	8-40			61	48-74
24	10-42	8-48		62	49-74
26	12-44			63	50-75
28	14-46	8-52		64	51-76
30	14-48		10-70	65	52-77
32	16-50	12-56		66	53-78
34	18-52			67	54-79
36	20-54	16-60		68	55-80
38	22-56			69	56-81
40	22-58	16-64	10-80	70	57-81
42	24-60			71	58-82
44	26-62	20-68		72	59-83
46	28-64			73	60-84
48	30-66	24-72		74	61-85
50	32-68		10-90	75	63-86
52	34-70	28-76		76	64-86
54	36-72			77	65-87
56	38-74	32-80		78	66-88
58	40-76			79	67-89
60	42-78	36-84	20-90	80	68-89
62	44-78			81	69-90
64	46-80	40-84		82	71-91
66	48-82			83	72-92
68	50-84	44-88		84	73-92
70	52-86		30-90	85	74-93
72	54-86	48-92		86	75-94
74	56-88			87	77-94
76	58-90	52-92		88	78-95
78	60-92			89	79-96
80	64-92	56-96	40-100	90	81-96
82	66-94			91	82-97
84	68-94	60-96		92	83-98
86	70-96			93	85-98
88	74-96	68-96		94	86-99
90	76-98		50-100	95	88-99
92	78-98	72-100		96	89-99
94	82-98			97	91-100
96	86-100	80-100		98	92-100
98	90-100			99	94-100
100	96-100	92-100	80-100	100	97-100

*If score is less than 50%, find % Score = 100-observed score and subtract each critical difference limit from 100.

14.2. Carney Schlauch (2007) Word Recognition Scoring Chart

Table 1. Limits (upper and lower) of 95% critical differences for percent scores on lists of length $n = 10$, 25, 50, and 100.

% score	$n = 50$	$n = 25$	$n = 10$	% score	$n = 100$	% score	$n = 100$
0	0–6	0–12	0–20	50	37–63	50	37–63
2	0–10			49	36–62	51	38–64
4	0–14	0–20		48	35–61	52	39–65
6	0–18			47	34–60	53	40–66
8	2–**20**	0–28		46	33–59	54	41–67
10	2–24		0–**40**	45	32–58	55	42–68
12	4–26	0–32		44	31–57	56	43–69
14	4–**28**			43	30–56	57	44–70
16	6–32	4–40		42	29–55	58	45–71
18	6–34			41	28–54	59	46–72
20	8–36	4–44	0–**50**	40	**28**–53	60	47–**72**
22	**10**–**38**			39	**27**–52	61	48–**73**
24	10–42	8–48		38	26–51	62	49–74
26	12–44			37	25–50	63	50–75
28	14–46	8–52		36	24–49	64	51–76
30	**16**–48		10–70	35	23–48	65	52–77
32	16–50	12–56		34	22–47	66	53–78
34	18–52			33	23–46	67	54–79
36	20–54	16–60		32	20–45	68	55–80
38	22–56			31	**20**–44	69	56–**80**
40	**24**–58	16–64	10–**70**	30	19–43	70	57–81
42	24–60			29	18–42	71	58–82
44	26–62	20–68		28	17–41	72	59–83
46	28–64			27	16–**39**	73	**61**–84
48	30–66	24–72		26	15–**38**	74	**62**–85
50	32–68		**20**–**80**	25	**15**–37	75	63–**85**
52	34–70	28–76		24	14–36	76	64–86
54	36–72			23	13–35	77	65–87
56	38–74	32–80		22	12–34	78	66–88
58	40–76			21	11–31	79	69–89
60	42–**76**	36–84	**30**–90	20	11–32	80	68–89
62	44–78			19	10–**30**	81	**70**–90
64	46–80	40–84		18	9–29	82	71–91
66	48–82			17	8–28	83	72–92
68	50–84	44–88		16	8–27	84	73–92
70	52–**84**		30–90	15	7–26	85	74–93
72	54–86	48–92		14	6–**24**	86	**76**–94
74	56–88			13	6–23	87	77–94
76	58–90	52–92		12	5–22	88	78–95
78	**62**–**90**			11	4–21	89	79–96
80	64–92	56–96	**50**–100	10	4–19	90	81–96
82	66–94			9	3–18	91	82–97
84	68–94	60–96		8	**3**–17	92	83–**97**
86	**72**–96			7	2–15	93	85–98
88	74–96	68–100		6	1–14	94	86–99
90	76–98		**60**–100	5	1–12	95	88–99
92	**80**–98	72–100		4	1–11	96	89–99
94	82–100			3	0–9	97	91–100
96	86–100	80–100		2	0–**7**	98	**93**–100
98	90–100			1	0–6	99	94–100
100	94–100	88–100	80–100	0	0–3	100	97–100

Note. The upper and lower scores in the 95% critical interval are shown for various list sizes. Scores on successive administrations of a word recognition test may be considered to be "significantly different," at the .05 level, if they exceed the upper limit or fall below the lower limit. Discrepancies between these simulated values and those in Thornton and Raffin (1978) are coded as follows: numerals in boldface indicate that the critical interval is narrower on that side than in the previous table; underlined numerals indicate that the critical interval is wider on that side than in the previous table.

14.3. TFI Calculations

TINNITUS FUNCTIONAL INDEX

Today's Date _____ (Month / Day / Year) Your Name _____ (Please Print)

Please read each question below carefully. To answer a question, select *ONE* of the numbers that is listed for that question, and draw a *CIRCLE* around it like this: (10%) or (1).

I — Over the PAST WEEK...

1. What percentage of your time awake were you consciously **AWARE OF** your tinnitus?
   Never aware ► 0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100% ◄ *Always aware*
2. How **STRONG** or **LOUD** was your tinnitus?
   Not at all strong or loud ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Extremely strong or loud*
3. What percentage of your time awake were you **ANNOYED** by your tinnitus?
   None of the time ► 0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100% ◄ *All of the time*

SC — Over the PAST WEEK...

4. Did you feel **IN CONTROL** in regard to your tinnitus?
   Very much in control ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Never in control*
5. How easy was it for you to **COPE** with your tinnitus?
   Very easy to cope ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Impossible to cope*
6. How easy was it for you to **IGNORE** your tinnitus?
   Very easy to ignore ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Impossible to ignore*

C — Over the PAST WEEK, how much did your tinnitus interfere with...

7. Your ability to **CONCENTRATE**?
   Did not interfere ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Completely interfered*
8. Your ability to **THINK CLEARLY?**
   Did not interfere ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Completely interfered*
9. Your ability to **FOCUS ATTENTION** on other things besides your tinnitus?
   Did not interfere ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Completely interfered*

SL — Over the PAST WEEK...

10. How often did your tinnitus make it difficult to **FALL ASLEEP** or **STAY ASLEEP**?
    Never had difficulty ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Always had difficulty*
11. How often did your tinnitus cause you difficulty in getting **AS MUCH SLEEP** as you needed?
    Never had difficulty ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *Always had difficulty*
12. How much of the time did your tinnitus keep you from **SLEEPING** as **DEEPLY** or as **PEACEFULLY** as you would have liked?
    None of the time ► 0 1 2 3 4 5 6 7 8 9 10 ◄ *All of the time*

TINNITUS FUNCTIONAL INDEX PAGE 2

Please read each question below carefully. To answer a question, select *ONE* of the numbers that is listed for that question, and draw a *CIRCLE* around it like this: (10%) or (1).

A	**Over the PAST WEEK, how much has your tinnitus interfered with...**	*Did not interfere*									*Completely interfered*
13.	Your ability to **HEAR CLEARLY**?	0	1	2	3	4	5	6	7	8	9	10
14.	Your ability to **UNDERSTAND PEOPLE** who are talking?	0	1	2	3	4	5	6	7	8	9	10
15.	Your ability to **FOLLOW CONVERSATIONS** in a group or at meetings?	0	1	2	3	4	5	6	7	8	9	10
R	**Over the PAST WEEK, how much has your tinnitus interfered with...**	*Did not interfere*									*Completely interfered*
16.	Your **QUIET RESTING ACTIVITIES**?	0	1	2	3	4	5	6	7	8	9	10
17.	Your ability to **RELAX**?	0	1	2	3	4	5	6	7	8	9	10
18.	Your ability to enjoy "**PEACE AND QUIET**"?	0	1	2	3	4	5	6	7	8	9	10
Q	**Over the PAST WEEK, how much has your tinnitus interfered with...**	*Did not interfere*									*Completely interfered*
19.	Your enjoyment of **SOCIAL ACTIVITIES**?	0	1	2	3	4	5	6	7	8	9	10
20.	Your **ENJOYMENT OF LIFE**?	0	1	2	3	4	5	6	7	8	9	10
21.	Your **RELATIONSHIPS** with family, friends and other people?	0	1	2	3	4	5	6	7	8	9	10
22.	How often did your tinnitus cause you to have difficulty performing your **WORK OR OTHER TASKS**, such as home maintenance, school work, or caring for children or others?	*Never had difficulty* ►	0	1	2	3	4	5	6	7	8	9	10 ◄ *Always had difficulty*
E	**Over the PAST WEEK...**												
23.	How **ANXIOUS** or **WORRIED** has your tinnitus made you feel?	*Not at all anxious or worried* ►	0	1	2	3	4	5	6	7	8	9	10 ◄ *Extremely anxious or worried*
24.	How **BOTHERED** or **UPSET** have you been because of your tinnitus?	*Not at all bothered or upset* ►	0	1	2	3	4	5	6	7	8	9	10 ◄ *Extremely bothered or upset*
25.	How **DEPRESSED** were you because of your tinnitus?	*Not at all depressed* ►	0	1	2	3	4	5	6	7	8	9	10 ◄ *Extremely depressed*

The possible responses to items except 1 and 3 are 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. The possible responses to items 1 and 3 are 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Prior to computing the TFI total score, the responses for items 1 and 3 are transformed from a percentage scale to a 0 to 10 scale, by dividing the values by 10.

The TFI total score is then defined as a sum of the 25 items, after items 1 and 3 are transformed. The total TFI score is calculated as the sum of the 25 scores, divided by the number of non-missing scores, multiplied by 10. The total score ranges from 0 to 100, with higher scores representing greater perceived handicap.

The TFI total score is not valid if 7 or more items are omitted. To be valid as a measure of tinnitus severity, at least 19 items must be completed.

TFI Subscales

The 25 items can also be grouped into 8 subscales: intrusive, sense of control, cognitive, sleep, auditory, relaxation, quality of life, and emotional.

- The intrusive group includes items 1, 2, and 3.
- The sense of control group includes items 4, 5, and 6.
- The cognitive group includes items 7, 8, and 9.
- The sleep group includes items 10, 11, and 12.
- The auditory group includes items 13, 14, and 15.
- The relaxation group includes items 16, 17, and 18.
- The quality of life group includes items 19, 20, 21, and 22.
- The emotional group includes items 23, 24, and 25.

The subscale scores will be computed by summing the points for each question included in the relevant subscale, dividing by the number of non-missing scores within the subscale, multiplied by 10. Each subscale score has a range of 0 to 100. Each TFI subscale score is not valid if 2 or more items are omitted. To be valid subscale scores, no more than 1 item can be omitted.