Clinical Study Protocol

A Phase 2, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Multicenter Study to Evaluate the Efficacy of FX-322 Administered by Intratympanic Injection in Adults with Acquired Sensorineural Hearing Loss

NCT Number: 05086276

Protocol Date: 05 Jan 2022

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CLINICAL STUDY PROTOCOL

A Phase 2, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Multicenter Study to Evaluate the Efficacy of FX-322 Administered by Intratympanic Injection in Adults with Acquired Sensorineural Hearing Loss

Protocol Number:	FX-322-208
Investigational Product:	FX-322
Phase:	2
Sponsor:	Frequency Therapeutics 75 Hayden Ave – Suite 300 Lexington, MA 02421 USA
Protocol Date:	05 Jan 2022
Protocol Version:	3.0

CONFIDENTIAL

1 PROTOCOL APPROVAL SIGNATURES

Protocol Title: A Phase 2, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Multicenter Study to Evaluate the Efficacy of FX-322 Administered by Intratympanic Injection in Adults with Acquired Sensorineural Hearing Loss

Protocol Number: FX-322-208

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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.

Sponsor Signature

2 SYNOPSIS

Protocol Number:
FX-322-208

Title:
A Phase 2, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Multicenter Study to Evaluate the Efficacy of FX-322 Administered by Intratympanic Injection in Adults with Acquired Sensorineural Hearing Loss

Investigational Product:
FX-322

Study Centers:
Approximately 25 centers in the US

Phase:
Phase 2

Objectives:
Primary Objective

- To assess efficacy following a single dose of FX-322 versus Placebo, in subjects with acquired sensorineural hearing loss.

Secondary Objective(s)

- To assess safety following a single dose of FX-322 or Placebo in subjects with acquired sensorineural hearing loss.

Endpoints:

Primary Efficacy Endpoint

- Word Recognition in quiet (WR) improvements through Day 90

Secondary Endpoint(s)

- Words-in-Noise improvements through Day 90
- Audiometry
    - Standard Pure Tone (air 0.25-8 kHz; bone 0.5-4 kHz)
    - Extended High Frequency (air 9-16 kHz)
- [redacted] Qualitative Questionnaire
- Tinnitus Functional Index (TFI) Questionnaire
- Research Assessment on [redacted] Quality of Life [redacted] Questionnaire
- Patient Global Impression of Change (PGI-C) Hearing Loss Scale
- Patient Global Impression of Change (PGI-C) Daily Impacts Scale

Exploratory Endpoint(s)

- Audiometry, speech perception measure improvements, and safety assessments beyond Day 90

Safety Endpoint(s)

- Adverse events
- Changes in:
    - Otoscopy
    - Tympanometry
    - C-SSRS

Study Design:

This is a Phase 2, prospective, randomized, double-blind, placebo-controlled, single-dose, multicenter study to evaluate the efficacy of FX-322, administered by intratympanic injection, in adults with acquired sensorineural hearing loss (SNHL).

Previous human studies in subjects 18 to 65 years inclusive (FX-322-103, FX-322-201, FX-322-111) and subjects 66-85 inclusive (FX-322-112) demonstrated that a single dose FX-322 was well tolerated in patients with acquired SNHL with no treatment-related serious adverse events. Adverse events in these studies were generally common to and associated with the intratympanic injection procedure with mild and transient discomfort in patients both with the drug and the placebo (McLean et al., 2021).

In previous single-dose human studies in subjects 18-65 years inclusive (FX-322-201, FX-322-111) it was demonstrated that treatment with FX-322 was associated with statistically significant and clinically meaningful improvements in auditory measures 90 days after a single administration of FX-322, compared to either placebo or untreated ears.

We intend to further establish efficacy with a single dose of FX-322 in subjects 18 to 65 years inclusive with acquired SNHL.

The study will have two Parts as described below:

Part A: Screening/Start of Lead-in/Visit 1Lead-in/Visit 2

Part B: Randomization, Treatment, Follow-up, and Observation

Part A

Screening/Start of Lead-In: Visits can occur 30-40 days prior to study drug administration [Visit 3 (Day 1)]. All subjects will be evaluated to determine study eligibility. For the purpose of ensuring the integrity of the trial and minimizing bias, certain study design details are further described within a separate, restricted-access unmasked protocol addendum. As part of recording the medical history of subjects, concurrent use of hearing aids during the study will be documented, as well as hearing loss etiology and laterality (unilateral, bilateral). Eligible subjects will be required to return for Lead-in/Visit 2 (14-18 days after Visit 1) to complete assessments. Subjects will be instructed to contact the Investigator or relevant staff if a medical change has occurred during the lead-in period (Visit 1 to pre-dose Visit 3). Eligible subjects will return for Visit 3 and will be evaluated to determine whether they meet appropriate Inclusion/Exclusion criteria required for randomization.

Part B

Randomization, Treatment, Follow-up, and Observation: Approximately 124 subjects are planned to be randomized in this study. The subjects will be randomized to receive FX-322 or placebo according to the different treatment group assignments listed below:

Treatment Group	# of Subjects	Day 1
1	62	FX-322
2	62	Placebo

Once a subject has been identified as meeting all Inclusion/Exclusion criteria, including the criteria described within the unmasked addendum which will be determined via the electronic data capture (EDC) system, the subject will be randomized. FX-322 or placebo will be administered at Visit 3 (Day 1). The syringes containing either placebo or FX-322 will be obscured to maintain the blind for the injecting Otolaryngologist.

Each subject will be placed in the supine position. Topical anesthesia (with the exception of phenol) will be administered directly 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 20-30 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), monitoring of audiology, tympanometry, otoscopic exams, and C-SSRS.

Follow up: Subjects will be required to return to clinic for safety, otologic, and audiologic assessments at Days 30 (Visit 4), 60 (Visit 5), and 90 (Visit 6) following the study injection. Unscheduled visits may occur as needed.

Observation: Subjects will return to clinic for safety, otologic, and audiologic assessments at Days 180 (Visit 7) and 270 (Visit 8).

Number of Subjects to be Randomized:

Approximately 124 randomized subjects in order to obtain a target of 112 evaluable subjects.

Study Duration:

Part A: 30-40 days

Part B: 90 days

Study Population:

Male and female adults (18-65 years inclusive), otherwise healthy with acquired sensorineural hearing loss, with screening PTA values between 35-85 dB. Subject must have a medical history that is consistent with adult-onset SNHL that is acquired based on medical histories consistent with exposure to noise (Johnson et al., 2017) or as a result of sudden sensorineural loss.

Statistical Analysis:

Sample Size: The sample size of 112 evaluable subjects, in a 1:1 allocation ratio of FX-322 and Placebo provides 80% to show improvements in WR.

Statistical Methods: Descriptive summaries will be provided for patient disposition, demographic and baseline disease data by treatment group.

Efficacy analysis of continuous endpoints will be examined via a Mixed Model for Repeated Measures (MMRM). Inferential analyses for categorical endpoints will be analysed using univariate methods and/or Generalized Estimating Equations (GEEs) (or appropriate longitudinal methods as detailed in the SAP). When appropriate, models will include adjustments for baseline as well as the stratification factors used at randomization and clinically relevant confounders as identified in the SAP. Departures of these models from the statistical assumptions will be assessed and alternative approaches maybe employed.

Summary statistics for efficacy endpoints will be provided by treatment group over the study and at specific timepoints. Comparisons between FX-322 and placebo and 95% confidence interval of the difference will also be calculated.

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. Audiology, 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 all planned statistical analyses and methods will be provided in the SAP, and will be finalized prior to study blind break for the primary analysis.

3 PROTOCOL AMENDMENT

3.1 Protocol Version 3.0 Amendments

Item No.	Change	Section and Page Number(s)
1.	Updated date and version of protocol.	Title page, pg. 1 Section 1, Signature page, pg. 2 Section 20, Investigator Signature page, pg. 54 Footer, all pages
2.	Clarified primary and secondary endpoints to show improvement through Day 90. Added Exploratory Endpoints.	Section 2, Synopsis, pgs. 3-4 Section 9, Study Objectives and Endpoints, pgs. 22-23
3.	Removed 'Day -40 to Day -30' from Screening/Start of Lead-in/Visit 1 and removed 'Day -15' from Lead-in/Visit 2. Added Observational visits (Visit 7 and Visit 8). Updated the visit windows for Visits 2 and 3.	Section 2, Synopsis, pgs. 4-5 Section 10.2, Description, pgs. 23-24 Section 12, Timing of Study Procedures, pgs. 36-38 Section 12.9, Duration of Treatment, pg. 39 Section 13, Study Assessments, pgs. 39-41 Section 14, Adverse Events, pg. 42
4.	Clarified that summary statistics for efficacy endpoints will be provided by the treatment group over the study and at specific timepoints. Further clarified primary efficacy analysis.	Section 2, Synopsis, pg. 6 Section 15.5, Efficacy Analyses, pg. 47
5.	Updated protocol to confirm that interim analysis is no longer planned.	Section 2, Synopsis, pg. 6 Section 10.8.1, Blinding, pg. 33 Section 15.4, Interim Analyses, pg. 47
6.	Updated Introduction to align with Investigator's Brochure, v7.0.	Section 7, Introduction, pgs. 19-21

Item No.	Change	Section and Page Number(s)
		Section 8.1, Known Potential Benefits, pg. 21
7.	Added reference for McLean et al., 2021.	Section 8.2, Known Potential Risks, pg. 22 Section 19, References, pg. 52
8.	Updated Study Design schematic.	Section 10.1.2 Study Design, pg. 25
9.	Updated Schedule of Assessments to clarify visit windows and add Observational Visits 7 and 8.	Section 10.2.2, Schedule of Assessments, pgs. 26-27
10.	Clarified end of study definition to be considered a subject who has completed all follow-up visits, including Visit 6 (Day 90).	Section 10.4, End of Study Definition, pg. 28
11.	Updated protocol to allow for rescreening with sponsor approval.	Section 10.5.5, Screen Failures, pg. 30
12.	Updated protocol to confirm that all unused and used study drug 'should' be retained at the site until inventoried by the site monitor, 'in compliance with the site's SOPs'.	Section 10.7.4, Disposal, Return or Retention of Study Drug, pg. 33
13.	Changed bilateral 'NIHL' to bilateral 'hearing loss'.	Section 10.8.4, Selection and Timing of Dose for Each Subject, pg. 34
14.	Updated Prohibited Medication/Therapy section to clarify that oral and intratympanic steroids are prohibited through Visit 6 (Day 90).	Section 10.9.1, Prohibited Medication/Therapy, pg. 35
15.	Updated Discontinuation details to include observational visits and confirm early termination procedures should be Visit 6 (Day 90) assessments.	Section 11.1, Discontinuation, pg. 35
16.	Further defined who will be considered Lost to Follow-up.	Section 11.2, Lost to Follow-up, pg. 35

Item No.	Change	Section and Page Number(s)
17.	Clarified use of center frequencies for audiometry.	Section 13.1.4, Extended High Frequency Audiometry, pg. 40
18.	Clarified that subjects should rest for at least 5 minutes prior to vital signs.	Section 13.2.3, Vital Signs, pg. 41
19.	Clarified that AESIs will be captured through Visit 6 (Day 90) only.	Section 14, Adverse Events, pg. 44
20.	Removed 'clinical laboratory measurements' and added 'pregnancy tests.' Removed details of partial date imputations. This will be captured in the Statistical Analysis Plan.	Section 15.6, Safety Analyses, pg. 48

3.2 Protocol Version 2.0 Amendments

Item No.	Change	Section and Page Number(s)
1.	Updated date and version of protocol.	Title page, pg. 1 Section 1, Signature page, pg. 2 Section 22, Investigator Signature page, pg. 49 Footer, all pages
2.	Added Patient Global Impression of Change Hearing Loss and Daily Impacts.	Section 2, Synopsis – Secondary Endpoints, pg. 3 Section 9, Study Objectives and Endpoints, pg. 18 Section 10.2.2, Schedule of Assessments, pg. 22 Section 12.6, Follow-up Visit, pg. 33 Section 13.1, Efficacy Measurements Assessed, pgs. 35-36

Item No.	Change	Section and Page Number(s)
		Section 23.5 and 23.6, Appendix, pgs. 58-59
3.	Added safety and efficacy information about studies FX-322-111 and FX_322-112 from Investigator's Brochure v7.0.	Section 2, Synopsis – Study Design, pg. 4 Section 7, Introduction, pg. 16 Section 10, Investigational Plan, pg. 19 Section 10.3, Scientific Rationale for Study Design, pg. 24
4.	Added PGI-S, PGI-C, SAP, and SRT to List of Abbreviations and Definitions of Terms.	Section 6, List of Abbreviations and Definition of Terms, pgs. 14-15
5.	Study Design schematic updated	Section 10.1.2 Study Design, pg. 21
6.	Added Speech Reception Threshold (SRT) as assessment.	Section 10.2.2, Schedule of Assessments, pg. 22 Section 12, Timing of Study Procedures, pgs. 32-34 Section 13.3, Other Study Assessments, pg. 37
7.	Removed 'SL' from 'W-22 SL'.	Updated throughout protocol
8.	Added Patient Global Impression of Severity Hearing Loss and Daily Impacts.	Section 10.2.2, Schedule of Assessments, pg. 22 Section 12.3, End of Lead-In/Treatment Visit 3 (Day 1), pg. 32 Section 13.3, Other Study Assessments, pg. 37 Section 23.3 and 23.4, Appendix, pgs. 56-57
9.	Updated Schedule of Assessments to add Inclusion/Exclusion assessment at Visit 2 (Day -15), clarify that Visit 6 procedures should be conducted for subjects who early terminate from the	Section 10.2.2, Schedule of Assessments, pgs. 22-23

Item No.	Change	Section and Page Number(s)
	study, added SRT, PGI-S, and PGI-C.	
10.	Updated Inclusion #6 to note examples of two forms of contraception that would include the use of a condom.	Section 10.5.2, Inclusion Criteria, pg. 24
11.	Clarified procedures to be conducted for Early Termination visits.	Section 11.1, Discontinuation, pg. 31
12.	Updated Visit 2 (Day -15) procedures to include reassessment of Inclusion/Exclusion eligibility.	Section 12.2, Lead-in Visit 2 (Day -15), pg. 32
13.	Added SRT and PGI-S scales to Section 13.3 'Other Study Assessments' and updated Section 13 from 'Efficacy and Safety Assessments' to 'Study Assessments'.	Section 13, Study Assessments, pgs. 34-37
14.	Clarified that [redacted] Qualitative Questionnaire is completed by the subject.	Section 13.1.6, [redacted] Qualitative Questionnaire, pg. 35
15.	Clarified that adverse events should be collected starting from the time of study treatment and new adverse events should be documented for worsening events.	Section 14, Adverse Events, pgs. 37-38
16.	Added references for: Johnson, et al 2017, Meikle et al 2012, Posner et al 2011, US FDA Oct 2018 and alphabetized all references.	Section 2, Synopsis – Study Population, pg. 5 Section 13.1.5, Tinnitus Functional Index, pg. 35 Section 13.2.6, Columbia Suicide Severity Rating Scale, pg. 36 Section 21, References, pgs. 47-48
17.	Removed 'Baseline' from Visit 3 (Day 1). Updated references to	Updated throughout protocol

Item No.	Change	Section and Page Number(s)
	Visits by visit number instead of days.	

4 TABLE OF CONTENTS

5 LIST OF TABLES

6 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or Term	Explanation
AE	Adverse Event
AESI	Adverse Event of Special Interest
ASHA	American Speech-Language-Hearing Association
BMI	Body mass index
CNC	Consonant-nucleus-consonant
CONSORT	Consolidation Standards of Reporting Trials
CRF	Case Report Form
CSR	Clinical Study Report
C-SSRS	Columbia Suicide Severity Rating Scale
dB	Decibel
DMSO	Dimethyl sulfoxide
EDC	Electronic Data Capture System
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GSK	Glycogen Synthase Kinase
HDAC	Histone deacetylase
HCG	Human chorionic gonadotrophin
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LGR5+	Leucine-rich repeat-containing G-protein coupled receptor 5 positive
MedDRA ®	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model for Repeated Measures
NIHL	Noise Induced Hearing Loss
NU-6	Northwestern University Auditory Test No. 6
PE	Physical exam
PGI-S	Patient Global Impression of Severity
PGI-C	Patient Global Impression of Change
PI	Principal Investigator
PK	Pharmacokinetics
PPAS	Per Protocol Analysis Set
PTA	Pure Tone Average
[redacted]	Research Assessment on [redacted] Quality of Life
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SfAS	Safety Analysis Set
SL	Sensation Level
SNHL	Sensorineural hearing loss
SNR	Signal-to-noise ratio
SRT	Speech Reception Threshold
SSNHL	Sudden Sensorineural hearing loss

Abbreviation or Term	Explanation
SOP	Standard Operating Procedure
SPL	Sound pressure level
SUSAR	Suspected Unexcepted Serious Adverse Reaction
TFI	Tinnitus Functional Index
TM	Tympanic membrane
WHO	World Health Organization
WIN	Words-in-Noise
WR	Word Recognition in quiet

7 INTRODUCTION

Worldwide, an estimated 1.5 billion people currently experience hearing loss due to exposure to damaging levels of sound, with an anticipated 2.5 billion afflicted by 2050, including 1.1 billion young people at risk for hearing loss due to loud sounds over long periods (WHO 2021). According to the US National Institutes of Health, approximately 90 percent of those with hearing loss are affected by SNHL. Excessive sound levels or loud sounds for extended periods of time hyperstimulate cochlear hair cells, which can lead to increased production of reactive oxygen species and oxidative cell death. Structural damage to hair cells results in SNHL, which can be characterized by an attenuation and distortion of response to incoming auditory stimuli. 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 device options, however, do not address the cause of SNHL, whereas the proposed drug product FX-322 is designed to activate cochlear progenitor cells leading to the development of new sensory hair cells and new progenitor cells, and these regenerative mechanistic steps translate to a restoration of hearing function in patients with SNHL.

SNHL accounts for about 90% of all cases of hearing loss (Li 2017). Leading causes include noise exposure, ototoxic medications, advanced age, inherited, and autoimmune disorders. SNHL is usually irreversible and managed with hearing aids or cochlear implants. Noise is a major occupational and environmental hazard, causing hearing loss, sleep disturbance, fatigue, and hypertension (Hong 2013). SNHL has long been recognized as the primary and direct health effect of excessive noise exposure (Basner 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 2011). The World Health Organization reported that 16% of the disabling hearing loss in adults is attributable to occupational noise exposure (Nelson 2005). Hearing loss is associated with increased disability, dementia, clinically relevant depression, anxiety, stress and other mental health disorders, especially in the elderly (Choi 2016) (McGilton 2016) (Deal 2016) (Brody 2018) (Curhan 2019) (Hsu 2016) (Cosh 2019) (Amieva 2018) (Jayakody 2018).

FX-322 intratympanic injection is indicated for improvement in speech perception in patients with acquired sensorineural hearing loss (SNHL). FX-322 was developed to improve how a patient with SNHL feels, functions or survives, assessed using a reliable measure of speech perception. FX-322 is a combination of the GSK3 inhibitor, laduviglusib and sodium valproate. The drug product contains [redacted] FX-322 has been developed as the maximal feasible dose (MFD) that allows the highest amount of the actives which can be contained in the formulation without affecting its gelation properties. [redacted] is contained in several pharmaceutical products approved for human use including at 15.1% (w/w) for intratympanic use.

FX-322 has been shown to target cellular mechanisms, including GSK and HDAC inhibition, which act synergistically to induce a regenerative response in cochlear tissue by causing asymmetric division of the Lgr5+ progenitor cells to become inner ear hair cells while retaining progenitor cells (which also function as a defined subset of supporting cells) (McLean et al., 2017) and thus may restore hearing function in patients with acquired SNHL. 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.

[redacted] FX-322 is a combination of laduviglusib and sodium valproate [redacted] FX-322 is administered by intratympanic injection into the middle ear and deposited onto the surface of the oval and round window membranes where the active ingredients diffuse into the cochlea of the inner ear and are absorbed.

Minimal systemic exposure occurred after a single intratympanic injection of FX-322 in three 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, FX-322-110, was a safety and tolerability study conducted in patients with hearing loss with a planned cochlear implant surgery to measure concentrations of FX-322 in cochlear fluid and plasma post intratympanic injection. FX-322 was well tolerated, as all AEs recovered without sequelae and no serious or severe adverse events were observed. FX-322 showed acceptable local and systemic acute tolerability and very limited systemic exposure after intratympanic injection.

The third clinical trial was a Phase ½ (FX-322-201) randomized, double-blind, placebo-controlled single-dose study in adults with mild to moderately severe 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. The most frequently reported TEAEs that were reported, such as ear discomfort and pain, were all transient, generally mild and typical of the intratympanic injection procedure and not related to the material injected. One subject treated with FX-322H was observed with a pinhole tympanic membrane perforation on Day 15 that resolved by Day 30. There were no other TEAEs observed in the otoscopic examinations. No clinically significant safety issues were observed in ECGs, physical examinations, vital sign measurements, or 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 measures of hearing function for patients treated with FX-322 versus patients given placebo. FX-322 patients showed an improvement in hearing function from baseline to

Day 90. The notable improvements in these measures of hearing function were generally observed in FX-322 patients within 15-30 days of treatment, they were sustained for 90 days, and when responders were retested 1-2 years later the improvements in auditory function were maintained.

Preliminary results are available from a recently completed open-label, single-dose study of FX-322 (FX-322-111) designed to evaluate the impact of injection conditions on tolerability. In the multi-center, randomized study, subjects with mild to severe SNHL (n=33) were injected in one ear with FX-322, with the untreated ear as the control. Hearing function was tested over the course of 90 days following dosing. At this timepoint, thirty-four percent (34%) of subjects achieved a ten percent (10%) or greater absolute improvement in word recognition scores in the treated ear, which was clinically meaningful and statistically significant compared to the untreated ear ($p < 0.05$).

A Phase 1b (FX-322-112) randomized, double blind, placebo controlled, single dose, multicenter, safety study in adults with age related sensorineural hearing loss did not show significant treatment effect with FX-322 administration compared to placebo. No subjects enrolled in this study had either noise induced or sudden sensorineural hearing loss, conditions where FX-322 associated hearing benefits were observed in prior studies. Results did show a favorable safety and tolerability profile.

Frequency has completed a pre-defined interim analysis of Study FX-322-202. The interim analysis does not replicate the results we have seen from our other single dose trials, including FX-322-201, FX-322-201-2, and FX-322-111. The FX-322-202 trial may be flawed due to bias that may have been introduced to the trial from a subject who posted the informed consent on social media, causing potential subjects to consciously or unconsciously adjust their baseline evaluation scores with intentional incorrect responses to qualify for the study. Unfortunately, these factors may have compromised the ability of study FX-322-202 to precisely and accurately assess any treatment effects of FX-322 with multiple injections.

As a result, efficacy results were confounded by bias issues affecting patient inclusion/exclusion and a potential placebo effect.

8 RISK/BENEFIT ASSESSMENT

8.1 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 in a number of studies that in humans with stable mild to moderately severe SNHL, a single intratympanic dose of FX-322 may improve hearing function to some degree in humans.

8.2 Known Potential Risks

The risks of intratympanic injection of FX-322 have been carefully studied in nonclinical toxicology studies, as well as in a number of 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 of single intratympanic injections 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; McLean et al., 2021).

8.3 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.

9 STUDY OBJECTIVES AND ENDPOINTS

Primary Objective

- To assess efficacy following a single dose of FX-322 versus Placebo, in subjects with acquired sensorineural hearing loss.

Secondary Objective(s)

- To assess safety following a single dose of FX-322 or Placebo in subjects with acquired sensorineural hearing loss.

Endpoints:

Primary Efficacy Endpoint

- Word Recognition in quiet (WR) improvements through Day 90

Secondary Endpoints

- Words-in-Noise improvements through Day 90
- Audiometry
    - Standard Pure Tone (air 0.25-8 kHz; bone 0.5-4 kHz)
      Extended High Frequency (air 9-16 kHz)
- [redacted] Qualitative Questionnaire
- Tinnitus Functional Index (TFI) Questionnaire
- Research Assessment on [redacted] Quality of Life [redacted] Questionnaire
- Patient Global Impression of Change (PGI-C) Hearing Loss Scale
- Patient Global Impression of Change (PGI-C) Daily Impacts Scale

Safety Endpoint(s)

- Adverse events
- Changes in:

- Otoscopy
- Tympanometry
- C-SSRS

Exploratory Endpoint(s)

- Audiometry, speech perception measure improvements, and safety assessments beyond Day 90

10 INVESTIGATIONAL PLAN

10.1 Overall Study Design and Plan:

10.2 Description

This is a Phase 2, prospective, randomized, double-blind, placebo-controlled, single-dose, multicenter study to evaluate the efficacy of FX-322, administered by intratympanic injection, in adults with acquired sensorineural hearing loss (SNHL).

Previous human studies in subjects 18 to 65 years inclusive (FX-322-103, FX-322-201, FX-322-111) and subjects 66-85 inclusive (FX-322-112) demonstrated that a single dose of FX-322 was well tolerated in patients with acquired SNHL with no treatment-related serious adverse events. Adverse events in these studies were common to and associated with the intratympanic injection procedure with mild and transient discomfort in patients both with the drug and the placebo.

In previous single-dose human studies in subjects 18-65 years inclusive (FX-322-201, FX-322-111) it was demonstrated that treatment with FX-322 was associated with statistically significant and clinically meaningful improvements in auditory measures 90 days after a single administration of FX-322, compared to either placebo or untreated ears.

We intend to further establish efficacy with a single dose of FX-322 in subjects 18 to 65 years inclusive with acquired SNHL.

The study will have two Parts as described below:

Part A: Screening/Start of Lead-in/Visit 1, Lead-in/Visit 2

Part B: End of Lead-in/Treatment/Visit 3 (Day 1), Randomization (Double-blind), Follow-up (Days 30, 60 and 90), and Observation (Days 180 and 270)

Part A

Screening/Start of Lead-In: Visit can occur 30-40 days prior to study drug administration [Visit 3 (Day 1)]. All subjects will be evaluated to determine study eligibility. For the purpose of ensuring the integrity of the trial and minimizing bias, certain study design details are further described within a separate, restricted-access unmasked protocol addendum. As part of recording the medical history of subjects, concurrent use of hearing aids during the study will be documented, as well as hearing loss etiology and laterality (unilateral, bilateral). Eligible subjects will be required to return for Lead-in Visit 2 (14-18 days after Visit 1) to complete assessments. Subjects will be instructed to contact the Investigator or relevant staff if a medical change has occurred during the lead-in period (Visit 1 to pre-dose Visit 3).

Eligible subjects will return for Visit 3 and will be evaluated to determine whether they meet appropriate Inclusion/Exclusion criteria required for randomization.

Part B

Randomization, Treatment, and Follow-up: Approximately 124 subjects are planned to be randomized in this study. The subjects will be randomized to receive FX-322 or placebo according to the different treatment group assignments listed below:

Table 1: FX-322 Group Dosing Schedule

Treatment Group	# of Subjects	Day 1
1	62	FX-322
2	62	Placebo

Once a subject has been identified as meeting all Inclusion/Exclusion criteria, including the criteria described within the unmasked addendum which will be determined via the electronic data capture (EDC) system, the subject will be randomized. FX-322 or placebo will be administered on Visit 3 (Day 1). The syringes containing either placebo or FX-322 will be obscured to maintain the blind for the injecting Otolaryngologist.

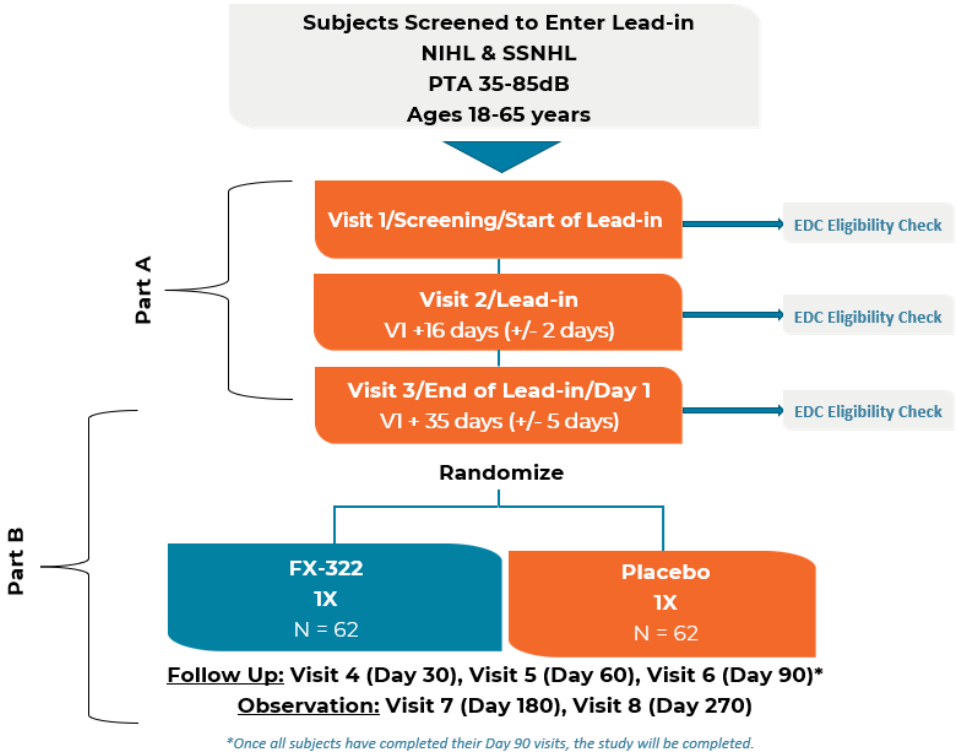
Each subject will be placed in the supine position. Topical anesthesia (with the exception of phenol) will be administered directly to the tympanic membrane. Under a microscope, a 25-gauge needle will be used to inject FX-322 or placebo into the middle ear at the junction of the posterior inferior and 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 20-30 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), monitoring of audiology, tympanometry, otoscopic exams, and C-SSRS.

Follow up: Subjects will be required to return to clinic for safety, otologic, and audiologic assessments at Days 30, 60, and 90 following the study injection. Unscheduled visits may occur as needed. See the Schedule of Assessments for more information.

Observation: Subjects will return to clinic for safety, otologic, and audiologic assessments at Days 180 and 270.

10.2.1 Study Design

Figure 1: Study Design



10.2.2 Schedule of Assessments

Table 2: Schedule of Assessments

Visit	Screening/ Start of Lead-In	Lead-In Visit	End of Lead-In/ Treatment	In-Clinic Follow-up	In-Clinic Follow up	In-Clinic Follow-up / ET[e]	In-Clinic Observation	In-Clinic Observation	Un-scheduled Visit
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**UNS**
Assessment/Day	**Day -40 to -30**	**Visit 1 + 16 days**	**Visit 1 + 35 days**	**Day 30**	**Day 60**	**Day 90**	**Day 180**	**Day 270**	
Visit Window	**N/A**	**+/- 2 days**	**+/- 5 days**	**+/- 5 days**	**+/- 5 days**	**+/- 5 days**	**+/- 7 days**	**+/- 7 days**	**N/A**
Informed Consent	X								
Inclusion/ Exclusion Criteria	X[a]	X[a]	X[b]						
Eligibility Assessment by EDC	X	X	X[b]						
Demographics	X[a]								
Medical History	X[a]	X[a]	X[b]						
Concomitant Medication	X[a]	X[a]	X[b, c]	X	X	X	X	X	X
Physical Examination including weight and height	X								X[d]
Vital Signs (body temperature, pulse rate, bp)	X	X	X[b]	X	X	X			X[d]
Tympanometry	X		X[b]	X	X	X	X	X	X[d]
Standard Pure Tone Audiometry	X	X	X[b]	X	X	X	X	X	X[d]
Speech Reception Threshold (SRT)	X	X	X	X	X	X	X	X	X[d]
Extended High Frequency Audiometry	X	X	X[b]	X	X	X	X	X	X[d]
Word recognition, quiet (Maryland CNC)	X	X	X[b]	X	X	X	X	X	X[d]
Word recognition (W-22)	X	X	X[b]	X	X	X	X	X	X[d]
Words in noise (WIN)	X	X	X[b]	X	X	X	X	X	X[d]

Visit	Screening/ Start of Lead-In	Lead-In Visit	End of Lead-In/ Treatment	In-Clinic Follow-up	In-Clinic Follow up	In-Clinic Follow-up / ET[e]	In-Clinic Observation	In-Clinic Observation	Un-scheduled Visit
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**UNS**
Assessment/Day	**Day -40 to -30**	**Visit 1 + 16 days**	**Visit 1 + 35 days**	**Day 30**	**Day 60**	**Day 90**	**Day 180**	**Day 270**	
Visit Window	**N/A**	**+/- 2 days**	**+/- 5 days**	**+/- 5 days**	**+/- 5 days**	**+/- 5 days**	**+/- 7 days**	**+/- 7 days**	**N/A**
Tinnitus Functional Index			X[b]			X	X	X	X[d]
[redacted] Questionnaire	[redacted]								
[redacted] Qualitative Questionnaire	[redacted]								
Columbia Suicide Severity Rating Scale			X[b]	X	X	X	X	X	X[d]
PGI-S Hearing Loss Scale			X[b]						
PGI-S Daily Impacts Scale			X[b]						
PGI-C Hearing Loss Scale						X			
PGI-C Daily Impacts Scale						X			
Otoscopy	X		X[b]	X	X	X	X	X	X[d]
Urine Pregnancy Test (women of childbearing potential only)	X		X[b]			X			X[d]
Randomization by EDC			X[b]						
Study Medication (FX-322 or placebo)			X						
Adverse Events			X[c]	X	X	X	X	X	X

a – Performed during the Screening Visit and again if a subject notes a change in their medical status during the lead in period
b – Assessment performed prior to injection
c – Assessments performed after injection
d – Perform at Investigator Discretion
e – If a subject discontinues prior to Visit 6 (Day 90), all efforts should be made to complete the Visit 6 (Day 90) In-Clinic Follow-up/Early Termination (ET) Visit as soon as possible and, whenever possible, prior to starting any new medication or treatment.

10.3 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 well tolerated and was associated with improvements in speech intelligibility as measured by WR and WIN, data that suggest FX-322 may improve hearing function to some degree in humans.

Previous human studies in subjects 18 to 65 years inclusive (FX-322-103, FX-322-201, FX-322-111) and subjects 66-85 inclusive (FX-322-112) demonstrated that a single dose FX-322 was well tolerated in patients with acquired SNHL with no treatment-related serious adverse events. Adverse events in these studies were common to and associated with the intratympanic injection procedure with mild and transient discomfort in patients both with the drug and the placebo.

In previous single-dose human studies in subjects 18-65 years inclusive (FX-322-201, FX-322-111) it was demonstrated that treatment with FX-322 was associated with statistically significant and clinically meaningful increases in auditory measures 90 days after a single administration of FX-322, compared to either placebo or untreated ears. We intend to further establish efficacy with a single dose of FX-322 in subjects 18 to 65 years inclusive with acquired SNHL.

10.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all follow-up visits, including Visit 6 (Day 90).

10.5 Selection of Study Population

10.5.1 Number of Planned Subjects

Approximately 124 subjects are planned to be randomized 1:1 to FX-322 or Placebo.

10.5.2 Inclusion Criteria

To be eligible for study entry subjects must satisfy all 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 at Screening.
3. Documented medical history consistent with acquired, adult onset, sensorineural hearing loss associated with noise-induced SNHL (NIHL) or idiopathic sudden SNHL (SSNHL) (documented audiogram at least 6 months prior to screening required).
4. A pure tone average at the Screening Visit of 35-85 dB at 500Hz, 1000Hz, 2000Hz, and 4000Hz in the ear to be injected.
5. Ability to communicate well with the Investigator and is willing to comply with and complete all the study procedures.

6. 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 and condom or an intrauterine device and condom) 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.
7. Have met additional masked criteria as determined by EDC.

10.5.3 Exclusion Criteria

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

1. Subject has previously been randomized in a FX-322 clinical trial.
2. 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.
3. 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.
4. Active chronic middle ear disease or a history of major middle ear surgery, as an adult, in the ear to be injected.
5. Subject has had an intratympanic injection in either ear within 3 months of the screening visit.
6. Evidence of or previous diagnosis of auditory neuropathy, traumatic brain injury, “central” hearing loss, or genetic hearing loss.
7. History of chronic, recurrent clinically significant vestibular symptoms.
8. History of Bilateral Sudden Sensorineural Hearing Loss or Recurrent Sudden Sensorineural Hearing Loss.
9. History of clinically significant systemic autoimmune disease (e.g. rheumatoid arthritis, Sjogren’s syndrome, multiple sclerosis, psoriasis).
10. History of head or neck radiation, treatment, or exposure to platinum based chemotherapy drugs or aminoglycosides.
11. Exposure to another investigational drug within 28 days prior to screening visit.
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. Positive urine pregnancy test or breast-feeding.
14. 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.

10.5.4 Withdrawal of Subjects From Study Assessments

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

- Adverse Event
- Death
- Lack of Efficacy

- Lost to Follow-up
- Other
- Physician Decision
- Pregnancy
- Progressive Disease
- Protocol Violation
- Recovery
- Study Terminated by Sponsor
- Technical Problem
- Withdrawal by Subject

Subjects are free to withdraw from the study at any time without providing reason(s) for withdrawal and without prejudice to further treatment. Should a subject withdraw early from the study the reason(s) for study withdrawal will be documented on the case report form (CRF). 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.

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 CRF at the conclusion of the pregnancy.

Non-childbearing potential:

Subjects that are deemed non-childbearing potential should have documented hysterectomy, bilateral oophorectomy, or bilateral tubal ligation, or be in menopause (no menstrual cycle for at least a year).

10.5.5 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 informed consent, demography, and screen failure details.

Rescreening may be permitted in the FX-322-208 study with sponsor approval.

10.6 Investigational Products

10.6.1 Investigational Products Administered

Approximately 124 subjects are planned to be randomized in this study. The subjects will be randomized 1:1 to receive FX-322 or placebo.

Table 1: FX-322 Group Dosing Schedule

Treatment Group	# of Subjects	Day 1
1	62	FX-322
2	62	Placebo

Topical anesthesia (with the exception of phenol) will be administered directly to the tympanic membrane. After approximately 10 minutes, under a microscope, a 25-gauge needle will be used to inject of FX-322 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 20-30 minutes. The injections will be performed at an appropriate location designated by a Board Certified Otolaryngologist trained and experienced in performing intratympanic injections.

10.6.2 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. FX-322 is in the form of a poloxamer thermo-sensitive gel matrix.

10.7 Preparation and Dispensing

The FX-322 investigational product is a [redacted] FX-322 is prepared by [redacted]

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

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

Table 3: **FX-322 Study Drug**

Active Ingredients	**Total in** [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. In a [redacted] injection, placebo contains [redacted]

Table 4: **Dosages of Active Agents to be Injected in Humans**

	FX-322 **Total** (in a [redacted] injection)
FX03	0.628 mg
FX00	17.72 mg

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

10.7.1 Packaging and Labeling

For the injection, the study drug will be provided in a kit labelled for the FX-322-208 study. The [redacted] will be taken from general stock at the site. Topical anesthetic will be provided to the site. More information can be found in the Pharmacy Manual. The study drug will be prepared and drawn into a sterile 1 mL tuberculin syringe. The syringe will be labelled clearly with the details for each randomized patient. The dose [redacted] will be filled to the [redacted] mark. Refer to the Pharmacy Manual for further instructions.

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

10.7.2 Supply, Storage and Handling

Empty vials and containers should be retained until the conclusion of the study, after the site monitor has performed accountability and in compliance with the site's SOP.

10.7.3 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. 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.

10.7.4 Disposal, Return or Retention of Study Drug

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

10.8 Randomization and Blinding

10.8.1 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 and will be un-blinded.

In addition to being blinded to the study drug assignment, Investigators and site personnel will be masked to certain study design elements, selection criteria, procedures, and statistical methods. The pharmacy staff will also be masked to certain study design elements, selection criteria, procedures, and statistical methods. These criteria will be specified in an unmasked addendum to this protocol. The EDC system will be programmed to determine eligibility of subjects and randomize eligible subjects as per these design elements.

10.8.2 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 RTSM 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.

Upon completion of the study and unblinding, subjects who received placebo and completed study visits through Day 90, may have the opportunity to receive FX-322 as part of an open-label extension. Further details will be provided in a separate protocol.

10.8.3 Method of Assigning Subjects to Treatment Groups

Subjects will be randomized 1:1 to one of two groups (FX-322 or placebo). A single injection of FX-322 or placebo will be administered on Day 1.

10.8.4 Selection and Timing of Dose for Each Subject

For subjects with bilateral hearing loss, if both ears meet the inclusion and exclusion criteria as determined by the Investigator, EDC will select the study ear at Visit 3 (Day 1). Subjects will be administered a total of 1 dose of FX-322 or placebo. Study injection will take place on Day 1.

10.9 Prior and Concomitant Therapy

At each study visit the study personnel 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 CRF. 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.

10.9.1 Prohibited Medication/Therapy

Oral and intratympanic steroids are prohibited through the follow up period [through Visit 6 (Day 90)]. The subject's best medical care should guide the investigator in the management of conditions that develop during the observational period after 90 days. Aminoglycosides and platinum-based chemotherapies are excluded during the study.

10.9.2 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.

10.9.3 Treatment Compliance

Treatment will be administered by the Investigator to each subject on Day 1 only, therefore compliance will not be recorded if the subject has their study treatment.

11 DISCONTINUATION AND LOST TO FOLLOW-UP

11.1 Discontinuation

If a clinically significant finding is identified (including, but not limited to changes from baseline) after randomization, 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 discontinuation will include the following:

- All efforts will be made for the subject to complete all of the follow up and observational visits.
- If the subject does not wish to continue the follow up period, all efforts will be made for the subject to complete all designated assessments collected at the Visit 6 (Day 90)/Follow-up ET Visit.

11.2 Lost to Follow-up

A subject will be considered lost to follow-up if he or she fails to return for 2 or more scheduled visits (or the final visit in the follow-up) 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.

12 TIMING OF STUDY PROCEDURES

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

12.1 Screening/Start of Lead-In Visit (Visit 1; [Day -40 to -30])

The following procedures will be performed at the Screening Visit:

- Obtain signed Informed Consent
- Assess for eligibility (against the Inclusion and Exclusion criteria)
- 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 (blood pressure, body temperature, and heart rate)
- Collect urine sample for pregnancy test, if applicable
- Perform otoscopy, standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry
- Enter audiologic assessment results into EDC and confirm eligibility

After the Screening Period (signing the Informed Consent Form and completing all Screening Visit assessments), the subject will enter a Lead-In period of at least 30 days (period can be extended to 39 days but cannot exceed).

12.2 Lead-In Visit (Visit 2; [14-18 days after Visit 1])

The following procedures will be performed at the Lead-in Visit:

- Collect concomitant medications
- Reassess for eligibility against the Inclusion and Exclusion criteria
- Record any changes in medical history that have occurred since the previous visit
- Record vital signs (blood pressure, body temperature, and heart rate)
- Perform standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), and WIN testing (NU-6)
- Enter audiologic assessment results into EDC and confirm eligibility

12.3 End of Lead-In/Treatment (Visit 3, 30-40 days after Visit 1 [Day 1])

The following procedures will be performed at End of Lead-In/Treatment/Visit 3 (Day 1):

- Before injection of study drug:
    - Reassess for eligibility against the Inclusion and Exclusion criteria

    - Record any changes in medical history that have occurred since the previous visit
    - Collect concomitant medications and document topical anesthetic used on study ear
    - Perform a urine pregnancy test, if applicable
    - Perform standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry
    - Perform otoscopy
    - Enter audiologic assessment results into EDC and confirm eligibility
    - Record vital signs
    - Perform Tinnitus Functional Index and Columbia Suicide and Severity Scale
    - Perform [redacted]
    - Perform PGI-S Hearing Loss Scale
    - Perform PGI-S Daily Impacts Scale
    - When all the above procedures have been performed and the Investigator and EDC 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
    - Perform [redacted] Qualitative Questionnaire

12.4 Follow-up (Visit 4, [Day 30 +/- 5 days])

The Follow-up Visit (Visit 4) will take place at Day 30 (± 5 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 Columbia Suicide and Severity Scale
- Perform otoscopy, standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry
- Record vital signs

12.5 Follow-up (Visit 5, [Day 60 +/- 5 days])

The Follow-up Visit (Visit 5) will take place at Day 60 (± 5 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 Columbia Suicide and Severity Scale
- Perform otoscopy, standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry
- Record vital signs

12.6 Follow-up (Visit 6, [Day 90 +/- 5 days])

The Follow-up Visit (Visit 6) will take place at Day 90 (± 5 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 Tinnitus Functional Index and Columbia Suicide and Severity Scale
- Perform [redacted]
- Perform PGI-C Hearing Loss Scale
- Perform PGI-C Daily Impacts Scale
- Perform otoscopy, standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry
- Record vital signs
- Collect urine sample for pregnancy test, if applicable

12.7 Observation (Visit 7, [Day 180 +/- 7 days])

The Observational Visit (Visit 7) will take place at Day 180 (± 7 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 Tinnitus Functional Index and Columbia Suicide and Severity Scale
- Perform otoscopy, standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry

12.8 Observation (Visit 8, [Day 270 +/- 7 days])

The Observational Visit (Visit 8) will take place at Day 270 (± 7 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 Tinnitus Functional Index and Columbia Suicide and Severity Scale
- Perform otoscopy, standard pure tone audiometry, speech reception threshold, extended high frequency audiometry, word recognition testing (Maryland CNC and W-22), WIN testing (NU-6), and tympanometry

12.9 Duration of Treatment

The duration of treatment will be 1 day at Visit 3 (Day 1) with up to 9 months of follow-up and observation after the study treatment.

13 STUDY ASSESSMENTS

13.1 Efficacy Measurements Assessed

13.1.1 Word Recognition in Quiet (WR)

Word recognition in quiet will be measured with recorded Maryland CNC and W-22 word lists. The Maryland CNC Word lists were devised by Lehiste and Peterson (1959) and revised to give more uniform distribution of word familiarity by eliminating rare words and proper nouns (Peterson & Lehiste, 1962). Normative studies were performed by Causey et al., (1984). The W-22 word list was devised by Hirsh et al. (1952). W-22 words are comprised of speech sounds with the same relative frequency as English speech. The tests 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 Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8. For Visit 1, testing will be performed at 30dB SL regarding the pure tone average of 0.5, 1, and 2 kHz at Screening/Visit 1. For Visit 2 and all subsequent visits (Visits 3, 4, 5, 6, 7, and 8) testing will be performed at 30dB SL regarding the pure tone average of 0.5, 1, and 2 kHz at Visit 2. If 30dB SL is at an uncomfortable listening level for the participant, testing will be performed at the participant's most comfortable listening level. Word lists will be rotated at each visit. Audio recordings of subject responses during audiologic testing will be reviewed by a surveillance team for quality assurance. Additional details can be found in the Audiology Manual of Procedures.

13.1.2 Words-In-Noise Testing (WIN)

The Words-in-Noise Test (WIN), using the NU-6 list, 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 Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8. Testing will be performed at 80dB SPL. Word lists will be rotated at each visit. Audio recordings of subject responses during audiologic testing will be reviewed by a surveillance team for quality assurance. Additional details can be found in the Audiology Manual of Procedures.

13.1.3 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 Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8. This will be performed on a calibrated audiometer by a licensed audiologist. The following frequencies will be obtained: Air: 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, and 8000 Hz; Bone: 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, and 4000 Hz. Audio recordings of subject responses during audiologic testing will be reviewed by a surveillance team for quality assurance. Additional details can be found in

the Audiology Manual of Procedures.

13.1.4 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 Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8. This will be performed on a calibrated audiometer by a licensed audiologist. The following frequencies will be obtained: Air: 9000 Hz, 10000 Hz, 11200 Hz, 12400 Hz, 14000 Hz, and 16000 Hz. Because some audiometers use center frequencies that vary by small amounts, sites should use the threshold at the closest listed center frequency (e.g., 11000 Hz may be used for 11200 Hz; 12500 Hz may be used for 12400 Hz). Audio recordings of subject responses during audiologic testing will be reviewed by a surveillance team for quality assurance. Additional details can be found in the Audiology Manual of Procedures.

13.1.5 Tinnitus Functional Index (TFI)

The Tinnitus Functional Index (Meikle et al 2012) 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 answers to each of the 25 questions using a scale of 0-10. The TFI will be given to the subject to complete at Visit 3, Visit 6, Visit 7, and Visit 8.

13.1.6 [redacted] Qualitative Questionnaire

This questionnaire records subject experience [redacted]

13.1.7 Research Assessment on [redacted] Quality of Life [redacted]

This questionnaire is a patient reported outcome measure. [redacted]

13.1.8 Patient Global Impression of Change Hearing Loss Scale

This questionnaire is a patient reported outcome measure. The subject will select their response to best describe the overall change in their hearing loss since they were administered the study medication. The scale will be given to all subjects to complete at Visit 6.

13.1.9 Patient Global Impression of Change Daily Impacts Scale

This questionnaire is a patient reported outcome measure. The subject will select their response to best describe the overall change in the impact of their hearing loss on daily activities since they were administered the study medication. The scale will be given to all subjects to complete at Visit 6.

13.2 Safety Measurements Assessed

13.2.1 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 Visit 1, Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8. 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.

13.2.2 Concomitant Medication

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

13.2.3 Vital Signs

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

13.2.4 Physical Exam (PE)

A complete physical examination will be performed by a licensed provider at Visit 1.

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.

13.2.5 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 Visit 1, Visit 3, Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8.

13.2.6 Columbia Suicide Severity Rating Scale (C-SSRS)

This scale is used to determine if any suicide ideation or intention is present (Posner et al 2011). 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 Visit 3 (Baseline/Screening version), Visit 4, Visit 5, Visit 6, Visit 7, and Visit 8 (Since Last Visit version).

13.3 Other Study Assessments

13.3.1 Speech Reception Threshold (SRT)

Speech reception threshold is being used as a cross-check measure for the results of pure-tone audiometry. The pure tone average (PTA) shall be compared to the SRT to alert the tester to possible concerns about the accuracy of the pure tone results.

13.3.2 Patient Global Impression of Severity (PGI-S) Hearing Loss

This questionnaire is a patient reported outcome measure. The subject will select their response to best describe the severity of their hearing loss over the past week. The scale will be given to all subjects to complete at Visit 3.

13.3.3 Patient Global Impression of Severity (PGI-S) Daily Impacts

This questionnaire is a patient reported outcome measure. The subject will select their response to best describe the impact of their hearing loss on their daily activities over the past week. The scale will be given to all subjects to complete at Visit 3.

14 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 on the CRF under specific 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 study treatment (Visit 3) through the end of the observational period, or final study visit. AEs should be reported on the appropriate page of the CRF.

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 worsening 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.

Action Taken Regarding Study Drug

Not Applicable:	Adverse event began after study drug injection was complete.
Dose Not Changed:	An adverse event was experienced at the time of study drug injection, but no action was taken with the study drug in relation to the specific adverse event.
Drug Withdrawn:	Study drug injection was stopped permanently in relation to the specific adverse event.

Adverse Events of Special Interest (Audiometric and Otoscopic)

If any of the following criteria are met through Visit 6 (Day 90), the event will be recorded as an adverse event of special interest (AESI) per ASHA guidelines (ASHA 1994):

- Asymmetric loss of hearing greater than or equal to 20 dB at any one frequency in the treated ear compared to Visit 3.

- Asymmetric loss of hearing greater than or equal to 10 dB at two adjacent frequencies in the treated ear compared to Visit 3.

- Asymmetric loss of response at three consecutive test frequencies where responses were previously obtained in the treated ear compared to Visit 3.

For word recognition testing, a follow up visit score through Visit 6 (Day 90) in the treated ear that falls below the lower limit of the 95% confidence interval of the Visit 3 word recognition score will be considered an AESI (Carney and Schlauch, 2007).

Additionally, if the subject experiences a perforation greater than 25% of the tympanic membrane in the treated ear through Visit 6 (Day 90) this will be recorded as an AESI.

If any subjects in the study experience the same AESI through Visit 6 (Day 90), the Medical Monitor will discuss these with the Principal Investigators and each subject's condition will be discussed.

Follow-up of Adverse Events

All AEs will be followed until the end of study participation and SAEs 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 CRF.

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 CRF 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 (see definitions above)
- Date of first occurrence and date of resolution (if applicable)
- Action taken (see definitions above)
- Causal relationship (see definitions above)
- Outcome of event (unknown, recovered, not yet recovered, recovering/resolving, recovered with sequelae, deaths [with date and cause reported])

14.1 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 CRF).
- 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.

- Important Medical Event

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 CRF 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 CRF is completed, an EDC email notification is sent to [redacted] Additional details regarding the SAE reporting process can be found in the Investigator Site File.

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 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 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 10.5.4.

14.2 Expedited Safety Reporting

All serious and unexpected suspected adverse reactions (SUSARs) will be the subject of expedited reporting. The sponsor 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.

15 STATISTICAL METHODS

All analyses, data listings, and reports will be detailed in the Statistical Analysis Plan (SAP). This will be finalized prior to unblinding of the study. Additional reports and/or analyses will be considered ad-hoc or post-hoc in nature and detailed in the CSR.

In general, descriptive statistics are defined for continuous variables as the number of subjects (n), mean, standard deviation, minimum, median, and maximum; and as count and percentage for categorical variables.

15.1 Sample Size

The sample size of 112 evaluable subjects, in a 1:1 allocation ratio of FX-322 and Placebo, was selected to provide an overall statistical power of at least 80% to detect a difference in the primary endpoint for word recognition. The target enrolment of 124 subjects is an adjustment for an assumed 10% study dropout rate.

15.2 Analysis Sets

- Full Analysis Set (FAS)

The Full Analysis Set is defined as all randomized subjects who receive one dose of study drug (regardless of whether a full dose was administered) in the qualified ear. Subjects will be analysed according to their randomized treatment group. The FAS will be the primary analysis set for all efficacy endpoints.

- Per Protocol Analysis Set (PPAS)

The Per Protocol Analysis Set is defined as all randomized subjects receiving one full dose of study drug in the qualified ear, treated per the randomization treatment schedule, and without major protocol deviations that could interfere with interpretation of the results. If different from the FAS, the PPAS may be used in additional sensitivity analyses. Details will be provided in the SAP.

- Safety Analysis Set (SfAS)

The Safety Analysis Set (SfAS) will include all subjects who receive one dose of study drug (regardless of whether a full dose was administered) in any ear and will be analyzed according to the actual treatment group regardless of their randomized treatment assignment. For safety assessments completed by ear for each subject, actual treated and actual untreated ears will be summarized separately. The SfAS will be used for the analysis of safety.

15.3 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 group.

15.4 Interim Analyses

No unblinded interim analysis is planned.

The blinded data will be continuously monitored for safety. Blinded efficacy data monitoring will be conducted on an on-going basis in order to understand patterns of missingness in real-time and to monitor data integrity.

The sponsor reserves the option to conduct an interim analysis for the potential purposes of sample re-estimation and/or futility. Should the sponsor choose to utilize this option, the protocol and SAP would each be prospectively amended. If an interim analysis is to be conducted, the sponsor intends to keep it blinded to an independent Data Monitoring Committee, as well as to Frequency. If an interim analyses is conducted, there would be prespecified criteria around sample size re-estimation based on pooled variance estimates, as well as futility analysis.

15.5 Efficacy Analyses

Primary efficacy analysis will be conducted after all subjects have either completed follow-up visits or early discontinued from study. Primary analysis will be performed in an unblinded fashion for analysis purpose while subjects not completing observation visits will continue to be followed as being blinded to treatment assignment.

Efficacy endpoints will be summarized descriptively at each study timepoint. Calculations will include the observed data at each timepoint, and if relevant, the change from baseline (baseline defined as the average across the pre-treatment timepoints).

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. For descriptive analyses, subjects with missing data will be excluded for a particular calculation.

Inferential statistical methods examining continuous endpoints will be examined via a Mixed Model for Repeated Measures (MMRM). Inferential analyses for categorical endpoints will be analysed using univariate methods and/or Generalized Estimating Equations (GEEs) (or appropriate longitudinal methods as detailed in the SAP). When appropriate, models will include adjustments for baseline as well as the stratification factors used at randomization and clinically relevant confounders as identified in the SAP. Departures of these models from the statistical assumptions will be assessed and alternative approaches may be employed.

Efficacy endpoints will be analysed using the FAS, and complete details of these analyses will be included in the SAP.

15.6 Safety Analyses

Safety will be evaluated using the safety analysis set and will include the incidence of adverse events (AEs) and serious adverse events (SAEs). Additionally, descriptive statistics will be provided by actual treatment group for vital signs, pregnancy tests, concomitant medications, otology, questionnaire responses, and tympanometry and otoscopic assessments.

15.6.1 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 and AESIs will include summaries by treated vs. non-treated ear.

15.6.2 Prior and Concomitant Medications

Prior and concomitant medications will be coded using the World Health Organization's (WHO) Drug Dictionary. The incidence of subjects using concomitant medications will be tabulated by Anatomical Therapeutic Chemical (ATC), preferred term, and dosing group. Prior medications will exclusively be displayed in line listings.

Concomitant medications are defined as all medications that started on or after the administration of study drug, or were ongoing at the time of study drug administration. Prior medications are all recorded medications that started and stopped prior to administration of study drug. In the event that a subject begins a previously ended medication (prior medication) following dose of study drug, the post-dose use will be considered concomitant while the prior use will still be reported.

15.7 Handling of Missing Data and Subject Withdrawals

With the exception of partial dates to assess AEs and concomitant medications, safety data will not be imputed. In general, safety endpoints will not be imputed and missing FAS analysis data will be excluded (outside of model-specific, required assumptions). Details and any exceptions will be provided in the SAP.

16 QUALITY ASSURANCE AND QUALITY CONTROL

16.1 Audit and Inspection

Study center(s) 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.

16.2 Monitoring

Data for each subject will be recorded on a CRF. 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 CRF 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 adherence 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 CRFs.

16.3 Data Management and Coding

The sponsor 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 the sponsor/CRO.

Study centers will enter data directly into the CRF. All data must be verifiable against source documents at the study center. Any changes to the data entered into the CRF 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 for clarification. Subsequent modifications to the CRFs will be documented.

17 RECORDS AND SUPPLIES

17.1 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. 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.

17.2 Financing and Insurance

Financing and insurance of this study will be handled by the Sponsor.

18 ETHICS

18.1 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.

18.2 Regulatory Authorities

Relevant study documentation will be submitted to the regulatory authorities of the participating country (USA only), according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the United States regulatory authorities will be notified that the study has ended.

18.3 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.

18.4 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, audio recordings, 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.

18.5 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.

18.6 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.

19 REFERENCES

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20 INVESTIGATOR SIGNATURE PAGE

Protocol Title: A Phase 2, Prospective, Randomized, Double-Blind, Placebo-Controlled, Single-Dose, Multicenter Study to Evaluate the Efficacy of FX-322 Administered by Intratympanic Injection in Adults with Acquired Sensorineural Hearing Loss

Protocol Number: FX-322-208

Protocol Version: 3.0, 05 Jan 2022

Confidentiality and GCP Compliance Statement

I, the undersigned, have reviewed this protocol (and amendments), 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 CRFs and 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.

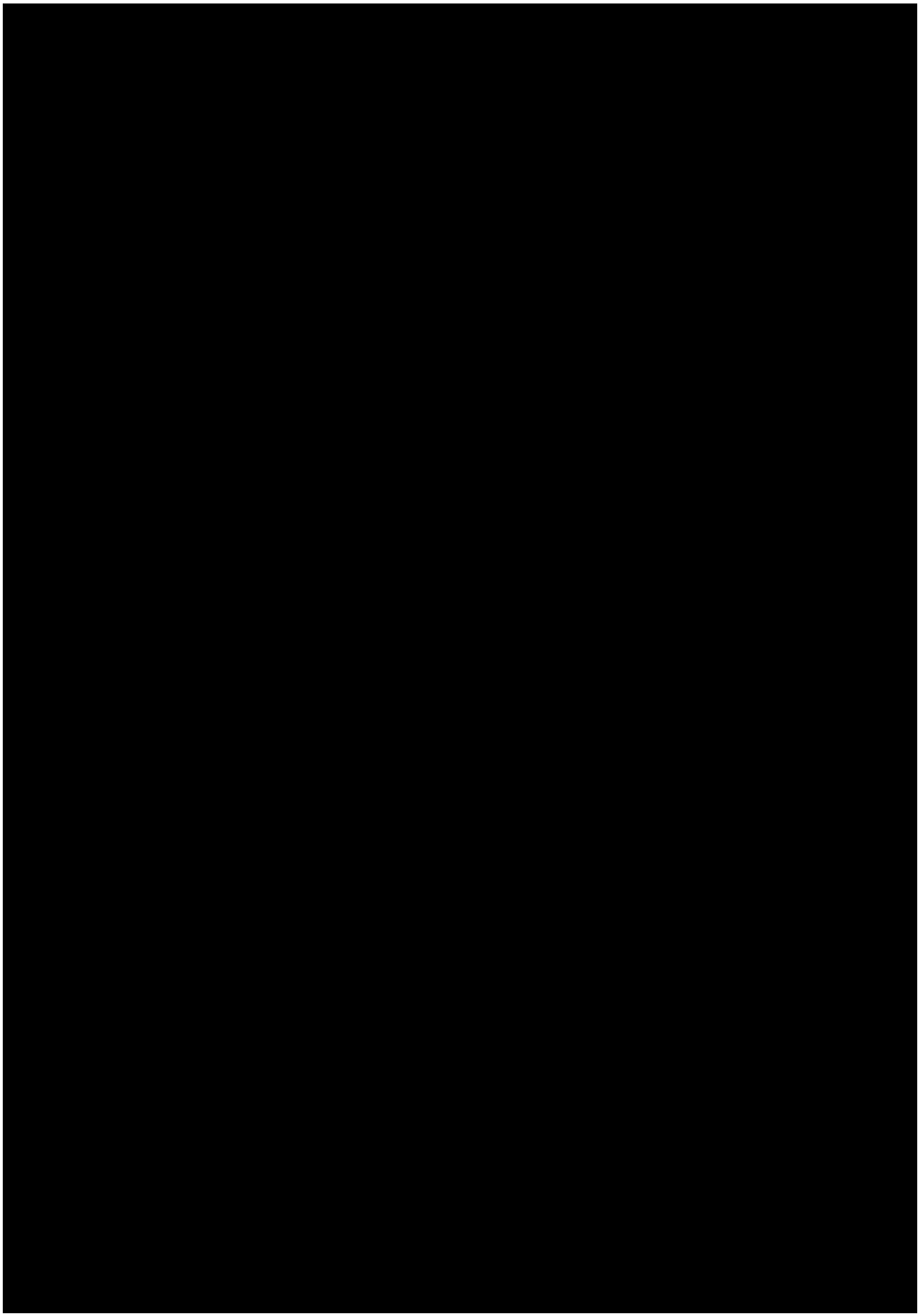
21 APPENDICES

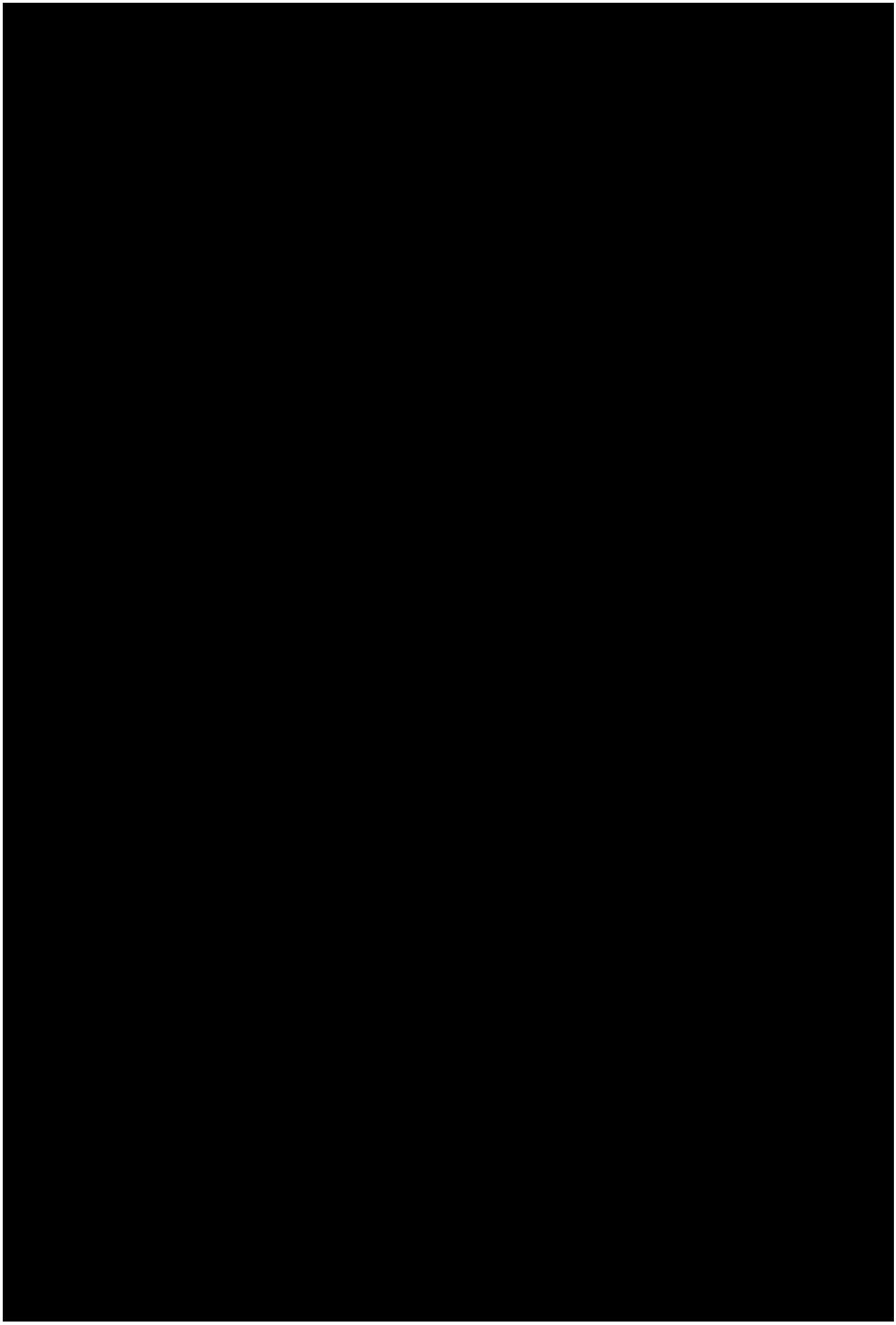
21.1 Appendix A: [redacted] Qualitative Questionnaire [redacted]

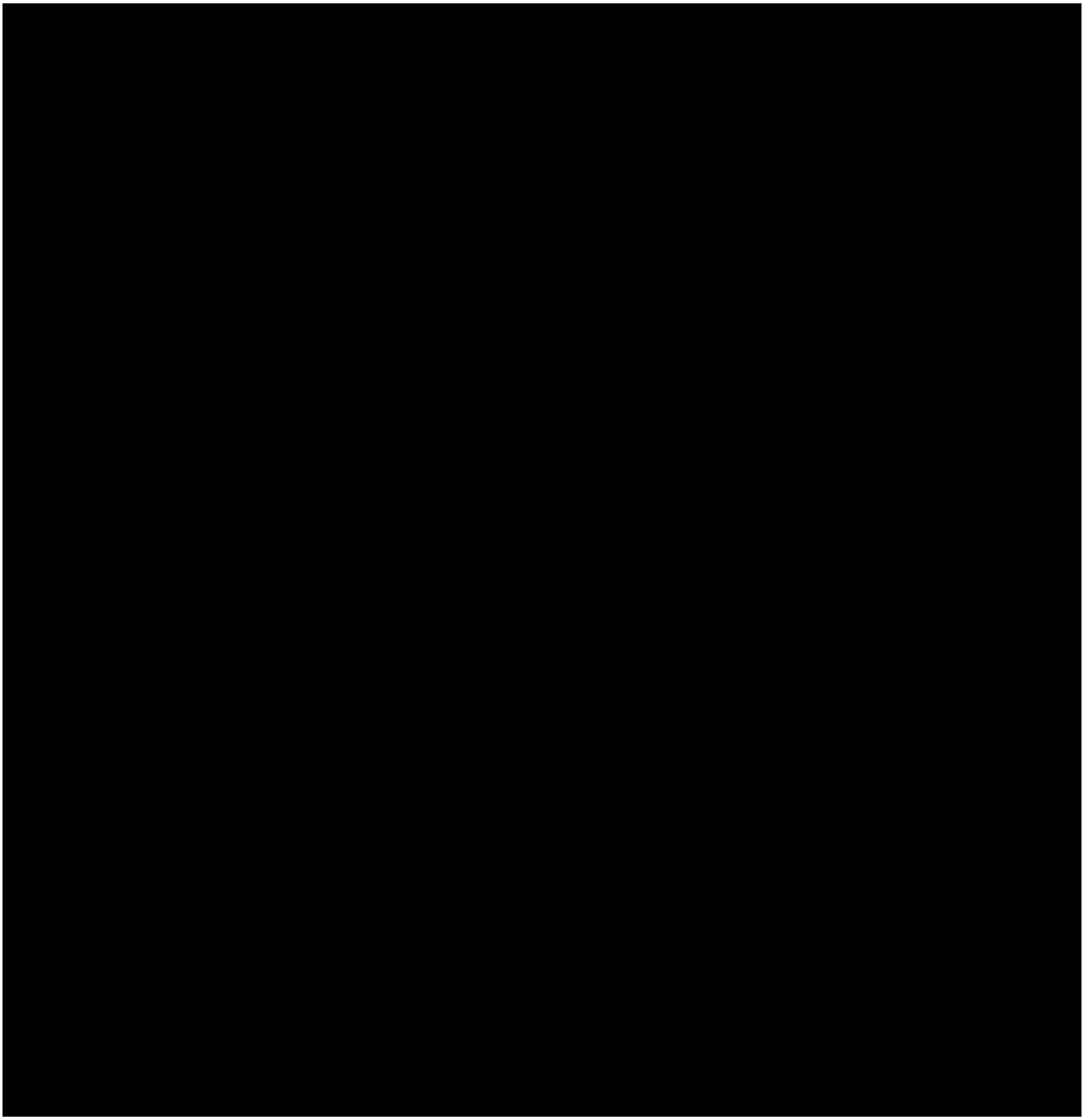
[redacted]

21.2 Appendix B: Research Assessment on [redacted] Quality of Life [redacted]

[redacted]







21.3 Appendix C: Patient Global Impression of Severity (PGI-S) Hearing Loss Scale

Instructions: Please choose the response below that best describes the *severity of your hearing loss* over the past week.

☐	None
☐	Mild
☐	Moderate
☐	Severe
☐	Very Severe

21.4 Appendix D: Patient Global Impression of Severity (PGI-S) Daily Impacts Scale

Instructions: Please choose the response below that best describes the *impact of your hearing loss on your daily activities* over the past week.

☐	None
☐	Mild
☐	Moderate
☐	Severe
☐	Very Severe

21.5 Appendix E: Patient Global Impression of Change (PGI-C) Hearing Loss Scale

Instructions: Please choose the response below that best describes overall *change in your hearing loss since you were administered the study medication*.

☐	Much better
☐	A little better
☐	No change
☐	A little worse
☐	Much worse

21.6 Appendix F: Patient Global Impression of Change (PGI-C) Daily Impacts Scale

Instructions: Please choose the response below that best describes overall *change in the impact of your hearing loss on your daily activities since you were administered the study medication*.

☐	Much better
☐	A little better
☐	No change
☐	A little worse
☐	Much worse