CLINICAL STUDY PROTOCOL

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

Protocol Number:	FX-322-202
EudraCT Number:	N/A
National Clinical Trial (NCT) Identified Number	NCT04120116
Investigational Product:	FX-322
Phase:	2a
Sponsor:	Frequency Therapeutics 19 Presidential Way Woburn. MA 01801 USA
Protocol Date:	8 February 2021
Protocol Version:	5.0

1 PROTOCOL APPROVAL SIGNATURES

Protocol Title: 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

Protocol Number: FX-322-202

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and applicable United States (US) Code of Federal Regulations (CFR). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

2 SYNOPSIS

Protocol Number:

FX-322-202

Title:

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

Investigational Product:

FX-322

Study Centers:

Approximately 15 centers in the US

Phase:

Phase 2a

Objectives:

Primary Objective(s)

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.

Endpoints:

Efficacy Endpoint(s)

- Mean changes from Screening in:
    - Word Recognition in quiet (WR)
    - Words-In-Noise testing (WIN)
    - Standard Pure Tone Audiometry (air 0.25-8 kHz; bone 0.5-4 kHz)

Additional Exploratory Efficacy Endpoint(s)

- Mean changes from Screening in:
    - Extended High Frequency Audiometry (air 9-16 kHz)
    - Tinnitus Functional Index (TFI)
    - Hearing Handicap Inventory for Adults (HHIA)
    - Hearing Screening Inventory (HSI)

Safety Endpoint(s)

- Adverse events
- Changes from Screening in:
    - Otoscopy
    - Tympanometry

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 (no changes in air conduction greater than 10 dB at a single frequency or greater than 5dB at two contiguous frequencies from the prior audiogram to the Screening audiogram).

The study will have 3 phases: Screening, Treatment, and Follow-up.

Screening: Can occur up to 30 days prior to study drug administration (Baseline/Treatment Day 1). All subjects will be screened to determine study eligibility. As part of recording the medical history of subjects, concurrent use of hearing aids during the study will be documented. All subjects will return for the Baseline visit and evaluated to ensure they continue to meet appropriate inclusion/exclusion criteria applicable at that visit.

Baseline/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 (ear with the worst Screening pure tone average) 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.

Follow up: Subject will be required to return to clinic 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.

Number of Subjects:

Approximately 96 evaluable subjects

Study Duration:

Screening phase: up to 30 days before first study drug administration (Baseline Visit).

Treatment phase: 21 days (4 treatment visits 7 days apart)

Follow-up phase: 7 months from Baseline (Day 1).

Study Population:

Male and female adults (18 to 65 years inclusive), otherwise healthy with stable sensorineural hearing loss.

Statistical Analysis:

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.

Statistical Methods: Descriptive summaries will be provided for patient disposition, demographic and baseline disease data. Summary statistics for efficacy endpoints will be provided for each randomized treatment group and timepoint. Comparisons of each FX-322 group to placebo along with 95% confidence intervals of the difference will also be calculated. Additionally, comparisons among the 3 FX-322 groups will also be conducted as well as a pooled FX-322 group comparison to placebo group. The Full Analysis Set defined as all randomized subjects exposed to at least one dose of study drug, will comprise the analysis set for the exploratoration of efficacy. Additional sensitivity analyses will be conducted using a Per Protocol Analysis Set. Subjects will be analysed by their randomized treatment group for both the Full Analysis and Per Protocol Analysis Set analyses.

The incidence of treatment-emergent adverse events will be presented by preferred term and body system using the Medical Dictionary for Regulatory Activities (MedDRA®). Tabulations will be provided by seriousness, severity, and relationship to study drug. Laboratory, tympanometry and otologic data will be presented as summary statistics at each timepoint as well as changes from baseline and/or shift tables. All subjects exposed to study drug will be included in the Safety Analysis Set and included in the safety analyses according to the actual treatment group regardless of the randomized assignment.

Details of the planned statistical analyses and methods will be provided in the Statistical Analysis Plan (SAP) which will be finalized prior to database lock and study blind break

PROTOCOL Version 5.0 Amendments

Item No.	Change	Section and Page Number(s)
1	Updated date and version of protocol.	Title Page, pg.1 Footer, every page
2	Updated language regarding data monitoring at the Day-90 Interim Analysis	Section 14.1.6 Interim Analysis pg. 41.

PROTOCOL Version 4.0 Amendments

Item No.	Change	Section and Page Number(s)
1	Updated date and version of protocol.	Title Page, pg.1 Footer, every page
2	Updated information regarding blinding roles on the study	Section 8.7.1 Blinding pg. 27.
3	Updated language to conduct data monitoring following the Day 90 visit	Section 14.1.6 Interim Analysis pg. 41.

PROTOCOL Version 3.0 Amendments

Item No.	Change	Section and Page Number(s)
1	Updated date and version of protocol.	Title Page, pg.1 Footer, every page
2	Updated visit type from clinic to phone.	Synopsis, pg. 4 Section 8.1 Overall Study Design and Plan, pg. 17. Section 8.1.2 Schedule of Assessments, pg. 18. Section 10.1.4 – 10.1.9 Visits, pgs. 30-31.
3	Removed assessments from visits	Section 11 Efficacy Measures pg. 32. Section 12.1.7 Otoscopy, pg 34. Section 12.1.8 Tympanometry, pg 34.
4	Updated language around interim analysis	Section 14.1.6 Interim Analysis pg. 41.
5	Updated how missing data will be handled	Section 14.2 Handling of Missing Subject Data and Subject Withdrawls pg. 42.
6	Updated text for grammatical and capitalization consistency	Throughout Protocol

PROTOCOL Version 2.0 Amendments

Item No.	Change	Section and Page Number(s)
1	Updated date and version of protocol.	Title Page, pg.1 Footer, every page
2	Added information and signature for Sponsor Chief Medical Officer	Sponsor Signature, pg. 2
3	Defined "ear with worst hearing" as ear with the worst Screening pure tone average	Section 2 Synopsis, pg. 4 Section 8.1, Overall Study Design and Plan, pg. 15 Section 8.7.5, Selection and Timing of Dose for Each Subject, pg. 26
4	Clarified randomization should be ≥50% correct, <50% correct for the WR scores	Section 8.1 Overall Study Design and Plan, pg. 15 Section 8.7.3 Method of Assigning Subjects to Treatment Groups, pg. 25
5	Updated stability criteria for clarification	Section 8.1 Overall Study Design and Plan, pg. 15 Section 8.4.2. Inclusion Criteria, pg. 19
6	Updated Exclusion Criteria #4 to allow for clarification of definition of conductive hearing loss	Section 8.4.3. Exclusion Criteria, pg. 20
7	Removed EMLA brand name to allow for use of generic topical anesthesia	Section 2 Synopsis, pg. 4 Section 8.5.1, Investigational Product Administered, pg. 22 Section 8.6.1 Packaging and Labeling, pg. 24
8	Updated language to allow for subjects to be rescreened	Section 8.4.5. Screen Failures, pg. 22
9	Added guidance for which prior audiogram should be used to confirm Inclusion/Exclusion criteria	Secition 10.1.1 Screening, pg. 28
10	Removed alcohol from urine drug testing	Section 10.1.1 Screening, pg. 28 Section 12.1.2 Clinical Laboratory Evaluation, pg 33
11	Clarified that the WR AESI should be recorded for the treated ear only	Section 13 Adverse Events, Adverse Events of Special Interest, pg. 36
12	Updated descriptive statistics to reflect change in Inclusion Criteria	Section 14.1.1. Descriptive Statistics, pg. 37
13	Updated text for grammatical and capitalization consistency	Throughout Protocol

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
AESI	Adverse Event of Special Interest
ASHA	American Speech-Language-Hearing Association
BMI	Body mass index
BPPV	Benign Paroxysmal Positional Vertigo
NIHL	Noise induced hearing loss
CONSORT	Consolidation Standards of Reporting Trials
CRO	Contract Research Organization
C-SSRS	Columbia Suicide and Severity Rating Scale
dB	Decibel
DMSO	Dimethyl sulfoxide
eCRF	Electronic Case Report Form
EDC	Electronic data capture
FAS	Full Analysis Set
FDA	Federal Drug Administration
GCP	Good Clinical Practice
GLMM	Generalized Linear Mixed Models
GSK	Glycogen Synthase Kinase
HDAC	Histone deacetylase
HCG	Human chorionic gonadotrophin
HHIA	Hearing Handicap Inventory in Adults
HSI	Hearing Screening Inventory
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
LGR5+	Leucine-rich repeat-containing G-protein coupled receptor 5 positive
MAR	Missing at Random
MedDRA ®	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model for Repeated Measures
PE	Physical exam
PI	Principal Investigator
PK	Pharmacokinetics
PPAS	Per Protocol Analysis Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SfAS	Safety Analysis Set
SNHL	Sensorineural hearing loss
SOP	Standard Operating Procedure
SUSAR	Suspected Unexcepted Serious Adverse Reaction
TFI	Tinnitus Functional Index
TM	Tympanic membrane
WHO	World Health Organization
WIN	Words-In-Noise Testing
WR	Word Recognition in quiet

5 INTRODUCTION

Worldwide, an estimated 1.1 billion people are at risk for hearing loss due to exposure to damaging levels of sound (WHO, 2015). The WHO also reported that 16% of the disabling hearing loss in adults is attributable to occupational noise exposure (Nelson et al, 2005). The hearing loss population shows higher rates of dementia, depression, and other mental health disorders, especially in the elderly (Choi et al, 2016; McGilton et al, 2016; Deal et al, 2016). Sensorineural hearing loss (SNHL) accounts for about 90% of all cases of hearing loss (Li et al, 2017). Leading causes include noise exposure, ototoxic medications, advanced age, inherited, and autoimmune disorders. Noise is a major occupational and environmental hazard, causing hearing loss, sleep disturbance, fatigue, and hypertension (Hong et al, 2013). SNHL has long been recognized as the primary and direct health effect of excessive noise exposure (Basner et al, 2015). In the United States, an estimated 48 million people or 20.3% of the population 12 years or older has hearing loss in one or both ears (Lin et al, 2011).

Excessive sound levels or loud sounds for extended periods time hyperstimulate cochlear hair cells, which can lead to increased production of reactive oxygen species and oxidative cell death. Presently, no curative treatments exist, but rather assistive devices such as hearing aids and cochlear implants are used to address the symptoms of SNHL. These options, however, do not address the underly biological deficit of SNHL and are not sufficient for normal quality of life in individuals with SNHL.

FX-322 is a fixed ratio dose combination of two small molecules: a glycogen synthase kinase (GSK) inhibitor, FX03, and Valproate Sodium (an HDAC inhibitor approved in multiple countries including the US for human use as Depacon®).

The active ingredients in FX-322 target two cellular mechanisms, histone deacetylase (HDAC) inhibition and GSK-3 inhibition, which act synergistically to induce a regenerative response in cochlear tissue. The combination of the mechanisms was shown to enable Leucine-rich repeat-containing G-protein coupled receptor 5 positive (Lgr5+) progenitor cells to asymmetricly divide to create a copy of themselves and a daughter cell that can become a hair cell (McLean et al, 2017). Thus, FX-322 could serve as a treatment for hearing loss by restoring lost hair cells.

In preclinical experiments, a fixed ratio dose combination of FX03 and Valproate Sodium demonstrates:

- Expansion of the Lgr5+ progenitor cells and subsequent conversion into hair cells using newborn and adult cells. Substantial Lgr5+ cell expansion occurs with the combination of agents but not with the individual agents.
- Expansion of progenitor cells from adult primate inner ear, and expansion and subsequent conversion of progenitor cells into hair cells from the adult human inner ear.
- Formation of new hair cells in ototoxin damaged mouse cochlear explants.
- Significant improvement in hearing and hair cell numbers in an adult mouse model of noise-induced hearing loss.

Based on these findings, FX-322 is formulated as a fixed ratio dose combination, which also contains [redacted]

[redacted] FX-322 will be administered by an intratympanic injection into the middle ear onto the round window membrane area, enabling the active ingredients to locally diffuse into the cochlea.

Minimal systemic exposure occurred after a single intratympanic injection of FX-322 in two previous clinical trials. In the first of these trials, FX-322-103, nine patients undergoing cochlear implantation surgery received a single unilateral intratympanic injection of FX-322 (6 patients) or placebo (3 patients) to study tolerability, systemic drug exposure, and cochlear drug exposure. The results demonstrated acceptable local and systemic acute tolerability and very limited systemic exposure that was unmeasurable within 24 hours post injection. In addition, the cochlear FX-322 drug concentration data demonstrated the drug was present at 4 hours after intratympanic injection in one patient but unmeasurable after 24 hours in the perilymph in two subjects.

The second clinical trial was a Phase 1/2 (FX-322-201) randomized, double-blind, placebo-controlled single-dose study in adults with stable sensorineural hearing loss. The objectives of this study were to assess: 1) the systemic safety of two dose levels of FX-322; 2) the plasma pharmacokinetic profile of FX-322; and 3) the effect of FX-322 on otologic and audiologic measures. After intratympanic injection of FX-322, there were no serious adverse events (SAEs) or AEs that led to a subject withdrawal or death and the most common AEs were considered associated with the intratympanic injection procedure. No clinically significant safety issues were observed on otologic or audiologic measures. Additionally, the systemic PK profile of FX-322 was minimal and dose-proportionate with both drugs cleared from the circulation within approximately 24 hours. Over the course of the study, a post-hoc analysis showed a statistically significant improvement in WR for patients treated with FX-322 versus patients given placebo. FX-322 patients showed an improvement in WR scores from baseline to Day 90. In addition, the FX-322 group showed a clinically meaningful improvement in WIN scores from baseline to Day 90 as determined by Wilson and McArdle, 2007. Four of the FX-322 patients showed a clinically meaningful improvement in WR according to established audiological definitions (Thorton and Raffin, 1978), with two of these patients demonstrating WIN improvements that are defined as clinically meaningful (Wilson and McArdle, 2007). The notable improvements in these measures of hearing function were generally observed in FX-322 patients within 15-30 days of treatment and were sustained for 90 days. There was no difference in treatment effect observed between the two dose volumes of FX-322.

6 RISK/BENEFIT ASSESSMENT

6.1 Known Potential Benefits

The active ingredients in FX-322 target two cellular mechanisms, histone deacetylase (HDAC) inhibition and GSK-3 inhibition, which act synergistically to induce a regenerative response in cochlear tissue. FX-322 could serve as a treatment for patients with SNHL because the active ingredients have demonstrated 1) the ability to induce progenitor cell expansion and subsequent conversion into new hair cells across species, including human, 2) restoration of hair cells in ototoxin-damaged mouse cochlear implants, and 3) a significant improvement in hearing and hair cell numbers in an adult mouse model of noise-induced hearing loss.

Most recently it has been shown that in humans with stable SNHL, a single intratympanic dose of FX-322 was associated with improvements in speech intelligibility as measured by

WR and WIN, data that suggest FX-322 may restore hearing function to some degree in humans.

6.2 Known Potential Risks

The risks of intratympanic injection of FX-322 have been carefully studied in nonclinical toxicology studies, as well as in two separate clinical trials. Based on these studies, the risks appear to be similar to those experienced with intratympanic injections of other materials, such as steroids, that are not FDA-approved. The risks are characterized as persistent eardrum perforation, pain or bleeding with injection, temporary dizziness, middle ear infection, and conductive or sensorineural hearing loss. A published clinical trial using multiple intratympanic injections of a steroid in SSNHL patients has shown the majority of adverse events to be associated with the injection procedure, with eardrum perforations reported at less than 4% (Rauch et al., 2011).

6.3 Assessment of Potential Risks and Benefits

Based on the very low potential for risk, which is primarily associated with the intratympanic injection procedure, compared to the potential benefit which is the possibility of restoration of hearing, the risk/benefit profile is considered acceptable to conduct the study described herein.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary Objective(s)

1. To assess the exploratory efficacy by audiologic response following single and repeated 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.

7.2 Endpoints

Efficacy Endpoint(s)

- Mean changes from Screening in:
    - Word Recognition in quiet (WR)
    - Words-In-Noise testing (WIN)
    - Standard Pure Tone Audiometry (air 0.25-8 kHz; bone 0.5-4 kHz)

Additional Exploratory Efficacy Endpoint(s)

- Mean changes from Screening in:
    - Extended High Frequency Audiometry (air 9-16 kHz)
    - Tinnitus Functional Index (TFI)
    - Hearing Handicap Inventory for Adults (HHIA)
    - Hearing Screening Inventory (HSI)

Safety Endpoint(s)

- Adverse events
- Changes from Screening in:
    - Otoscopy
    - Tympanometry

8 INVESTIGATIONAL PLAN

8.1 Overall Study Design and Plan: Description

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 (no changes in air conduction greater than 10 dB at a single frequency or greater than 5dB at two contiguous frequencies from the prior audiogram to the Screening audiogram in the study ear).

In a previous human study, Study FX-322-201, it was demonstrated that a single dose FX-322 was well-tolerated in patients with hearing loss with no drug-related serious adverse events. The only ear related adverse events in the study were consistent with those experienced with a standard intratympanic injection. Additionally, FX-322 was shown to be associated with improvements in hearing function in some patients that were considered clinically meaningful, as defined by several key measures of audibility and intelligibility.

Screening: Can occur up to 30 days prior to study drug administration (Baseline/Treatment Day 1). All subjects will be screened to determine study eligibility. As part of recording the medical history of subjects, concurrent use of hearing aids will be documented. All subjects will return for the Baseline visit and evaluated to ensure they continue to meet inclusion/exclusion criteria applicable at that visit.

Baseline/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

Subjects will be stratified at randomization by baseline (last assessment before study drug administration Word Recognition (>=50% correct, <50% correct) and etiology of hearing loss (Sudden vs Noise).

Once a subject has been identified as meeting all inclusion/exclusion criteria, the qualifying ear with the worse hearing (ear with the worst Screening pure tone average) will be selected, unless a rationale exists to treat the other ear. 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.

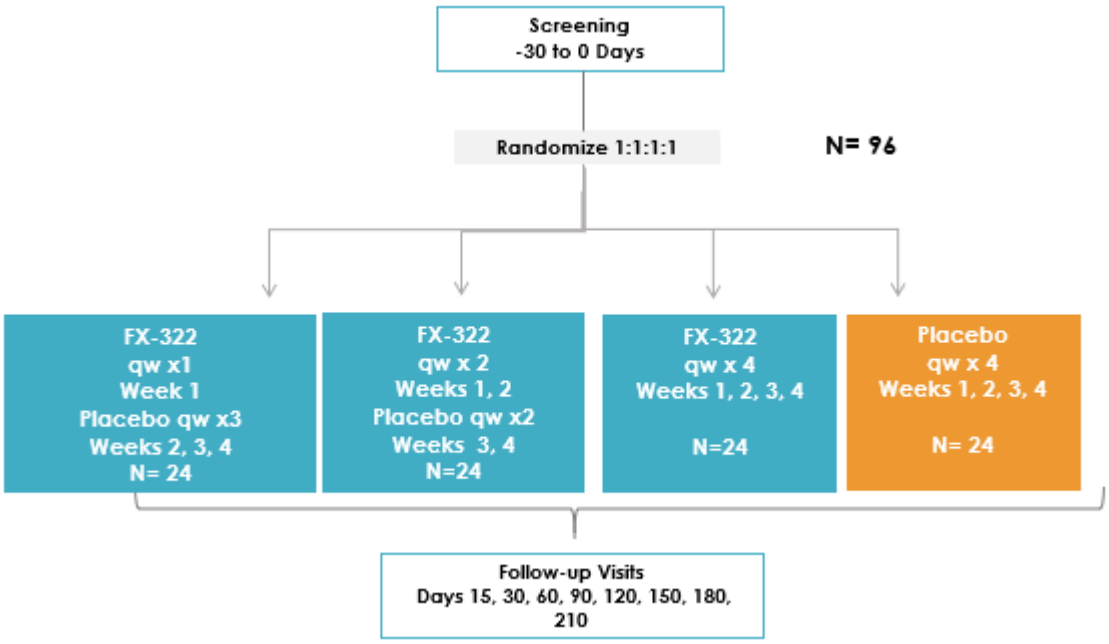
Each subject will be placed in the supine position. Topical anesthetic cream will be administered to the tympanic membrane. Under a microscope, a 25-gauge needle will be used to inject FX-322 or placebo into the middle ear at the junction of the posterior inferior and posterior superior quadrant with 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.

Follow up:

Subjects will be required to return to clinic 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.

Unscheduled visits may occur as needed. See the Schedule of Assessments for more information.

8.1.1 Study Design



8.1.2 Schedule of Assessments

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

8.2 Scientific Rationale for Study Design

The active ingredients in FX-322 target two cellular mechanisms, histone deacetylase (HDAC) and GSK-3 inhibition, which act synergistically to induce progenitor cell expansion and new hair cell formation in several ex vivo test systems. In vivo, significant improvements in hearing and increases in hair cell counts were observed in an adult mouse model of noise-induced hearing loss after a single treatment. Most recently, it has been shown that in humans with stable SNHL, a single intratympanic dose of FX-322 was associated with improvements in speech intelligibility as measured by WR and WIN, data that suggest FX-322 may restore hearing function to some degree in humans. Taken together, these data provide justification to evaluate the safety and efficacy of multiple intratympanic injections of FX-322 in patients with stable SNHL.

8.3 End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Assessments, Section 8.1.2.

8.4 Selection of Study Population

8.4.1 Number of Planned Subjects

Approximately 96 subjects are planned to enter the study, and 96 subjects are expected to complete the study.

8.4.2 Inclusion Criteria

To be eligible for study entry subjects must satisfy all of the following criteria:

1. Subject has read and voluntarily signed the Informed Consent Form (ICF) after all questions have been answered and prior to any study-mandated procedure.
2. Adult aged 18-65 years inclusive.
3. Established diagnosis of stable sensorineural hearing loss by standard audiometric measures for ≥ 6 months prior to the Screening visit (no changes in air conduction greater than 10 dB at a single frequency or greater than 5dB at two contiguous frequencies from the prior audiogram to the Screening audiogram in the study ear).
4. Documented medical history consistent with hearing loss being caused by noise exposure or sudden sensorineural hearing loss (documented audiogram at least 6 months prior to screening required).
5. A pure tone average of 26-70 dB at 500Hz, 1000Hz, 2000Hz, and 4000Hz in the ear to be injected.
6. Subjects with a 15% - 85% correct score on Word Recognition test (8 – 42 words correct out of 50) at Screening in the study ear.
7. Ability to communicate well with the Investigator and is willing to comply with and complete all the study procedures.
8. Female subjects must be of non-childbearing potential or will need to utilize two methods of highly effective contraception during the study participation (e.g. hormonal contraception or an intrauterine device and condoms) or remain abstinent. Male subjects

should use condoms with spermicide during the course of the study or remain abstinent. Subjects should not donate sperm or ova during the study period.

8.4.3 Exclusion Criteria

Subjects will be excluded from the study if one or more of the following criterion are applicable:

1. Subject has previously participated in a FX-322 clinical trial.
2. Subjects currently on any medication consisting of valproic acid, valproate sodium, or divalproex sodium.
3. Perforation of tympanic membrane or other tympanic membrane disorders that would interfere with the delivery and safety assessment of an intratympanic medication or reasonably be suspected to affect tympanic membrane healing after injection in study ear. This includes a current tympanostomy tube.
4. Any conductive hearing loss of greater than 15 dB at a single frequency or greater than 10dB at two or more contiguous octave frequencies in the study ear at the Screening visit or on the prior audiogram (if the Investigator feels there is not a true conductive hearing loss, the Medical Montior should be consulted).
5. Active chronic middle ear disease or a history of major middle ear surgery, as an adult, in the ear to be injected.
6. Subject has had an intratympanic injection in either ear within 6 months of the screening visit.
7. History of clinically significant vestibular symptoms at the discretion of the investigator. For example, BPPV may be considered acceptable whereas Meniere's would not.
8. History of clinically significant systemic autoimmune disease (e.g. rheumatoid arthritis, Sjogren's syndrome, multiple sclerosis, psoriasis).
9. History of head or neck radiation treatment or exposure.
10. History of platinum-based chemotherapy treatment.
11. Exposure to another investigational drug within 28 days prior to injection of study drug.
12. Evidence of any active or chronic disease or condition that could interfere with, or for which the treatment of might interfere with, the conduct of the study, or that would pose an unacceptable risk to the subject in the opinion of the investigator following a detailed medical history, physical examination, and vital signs (systolic and diastolic blood pressure, pulse rate, body temperature).
13. History of substance abuse within 2 years of the Screening Visit.
14. Positive test for drugs of abuse at screening. In cases when the Investigator feels the subject should not be excluded for a positive drug test, the Sponsor will be consulted and determine final eligibility.
15. Positive urine pregnancy test or breast-feeding.
16. Any known factor, condition or disease that, in the view of the Investigator, might interfere with treatment compliance, study conduct or interpretation of the results (e.g. previous high-dose aminoglycoside treatment).

8.4.4 Withdrawal of Subjects From Study Drug and/or Study Assessments

Subjects may discontinue study drug or withdraw from the study for any of the following reasons:

- Adverse Event,

- Death,
- Lack of Efficacy,
- Lost to Follow-up,
- Non-Compliance with Study Drug,
- Other,
- Physician Decision,
- Pregnancy,
- Progressive Disease,
- Protocol Violation,
- Recovery,
- Study Terminated by Sponsor,
- Technical Problem,
- Withdrawal by Subject

Subjects are free to discontinue study drug and/or withdraw from the study at any time without providing reason(s) for withdrawal and without prejudice to further treatment. Subjects who discontinue study drug should be encouraged to continue to remain in the study and follow the per protocol assessments. Should a subject withdraw early from the study the reason(s) for study withdrawal will be documented in the electronic case report form (eCRF). All reasonable efforts will be made to have the subject return for one final assessment. Subjects withdrawing from the study will be encouraged to complete the same final evaluations as subjects completing the study according to this protocol, particularly safety evaluations. The aim is to record data in the same way as for subjects who completed the study.

Reasonable efforts will be made to contact subjects who are lost to follow-up. These efforts must be documented in the subject's file. Refer to Section 8.4.4 for details.

Pregnancy

Subjects will be instructed that known or suspected pregnancy occurring during the study, in subjects or female partners of male subjects, should be confirmed and reported to the investigator. All subjects will be followed until the end of the study, completing study assessments as appropriate during pregnancy. The investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject is subsequently found to be pregnant after inclusion in the study, any pregnancy will be followed to term, and the status of mother and child will be reported to the sponsor after delivery.

Full details will be recorded on the pregnancy page of the eCRF if the subject has completed the study.

8.4.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal

information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Rescreening is allowed for subjects in the Phase 2a study FX-322-202. Rescreening must occur on a different day than the original Screening Visit. Subjects will need to sign a new Informed Consent Form and will be assigned a new Screening number. All assessments will need to be performed again at the new Screening visit.

8.5 Investigational Products

8.5.1 Investigational Products Administered

Subjects will be randomized 1:1:1:1 to four treatment groups to receive multiple doses of [redacted] 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).

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

Topical anesthetic cream will be administered to the tympanic membrane. After 10 minutes, under a microscope, a 25-gauge needle will be used to inject of 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.

8.5.2 Identity of Investigational Products

The investigational drug is FX-322 which is a fixed ratio dose combination of 2 small molecules: a glycogen synthase kinase (GSK) inhibitor (FX03) and valproate sodium (FX00), a histone deacetylase (HDAC) inhibitor. [redacted]

8.6 Preparation and Dispensing

The FX-322 investigational product is a sterile liquid for intratympanic injection only. [redacted]

An osmolarity matched placebo for FX-322 is a sterile liquid for intratympanic injection only. [redacted]

The components in the study drug and placebo are shown in the following tables:

Table FX-322 Study Drug:

Active Ingredients	**Total** [redacted]
FX03	[redacted]
Valproate Sodium	[redacted]
Inactive Ingredients	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

The matched placebo has been developed with similar pH, osmolality and gelation as FX-322. [redacted]

Dosages of Active Agents to be Injected in Humans

	FX-322 **Total** [redacted]
FX03	[redacted]
FX00	[redacted]

Frequency Therapeutics will supply sterile FX-322 and placebo, as a sealed sterile vial containing [redacted] and another sterile vial containing [redacted]. The placebo or FX-322 is prepared by [redacted]

Each box will be labelled Study drug in compliance with applicable local regulations. FX-322/Placebo and [redacted] vials will be packaged in separate boxes.

Detailed instructions on compounding the study drug will be provided in the pharmacy manual.

All study drug will be transported, received, stored and handled strictly in accordance with the product label, the instructions provided to the investigational sites, the sites standard operation procedures and applicable regulations.

8.6.1 Packaging and Labeling

For the injection, the study drug or placebo will be provided in a kit with 4 study vials labelled for each injection (Injection 1, Injection 2, Injection 3, and Injection 4). The subject will be assigned just one kit for all of the study treatments. The [redacted] will be taken from general stock at the site. Topical anesthetic cream will be provided to the site. More information can be found in the Pharmacy Manual. The study drug and placebo will be prepared in a sterile 1 mL tuberculin syringe with a sterile cap. The syringe will be labelled clearly with the details for each randomized patient. Two syringes per patient will be provided in case a back-up syringe is needed for injection. The dose [redacted] will be filled to the [redacted] mark. The FX-322 and placebo preparations drawn into the syringes must be warmed in a heating unit. Refer to the Pharmacy Manual for further instructions.

The label(s) for the investigational product will include sponsor name, address and telephone number, the protocol number, investigational product name, dosage form, amount of investigational product per container, lot number, unique kit number, storage conditions, and required caution statements and/or regulatory statements, as applicable.

8.6.2 Supply, Storage and Handling

The vials must be stored as indicated in the Pharmacy Manual. Empty vials and containers may be destroyed at the conclusion of the study, after the site monitor has performed accountability and in compliance with the site's SOP.

8.6.3 Compliance and Drug Accountability

Accountability for the study drug at the study site is the responsibility of the Investigator, or their designee. The Investigator will ensure that the study drug is used only in accordance with this protocol. In order to maintain the blinding of the study drug, the Investigator may choose to assign some of the drug accountability responsibilities to a unblinded pharmacist or other appropriate individual. This individual will not perform any other study procedures other than study drug preparation and accountability. Drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each subject, and return (of unused vials) to Frequency Therapeutics (or destruction, if approved by Frequency Therapeutics) will be maintained by the clinical site. These records will adequately document that the subjects were provided the doses as specified in the protocol and should reconcile all study drug received from Frequency Therapeutics or its designee. Accountability records will include dates, quantities, batch/serial numbers, and subject identification numbers. The site monitor will review drug accountability at the site on an ongoing basis during monitoring visits.

8.6.4 Disposal, Return or Retention of Study Drug

All unused and used study drug will be retained at the site until inventoried by the site monitor. All used, unused or expired study drug will be returned to Frequency Therapeutics or if authorized, disposed off at the study site in accordance with governing regulations and documented.

8.7 Randomization and Blinding

8.7.1 Blinding

The subjects, all Frequency Therapeutics staff and representatives, Investigators and site personnel involved in administering study assessments will be blinded to the study drug assignment. The otolaryngologist will be blinded to treatment. The pharmacy staff who prepare the study drug will be unblinded and will not perform any other roles on the study other than drug preparation and accountability. The independent statistician and/or independent statistical programmer who is not otherwise involved with the study will generate the randomization schedule, will be un-blinded, and will perform the Day 90 data monitoring review. There will be a designated unblinded team from Frequency Therapeutics that will manage the implementation and distribution of the Interim Analysis. This team will not perform any other roles on the study at the time of this analysis or for the rest of the study.

8.7.2 Un-blinding

Only in the case of emergency, when knowledge of the study drug administered is essential for the clinical management or welfare of the subject, may the Investigator un-blind a subject's treatment group assignment. Under such conditions, the identity of the study drug will be obtained either via the unblinding functionality within the IRT system or by contacting the CRO.

If possible, the Medical Monitor and Frequency Therapeutics should be consulted prior to breaking the blind. If the blind is broken for any reason, the Investigator must notify Frequency Therapeutics and the Medical Monitor immediately of the un-blinding incident without revealing the subject's study treatment group assignment to Frequency Therapeutics and the Medical Monitor. In the event that the treatment group assignment is broken, the date, the signature of the person who broke the code and the reason for breaking the code must be recorded on the source documents. Any code-breaks that occur must be immediately reported to the Medical Monitor. Any subject whose treatment group assignment has been un-blinded will be followed up for safety purposes.

8.7.3 Method of Assigning Subjects to Treatment Groups

Subjects will be randomized 1:1:1:1 to one of four treatment groups. To protect the blind, all subjects will receive four injections. 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.

The randomization will be stratified for baseline Word Recognition Score (>=50% correct, <50% correct) and etiology of hearing loss (SSNHL vs. NIHL).

8.7.4 Selection of Doses in the Study

In this Phase 2a study (FX-322-202), four intratympanic injections will be administered as either placebo or the investigational product FX-322 and given on Days 1, 8, 15, and 21. [redacted]

[redacted] Weekly dosing of FX-322 did not produce any significant toxicological findings that were different from controls in the study. Additionally, study FX-322-201 demonstrated that a single dose of FX-322 was well-tolerated in patients with stable SNHL. Given that FX-322 drug product is formulated at maximally achievable concentration and that the systemic circulation and cochlear fluid pharmacokinetics show short half-lives for each of the FX-322 components, the weekly dosing schedule was selected as it allows for audiometric evaluations between doses and determination of the number of doses that provides the maximum progenitor cell effect and consequent improvement in hearing function.

The data from Phase 1/2 Study FX-322-201 support the hypothesis that activation of progenitor cells and consequent sensory hair cell formation and restoration of hearing function are driven by the concentration of the drug product applied to the round window membrane via intratympanic injection, and not by the volume of drug product delivered to the middle ear.

Based on the above, [redacted] will be used as the standard dose volume for all future clinical trials that study intratympanic administration of FX-322.

8.7.5 Selection and Timing of Dose for Each Subject

Once a subject has been identified who meets all inclusion/exclusion criteria, the ear with the worse hearing (ear with the worst Screening pure tone average) will be selected, unless a rationale exists to treat the other ear. The Investigator will need to discuss that rationale with the Sponsor prior to randomization. Subjects will be administered a total of 4 doses of FX-322 and/or placebo. Injections will take place on Days 1, 8, 15, and 21 as per treatment group.

8.8 Prior and Concomitant Therapy

At each study visit the study personal will question the subject about any medication taken, including vitamin supplements and herbal remedies. Any concurrent medications will be recorded in the subject's records and the eCRF. Any changes in doses or introduction of a new medication during the course of the study will be recorded. Any medications taken in the 14 days prior to the Screening visit will be recorded on the Concomitant Medication log.

8.8.1 Prohibited Medication/Therapy

Concomitant medications that are not allowed during the study are valproic acid (brand name (e.g. Depacon®, Depakote®, Depakene, Epival®, Valpro, and Epilim) or derivatives. Intratympanic injections of anything other than study medication are prohibited during the course of the study or within 14 days prior to the Screening Visit. Oral and intratympanic steroids are prohibited during the study, including the follow up period. Aminoglycosides and platinum-based chemotherapies are excluded during the study.

8.8.2 Rescue Medication

No rescue medication will be provided as no FDA approved treatment for sensorineural hearing loss exists. The Investigator should treat any adverse events as medically appropriate and per their standard of care.

8.8.3 Treatment Compliance

Treatment will be administered by the Investigator to each subject on Day 1, 8, 15 and 21 as per the treatment schedule for each of the subjects randomized to one of four groups. If a subject misses a treatment, that treatment will not be made up and subject will return for the next scheduled treatment.

9 DISCONTINUATION AND LOST TO FOLLOW-UP

9.1 Discontinuation of Study Intervention

Discontinuation from FX-322 does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- All efforts will be made for the subject to complete all of the follow up visits.
- If the subject does not wish to continue the follow up period, all efforts will be made for the subject to complete all assessments collected at the last follow up visit (see Section 8.4.4).

9.2 Lost to Follow-up

A subject will be considered lost to follow-up if he or she fails to return for 2 scheduled visits (or the final visit) and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 5 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

10 TIMING OF STUDY PROCEDURES

Subjects will provide written informed consent before any study related procedures are performed.

The planned study assessments are in Section **Error! Reference source not found.**.

10.1 Pre-treatment

10.1.1 Screening Visit (Visit 1; Day -30 to 0)

The following procedures will be performed at the Screening Visit:

- Obtain signed Informed Consent.
- Assess for eligibility (against the inclusion and exclusion criteria).
- Obtain prior audiogram. The audiogram must be performed at least 6 months prior to the Screening Visit. If there are multiple audiograms present in the subject's records, the most recently performed audiogram at least 6 months old should be used.
- Collect full medical history, including targeted hearing loss history, concomitant illnesses/diseases, and concomitant medications.
- Record demographic data, including ethnic origin, date of birth, and sex.
- Perform a physical examination, including body weight and height.
- Record vital signs (supine blood pressure, body temperature, and heart rate).
- Collect blood sample for safety laboratory assessments.
- Collect urine sample for pregnancy test, if applicable.
- Collect urine sample for drug screening (cocaine, narcotics, benzodiazepines, tetrahydrocannabinol, barbiturates, and amphetamines).
- Perform otoscopy, standard pure tone audiometry, extended high frequency audiometry, word recognition testing, WIN testing, and tympanometry

10.1.2 Baseline/Treatment Visit (Visit 2a, [Day 1])

The following procedures will be performed at the Baseline/Treatment Visit:

- Before injection of study drug:
    - Reassess for eligibility against the inclusion and exclusion criteria.
    - Collect blood sample for biomarker analysis.
    - Collect concomitant medications.
    - Record vital signs.
    - Perform a urine pregnancy test, if applicable.
    - Perform otoscopy.
    - Record any changes in medical history that have occurred since the previous visit.
    - Administer the Tinnitus Functional Index, C-SSRS, Hearing Handicap Inventory for Adults, and Hearing Screening Inventory.
    - When all the above baseline procedures have been performed and the Investigator has confirmed the subject's eligibility for the study, the subject will be randomized. Each subject will receive a unique randomization number.
- Inject FX-322 or placebo via intratympanic injection to the study ear.
- After study drug injection:
    - Record any adverse events that have occurred since study treatment.
    - Collect concomitant medications.

10.1.3 Additional Treatment Visits (Visits 2b, 2c, 2d, [Days 8, 15 and 21] +/- 2 days)

The additional treatment visits (Visits 2b, 2c, and 2d) will take place 7 days apart on Days 8, 15 and 21 after the Baseline/Treatment visit. The following procedures will be performed at these visits:

- Before injection of study drug:
    - Collect concomitant medications.
    - Record any adverse events that have occurred since last study visit.
    - Record vital signs.
    - Perform otoscopy.
    - Perform urine pregnancy test.
- Inject FX-322 or placebo via intratympanic injection to the study ear as per treatment group.
- After study drug injection:
    - Record any adverse events that have occurred since study treatment.
    - Collect concomitant medications.

In addition to the above the following procedures will be performed only at Visit 2c, Day 15 prior to study injection:

- Perform standard pure tone audiometry, extended high frequency audiometry, word recognition testing, WIN testing, and tympanometry.

10.1.4 Follow-up (Visit 3, [Day 30 +/- 15 days])

The Phone Call Follow-up Visit (Visit 3) will take place at Day 30 (± 15 days). The following procedures will be assessed via phone at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.

10.1.5 Follow-up (Visit 4, [Day 60 +/- 15 days])

The Follow-up Visit (Visit 4) will take place at Day 60 (± 15 days). The following procedures will be performed at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.
- Perform otoscopy, standard pure tone audiometry, extended high frequency audiometry, word recognition testing, WIN testing, and tympanometry.
- Administer the Tinnitus Functional Index, C-SSRS, and Hearing Handicap Inventory for Adults.
- Record vital signs.

If the subject is not able to return to clinic for this visit due to any reason, a phone call visit will be performed. Any AEs and concomitant medication changes will be recorded along with the questionniares.

10.1.6 Follow-up (Visit 5, [Day 90 +/- 15 days])

The Follow-up Visit (Visit 5) will take place at Day 90 (± 15 days). The following procedures will be performed at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.
- Perform otoscopy, standard pure tone audiometry, extended high frequency audiometry, word recognition testing, WIN testing, and tympanometry.
- Record vital signs.

If the subject is not able to return to clinic for this visit due to any reason, a phone call visit will be performed. Any AEs and concomitant medication changes will be recorded.

10.1.7 Follow-up (Visit 6, [Day 120 +/- 15 days])

The Phone Call Follow-up Visit (Visit 6) will take place at Day 120 (± 15 days). The following procedures will be assessed via phone at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.
- Administer the Tinnitus Functional Index, C-SSRS, and Hearing Handicap Inventory for Adults.

10.1.8 Follow-up (Visit 7, [Day 150 +/- 15 days])

The Follow-up Visit (Visit 7) will take place at Day 150 (± 15 days). The following procedures will be performed at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.
- Perform otoscopy, standard pure tone audiometry, extended high frequency audiometry, word recognition testing, WIN testing, and tympanometry.
- Record vital signs.

If the subject is not able to return to clinic for this visit due to any reason, a phone call visit will be performed. Any AEs and concomitant medication changes will be recorded.

10.1.9 Follow-up (Visit 8, [Day 180 +/- 15 days])

The Phone Call Follow-up Visit (Visit 8) will take place at Day 180 (± 15 days). The following procedures will be assessed via phone at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.

10.1.10 Follow-up (Visit 9, [Day 210 +/- 15 days])

The Follow-up Visit (Visit 9) will take place at Day 210 (± 15 days). The following procedures will be performed at the Follow-up Visit:

- Record any AEs that have occurred since the last visit and any changes in concomitant medication.

- Perform otoscopy, standard pure tone audiometry, extended high frequency audiometry, word recognition testing, WIN testing, and tympanometry.
- Administer the Tinnitus Functional Index, C-SSRS, Hearing Handicap Inventory for Adults, and Hearing Screening Inventory.
- Collect blood sample for safety laboratory assessments.
- Collect blood sample for biomarker analysis.
- Record vital signs.

10.2 Duration of Treatment

The duration of treatment will be 4 weeks with 6 months of follow up after the treatment period.

11 EFFICACY ASSESSMENTS

11.1 Efficacy Measurements Assessed

11.1.1 Word Recognition in Quiet (WR)

Word recognition in quiet will be measured with recorded CNC word lists. The test will consist of 50 words presented to the subject and the percentage of words correctly identified will be recorded. Word recognition will be performed at Screening, Day 15, 60, 90, 150, and 210 Visits. Testing will be performed at 30dB 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 Audiology Manual of Procedures.

11.1.2 Words-In-Noise Testing (WIN)

The Words-in-Noise Test (WIN) was developed as an instrument to quantify the ability of listeners to understand monosyllabic words in background noise using multitalker babble (Wilson, 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. This test is conducted by presenting 2 lists of 35 recorded words to each ear. Overall accuracy will be recorded as the number of words identified correctly. This test will occur at Screening, Day 15, 60, 90, 150, and 210. Testing will be performed at 80dB SPL. Word lists will be rotated at each visit. Additional details can be found in the Audiology Manual of Procedures.

11.1.3 Standard Pure Tone Audiometry

Standard Pure Tone Audiometry will be measured to determine a subject's threshold for hearing at various frequencies and will be performed at the Screening visit and Day 15, 60, 90, 150, and 210. This will be performed on a calibrated audiometer by a licensed audiologist. The following frequencies will be obtained: Air: 250 Hz, 500 Hz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 6 kHz, and 8 kHz; Bone: 500 Hz, 1 kHz, 2 kHz, 3kHz, and 4 kHz.

11.2 Exploratory Efficacy Measurements Assessed

11.2.1 Extended High Frequency Audiometry

Extended high frequency audiometry will be performed to determine a subject's threshold for hearing at frequencies beyond those in standard pure tone audiometry and will be performed

at the screening visit and Day 15, 60, 90, 150, and 210. This will be performed on a calibrated audiometer by a licensed audiologist. The following frequencies will be obtained: Air – 9 kHz, 10 kHz, 11.2 kHz, 12.4 kHz, 14 kHz, and 16 kHz.

11.2.2 Tinnitus Functional Index (TFI)

The Tinnitus Functional Index has eight subscales that address 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. The subject will report (either directly onto the questionnaire in person, via web, or over the phone) answers to each of the 25 questions using a scale of 0-10. The TFI will be given to the subject to complete at the Baseline, Day 60, Day 120, and Day 210 visits.

11.2.3 Hearing Handicap Inventory for Adults (HHIA)

The Hearing Handicap Inventory for Adults (HHIA), is a 25-item self-assessment scale composed of two subscales (emotional and social/situational). The subject will report (either directly onto the questionnaire in person, via web, or over the phone) one of the following answers for each item on the scale: Yes, Sometimes, No. The HHIA will be given to the subject to complete at the Baseline, Day 60, Day 120, and Day 210 visits.

11.2.4 Hearing Screening Inventory (HSI)

The Hearing Screening Inventory is a 12 item self report inventory to assess hearing impairment. The subject will self report one of the following answers, for each question from 1 to 8: Never, Seldom, Occasionally, Frequently, Always, and for each question from 9 to 12: Good, Average, Slightly below average, Poor, Very Poor. The HSI is designed to assess subjective change during the study and will be given to the subject to complete at the Baseline and Day 210 visits.

12 SAFETY ASSESSMENTS

The planned schedule of assessments is in Section **Error! Reference source not found.**.

12.1 Safety Measurements Assessed

12.1.1 Concomitant Medication

Subjects will be asked about concomitant medications at time points outlined in the Table of Assessments. All concomitant medication information will be recorded in the eCRF.

12.1.2 Clinical Laboratory Evaluation

Blood samples will be collected for safety laboratory assessments for clinical chemistry and a complete blood count.

Urine drug screen (cocaine, narcotics, benzodiazepines, tetrahydrocannabinol, barbiturates, and amphetamines) will be performed at Screening only.

Screening for pregnancy will be performed (urine β-HCG) as outlined in Table 1.

12.1.3 Vital Signs

Vital signs (temperature, blood pressure and heart rate) will be recorded at time points outlined in Table 1 and will be performed in a standardized manner, i.e., after the subject has rested in the sitting position for 5 minutes.

12.1.4 Physical Exam (PE)

A complete physical examination will be performed by a licensed provider at Screening.

Complete physical examinations include: general appearance, head, ears, eyes, nose, throat, dentition, thyroid, chest (heart, lungs), abdomen, skin, neurological, extremities, back, neck, musculoskeletal, and lymph nodes and any pertinent system based on any prior findings. Physical examinations may be performed at various unscheduled time points, if deemed necessary by the Investigator.

12.1.5 Columbia Suicide and Severity Rating Scale (C-SSRS)

This scale is used to determine if any suicide ideation or intention is present. The questionnaire is read aloud to the subject and responses are recorded by the trained staff member. If any ideation or intention is present, the Investigator will interview the subject and have them follow up with their primary healthcare provider. The C-SSRS will be completed at the Baseline, Day 60, Day 120, and Day 210 visits.

12.1.6 Body Mass Index (BMI)

BMI will be calculated by dividing the subject's body weight in kilograms by the subject's height in meters squared (BMI = kg/m^2). Body height (centimeter) and body weight (kilograms) will be measured at the time points delineated in the Schedule of Assessments.

12.1.7 Otoscopy

Microscopic otoscopy will be included to specifically record any abnormalities of the external ear canal, tympanic membrane and middle ear and will be performed at Screening, prior to injection on Day 1, 8, 15, and 21 and at the Day 60, 90, 150, and 210 visits.

12.1.8 Tympanometry

Tympanometry (an objective test of tympanic membrane mobility) tests the integrity of the tympanic membrane by varying air pressure in the ear canal. Middle ear compliance, peak pressure, and tympanogram type will be recorded. Tympanometry will be performed at the Screening, Day 15, and at the Day 60, 90, 150, and 210 Visits. The assessment will be performed by a licensed audiologist on a calibrated tympanometer. A print-out containing the tympanometry from the machine will be collected and stored in the source document.

13 ADVERSE EVENTS

Adverse Event Definition

An AE is defined as any untoward medical occurrence in a clinical study subject administered a medicinal product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not it is related to the medicinal (investigational) product. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction or the significant worsening of the indication under investigation that is not recorded elsewhere in the eCRF under specific efficacy assessments. Anticipated fluctuations of pre-existing conditions, including the disease under study that do not represent a clinically significant exacerbation or worsening need not be considered AEs.

It is the responsibility of the investigator to document all AEs that occur during the study. AEs will be elicited by asking the subject a nonleading question, for example, “Have you experienced any new or changed symptoms since we last asked/since your last visit?”. AEs will be recorded from the time of the first treatment through the end of the follow up period. AEs should be reported on the appropriate page of the eCRF.

Unexpected Adverse Event or Unexpected Suspected Adverse Reaction Definition

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

"Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Assessment of Severity

Each AE will be assigned a category by the investigator as follows:

Mild:	An AE that is easily tolerated by the subject, causes minimal discomfort and does not interfere with everyday activities.
Moderate:	An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed.
Severe:	An AE that prevents normal everyday activities; treatment or other intervention usually needed.

If there is a change in severity of an AE, it must be recorded as a separate event.

Assessment of Causality

Every effort will be made by the investigator to assess the relationship of the AE, if any, to the study drug. Causality should be assessed using the categories presented in the following table:

Not Related:	Clinical event with an incompatible time relationship to study drug administration, and that could be explained by underlying disease or other drugs or chemicals or is incontrovertibly not related to the study drug.
Unlikely:	Clinical event whose time relationship to study drug administration makes a causal connection improbable, but that could plausibly be explained by underlying disease or other drugs or chemicals.
Possible:	Clinical event with a reasonable time relationship to study drug administration, but that could also be explained by concurrent disease or other drugs or chemicals.
Related:	Clinical event with a reasonable time relationship to study drug administration and is unlikely to be attributed to concurrent disease or other drugs or chemicals.

Adverse Events of Special Interest (Audiometric and Otoscopic)

If any of the following criteria are met, the event will be recorded as an adverse event of special interest (AESI) per ASHA guidelines (ASHA 1994):

- Asymmetric loss of hearing greater than 20 dB at any one frequency in the treated ear compared to the Screening Visit.
- Asymmetric loss of hearing greater than 10 dB at two adjacent frequencies in the treated ear compared to the Screening Visit.
- Asymmetric loss of response at three consecutive test frequencies where responses were previously obtained in the treated ear compared to the Screening Visit.

For word recognition testing, a follow up visit score in the treated ear that falls below the lower limit of the 95% confidence interval of the baseline word recognition score will be considered an AESI (Thorton and Raffin 1978).

Additionally, if the subject experiences a perforation greater than 25% of the tympanic membrane in the treated ear this will be recorded as an AESI.

If any subjects in the study experience the same AESI, the Medical Monitor will discuss these with the Principal Investigators and each subject's condition will be discussed. The Medical Monitor, in consultation with the sponsor, will determine whether to proceed with any future doses if the subjects have not completed all 4 doses.

Action Taken with Study Treatment in Response to Adverse Event

The investigator will describe the action taken in the appropriate section of the eCRF, as follows:

- Dose Increased,
- Dose Not Changed,
- Dose Reduced,
- Drug Interrupted,
- Drug Withdrawn,

- Not Applicable,
- Unknown

Follow-up of Adverse Events

All Investigators should follow up subjects with AEs until the event is resolved or until, in the opinion of the investigator, the event is stabilized or determined to be chronic. Details of AE resolution must be documented in the eCRF.

Documentation and Reporting of Adverse Events

AEs should be reported and documented in accordance with the procedures outlined below. All AEs occurring during the study (after study treatment has begun) must be documented on the relevant eCRF pages. The following data should be documented for each AE:

- Description of the symptom event, or underlying diagnosis
- Classification of 'serious' or 'not serious'
- Severity
- Date of first occurrence and date of resolution (if applicable)
- Action taken (see definitions above)
- Causal relationship
- Outcome of event (unknown, recovered, not yet recovered, recovering/resolving, recovered with sequelae, deaths[with date and cause reported])

13.1 Serious Adverse Events

Serious Adverse Event Definition

An SAE is any untoward medical occurrence or effect that, at any dose,

- Results in death.
- Is life-threatening (an AE is life-threatening if the subject was at immediate risk of death from the event as it occurred, i.e., it does not include a reaction that might have caused death if it had occurred in a more serious form).
- Requires or prolongs inpatient hospitalization. (Complications occurring during hospitalization are AEs and are SAEs if they cause prolongation of the current hospitalization. Hospitalization for elective treatment of a pre-existing non--worsening condition is not, however, considered an AE. The details of such hospitalizations must be recorded on the medical history or physical examination page of the eCRF).
- Results in persistent or significant disability/incapacity. (An AE is incapacitating or disabling if it results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions).
- Results in a congenital anomaly/birth defect.

In addition, medical and scientific judgement is required to decide if prompt notification is required in situations other than those defined for SAEs above. This may include any event that the Investigator regards as serious that did not strictly meet the criteria above but may have jeopardized the subject or required intervention to prevent one of the outcomes listed

above, or that would suggest any significant hazard, contraindication, side effect, or precaution that may be associated with the use of the investigational product.

Reporting of Serious Adverse Events

Any SAE must be reported by the Investigator if it occurs during the clinical study, whether or not the SAE is considered to be related to the investigational product. The Serious Adverse Event eCRF must be completed, with as much information as is available, within 24 hours of when the investigative site becomes aware of the event. Once the eCRF is saved, this triggers an automatic email to [redacted] Additional contact details of the Medical Monitor can be found in the Site Operation Manual.

The Investigator should not wait to receive additional information to document fully the event before notification of a SAE, though additional information may be requested. Information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

Instances of death, congenital abnormality, or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the investigator at any time after cessation of study drug administration and linked by the investigator to this study, should be reported to the study monitor.

The sponsor and/or CRO will promptly notify all relevant investigators and the regulatory authorities of findings that could adversely affect the safety of subjects, impact on the conduct of the study or alter the independent ethics committee (IEC)/institutional review board (IRB) approval/favorable opinion of the study. In addition, CRO on behalf of the sponsor, will expedite the reporting to all concerned investigators, to the IRBs, where required, and to the regulatory authorities of all adverse reactions that are both serious and unexpected.

Details of the procedures to be followed if a pregnancy occurs are provided in Section 8.4.4.

13.2 Expedited Safety Reporting

All serious and unexpected suspected adverse reactions (SUSARs) will be the subject of expedited reporting. The sponsor and/CRO shall ensure that all relevant information about a SUSAR that is fatal or life-threatening is reported to the regulatory authorities and IRB within 7 days after knowledge by the sponsor of such a case and that relevant follow up information is communicated within an additional 8 days. All other SUSARs will be reported to the regulatory authorities and IRB within 15 days after knowledge by the sponsor of such a case. All Investigators should follow up SUSARs until the event is resolved or until, in the opinion of the Investigator, the event is stabilized or determined to be chronic. Post study SUSARs that occur after the subject has completed the clinical study must be reported by the investigator to the sponsor.

14 STATISTICAL METHODS

14.1 Statistical and Analytical Plans

14.1.1 Descriptive Statistics

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 (no changes in air conduction greater than 10 dB at a single frequency or greater than 5dB at two contiguous frequencies from the prior audiogram to the Screening audiogram). The primary objectives of the study are to explore the efficacy of FX-322 associated with various dosing schedules and to describe the local and systemic safety.

Subjects will be randomized to one of four treatment groups using a 1:1:1:1 allocation ratio. Subjects will be stratified at randomization by baseline (last assessment before study drug administration Word Recognition (above and below 50% correct) and etiology of hearing loss (Sudden vs Noise). For the analysis, baseline will be defined as the last measurement prior to the first exposure to study drug.

The exploration of efficacy will be descriptive as well as analytical to describe dose response trends among the four treatment groups (three FX-322 dosing schedules and placebo). Additional analyses will include comparisons of each individual FX-322 to placebo and between the three FX-322 groups as well as the pooled FX-322 group to placebo. These analyses will examine each timepoint and explore the longitudinal aspects of the study design.

Safety will be analysed descriptively.

Study data will also be provided in line listings.

Details of the statistical analysis will be provided in the Statistical Analysis Plan (SAP) which will be finalized prior to database lock and study blind break.

14.1.2 Sample Size

The sample size of approximately 24 subjects per treatment 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.

14.1.3 Analysis Sets

- Full Analysis Set (FAS)

The Full Analysis Set is defined as all randomized subjects who receive at least one dose of study drug. Subjects will be analysed according to their randomized treatment group. The FAS will be used to explore efficacy. Details will be provided in the SAP.

- Per Protocol Analysis Set (PPAS)

The Per Protocol Analysis Set is defined as subjects receiving all four doses of study drug or placebo 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. If different from the FAS, the PPAS may be used in additional sensitivity analyses. PPAS subjects will be analysed according to their randomized treatment group. Details will be provided in the SAP.

- Safety Analysis Set (SfAS)

The Safety Analysis Set will include all subjects exposed to study drug or placebo and will be analyzed according to the actual treatment group regardless of their randomized treatment assignment. The SfAS will be used for the analysis of safety. Both the treated and untreated ear will be included in the SfAS when the ear is the unit of the safety analysis.

14.1.4 Subject Disposition, Demographics and Baseline Disease Status

Descriptive statistics for subject disposition, demographics, and baseline disease status will be provided. Tabulations will be summarized by randomized treatment group and a pooled FX-322 group. Where applicable, summaries by the two stratification factors used at randomization will also be provided.

14.1.5 Efficacy Exploration

Efficacy endpoints will be summarized descriptively at each study timepoint including baseline. Calculations will include the observed data at each timepoint, and if relevant, the change from baseline. For continuous endpoints means, medians, standard deviations, minimums and maximum will be provided. For categorical endpoints the number and percent of subjects within each category will be calculated. Tabulations for selected endpoints will be presented by subgroups such as age, study site, duration of hearing loss, smoking status, and stratification factors used at randomization. Details will be provided in the SAP. For descriptive analyses, subjects with missing data will be excluded for a particular calculation.

For questionnaires, the analysis will follow the instructions for scoring and handling of missing data appropriate for the particular instrument.

Inferential statistical methods examining continuous endpoints will be examined via a Mixed Model for Repeated Measures (MMRM). Analyses will include pairwise contrasts and dose response. Inferential analyses for categorical endpoints will be analysed using univariate methods and/or Generalized Linear Mixed Models (GLMM). When appropriate, models will include adjustments for baseline as well as the stratification factors used at randomization. Departures of these models from the statistical assumptions will be assessed and alternative approaches maybe employed. Additionally, univariate models such as Analysis of Covariance and/or count models examining single timepoints of interest may also be examined. General methods for handling missing data for inferential analyses are described below (Section 14.2).

The inferential analyses will not be adjusted for multiple endpoints; however, for a given endpoint, adjusted p-values for pairwise comparisons to a control will be provided where

relevant along with the unadjusted p-values. Unadjusted 95% confidence intervals will also be provided. In some circumstances, adjusted confidence intervals may be calculated.

Efficacy endpoints will be analysed using the FAS. Further details regarding the exploratory analysis of efficacy will be outlined in the SAP.

14.1.6 Interim Analyses

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 in order 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.

14.1.7 Safety Analyses

Safety will be evaluated using the safety analysis set and will include the incidence of adverse events (AEs), serious adverse events (SAE)s, vital signs, clinical laboratory measurements, concomitant medications, otology, questionnaire responses, and tympanometry assessments. Summary descriptive statistics will be provided by actual treatment group, pooled FX-322 group, and overall using the SfAS. No safety data will be imputed except for partial dates if required to determine if an adverse event is treatment emergent or a medication concomitant with exposure to study drug. Details of partial date imputation will be provided in the SAP.

14.1.7.1 Adverse Events

The number and percentage of subjects reporting adverse events (AEs) will be summarized via the MedDRA system by organ class and preferred term. Data will be tabulated by severity, physician assessment of relationship to study drug, serious AEs, and AEs leading to death or study withdrawal. Adverse events of the ear will also be summarized by treated vs. non-treated ear.

14.1.7.2 Clinical Laboratory Evaluations

Clinical laboratory evaluations will be presented as summary statistics by treatment group for each timepoint as well as changes from baseline and/or shift tables. Pregnancy results will not be summarized but will be provided in line listings.

14.1.7.3 Vital Signs and Physical Examation

Vital signs and physical exams will be summarized via descriptive statistics similar to laboratory evaluations described above.

14.1.7.4 Prior and Concomitant Medications

Prior and concomitant medications will be coded 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 recorded medications that started and stopped prior to administration of 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 percent of subjects using concomitant medications will be tabulated by drug class and preferred drug name. Prior medications will be presented in line listings only. Concomitant medications will also be provided in line listings.

14.1.7.5 Tympanometry and Otoscopy

Tympanometry and otoscopic results will be presented by treatment group and ear (treated vs. untreated) at each visit with changes and/or shifts from baseline.

14.2 Handling of Missing Data and Subject Withdrawals

Except for partial dates to assess AESIs and concomitant medications, safety data will not be imputed. Imputation of efficacy data may be implemented based on the analysis of patterns of missingness in the visit assessments for subjects. Details of how this will be conducted and the imputation methods planned will be provided in the SAP.

15 QUALITY ASSURANCE AND QUALITY CONTROL

15.1 Audit and Inspection

Study centers and study documentation may be subject to Quality Assurance audit during the course of the study by the sponsor or its nominated representative. In addition, inspections may be conducted by regulatory authorities at their discretion.

15.2 Monitoring

Data for each subject will be recorded on an eCRF. Data collection must be completed for each subject who signs an informed consent form (ICF) and is administered study drug.

In accordance with GCP and ICH guidelines, the study monitor will carry out source document verification and request clarification at regular intervals to ensure that the data collected in the eCRF are accurate, complete and reliable. Study monitor has the responsibility of assessing the progress of the study, of checking that the informed consent forms have been signed by the patient, ensuring adhesion to and compliance with the study protocol and other study-related documents

The investigator must permit the monitor, the IEC/IRB, the sponsor's internal auditors, and representatives from regulatory authorities direct access to all study-related documents and pertinent hospital or medical records for confirmation of data contained within the eCRFs.

15.3 Data Management and Coding

The sponsor or CRO will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant standard operating procedures (SOPs) of the data management and biostatistics departments of sponsor or CRO.

Study centers will enter data directly into an electronic data capture (EDC) system by completing the eCRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study center. Data to be recorded directly on the eCRF will be identified and the eCRF will be considered the source document. Any changes to the data entered into the EDC system will be recorded in the audit trail and will be FDA CFR 21 Part 11 compliant.

Medical coding will use Medical Dictionary for Regulatory Activities (MedDRA) for concomitant diseases and AEs and WHO Drug for medications.

Missing or inconsistent data will be queried in EDC for clarification. Subsequent modifications to the database will be documented.

16 RECORDS AND SUPPLIES

16.1 Drug Accountability

On receipt of the study drug, the Investigator (or designee) will conduct an inventory of the supplies and verify that study drug supplies are received intact and in the correct amounts before completing a supplies receipt. The Investigator will retain the original of this receipt at the study center and return a copy to the study monitor. The monitor may check the study supplies at each study center at any time during the study.

It is the responsibility of the study monitor to ensure that the Investigator (or designee) has correctly documented the amount of the study drug received, dispensed, and returned on the dispensing log that will be provided. A full drug accountability log will be maintained at the study center at all times. The study monitor will arrange collection of unused study drug returned by the subject. The study monitor will also perform an inventory of study drug at the close-out visit to the study center. All discrepancies must be accounted for and documented.

16.2 Financing and Insurance

Financing and insurance of this study will be outlined in a separate agreement between CRO and the sponsor.

17 ETHICS

17.1 Independent Ethics Committee or Institutional Review Board

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the subjects, and any other relevant study documentation will be submitted to the appropriate IEC/IRB. Written approval of the study and all relevant study information must be obtained before the study center can be initiated or the study drug is released to the Investigator. Any necessary extensions or renewals of IEC/IRB approval must be obtained for changes to the study such as amendments to the protocol, the ICF or other study documentation. The written approval of the IEC/IRB together with the approved ICF must be filed in the study files.

The Investigator will report promptly to the IEC/IRB any new information that may adversely affect the safety of the subjects or the conduct of the study. The Investigator will submit written summaries of the study status to the IEC/IRB as required. On completion of the study, the IEC/IRB will be notified that the study has ended.

17.2 Regulatory Authorities

Relevant study documentation will be submitted to the regulatory authorities of the participating countries, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

17.3 Ethical Conduct of the Study

The investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines, and the applicable national and local laws and regulatory requirements.

17.4 Informed Consent

The process of obtaining informed consent must be in accordance with applicable regulatory requirement(s) and must adhere to GCP.

The Investigator is responsible for ensuring that no subject undergoes any study related examination or activity before that subject has given written informed consent to participate in the study.

The Investigator or designated personnel will inform the subject of the objectives, methods, anticipated benefits, potential risks, and inconveniences of the study. The subject should be given every opportunity to ask for clarification of any points s/he does not understand and, if necessary, ask for more information. At the end of the interview, the subject will be given ample time to consider the study. Subjects will be required to sign and date the ICF. After signatures are obtained, the ICF will be kept and archived by the investigator in the investigator's study file. A signed and dated copy of the subject ICF will be provided to the subject or their authorized representative.

It should be emphasized that the subject may refuse to enter the study or to withdraw from the study at any time, without consequences for their further care or penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give or who withdraw written informed consent should not be included or continue in the study.

If new information becomes available that may be relevant to the subject's willingness to continue participation in the study, a new ICF will be approved by the IEC(s)/IRB(s) (and regulatory authorities, if required). The study subjects will be informed about this new information and reconsent will be obtained.

17.5 Subject Confidentiality

Monitors, auditors, and other authorized agents of the sponsor and/or its designee, the IEC(s)/IRB(s) approving this research, and the United States FDA, as well as that of any other applicable agency(ies), will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

All personal data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with the Health Insurance Portability and Accountability Act (1), applicable to national and/or local laws and regulations on personal data protection.

Reporting and Publication, Including Archiving

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study (end of study defined as the date of the last visit of the last subject), all documents and data relating to the

study will be kept in an orderly manner by the investigator in a secure study file. This file will be available for inspection by the sponsor or its representatives. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years since the discontinuation of clinical development of the investigational product. It is the responsibility of the sponsor to inform the study center when these documents no longer need to be retained. The investigator must contact the sponsor before destroying any study related documentation. In addition, all subject medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

The sponsor must review and approve any results of the study or abstracts for professional meetings prepared by the investigator(s). Published data must not compromise the objectives of the study. Data from individual study centers in multicenter studies must not be published separately.

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19 APPENDIX 1

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.

20 INVESTIGATOR SIGNATURE PAGE

Protocol Title: 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

Protocol Number: FX-322-202

Confidentiality and GCP Compliance Statement

I, the undersigned, have reviewed this protocol (and amendments), including appendices, and I will conduct the study as described in compliance with this protocol (and amendments), GCP, and relevant ICH guidelines.

Once the protocol has been approved by the IEC/IRB, I will not modify this protocol without obtaining prior approval of Frequency Therapeutics and of the IRB. I will submit the protocol amendments and/or any ICF modifications to Frequency Therapeutics and IRB, and approval will be obtained before any amendments are implemented.

I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all eCRFs, laboratory samples, or source documents forwarded to the sponsor. Clinical information may be reviewed by the sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Frequency Therapeutics, to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.